XVI. EUCARPIA

Capsicum and Eggplant Meeting

KECSKEMÉT • HUNGARY • 12-14.SEPT.2016

in memoriam
Dr. Alain Palloix

PROCEEDINGS

Editors:
Katalin Ertsey-Peregi
Zsuzsanna Füstös
Gábor Palotás
Gábor Csilléry

Proceedings

of

XVIth EUCARPIA Capsicum and Eggplant Working Group Meeting
in memoriam Dr. Alain Palloix

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Table of contents

ORAL PRESENTATIONS

Opening session

Father of vitamin C, Albert Szent-Györgyi (1893-1986) and his time. Science, creativity and society behind a Nobel Prize winner (1937)

Miklós G. Fári[1,2], József Popp[2,3], Éva Domokos-Szabolcsy[1]

[1] *Department of Agricultural Botany, Plant Physiology and Biotechnology, University of Debrecen, Debrecen, Hungary*

[2] *Ereky Foundation, Debrecen, Hungary*

[3] *Faculty of Economics and Business, Institute of Sectoral Economics and Methodology, University of Debrecen, Debrecen, Hungary*

Abstract

From the beginning of the 20th century the basic biological research topics were discovery, isolation and characterisation of the "accessory food factors", or "vitamins". A few years ago the present authors and his co-workers sought to understand better the historical linkage between the father of vitamin C, Albert Szent-Györgyi's biochemical researches focused on, i.e. the high vitamin C content of pepper and the great figures of the physiological chemistry. Then, our studies expanded also toward the creation of the first concept "biotechnology", the contribution of his countryman, Karl Ereky who created the very first concept of biotechnology, two decades earlier the historical contribution of Szent-Györgyi. This study deals with the history of science and summarises the contribution of Hungarian experts dealing with pepper between 1849 and 1949. The study focuses on once known, but since forgotten fellow countrymen of this genius, researchers who were studying pepper and constituted the foundation of the pyramid at the top of which stands Albert Szent-Györgyi and his Nobel prize. Our aim was to find researchers, data and communications which shed new light on the forgotten prehistory of modern age pepper research (medical science, biochemistry, botany, genetics – breeding, industrial use and culture). We dedicate our work to the memory of Albert Szent-Györgyi, who passed away 30 years ago on 22nd October 1986 in Woods Hole, USA.

1. Introduction

The first half of the last century was one of the most intellectually productive cultures that the world has ever seen. From as early as 1898, following the famous paper of the British biochemist, *Sir William Crookes (1832-1919)*, the idea of leading scientists was that developed societies could not be supplied with sufficient food by means of the peasants' obsolete systems and tools. Instead, natural sciences and technologies should be purposefully applied to modern agricultural practice. Across the Europe and USA, the leading biochemists such as *F.G. Hopkins, J.C. Drummond* in Great Britain, *T.B. Osborn, L. Mendel, E.V. McCollum* in the USA, and *F. Haber, E. Fischer* and *E. Abderhalden* in Germany founded a very new branch of modern biology called "*physiological chemistry*". *Albert Szent-Györgyi*, the student of Hopkins also belongs to this group. In 1937, Szent-Györgyi was awarded the Nobel prize, a huge success in which pepper played a key role. Albert Szent-Györgyi passed away 30 years ago on 22nd October 1986 in Woods Hole, USA.

2. Aims and methods of research

This study deals with the history of science and summarises the contribution of Hungarian experts studying with pepper between 1849 and 1949. Our aim was to summarise the pepper-related prehistory of medical science, biochemistry, botany, genetics – breeding, industrial use and culture in the country of Albert Szent-Györgyi. We performed a systematic review during which we evaluated around 1 000 primary bibliographical sources in English, German, Spanish, Italian, Portuguese, French and grey literature in Hungarian. We were looking for answers to three questions: (1) What kind of pepper research events did take place between physicians and chemists in Hungary before Albert Szent-Györgyi appeared?; (2) What kind of impact did the Nobel prize of Albert Szent-Györgyi have on the ongoing pepper research at the time in Hungary?; (3) Was there any personal, professional and/or intellectual relationship between Károly Ereky who was the first ever to come up with biotechnology as a separate branch of science and Albert Szent-Györgyi?

3. Albert Szent-Györgyi, the discovery of vitamin C and the Nobel Prize

The 8th November 1937 issue of The Time was titled "Medicine: Pepper Prize". The famous journal communicated the following facts to the world: "*In Stockholm last week a committee of Swedish doctors was deciding whether to give the 1937 Nobel Prize ($40,000) for Medicine to: 1) Biochemist Albert Szent-Györgyi of the Hungarian University of Szeged who discovered that a certain acid (ascorbic) in the adrenal glands of healthy men and animals had the same beneficial effect as Vitamin C contained in oranges and lemons; 2) Biochemist Walter Norman Haworth of Birmingham (England) University, who analysed the chemical structures of Vitamin C and the ascorbic acid which Professor Szent-Györgyi isolated; or 3) Biochemist Paul Karrer...*"

Figure 1:
Szent-Györgyi Albert (1893-1986) and his "Pepper-Nobel-prize"

The then 44-year-old Szent-Györgyi received the following brief official notice from the Nobel Committee: "*for his discoveries in connection with the biological combustion processes, with special reference to vitamin C and the catalysis of fumaric acid*".

The solemn title of The Time was meant to announce the beginning of a new medical-biochemical era in medical science. Science eventually put an end to a 3-century-long riddle. Although it was not specified in the short explanation of the Nobel Committee, everyone knew that the key factors of research were sweet pepper and spice pepper, more specifically Szeged

pepper. Therefore, vitamin C became the first known representative of this era of vitamins: vitamin C is the first vitamin isolated in a crystalline form, it was the first vitamin whose chemical structure was explored and the first vitamin which was produced in an industrial process. Nowadays, the global production of vitamin C increases steadily, currently exceeding 600 million USD. According to certain reports, China represents 90% of the global vitamin C production. The market of functional vitamin C products produced with non-chemical synthesis also significantly increases. Albert Szent-Györgyi was one of the most successful medical biochemists of the 20th century science who managed to create something permanent. Szent-Györgyi had a long and adventurous life which reached across countries and continents and melted into the great changes of global politics, ideology and science of the 20th century, including the never before seen revolution of physiological science. The contemporaries of Szent-Györgyi also recognised his outstanding skills of creation; they considered him to be a brilliant researcher who is following his own way and an extremely intelligent and multi-sided personality.

Many authors wrote about the secret of Szent-Györgyi's exceptional success in many ways and thousands of studies were written about him. American cancer researcher *Ralph W. Moss* is the author of one of the most important of these studies titled "*Free radical: Albert Szent-Györgyi and the battle over vitamin C"* (MOSS, 1988), a captivating and stirring biography of Szent-Györgyi which is recommended for everyone dealing with biology today.

4. The beginning of analytical chemical and medical pepper research before Szent-Györgyi's Nobel Prize

According to Moss' biography, Szent-Györgyi conducted research on the border of biochemistry and medical science in several European countries and the USA since 1919. Sweet and spice pepper played a key role in the research and innovation Szent-Györgyi carried out in Szeged between 1931 and 1945. It goes without question that it was the most significant period in the history of science when chemists and physicians studied pepper as a team.

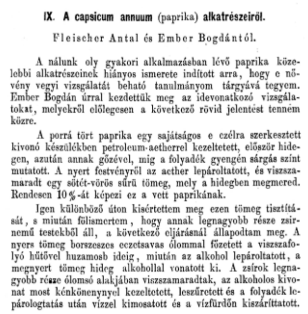
However, it seems that there are two forgotten examples of collaboration between chemist-analysts and medical physiologists between 1862 and 1877. One of these was the joint research of *Imre Poór (1823-1897)*, a dermatology professor and *Emil Felletár (1834-1917)* a pharmaceutical chemist on *Tinctura capsici* (pálinka with spice pepper) (POOR, 1865). Poór was probably the pioneer of the two of them, as he demonstrated the traditional observation that properly produced pepper pálinka (*Tinctura capsici*) has a fever reducing effect in malaria-type illnesses, based on a medical research performed in Budapest on more than 200 patients between 1861 and 1865. Poór described this series of experiments the following way (POOR, 1865): "*The observed medicinal effect as a result of taking pepper convinced me to widen the range of experiments and, since I also performed a successful experiment on myself when I was suffering from bouts of fever, I started to use pepper in my clinical practice and also outside of it, first against plain shivers and then against its various different forms. Based on my experiments performed on more than 200 patients suffering from fever during the subsequent three years, I make the following conclusions:*

1) On a strict diet, taking 15-60 grains of pepper per day eases intermittent fever bouts occurring every day or every three or even four days without acute and inflammatory complications. I do not remember any case in which pepper used for pure, that is, acute inflammatory complication-free intermittent fever was not effective. 2) The chronic inflammatory complications of intermittent fever, i.e., furunculous, impetigo, herpes, eczema and prurigo can be eased if pepper is used internally, as long as there is no organic degeneration in the internal viscera. In order to prove these statements, I have been showing

dermatology patients successfully cured with pepper to the Royal Budapest Physicians' Association …. The beneficial effect that we could elicit with pepper makes us suppose that pepper, more specifically, the ripened fruit of the pepper plant contains an effective alkaloid which is called capsaicin by those who wish to validate the word before the concept. My chemist friend Emil Felletár was kind enough to perform the chemical breakdown of the alkaloid and arrived at the conclusion that the small capsules of capsaicin hold a coniine-smelling alkaloid." (POOR, 1865; FELLETÁR, 1868).

In Hungary, the other medical-chemist pepper research took place in 1877 in Kolozsvár (Cluj-Napoca, Romania), performed by pharmaceutical chemist *Antal Fleischer (1845-1877)* and physician *Endre Hőgyes (1847-1906)* (FLEISCHER & EMBER, 1877; HŐGYES, 1877). In Cluj-Napoca, Antal Fleischer produced a pepper extract with lead acetate purification following its extraction from pulverised pepper with petroleum and drying (FLEISCHER & EMBER, 1877). Fleischer and his pharmaceutical student *Bogdán Ember* were also focusing on determining the chemical composition of the extract. As the chemical formula ($C_{25}H_{45}O_4$) suggests, the extract obtained by Fleischer is not capsaicin, but probably a pepper pigment cocktail unknown at the time. However, no subsequent chemical experiments were carried out by Fleischer, due to his early death. The product was named as *Fleischer's pepper oil* by Hőgyes, Fleischer's physician colleague and he was keen on continuing Fleischer's research.

Hőgyes, who was nominated for Nobel Prize in 1901 and was also experienced in bacteriology, was the first to perform modern and complex medical physiological trials on humans and animals using the pepper sample given to him by Fleischer.



IX. A capsicum annuum (paprika) alkatrészeiről.

Fleischer Antal és Ember Bogdántól.

A nálunk oly gyakori alkalmazásban lévő paprika közelebbi alkatrészeinek hiányos ismerete indított arra, hogy e növény vegyi vizsgálatát beható tanulmányom tárgyává tegyem. Ember Bogdán úrral kezdettük meg az idevonatkozó vizsgálatokat, melyekről előlegesen a következő rövid jelentést tennem közre.

A porrá tört paprika egy sajátságos e czélra szerkesztett kivonó készülékben petroleum-aetherrel kezeltetett, először hidegen, azután annak gőzével, mig a folyadék gyengén sárgás színt mutatott. A nyert festvényről az aether lepároltatott, és visszamaradt egy sötét-vörös sűrű tömeg, mely a hidegben megmered. Rendesen 10%-át képezi ez a vett paprikának.

Igen különböző úton kisértettem meg ezen tömeg tisztítását, s miután fölismertem, hogy annak legnagyobb része zsirnemű testekből áll, a következő eljárásnál állapodtam meg. A nyers tömeg borszeszes eczetsavas ólommal főzetett a visszafolyó hűtővel huzamosb ideig, miután az alkohol lepároltatott, a megnyert tömeg hideg alkohollal vonatott ki. A zsírok legnagyobb része ólomsó alakjában visszamaradtak, az alkoholos kivonat most kénkönenynyel kezeltetett, leszűretett és a folyadék lepárologtatás után vízzel kimosatott és a vízfürdőn kiszáríttatott.

Figure 2. Participants of the cooperation between chemists and physicians. Antal Fleischer's article from 1877: "On the components of Capsicum annum (pepper)" (left). The article of Endre Hőgyes (in the middle) from 1877, titled "Data on the physiological effect of the components of Capsicum annum (pepper) (right).

Hőgyes compared three samples: his research focused on the physiological impacts of spice pepper powder, capsaicin extracts received from foreign pharmacists and *Fleischer's purified pepper oil*. He examined the effect of these products on the digestive system, the skin, the subcutaneous tissue, the mucous membrane and varicose veins on himself, his assistant, dogs and rabbits, as well as on prepared frog heart and frog muscle. Hőgyes also tested a gelatine capsule product filled with pepper oil and concluded that Fleischer's extract has no bactericide effect (HŐGYES, 1877).

5. Genetic, physiological and medical biological pepper research in Szent-Györgyi's age

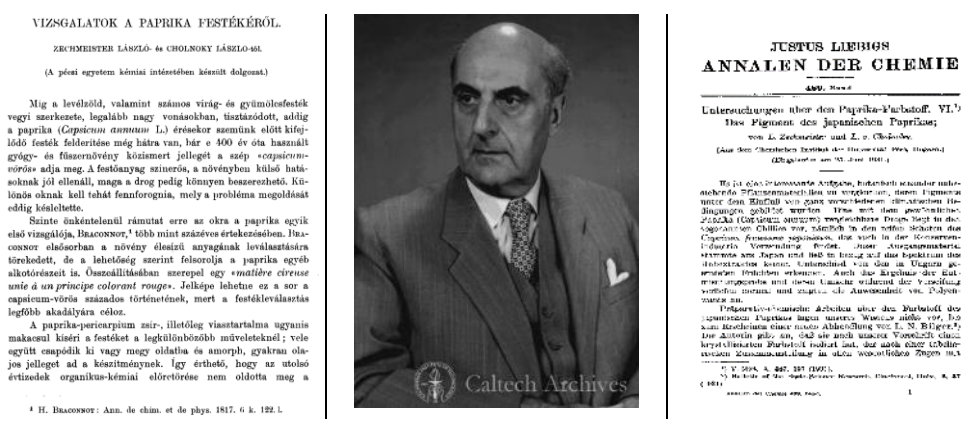
In Hungary, it is commonly known that Szent-Györgyi's Nobel Prize had an enormous impact on scientific research in general and especially on the Hungarian research and trade of spice pepper.

At the same time, one of the reasons for Szent-Györgyi's success concerning vitamin C was that intensive research and development activity had been carried out in relation to the so-called pepper cult decades before he appeared as a scientist in Hungary. One could also say that this situation was bound to end in huge success, but it also called for a really significant scientist. A scientist who also possessed knowledge which belonged to the top league of the international medical-biochemical scientific elite, as well as proper networking and original intuition.

It is interesting to note that at least two dozens of outstanding chemists were active in the age of Szent-Györgyi in Hungary. Of them, *László Zechmeister (1889-1972)* was also dealing with pepper and he was the first of those who revealed the pigments of pepper between 1927 and 1932 in a publication co-authored with *László Cholnoky (1899-1967)* in Hungarian in 1926 (ZECHMEISTER & CHOLNOKY, 1926). The name of *Ilona Banga (1906-1998)* has to be mentioned here because she was the biochemist who published the mass extraction of vitamin C from pepper in 1934 (BANGA & SZENT-GYÖRGYI, 1934). Professor *Norman Haworth (1883-1950)* in Birmingham also received a sample of the Szeged vitamin C, resulting in the other "Pepper Nobel prize" for the discovery of the ascorbic acid structure (SZENT-GYÖRGYI & HAWORTH, 1932).

Several of Szent-Györgyi's contemporary scientists were outstanding physicians dealing with the human physiological role of pepper. Professor *István Rusznyák (1889-1974)* revealed the value of bioflavonoid content of pepper in 1936 and named it vitamin P (RUSZNYÁK, SZENT-GYÖRGYI, 1936).

In addition, Szent-Györgyi had outstanding plant geneticist colleagues. Of them, *Barna Győrffy (1911-1970)* was the first to produce pepper polyploids with colchicine (GYŐRFFY, 1939) and the first to research the inheritance of the vitamin C content of pepper (GYŐRFFY, 1941; GYŐRFFY & PATKA, 1942).



VIZSGALATOK A PAPRIKA FESTÉKÉRŐL.

ZECHMEISTER LÁSZLÓ- és CHOLNOKY LÁSZLÓ-tól.

(A pécsi egyetem kémiai intézetében készült dolgozat.)

Míg a levélzöld, valamint számos virág- és gyümölcsfesték vegyi szerkezete, legalább nagy vonásokban, tisztázódott, addig a paprika (*Capsicum annuum* L.) érésekor szemünk előtt kifejlődő festék felderítése még hátra van, bár e 400 év óta használt gyógy- és fűszernövény közismert jellegét a szép «*capsicum-vörös*» adja meg. A festőanyag szinerős, a növényben külső hatásoknak jól ellenáll, maga a drog pedig könnyen beszerezhető. Különös oknak kell tehát fennforognia, mely a probléma megoldását eddig késleltette.

Szinte önkéntelenül rámutat erre az okra a paprika egyik első vizsgálója, BRACONNOT,[1] több mint százéves értekezésében. BRACONNOT elsősorban a növény élesítő anyagának leválasztására törekedett, de a lehetőség szerint felsorolja a paprika egyéb alkotórészeit is. Összeállításában szerepel egy «*matière cireuse unie à un principe colorant rouge*». Jelképe lehetne ez a sor a capsicum-vörös százados történetének, mert a festéklevalasztás legfőbb akadályára céloz.

A paprika-pericarpium zsír-, illetőleg viasztartalma ugyanis makacsul kíséri a festéket a legkülönbözőbb műveleteknél; vele együtt csapódik ki vagy megy oldatba és amorph, gyakran olajos jelleget ad a készítmények. Így érthető, hogy az utolsó évtizedek organikus-kémiai előretörése nem oldotta meg a

[1] H. BRACONNOT: Ann. de chim. et de phys. 1817. 6 k. 122. l.

JUSTUS LIEBIGS
ANNALEN DER CHEMIE

Untersuchungen über den Paprika-Farbstoff. VI.[1]
Das Pigment des japanischen Paprikas;
von L. Zechmeister und L. v. Cholnoky.

Figure 3. The first papers of László Zechmeister and László Cholnoky on pepper pigments (left and right) and László Zechmeister (in the middle)

6. Relationship between Albert Szent-Györgyi and Károly Ereky, the father of the term "biotechnology"

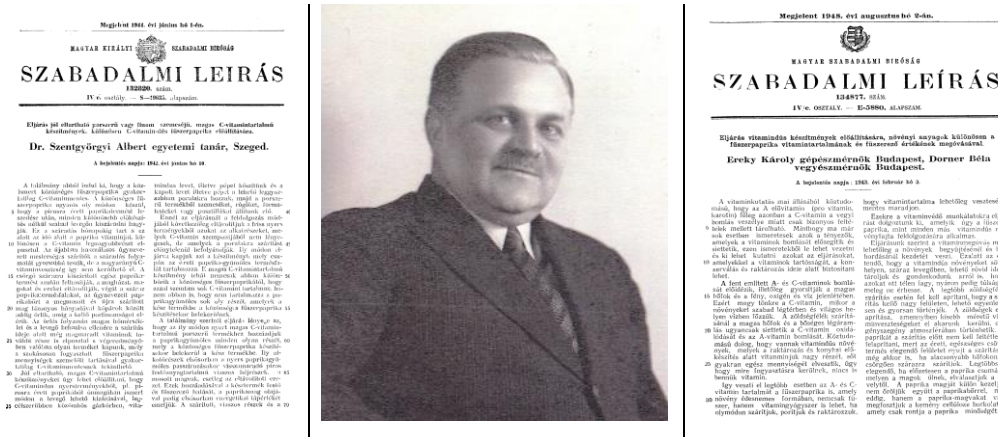
Science historian *Robert Bud* helped the name of *Károly Ereky (1878-1952)*, father of the word "biotechnology" to become widely known in a paper published by Nature (BUD, 1989), when the British scientist found the first book in the world dealing with biotechnology written by Ereky ("*Biotechnologie*", EREKY, 1919).

We found 438 publications by Ereky in Hungary, written in German, English, French and Hungarian and published between 1898-1945. These papers reshape the 20th century prehistory of biotechnology (FÁRI & KRALOVÁNSZKY, 2006). Ereky was the contemporary of Szent-Györgyi, who might know the name of Ereky. In the autumn of 1919, when Albert Szent-Györgyi moved to Budapest, Károly Ereky was the Minister of Food. We found a connection between Szent-Györgyi and Károly Ereky in relation to spice pepper. Szent-Györgyi's discoveries concerning the vitamin C content of pepper were followed by significant practical work. In 1932, the production of raw, high vitamin C content pepper mash "Vitapric" was launched. In October 1932, the pharmaceutical company Chinoin applied for a patent for the procedure of vitamin C extraction from pepper (ANONYMUS, 1932). Later, Szent-Györgyi obtained three patents for the production of vitamin products from pepper (SZENT-GYÖRGYI, 1936; 1941; 1942).

There is a logical connection between Szent-Györgyi's patent obtained in 1942 and Ereky's patents obtained between 1926 and 1928. The pepper processing procedure used by Szent-Györgyi in 1942 is the further development of the vegetable and fruit flour production process popularised by Ereky in England. Ereky also obtained a patent in 1943 – the same time as Szent-Györgyi – for a new grinding procedure of pepper (EREKY & DORNER, 1943). It is a further interesting fact that there is also an indirect connection between the Szent-Györgyi and Ereky families. *István Ereky (1876-1943)*, the older brother of Károly Ereky had been a professor colleague of Szent-Györgyi at the University of Szeged for 15 years. István Ereky knew Szent-Györgyi even in 1919. As a professor, he worked in Bratislava (Slovak for Pozsony) at the Erzsébet University, where Szent-Györgyi was the teaching assistant of professor *Géza Mansfeld (1882-1950)*. István Ereky became the rector of the university in 1938-1939, before Szent-Györgyi. In 1939, István Ereky, as the rector of the University, notified Szent-Györgyi that the University elected him to be the Upper House representative of the Hungarian National Assembly.

The intellectual relationship between Szent-Györgyi, the scientist and Ereky, the axiomatic-synthesising mechanical engineer-economist is edifying for succeeding generations. Both scientists supplied their ideas from the same scientific sources of knowledge, i.e. from the physiological chemistry of that time.

However, their orientation was different. One side the goal of *Szent-Györgyi* was to understand the "*fundamental aspects of the Living State*". On the other side, *Ereky*'s aspects were the "*uses of Life*". Both strategies were strengthened with considerable spectrum of knowledge, novelty and creativity. In addition, *Szent-Györgyi*'s work was basically permeated with sophisticated philosophy of science. *Ereky* focused on the economic philosophy of emerging biology-based technologies.



Megjelent 1944. évi június hó 1-én.

MAGYAR KIRÁLYI SZABADALMI BIRÓSÁG

SZABADALMI LEIRÁS

132020. szám.

IV/c. osztály. — S—19625. alapszám.

Eljárás jól eltartható porszerű vagy finom szemcsésjű, magas C-vitamintartalmú készítmények, különösen C-vitamin-dús fűszerpaprika előállítására.

Dr. Szentgyörgyi Albert egyetemi tanár, Szeged.

A bejelentés napja: 1942. évi június hó 10

Megjelent 1948. évi augusztus hó 2-án.

MAGYAR SZABADALMI BIRÓSÁG

SZABADALMI LEÍRÁS

134877. SZÁM.

IV/c. OSZTÁLY. — E-5880. ALAPSZÁM.

Eljárás vitaminokban készítmények előállítására, növényi anyagok különösen a fűszerpaprika vitamintartalmának és fűszerező értékének megóvásával.

Ereky Károly gépészmérnök Budapest, Dorner Béla vegyészmérnök Budapest.

A bejelentés napja: 1943. évi február hó 3.

Figure 4: The patent obtained by Károly Ereky (1878-1952, in the middle) for spice pepper grinding under protective gauze (1943, left) and Szent-Györgyi's patent (1942, right)

7. Chronology of pepper research in Hungary (1848-1949)

As it was already mentioned, the pioneering pepper research of Albert Szent-Györgyi had an intensive impact on his age. Consequently, we put together the brief chronology of significant Hungarian scientists and their work related to pepper between 1848-1949 (*Tables I and II*).

YEAR	EVENT	YEAR	EVENT
Medical Science		**Biochemistry**	
1848 - 1850	An unknown Hungarian produced and distributed the product *Tincturus paprikus* (pálinka with pepper, Tincturus Pepper) in the USA.	1868	In Budapest, chemist Emil Felletár researches the chemical composition of capsaicin in spice pepper.
1861 - 1864	In Budapest, dermatologist Imre Poór conducts medical research with *Tinctura capsici*.	1877	In Cluj-Napoca, chemist Antal Fleischer develops a new method for the separation of spice pepper pigments and determines the chemical formula of the sample.
1877 – 1878	In Budapest, physician Endre Hőgyes is the first to perform complex physiological-biological experiments with pepper extract on humans and animals. The pepper pigment was produced by chemist Antal Fleischer.	1893 - 1895	In Budapest, chemist Béla Bittó analyses the chemical composition of pepper varieties.
		1926 / 1931	In Pécs, László Zechmeister and László Cholnoky achieve new findings in the chemical research of pepper pigments
1933-1934	In Szeged, internist and clinical physician László Berkessy studies the effect of pepper on gastric functions.	1931 - 1933	In Szeged, American guest researcher Joe Sviberly of Hungarian origin performs biological experiments with hexuronic acid on guinea pigs. Szent-Györgyi concludes that hexuronic acid and vitamin C are the same.
1932	In Szeged, physician László Tokay diagnoses the illness "*capicismus*".		
1935	In Szeged, Ernő Obermayer summarises the findings of pepper research.	1932	In Szeged, Albert Szent-Györgyi detects the high vitamin C content of sweet pepper and starts to develop a mass extraction method.
1935	In Szeged, pulmonologist Ferenc Kováts diagnoses the reason for chronic bronchitis paprika splitters suffer from.	1933	In Birmingham, chemist Norman Haworth determines the chemical structure of vitamin C of the crystalline pepper received from Szeged.
1936	In Szeged, internist István Rusznyák researches the medical effect of the pepper mash "*Vitapric*".	1934	In Szeged, Ilona Banga is the first to produce a large quantity of vitamin C extract from sweet and spice pepper.
1939	In Leipzig, dietician Margarette Rauner publishes the book "*Das pepper*" which also describes the dietetic effects of Vitapric. The foreword is written by Szent-Györgyi.	1936	In Szeged, István Rusznyák and Albert Szent-Györgyi discover vitamin P in the pepper mash "*Vitapric*".
		1937	Albert Szent-Györgyi is awarded the "Pepper Nobel Prize"
1949	In Budapest, biochemist Miklós Jancsó and his wife are the first to report "capsaicin desensibilisation".	1938	In Pécs, L. Zechmeister is the editor of the first volume of the famous series "*Progress in the Chemistry of Organic Natural Products*".

Table I. The chronology of significant Hungarian scientists and their pepper-related work in medical biology and biochemistry (1848-1949)

YEAR	EVENT	YEAR	EVENT
Botany, Genetics, Breeding & Pepper Growing		1917	Establishment of the Pepper Experiment and Chemical Analysis Station in Kalocsa.
1905/ 1907	In Budapest, pharmacist Béla Augusztin performs research on the anatomy of pepper.	1922	The Hungarian Spice pepper Decree.
		1936	In Budapest, the educational film "*Pepper processing*" is made
1918	In Kalocsa, chemist Ernő Obermayer starts the breeding of spice pepper varieties from local resources.	1932-1939	*"Vitapric"* pepper mash experiments and large scale production from tomato type sweet pepper with Gábor Kamocsay (Hódmezővásárhely).
1926	The Biological Research Institute of Tihany was opened.	1938	László Benedek carries out experiments to colour the yolk of hen's egg with pepper
1928-1936	In Kalocsa, chemist Ferenc Horváth starts the breeding of capsaicin-free spice pepper and organises its production.	1936	The Hungarian patent of Albert Szent-Györgyi for the production of citrin from pepper
1933-1935	In Szeged, chemist István Szanyi examines the vitamin C content of Szeged spice pepper varieties.	1941	The Hungarian patent of Szent-Györgyi for preserving the vitamin content of pepper.
1933-1935	In Kalocsa, chemist Ferenc Horváth examines the vitamin C content of spice pepper varieties	1942	The patent of Albert Szent-Györgyi for the production of high vitamin content and medicinal spice pepper.
1935	László Benedek develops a new pigment detection method for spice pepper breeding.	1942	Establishment of Pepper Vitamin Production Plc. The enterprise was liquidated after World War II.
1936	In Budapest, the educational film "*Pepper production*" is made.	1943	The patent of Károly Ereky and Béla Dorner for preserving the vitamin and spice value of spice pepper.
1939	In Szeged, Béla Győrffy produces the first tetraploid pepper induced with colchicine.	1946	The open letter of Szent-Györgyi in Nature about the industrial support and the freedom of research in Hungary.
1941-1942	In Tihany, Barna Győrffy and Éva Patka are the first to research the possibility of sweet pepper breeding focusing on vitamin C content.	**Literature & Gastronomy**	
		1923	Geoffrey Moss' book "*Sweet pepper*" is published in London. The plot of the popular novel takes place in Budapest.
Industry & Processing Product Development		1932	Joe Pasternak's movie "Pepper" is made in Berlin. The leading character is Hungarian actress Gaál Franzisca.
1892	In Budapest, chemists Béla Bittó and Gyula Istvánffi develop scientific analytical methods against counterfeiting.	1934	In Budapest, Károly Gundel's famous book, "*The art of hospitality*", the golden book of Hungarian gastronomy is published. A separate section was awarded to spice pepper.

Table II. The chronology of significant Hungarian scientists and their pepper-related work in genetics, processing and culture (1892-1946)

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SESSION 1

Breeding strategies

Chairs: Paul Bosland, Giuseppe L. Rotino

Utilization of crop wild relatives in eggplant pre-breeding for adaptation to climate change

Jaime Prohens[1], Hemal Fonseka[2], Auguste Kouassi[3], Santiago Vilanova[1], Ramya Fonseka[4], Abou Kouassi[3], Pietro Gramazio[1], Francisco J. Herraiz[1], Tharanganee Welegama[2], Brice Kouassi[3], Ana Fita[1], Adrián Rodríguez-Burruezo[1], Mariola Plazas[1]

[1] *Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, València, Spain*

[2] *Horticultural and Crop Research and Development Institute, Peradeniya, Sri Lanka*

[3] *Laboratory of Genetics, Félix Houphouët-Boigny University, Abidjan, Ivory Coast*

[4] *Department of Crop Science, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka*

Abstract

Crop wild relatives represent sources of variation of interest for adaptation of eggplant (*Solanum melongena*) to climate change. We are using wild relatives for eggplant pre-breeding for tolerance to climate change, in particular to drought, by developing a set of introgression lines (ILs) from *S. incanum* into the genetic background of *S. melongena*. The ILs have also been hybridized to local varieties of Southeast Asia (Sri Lanka) and Western Africa (Ivory Coast) in order to transfer the introgressions to local varieties from these regions. In addition, we have initiated the development of two new collections of ILs between *S. melongena* on one side and *S. insanum* and *S. dasyphyllum* on the other. Three Sri Lankan and three Ivorian local varieties have been crossed with 26 accessions of 15 wild eggplant relatives from the primary, secondary and tertiary genepools. Interspecific hybrids have been obtained with all wild species evaluated, except with *S. sisymbriifolium*. For the interspecific hybrids with the the tertiary genepool species *S. torvum* and *S. elaeagnifolium*, which have resistance to many diseases (*S. torvum*) and tolerance to drought (*S. elaeagnifolium*), embryo rescue was needed. Interspecific hybrids have been backcrossed to the cultivated parents and first backcross generations have been obtained with nine species from the primary and secondary genepools. These materials are going to be tested and selected for tolerance to drought. Our works are aimed at an increased utilization of wild crop relatives in eggplant breeding and we hope that the materials obtained will contribute to broadening the genetic base of eggplant and to the development of a new generation of drought resistant eggplant varieties.

1. Introduction

Changes in the agricultural environments caused by climate change are expected to have a major impact on the productivity of crops. Eggplant (*Solanum melongena* L.) is one of the most important vegetables, especially in tropical and subtropical climates, which are areas expected to be severely affected by climate change [1,2]. One of the major challenges posed by climate change is increased drought stress, which may affect severely the yield of eggplant [3]. Amazingly, many wild relatives of eggplant grow in areas affected by drought, like desertic and semi-desertic areas [4]. Some of these wild relatives can be hybridized with eggplant, although with different degrees of success [5-8]. In order to develop new varieties of eggplant adapted to

drought, we are participating in an initiative for using wild relatives for adaptating crops to climate change [9]. Our pre-breeding programmes involve the development of materials of eggplant carrying introgressions from different wild relatives that allow the development of a new generation of eggplant varieties adapted to climate change. These include the development of sets of introgression lines (ILs) and advance backcross materials.

The development of sets of ILs is a powerful tool for breeders [10]. In the case of eggplant (*Solanum melongena* L.) there are some ILs obtained with the cultivated scarlet (*S. aethiopicum* L.) and with *S. linneanum* Hepper & P.-M.L. Jaeger that are resistant to *Verticillium* and *Fusarium* and that have a fruit composition similar to the cultivated eggplant [7,11]. However, to our knowledge, there are no sets of eggplant ILs with a complete coverage of the genome of the donor species. Therefore, the development of new sets of ILs in eggplant will provide breeders with pre-bred materials that can be of interest for selecting materials adapted to climate change challenges as well as to other traits of interest for breeders. Also, these materials are of great relevance for the genetic dissection of these traits [10].

The success of interspecific hybridization in eggplant with its wild relatives depends on the species involved, the specific accession of the wild species or of the cultivated eggplant, and the direction of the cross [5-8,12,13]. In this respect, hybrids with the only species of the primary genepool (*S. insanum* L.) [4] are easily obtained and many seeds are obtained per successful cross [8,14]. Regarding secondary genepool species, which comprise a large number of African and Asian species [4], there are many differences in the success of the hybridization and fertility of the hybrids [5-8,12,13]. Also, interspecific hybrids have been reported between eggplant and tertiary genepool species *S. torvum* Sw., although the hybrids were highly sterile [15].

Here we present the activities we have performed up to now for the development of pre-breeding materials of eggplant with introgressions from wild species, in particular obtaining complete sets of ILs with wild species from the primary and secondary genepools, as well as obtaining hybrids and backcrosses to a larger number of wild eggplant relatives from the primary, secondary and tertiary genepools. Earlier works performed dealing with obtaining interspecific hybrids reported in Plazas et al. [8] are updated here with new data.

2. Material and methods

2.1. Development of introgression lines

Advanced backcrosses (BC4 and BC5, as well as some BC2) of *S. melongena* accession ANS26 with introgressions of *S. incanum* MM577 that had been subjected to marker assisted selection were selfed in order to select plants homozygous for the desired *S. incanum* introgressions. In total, more than 400 individuals were genotyped with the same SNP and SSR markers used for the marker assisted selection of the BC materials. A selection was performed of homozygous plants for the desired fragments. These homozygous plants were subjected to a new cycle of selfing in order to obtain the ILs. In addition, they were crossed with two local varieties from Ivory Coast and Sri Lanka (accessions MEL1 and MEL5, respectively) in order to introgress desired parts of the genome of *S. incanum* in the genetic background of local varieties of the Occidental (Western Africa) and Oriental (Southeast Asia) types [16].

In order to develop new sets of introgression lines, interspecific hybrids between two *S. melongena* accessions and *S. insanum* and *S. dasyphyllum* have been backcrossed to the *S. melongena* parents in order to obtain the BC1 generation.

2.2. Interspecific hybridization and backcrossing

A total of six accessions of *S. melongena* (MEL1 to MEL6) have been used as cultivated parents for interspecific hybridization with 26 accessions of 15 wild species from the primary (*S. incanum*), secondary (*S. anguivi* Lam., *S. campylacanthum* Hochst. ex A. Rich, *S. dasyphyllum* Schumach. & Thonn., *S. insanum*, *S. lichtensteinii* Willd., *S. lidii* Sunding, *S. linnaeanum*, *S. pyracanthos* Lam., *S. tomentosum* L., *S. vespertilio* Aiton, and *S. violaceum* Ortega) and tertiary (*S. eleaegnifolium* Cav., *S. sisymbriifolium* Lam., and *S. torvum*) genepools. Accessions MEL1 to MEL3 are from Western Africa (Ivory Coast), while accessions MEL4 to MEL6 from Southeast Asia (Sri Lanka).

Reciprocal crosses between the six *S. melongena* accessions and the 26 accessions of wild relatives have been performed during 2014 and 2015 in three countries (Spain, Ivory Coast and Sri Lanka). Fruits obtained after crossing with primary and secondary genepool species were allowed to achieve physiological maturity, after which seeds were extracted. In the case of fruits obtained after crossing with tertiary genepool species, they were harvested after two weeks to one month after pollination and embryos were extracted and cultivated as indicated in Manzur et al. [17]. First backcrosses to *S. melongena* were obtained in 2015 with those hybrids that could be obtained in 2014. The procedure followed was the same than with interspecific hybridization.

Hybridity of putative interspecific hybrids was confirmed morphologically at the seedling stage and also with molecular SNP markers [8]. Pollen viability of *S. melongena* and interspecific hybrids was evaluated with fluorescein diacetate [18].

3. Results

3.1. Development of introgression lines

The selfed plants of the backcross generations BC2, BC4 and BC5 (i.e., the BC2S1, BC4S1 and BC5S1 generations) were genotyped with SNP markers that allowed detecting homozygous introgressions of *S. incanum*. As a result, over 60 plants that allowed a maximum coverage of the *S. incanum* genome were selected. The selected BC5S1, BC4S1 and BC2S1 plants represented a total of 39 ILs with introgressed fragments distributed in the 12 chromosomes of eggplant (Table 1). The number of ILs per chromosome ranged between one for chromosomes 10 and 11 and six for chromosome 9 (Table 1).

Chromosome	ILs	Hybrids with MEL1	Hybrids with MEL5
Chr. 1	3	2	3
Chr. 2	5	2	1
Chr. 3	4	1	1
Chr. 4	4	2	3
Chr. 5	2	2	2
Chr. 6	5	3	3
Chr. 7	3	1	1
Chr. 8	3	0	0
Chr. 9	6	3	0
Chr. 10	1	0	1
Chr. 11	1	0	0
Chr. 12	2	2	2
Total	39	18	17

Table 1:
Number of introgression lines (ILs) with introgressions of S. incanum MM577 in the genetic background of S. melongena ANS26 and hybrids between the ILs and local varieties of S. melongena MEL1 and MEL5

As a result of the hybridization of the selected plants with the desired introgressions of *S. incanum* in homozygosis, a total of 18 and 17 hybrids were obtained between the ILs and *S. melongena* accessions MEL1 and MEL5, respectively. Hybrids with ILs were obtained for all chromosomes, except 8 and 11 for both MEL1 or MEL5, and for chromosome 10 for MEL1 and chromosome 9 for MEL5 (Table 1).

3.2. Interspecific hybridization and backcrossing

A total of more than 2300 crosses have been made in Spain, Ivory Coast and Sri Lanka throughout 2014 and 2015. As a result, seed (or plantlets obtained through embryo rescue in the case of hybrids between *S. melongena* with tertiary genepool species *S. elaeagnifolium* and *S. torvum*) of a total of 91 interspecific hybrid combinations have been obtained between cultivated eggplant and wild relatives (Table 2).

	Interspecific hybrids (2014 & 2015)		First backcrosses (2015)	
Wild species	**Ivorian accessions (n=3)**	**Sri Lankan accessions (n=3)**	**Ivorian accessions (n=3)**	**Sri Lankan accessions (n=3)**
Primary genepool				
S. insanum (n=3)	9	9	9	9
Total primary genepool	**9**	**9**	**9**	**9**
Secondary genepool				
S. anguivi (n=2)	6	6	3	3
S. campylacanthum (n=4)	2	5	0	0
S. dasyphyllum (n=1)	3	3	3	1
S. incanum (n=1)	2	2	0	2
S. lichtensteinii (n=2)	6	5	4	3
S. lidii (n=1)	3	3	0	1
S. linnaeanum (n=2)	4	6	0	2
S. pyracanthos (n=1)	2	0	2	0
S. tomentosum (n=1)	3	2	2	0
S. vespertilio (n=1)	1	0	0	0
S. violaceum (n=1)	3	2	0	0
Total secondary genepool	**35**	**34**	**14**	**12**
Tertiary genepool				
S. elaeagnifolium (n=1)	1	0	0	0
S. sisymbriifolium (n=2)	0	0	0	0
S. torvum (n=3)	2	1	0	0
Total tertiary genepool	3	1	0	0
TOTAL	**47**	**44**	**23**	**21**

Table 2:
Number of interspecific hybrid combinations between S. melongena accessions from Ivory Coast and Sri Lanka with accessions of wild species form the primary, secondary and tertiary genepools and number of first backcross generations of the interspecific hybrids to the cultivated S. melongena accessions.

Of these interspecific hybrids between cultivated eggplant and wild species, 18 are hybrids with the primary genepool species *S. insanum*, 69 with the 11 secondary genepool species used, and four with two out of the three tertiary genepool species (*S. elaeagnifolium* and *S. torvum*) (Table 2). For the hybrids of *S. melongena* with the latter tertiary genepool species, the hybrid plants could be obtained only after embryo rescue. The number of hybrid combinations obtained

in Spain, Sri Lanka and Ivory Coast, was of 64, 65 and 48, respectively.

All possible hybrid combinations between the six accessions of *S. melongena* and the three accessions of the primary genepool species *S. insanum* and the accessions of secondary genepool species *S. anguivi* (two accessions), *S. dasyphyllum* (one accession) and *S. lidii* (one accession) were obtained. On the other hand only one hybrid combination was obtained with tertiary genepool species *S. elaeagnifolium*. A similar number of interspecific hybrids was obtained with accessions from Ivory Coast and Sri Lanka, with a total number of 47 and 44 hybrid combinations with accessions from Ivory Coast and Sri Lanka, respectively (Table 2). Fruits obtained after hybridization of *S. melongena* with *S. sisymbriifolium* did not contain viable embryos for in vitro cultivation and in consequence no hybrids were obtained.

The morphology of interspecific hybrids was different from either parents, although it was generally more similar to the wild parents. The genotyping of hybrid plants with SNP markers polymorphic among parents confirmed the hybridity status of putative interspecific hybrids.

Pollen viability of the cultivated *S. melongena* and wild species of the three tertiary genepools and of interspecific hybrids between *S. melongena* and the primary genepool species *S. insanum* was generally high with average values above 60%. For interspecific hybrids with secondary genepool species a wide range of variation was observed for pollen viability, ranging from low values in some interspecific hybrid combinations (<5%) to high (>80%) in others. No determinations of pollen fertility could be made for interspecific hybrids with tertiary genepool species as no pollen could be extracted from hybrid with *S. torvum* and hybrids with *S. elaeagnifolium* have not flowered at the time of writing this manuscript.

Regarding backcrosses, more than 2000 crosses have been made in 2015 in the three countries (Spain, Ivory Coast and Sri Lanka) in 2015 using the interspecific hybrids obtained in 2014. In total, 44 first backcross generations to the *S. melongena* parents have been obtained (Table 2). Of these, 18 first backcrosses are with hybrids with the primary genepool species *S. insanum*, and 26 with the eight out of the 11 secondary genepool species (Table 2). In this respect, for *S. campylacanthum* and *S. vespertilio*, no first backcrosses could be attempted as no interspecific hybrids were obtained in 2014. Regarding tertiary genepool species, first backcrosses could only be attempted with the interspecific hybrids between *S. melongena* and *S. torvum*, as the only hybrid combination with *S. elaeagnifolium* was obtained in 2015. The fruits obtained for the backcrosses with the interspecific hybrids with *S. torvum* did not contain viable embryos, and therefore no first backcross generations were obtained. The number of first backcross combinations obtained in Spain, Sri Lanka and Ivory Coast, was variable, with 31, 25 and 8 hybrid combinations obatined, respectively. Some combinations were obtained in several countries, while others were obtained in just one of the countries.

All possible first backcrosses of the 18 interspecific hybrids between *S. melongena* and the three accessions of the primary genepool species *S. insanum* to the respective *S. melongena* parents were obtained (Table 2). For the backcrosses involving interspecific hybrids with secondary genepool species for which seed was obtained, the number of first backcross combinations ranged between one for interspecific hybrids with *S. lidii* to seven for interspecific hybrids *S. lichtensteinii*. As occurred with the interspecific hybrids, a similar number of first backcross combinations was obtained with accessions from Ivory Coast and Sri Lanka, with a total number of 23 and 21 first backcross combinations, respectively (Table 2).

Discussion

The development of eggplant materials with introgressions from wild species is one of the ways of increasing the variation available to the breeder [5,7]. Given the need to adapt crops to climate change, the use of the wild species as sources of variation in a pre-emptive breeding strategy [19] is a way to address the formidable challenges posed by climate change [9]. In this respect, many wild relatives of eggplant grow in areas where they are subjected to drought stress [4] and in consequence represent sources of variation of interest.

In our pre-breeding programme, we have obtained a set of ILs covering all the chromosomes of *S. incanum*, which grows in desertic areas [20], and therefore is expected to be highly tolerant to drought. These ILs are of great interest for breeders, as they are readily usable in breeding programmes for adaptation to climate change or other traits of potential interest, for studying the genetic basis of domestication traits, as well as for obtaining subILs [10, 21]. The ILs have also been hybridized with local varieties from Western Africa (MEL1) and South East Asia (MEL5) as a way to obtain introgression materials in the genetic background of local varieties of genetically differentiated Occidental and Oriental types [16,22]. All these materials are going to be tested for tolerance to drought in order to select the most promising ILs for breeding. Also, the works we have initiated for developing new introgression lines with *S. insanum*, the closest wild relative to *S. melongena* [20] and with *S. dasyphyllum*, the wild ancestor of the cultivated gboma eggplant (*S. macrocarpon* L.) [23] will contribute to increase the materials of eggplant potentially tolerant to climate change stresses.

The fact that we have been able to hybridize cultivated eggplant with 12 wild relatives from the primary and secondary genepools and obtaining first backcross generations with nine of them confirms that eggplant is amenable to interspecific hybridization breeding [5-8,12,13] and that there are ample prospects of using many eggplant wild species for breeding. Amazingly, we have also obtained interspecific hybrids with the tertiary genepool species *S. elaeagnifolium*, which is a weed that thrives in areas with low rainfall [23], and with *S. torvum*, which is very vigorous and resistant to multiple diseases [6]. Although hybrids with *S. torvum* had been reported before [6,7,15], this is the first report, to our knowledge, of hybrids of *S. melongena* with *S. elaeagnifolium*. However, no backcrosses to *S. melongena* of these tertirary genepool interspecific hybrids have been obtained so far.

The results of the hybridization and backcrossing reveal that there are differences among genotypes of eggplant in the success of hybridization and backcrossing, which we already observed [8]. Also, some differences were observed among countries in the success of interspecific hybridization and backcrossing. This indicates that some materials of eggplant may be more appropriate for successful introgression breeding and that environmental conditions play a major role in the success of obtaining certain hybrid combinations.

Overall, our results reveal that there is ample potential for introgression and use in breeding of eggplant wild relatives. The materials obtained are of great value for introgression breeding of eggplant and we expect that after selection, we will be able to develop new eggplant varieties adapted to drought, which is a major stress associated to climate change.

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New hot interspecific hybrid variety between *Capsicum annuum* L. and *Capsicum chinense* Jacq.

Zoltán Timár[1], Gábor Palotás[1], Gabriella Palotás[1], János Szarka[2], Béla Ágoston[1], Gábor Csilléry[3]

[1] *Univer Product Plc., Kecskemét, Hungary*

[2] *Pirospaprika Ltd., Budapest, Hungary*

[3] *Budakert Ltd., Budapest, Hungary*

Corresponding address: zoltan.timar@univer.hu

Abstract

Hungary is considered as heavy user country of pepper products, both in powder and crushed/creamy food seasoning forms. The production and consumption of latter one's is based on local varieties. The fruits of Hungarian hot varieties contain 50-250 mg/kg total capsaicinoids which provide same level of pungency in case of crushed or creamy products and cca. 300-1.500 mg/kg in case of powder products. An excellent, *Xanthomonas*-resistant (*Bs2*) *C. annuum* line were crossed with the best *C. chinense* lines which have good fertility and earliness. We tested the capsaicinoid content, earliness and fertility of F1 hybrids in greenhouse and open field too. The total capsaicinoid content of the best F1 hybrid was 12.000 mg/kg in average (measured from powder). The yield was more than 20 tons/hectar in open field. The name of the new interspecific hybrid is „**Unijol F1".** It has got the national registration of varieties in 2015.

1. Introduction

The paprika (*Capsicum*) genus is a collective name for species whose fruit contains capsaicinoid alkaloids, which cause a burning sensation. The consumption of hot peppers in Hungary is an important element of traditional and modern cuisine culture alike. One of the objectives of breeding hot paprika is to achieve the highest possible capsaicinoid content, that is still in line with consumer needs, in any given variety. Beyond a certain level of pungency, producing products of stable quality poses challenges. Certain producers satisfy this need with added hot extracts.

The total capsaicinoid content of Hungarian hot varieties measured in raw products typically varies between 50-250 mg/kg, while in ground form, between approximately 300-1,500 mg/kg. The *Capsicum chinense* species contains lines with substantially higher capsaicinoid content than domestic varieties, however, their cultivation in Hungary is limited (short growing season period, low average temperature), and special conditions are needed to ensure optimal crop quantity.

Since Univer Product Plc.'s traditional products do not contain added hot extracts, the company started to breed species with particularly high capsaicinoid content in order to satisfy this new consumer need. These high-capsaicinoid content varieties must be adapted to the environmental and economical conditions of Hungarian cultivation.

2. Material and method

In recent years, we received various *Capsicum chinense* lines from the USDA, the NMSU (Prof. Bosland) and Pepperfriends (Italy). These lines were selected on the basis of phenotype, earliness, fertility and nutritional values. Observations and measurements were made for biologically mature crops from heated greenhouses. We determined the capsaicinoid content of the paprika lines and varieties available to us and selected the lines and parent lines for cross-breeding. We essentially categorised the *Capsicum chinense* lines into two groups. The first group's average capsaicinoid content measured in dry-matter is approximately 10,000 mg/kg, with crop yields of around 0.08-0.4 kg per plant. The second group's average capsaicinoid content is approximately 30,000-50,000 mg/kg, with crop yields of around 0.01-0.27 kg per plant. These *C. chinense* male parent lines were crossed with a *Xanthomonas*-resistant (*Bs2* gene) condiment female parent line from a hot *Capsicum annuum* species [1].

We studied a total of ten hybrid combinations and examined these in unheated greenhouses. When selecting the plants, the primary aspect was high capsaicinoid content (Figure 1). Further tests of the 5 best performing hybrid combinations were carried out under open field conditions, with 4 replications. The plants were planted in black plastic covered beds with a density of 45,000 plants/ha. The irrigation and fertilization of the plants was performed using drip tapes. As part of the open field experiment, we also tested the qualities (fruit size [2], stem removability) that are essential for economical cultivation (Figures 2 and 3). We also performed the examination of harvested fruits in terms of nutritional value (Figures 4 and 5).

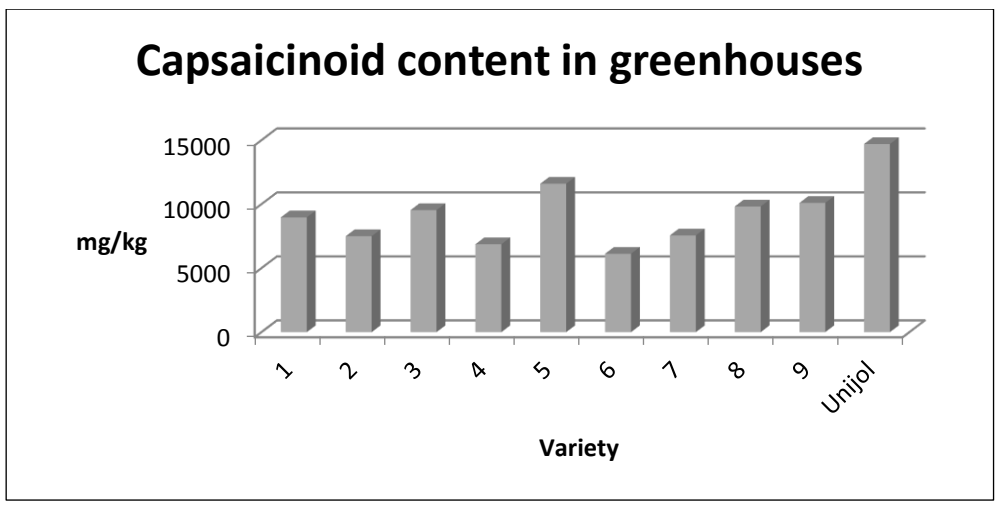

Figure 1:
Capsaicinoid content in greenhouses

Figure 2:
Crop from Unijol F1

Figure 3:
Crop from Variety "4"

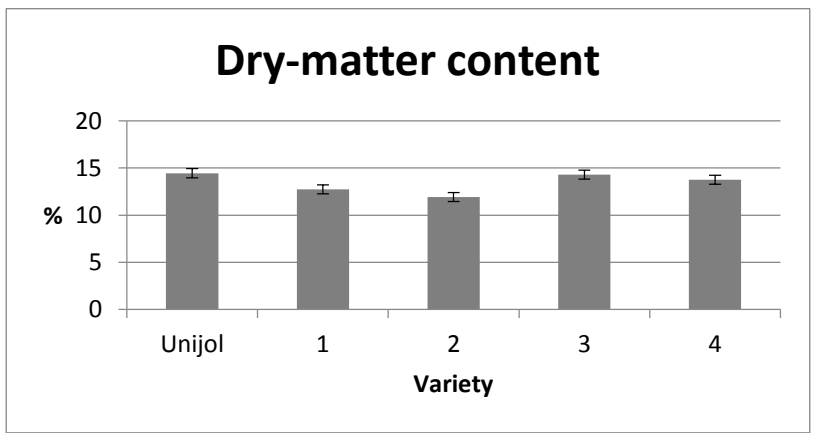

Figure 4:
Dry-matter content

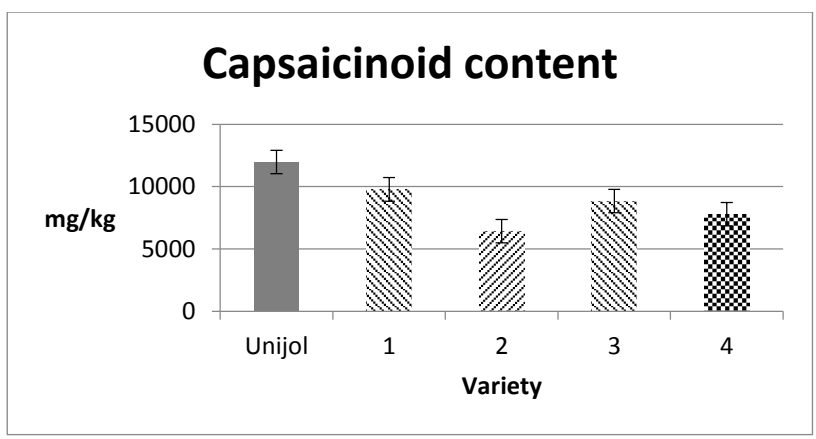

Figure 5:
Capsaicinoid content under open field conditions

3. Results

The capsaicinoid content of the hybrids under open field conditions varies between 6,413-11,968 mg/kg measured in dry-matter. The dry-matter content of crops varied between 11.92 % and 14.45 %, with no significant difference between the varieties. However, this was significantly lower than the values of traditional Hungarian condiment paprika varieties.

The total capsaicinoid content of the best performing hybrid combination measured in ground form was 11,968 mg/kg volt, significantly higher than the other varieties. In open field cultivation, crop yield was 20 t/ha. Its environmental needs are similar to that of traditional Hungarian condiment paprika, and does not require costly cultivation technology elements. It is resistant to the *Xanthomonas vesicatoria* bacterium, which has caused considerable damage under open field conditions. The new interspecific hybrid is called "Unijol F1", and it was entered into the national registration of varieties in 2015.

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Male sterility research in peppers at AVRDC – The World Vegetable Center

Shih-wen Lin, Hsueh-ching Shieh and Sanjeet Kumar

AVRDC – The World Vegetable Center, Shanhua, Tainan, Taiwan

Abstract

Male sterility is the most commonly used mechanism in hybrid breeding of a number of crop plants including peppers (*Capsicum annuum* L.). In hot pepper (syn. chili pepper) and sweet pepper, nuclear male sterility (genic male sterility, GMS) and cytoplasmic male sterility (CMS) are used to produce commercial hybrid seed. AVRDC has incorporated CMS (S-cytoplasm) cytoplasm in 13 hot pepper and six sweet pepper genotypes. Since 1999, more than 230 CMS lines and their maintainer lines have been introduced by public and private sector breeders in 34 countries. CMS lines were used either directly to develop commercial hot pepper hybrids, or maintainer lines were used to incorporate the CMS trait in locally available and adaptable hot pepper lines to generate new pairs of CMS and maintainer lines. Progress in pepper male sterility research at AVRDC is briefly described using selected examples.

1. Introduction

Peppers (*Capsicum* spp.) are important cash crops for smallholder farmers in a number of Asian, African and Central/South American countries. Hot pepper (syn. chili pepper) dominates today's world spice trade, and sweet pepper has become a popular vegetable. Peppers (dry and green) are cultivated on about 4 million ha and among the five cultivated species of the genus *Capsicum*, *C. annuum* (both hot and sweet peppers) is the most commonly cultivated. AVRDC – The World Vegetable Center has had an active international pepper breeding program for more than two decades to genetically improve *C. annuum* hot pepper and sweet pepper for several traits, including resistance to biotic and abiotic stresses and male sterility (Lin *et al.*, 2013). Male sterility is extensively used in hybrid breeding of several crop species. Seed companies use nuclear male sterility (genic male sterility, GMS) and cytoplasmic male sterility (CMS) for cost-effective seed production of hot and sweet pepper hybrid cultivars. The progress made on pepper male sterility research at the Center is presented and the successful use of AVRDC's CMS lines by public and private sector breeders is described.

2. Development of CMS lines

Male sterility research in pepper at the Center was initiated in 1996. Through conventional backcross breeding, new pairs of CMS lines and their maintainer lines were developed using a CMS line and its maintainer (received from Choong Ang Seed Company, Korea) as a donor for male sterile cytoplasm (S-cytoplasm) and the maintainer allele (*rf*), respectively. Two backcross generations were completed successfully each year, and by 1999, six sets of hot pepper CMS and their isoplasmic maintainer lines were available for distribution to international cooperators. Since then, the Center has converted 11 improved lines and eight germplasm accessions (originating from six countries) into CMS and maintainer lines, which include 13 hot pepper and six sweet pepper pairs with different fruit characteristics (Table 1).

CMS line	Maintainer line	Origin	Fruit position, color*, type, size (cm), weight (g)
Hot pepper			
AVPP9606-S	VI046844/PBC308	Malaysia	Pendent, G→R, cayenne, 11.6 × 2.2, 16.2
AVPP9706-S	AVPP9706/97-7644	AVRDC	Pendent, G→R, cayenne, 10.7 × 1.3, 6.6
AVPP9907-S	AVPP9907/9907-9611	AVRDC	Pendent, G→R, cayenne, 12.2 × 1.3, 7.4
AVPP9909-S	AVPP9909/9950-5574	AVRDC	Pendent, G→R, cayenne, 14.2 × 1.5, 11.2
AVPP9910-S	AVPP9910/9950-5633	AVRDC	Pendent, DG→R, cayenne, 14.0 × 1.7, 17.2
AVPP9911-S	AVPP9911/9946-2141	AVRDC	Erect, G→R, cayenne, 7.2 × 2.5, 16.0
AVPP0309-S	AVPP0309/9849-5765	AVRDC	Pendent, G→R, cayenne, 7.8 × 1.1, 3.8
AVPP0310-S	VI060629/PBC378-2	Indonesia	Pendent, G→R, cayenne, 9.2 × 1.9, 10.6
AVPP0516-S	VI037614/PBC380	Indonesia	Pendent, G→ R, paprika, 12.5 × 1.9, 12.0
AVPP0517-S	VI060632/PBC483	Sri Lanka	Semi-erect, G→R, cayenne, 9.6 × 1.0, 3.8
AVPP0709-S	VI060627/PBC362	Korea	Pendent, G→ R, cayenne, 12.3 × 1.7, 10.2
AVPP0710-S	VI046838/PBC292	USA	Pendent, G→R, cayenne, 14.5 × 3.3, 36.0
AVPP0711-S	VI060630/PBC534	Indonesia	Pendent, G→ R, cayenne, 15.9 × 2.0, 21.4
Sweet pepper			
AVPP9607-S	VI037597/PBC84	Peru	Pendent, G→R, bell, 9.3 × 6.6, 98.4
AVPP9820-S	AVPP9820/9847-4754	AVRDC	Pendent, G→R, long bell, 16.8 × 5.4, 115.0

AVPP9821-S	AVPP9821/9852-174-3	AVRDC	Pendent, G→R, bell, 9.7 × 7.2, 126.0
AVPP9908-S	AVPP9908/9946-2162	AVRDC	Pendent, G→R, bell, 6.7 × 6.0, 106.4
AVPP9912-S	AVPP9912/9946-2194	AVRDC	Pendent, LG→Y, bell, 9.3 × 8.5, 200
AVPP9913-S	AVPP9913/9946-2138	AVRDC	Pendent, G→R, bell, 8.7 × 6.7, 138.6

*G = green, R = red, Y = yellow (L = light and D = dark)

Table 1:
Cytoplasmic male sterile (CMS) peppers and their maintainer lines developed by AVRDC

3. Development of GMS line

Through continuous selfing and simple selection of a GMS-based sweet pepper hybrid ('Forever'), a stable F_6 inbred population segregating for 50% male sterile (*msms*) and 50% male fertile (*Msms*) progenies was created and used in sweet pepper hybrid breeding. Recently, we also initiated development of hot pepper GMS lines and development of molecular markers associated with *ms* genes to facilitate more efficient utilization of GMS in hybrid breeding and other purposes.

4. Validation of markers for S-cytoplasm and *Rf* locus

A number of molecular markers associated with S-cytoplasm (Kim *et al.,* 2005; 2007; Ji *et al.,* 2013) and the restorer-of-fertility *Rf* gene (Gulyas *et al.,* 2006; Lee *et al.,* 2008) are available in peppers. We have examined their validity in several CMS, maintainer and restorer lines and found co-dominant SCAR markers (S_{130} & N_{140}) for CMS (Ji *et al.,* 2013) and a dominant SCAR marker (CRF-S_{870}) for the *Rf* (Gulyas *et al.,* 2006) gene more useful for efficient germplasm characterization at the seedling stage with respect to type of cytoplasm (S or N) and genetic constitution at fertility restoration (*Rf* or *rf*) locus. In contrast, conventional germplasm characterization for these traits requires developing CMS-based F_1 progenies using a large number of test accessions as male parent (in the first season, 4-5 months) and evaluating these test-cross F_1 plants for their fertility restoration ability (in the second season, another 4-5 months). Since independently isolated and commercially used S-cytoplasms in hot pepper are known to be genetically similar (Kumar *et al.*, 2009), we are using both validated SCAR markers to rapidly analyze pepper cultivars (hybrids, improved varieties, and landraces) of different origins for types of cytoplasm and fertility restoration locus. Obtaining this strategic information is critical, as it will reveal the extent of cytoplasmic variability in widely grown cultivars, and anticipate any possible risk of **vulnerability** associated with monopolistic use and/or existence of a single source of cytoplasm in pepper.

5. Marker-assisted restorer breeding in sweet pepper

Lack of a strong and stable *Rf* allele in sweet pepper has been a major hurdle in commercial exploitation of CMS technology in sweet pepper hybrid breeding. The CRF-S_{870} marker linked with the *Rf* gene (Gulyas *et al.,* 2006) was validated in a strong restorer hot pepper inbred line (AVPP9905). The CRF-S_{870} was then successfully used in marker-assisted backcrossing to

transfer the *Rf* allele from hot pepper line AVPP99005 to several sweet pepper genotypes. The introgression of the *Rf* allele in BC_4F_2 sweet pepper plants was confirmed (Lin *et al.,* 2015). We intend to advance segregating backcross progenies possessing the *Rf* allele to generate BC_4F_7 sweet pepper CMS and restorer inbred lines, and then increase the seed for international distribution.

6. Dissemination and use of CMS lines

Since 1999, the Center's CMS lines and their maintainer lines have been distributed in 34 countries (Fig. 1). Public and private sector breeders have successfully used CMS lines directly as parents for hybrid combinations or as a donor source of S-cytoplasm and maintainer allele (*rf*) to create new pairs of CMS and maintainer lines in the genetic background of locally adaptable inbred lines with desirable horticultural traits. The most noticeable successes with these CMS lines have been achieved by seed companies in India for commercial hybrid seed production (Reddy *et al.,* 2015). The use of CMS lines reduces the cost of hybrid seed production by at least 50% compared with conventional hybrid seed production, which requires skilled labor for manual emasculation and pollination (Lin *et al.,* 2013). Recently, potential crosses based on the Center's CMS lines have been developed for further evaluation (Singh et al., 2014). CMS lines also have been successfully used to validate CMS markers associated with S-cytoplasm (Kumar *et al.,* 2009), examining distribution of *Rf* allele associated markers in India (Kumar *et al.,* 2007), and experimental hybrid development in Egypt.

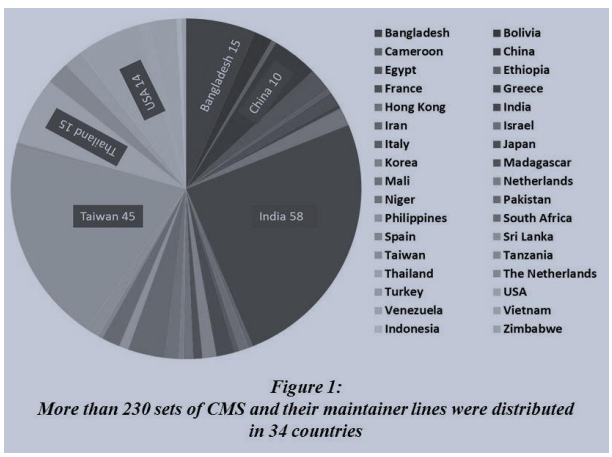

Figure 1:
More than 230 sets of CMS and their maintainer lines were distributed in 34 countries

7. Acknowledgements

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Sweet pepper breeding against bacterial spot (*Xanthomonas euvesicatoria*) in Serbia

Dario Danojevic, Sladjana Medic-Pap, Maja Ignjatov

Institute of Field and Vegetable Crops, Novi Sad, Serbia

Abstract

Bacterial spot of pepper (BS) caused by *Xanthomonas euvesicatoria* is one of the most important pepper diseases in Serbia. Growing of resistant pepper genotypes is the most efficient way of BS control. The study was started in 2013, when eight pepper varieties from the Institute of Field and Vegetable Crops, Novi Sad (IFVCNS), Cal wonder and isogenic line from Early Cal wonder (ECW-20R) were inoculated with *X. euvesicatoria* strain P8 in the field conditions. Intensity of infection was evaluated according to the Horsfall-Barratt (HB) scale. Nonparametric Kruskal-Wallis test was used for statistical analysis. All tested varieties were susceptible, except ECW-20R. In the same year, several IFVCNS pepper varieties were crossed with ECW-20R and also with line 30. After crossing 3 hybrids were sown in a greenhouse in October 2013 to obtain F2 generation. Those F2 transplants with resistant parents and Cal Wonder were planted 2014 in open field and inoculated. In the other field, IFVCNS varieties (Amfora, Una, Matica), line 30, ECW-20R and their 6 hybrids were evaluated under natural infection. Hybrids showed a significant resistance in relation to susceptible parents, except combination Matica x ECW-20R. Seeds of selected plants (F2 and F1 generation) from those two fields were sown for transplants producing in 2015 in order to obtain F3 and F2 generation. Transplants were inoculated in a greenhouse. Healthy plants were transplanted in the open field and after three months evaluated again. Progenies from ECW-20R were more resistant than progenies from line 30.

1. Introduction

Bacterial spot (BS), caused by *Xanthomonas euvesicatoria* (Xe-Group A) is a widespread and it is considered as one of the most common pepper diseases in Serbia (Ignjatov et al. 2010). Pathogen is seed-borne and during past few years, disease appeared regularly caused great damage and complete death of the plants (Ignjatov et al. 2010). Pepper ranked as the second highest vegetable crop in Serbia (grown on about 20.000 ha of open field and plastic covered greenhouses) and BS is considered to be an economically very important disease. Presence of diseased plants in certified pepper production fields affects the certification eligibility of the crop, as defined by certification rules and regulations. When weather conditions are favorable for disease development, pepper producers do not have adequate resources to control this pathogen. Copper based bactericides registered in our country are not effective enough (Šević et al. 2015). Breeding programmes for BS-resistance are considered as one of the most effective strategic measures for controlling the disease. However, the development of resistance has been limited by the high degree of genetic and phenotypic diversity within the *Xanthomonas* species complex. Resistance to BS of pepper is conferred by single dominant genes *Bs1*, *Bs2*, *Bs3* and *Bs4* (APS-ISF, 2010). So far, 11 physiological races of *X. euvesicatoria* were described around the world. Four of them (P1, P3, P7, P8) are recorded in Serbia, with P8 predominating (Obradović et al. 2004; Ignjatov et al. 2015). In Serbian growing conditions the most cultivated pepper genotypes showed various degree of susceptibility to *X. euvesicatoria* (pepper race 8) (Ignjatov et al. 2012). Considering that the most of the studied genotypes were sensitive to *X. euvesicatoria* (P8), with exception of the isogenic line ECW-20 carrier of *Bs2* resistance gene,

transfer of this gene into commercial varieties of pepper would be significant contribution to control of this economically important disease. At the Institute of Field and Vegetable Crops, Novi Sad (IFVCNS) eleven pepper varieties with different fruit types were developed (Gvozdenović et al. 2002). So far IFVCNS pepper varieties were not resistant to bacterial spot. Since 2013 we have started breeding program to develop new varieties resistant to this disease because of the market demands.

2. Materials and Methods

The study was started in 2013, when eight pepper varieties (Amfora, Anita, Atina, Matica, Novosađanka, Plamena, Una and Vranjska) from the Institute of Field and Vegetable Crops, Novi Sad, Serbia (IFVCNS), Cal Wonder and isogenic line from Early Cal Wonder (ECW-20R) were sown on 3 April 2013 in plastic tunnel. Young plants were transplanted two months later to the open field. Spacing between plants was 70 x 25 cm in all trials. Copper products or other bactericides were not applied in the trials. The inoculation was done a month and a half after transplanting of the plants. Plants were inoculated with *X. euvesicatoria* strain P8 with concentration 10^6 CFU/ml and covered with foil overnight. (Figure 1).

Figure 1:
Inoculation with X. euvesicatoria in field conditions

Strain was isolated from pepper (variety Amfora) in 2011 locality Rimski Šančevi (near Novi Sad). Prior to the inoculation plants were irrigated by sprinklers to obtain optimal conditions for infection. The leaf spot evaluation was done three times. Data from third evaluation in the beginning of October were shown. In the same year, several IFVCNS pepper varieties were crossed with ECW-20R and line 30. After crossing 3 hybrid combination (Anita x ECW-20R, Amfora x ECW-20R and Matica x ECW-20R) were sown in the end of October 2013 in a greenhouse to obtain seed for F2 generation during winter period. Those F2 seed with ECW-20R resistant parents and Cal Wonder were sown in the beginning of April 2014 in plastic tunnel. Plants were transplanted in the beginning of June in open field and inoculated 40 days

later according to the same procedure. The leaf spot evaluation was carried out three times and final evaluation which was done in the end of September was shown (Figure 2, 3 and 4).

Figure 2:
Inoculated plant of Cal Wonder variety in 2014

Figure 3:
Inoculated plant of ECW-20R in 2014

Amfora, Una, Matica, line 30, ECW-20R and their 6 hybrids were sown in plastic tunnel together with plants for artificial inoculation. These plants were transplanted in the end of May in the other field. The intensity of bacterial leaf spot on these plants was evaluated under natural infection. The final evaluation in the end of September 2014 was shown.

Seeds of selected plants (F2 and F1 generation) from those two fields were sown on 6 April 2015 for producing transplants in order to obtain F3 and F2 generation. Transplants were inoculated in a greenhouse 40 days later. Evaluation of plants was done 18 days after inoculation. Plants without bacterial leaf spot were transplanted in the beginning of June to the open field and after three months evaluated again.

Figure 4:
Pepper line 30 after inoculation in 2014

Intensity of infection was evaluated according to the Horsfall-Barratt (HB) scale. Nonparametric Kruskal-Wallis test was used for statistical analysis because values from HB scale were not normally distributed. Data were analyzed using STATISTICA for Windows version 12.

3. Results and Discussion

According to results in 2013 all tested varieties were moderately susceptible (medians 5-6), while ECW-20R was resistant (Figure 5). Number of evaluated plants was recorded on the upper part of each graph as a second number in the bracket.

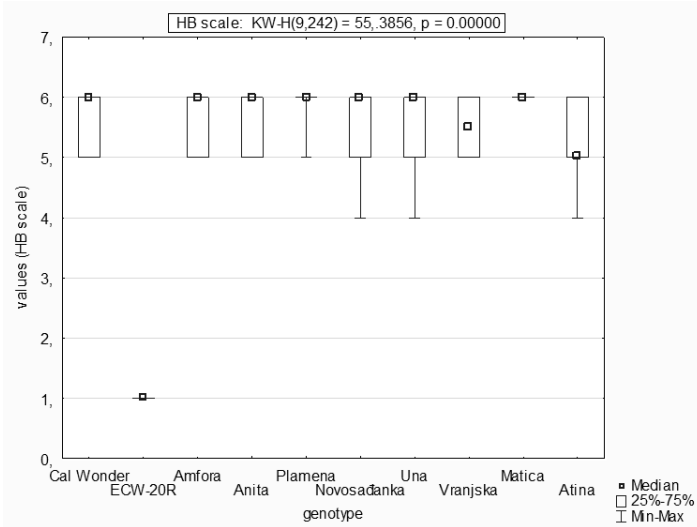

Figure 5:
HB values of pepper genotypes 84 days post inoculation (DPI) with X. euvesicatoria in 2013

Since a large amount of precipitation in 2014 we did not performed artificial infection in a selection plot for F1 progenies, because there were favorable conditions for the development of BS. Hybrids developed by crossing susceptible parents with resistant parents (ECW-20R and line 30) were more resistant comparing to susceptible parents in the condition of natural infection except combination Matica x ECW-20R (Figure 6). Researched the heritability to BS, Riva et al. (2004) reported that this parameter for reaction to diseases were somewhat variable as depend on the population and the environment studied. Our research confirmed this claim because values in HB scale for Matica x ECW-20R F1 varied from 1-4.

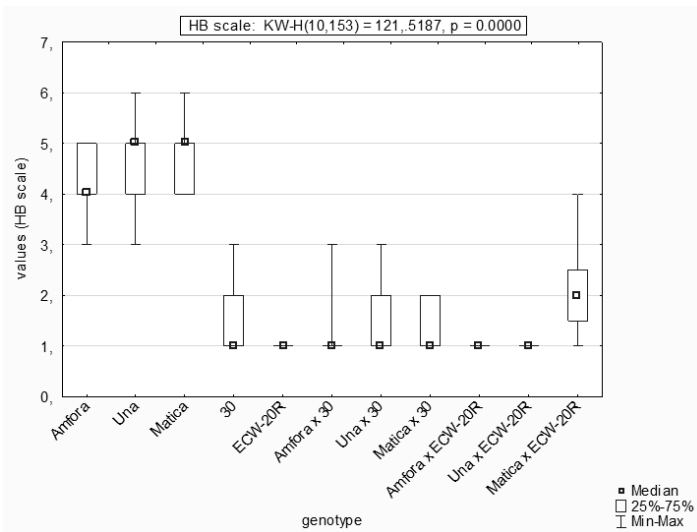

Figure 6:
HB values of pepper genotypes in natural infection with X. euvesicatoria in 2014

All ECW-20R plants were resistant (median 1) in the conditions of artificial inoculation in 2014, while in other genotypes there were plants with different level of resistance (Figure 7). Especially in F2 generation as it was expected the values ranged from 1-4 or 2-6. There was no significant difference between line 30 and ECW-20R, but line 30 was more variable.

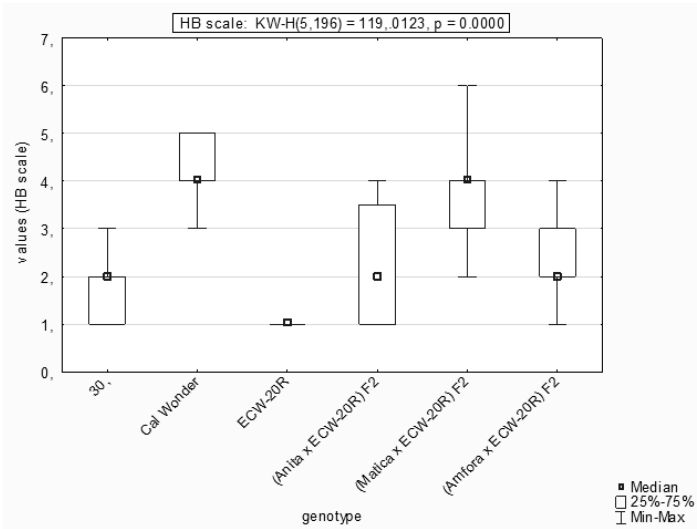

Figure 7:
HB values of pepper genotypes 73 DPI with X. euvesicatoria in 2014

Moderately susceptible were Cal Wonder and (Matica x ECW-20R) F2. Plants (Anita x ECW-20R) F2 were between those two groups. Since there were no plants with grade 1 in the progeny (Matica x ECW-20R) F2 this combination was discarded from further breeding.

ECW-20R was the most resistant genotype in 2015 trial (Figure 8). Plants from combination (Una x ECW-20R) F2, (Anita x ECW-20R) F3 and (Amfora x ECW-20R) F3 were not significantly different from ECW-20R. According to results from artificial infection progenies from the combination of susceptible parents and ECW-20R were more resistant (medians 1) than progenies from line 30 (medians 3 and 5). Line 30 was more susceptible (median 4) in 2015 than in 2014 (median 2). This could be explained that inoculation in 2015 was done when plants were in younger phase.

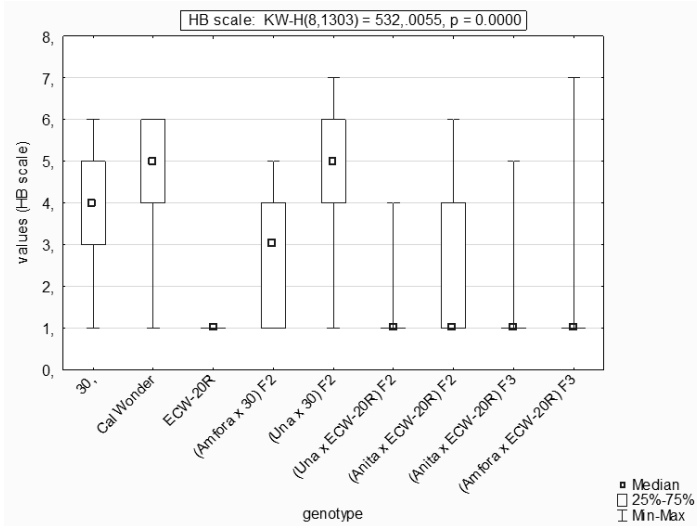

Figure 8:
HB values of pepper genotypes 18 DPI with X. euvesicatoria in 2015

Three months after inoculation median values were lower because of discarded susceptible plants (Figure 9). Also in repeated BS evaluation progenies from ECW-20R were more resistant (medians 1) than line 30 and progenies from line 30 (medians 2). At the moment ECW-20R (*Bs*$_2$ gene) is good donor for BS resistance in Serbia conditions. This gene *Bs*$_2$ is still functioning in Korea and may be used in breeding for resistance (Wai et al. 2015).

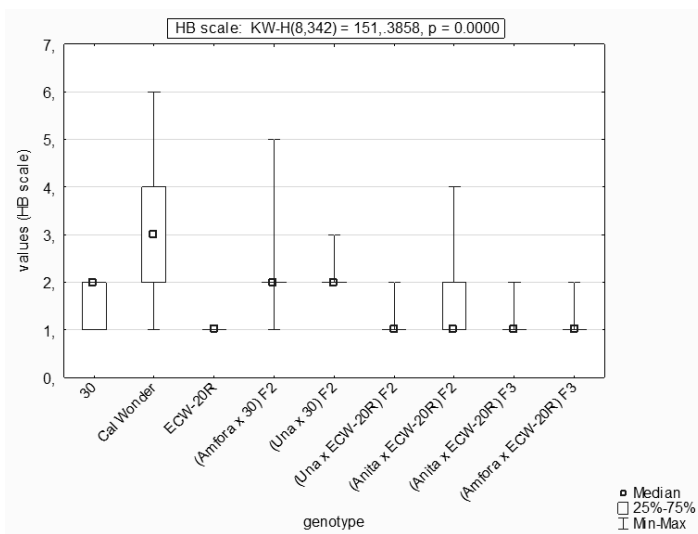

Figure 9:
HB values of selected pepper genotypes 90 DPI with X. euvesicatoria in 2015

4. Conclusion

In further selection process, plants will be also inoculated and measured for fruit traits and yield. Progenies from line 30 will not be used in breeding program because of lower resistance than progenies from ECW-20R. Backcross breeding with IFVCNS varieties will be applied if fruits traits from selected plants will not be commercially suitable. Also we plan to use marker assisted selection to speed up the breeding process.

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What kind of root should a pepper plant have?

Gábor Csilléry[1], Gianni Gatto[2], Miklós Gábor Fári[3,4]

[1] *Budakert Ltd., Budapest, Hungary*

[2] *Esasem S.p.A, Casaleone, Italy*

[3] *Department of Agricultural Botany, Plant Physiology and Biotechnology, University of Debrecen, Debrecen, Hungary*

[4] *Ereky Foundation, Debrecen, Hungary*

Abstract

The root of the pepper is the inconspicuous part of the plant that grows into the soil, thus it is rarely investigated during the breeding. It has substantial importance in intense cultivation in growth media being the organ of water and nutrient uptake. Based on the morphology and biological functions, the structure (i.e. the phenome) of the root can be accurately characterised with the tools of *phenotyping*. By investigating the *phenome*, special root traits ('phenes') of certain genotypes can be described. We here documented *three different pepper root phenomes* during our cross breeding works aiming at the resistance against *Meloidogyne* species using different *Capsicum annum* genotypes (HD322, HD330, HD107, HD149 and CM334), varieties (Mississippi Nemaheart, Carolina Cayenne, Carolina Hot, Charleston Belle, Carolina Wonder, Jalapeno, Serrano, Ancho and Mulato) and wild species (*C. chinense, C. frutescens* and *C. baccatum*). *One-whorled, multilateral fibrous root system (OWM)* develops form one single whorl (or quite closely adjacent whorls). *Multi-whorled, bilateral fibrous root system (MWB)*: roots of mostly the same order derive in several whorls without any conspicuously thicker conductive-anchoring roots. *Multi-whorled, multilateral fibrous root system (MWM)*: no conductive-anchoring root is present but only fibrous roots with an admirably large absorptive surface. This type presumably enables proper water and nutrient uptake from the soil. We observed that this latter delicate, dense fibrous root system can also ensure similarly efficient protection as the thick, wiry roots of the OWM. The pinnate, multi-whorled bilateral root system was mostly the indicator of admirable resistance, in contrast with the entangled, bent roots. We observed some traits (wizening fruit, fragile root base) among the progenies in crossings of HD322, HD330 which indicate that deleterious characteristics may be linked to the genes of resistance.

1. Introduction

It has substantial importance in intense cultivation in growth media being the organ of water and nutrient uptake. The root of the pepper is the inconspicuous part of the plant that grows into the soil (Leskovar et al., 1989; Leskovar et al., 1990; Leskovar, 1998; Niu et al., 2010; Goreta et al., 2007). There has been an era of great progress in root biology in general and the plant sciences in particular. Water shortages are responsible for the greatest crop losses around the world and are expected to worsen, heightening international interest in crop drought tolerance. Within the U.S. alone, about 67% of crop losses over the last 50 years have been due to drought. Actually, one of the key questions is that "which root traits help most and under what conditions for the stable crop production"? (Comas et al., 2013). Therefore, the plant roots are getting not to be the former "hidden part" of the plant Kingdom. Based on modern research techniques, our knowledges reflects to these developments on root science, as the following (Eshel, A. and Beeckman, T., 2013):

- Basics of root research and their evolution and role in the global context of soil development and atmosphere composition;
- New understandings about roots gained in the post-genomic era, for example, how the development of roots became possible, and the genetic basis required for this to occur;
- The mechanisms that determine root structure, with chapters on cellular patterning, lateral root and vascular development, the molecular basis of adventitious roots, and other topics;
- Plant hormone action and signaling pathways that control root development, including strigolactones and brassinosteroids;
- Soil resource acquisition from agricultural and ecological perspectives;
- Root response to stress, with chapters that address the impact of the genomic revolution on this topic;
- Root-rhizosphere interactions, from beneficial microorganisms to detrimental nematodes.

Recent advances in root biology are also making it possible to genetically design root systems with enhanced soil exploration and resource capture. These cultivars would have substantial value for improving global food security, where yields are limited by drought, low soil fertility, biotic and abiotic stress, pest and microbes, and would enhance the sustainability of intensive agriculture. Many of the „phenes controlling soil resource capture" are related to root architecture (Lynch and Brown, 2012). Based on the morphology and biological functions, the structure (i.e. the phenome) of the root can be accurately characterised with the tools of *root phenotyping*. By investigating the *phenome*, special root traits ('phenes') of certain genotypes can be described (Lynch and Brown, 2012).

Nevertheless, the pepper root system is rarely investigated yet during the breeding. We assume that, similarly to those of other species (maize, bean etc., Lynch and Brown, 2012), *phenes of the pepper root system architecture* are also under genetic control and in lucky cases they can be altered according to the special needs of breeding with the methods of biotechnology and breeding. For the first time, we took notice of *different pepper root phenomes* during our breeding works aiming at the resistance against *Meloidogyne* species.

2. Materials and Methods

We got sources of the highest resistance for *Meloidogyne* species from Dr. Alain Palloix (INRA, France).

Successors of hybrid HD (HD322, HD330) made from PM217 line contained the monogenic dominant Me1 and Me2 genes. Successors of hybrid HD (HD107, HD149) made from PM687 line contained the monogenic dominant M3 and M4 genes and the CM334 included monogenic dominant ME7 gene. From the USDA (Dr. R. Fery, USA) we got several lineages (Mississippi Nemaheart, Carolina Cayenne, Carolina Hot, Charleston Belle, Carolina Wonder) containing the dominant gene *N*. Besides the Mexican CM334, several other 'ancient cultivars' deriving from Mexico were also included in our experiments (Jalapeno, Serrano, Ancho, Mulato). We studied some parcels of the wild species *Capsicum chinense, C. frutescens* and *C. baccatum*, as well. Between 1995 and 2008, our experiments were carried out at two sites. In Italy our pepper research materials were planted at Casaleone, under unheated plastic tunnels (sandy soil, one generation annually). In Hungary we conducted our investigation at Cserkeszőlő, under thermal

water-heated plastic tunnels (sandy soil, two generations annually). From 2008, experiments have been carried out only in Italy.

3. Results

Based on our experimental observations of 20 years breeding works aiming at the resistance against *Meloidogyne* species, we discovered and documented at least three types of root systems, or *three different pepper root phenomes: OWM (one-whorled, multilateral fibrous root system); MWB (multi-whorled, bilateral fibrous root system and MWM (multi-whorled, multilateral fibrous root system).* There is no or limited data about genetics of showed pepper root phenomes (Fig. 1 - 3)

3.1. One-whorled, multilateral fibrous pepper root system (OWM, Fig. 1.)

This root develops form one single whorl, or quite closely adjacent whorls. The proportion of absorptive, fine roots and conductive-anchoring roots is remarkably shifted to its progenitor root type (cv. Ancho). This root is like a root of fruit sapling or a type of corn root. Considering our visual observation and robust feature it can be supposed that the pepper root phenome is related to OWM gene/s.

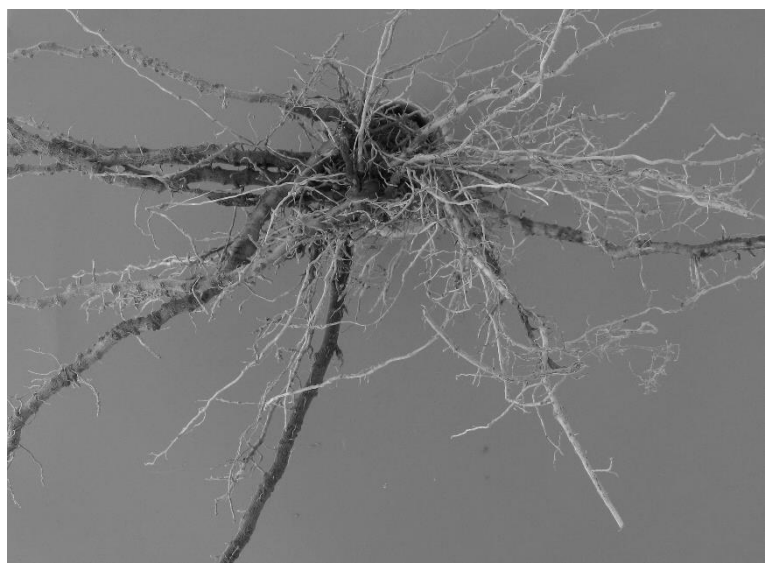

Figure 1:
One-whorled, multilateral fibrous pepper root system (OWM)

3.2. Multi-whorled, bilateral fibrous root system (MWB, Fig. 2.)

Roots of mostly the same order derive in several whorls without any conspicuously thicker conductive-anchoring roots. Possibly, the nutrient uptake of this type may be better due to the presence of the homogenous mass of thin fibrous roots. Considering our visual observation and robust feature it can be supposed that the pepper root phenome is related to MWB gene/s.

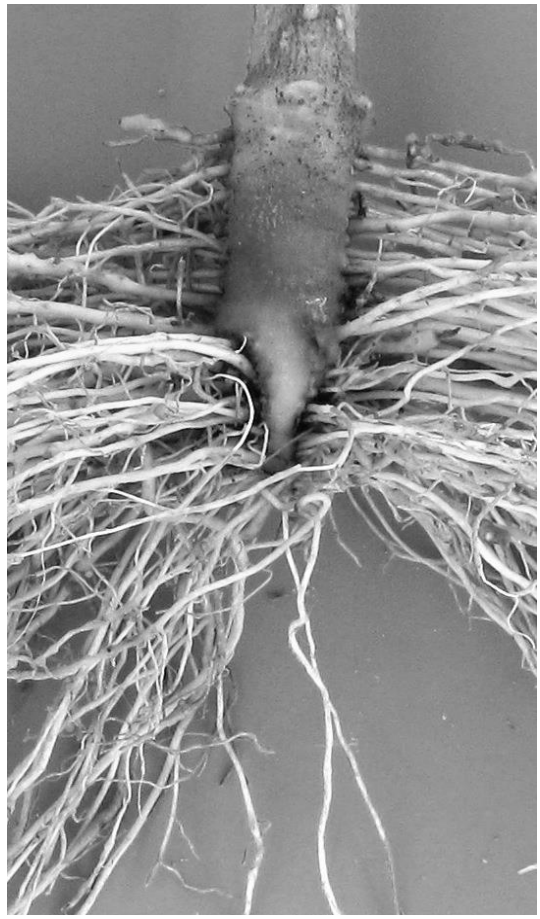

Figure 2:
Multi-whorled, bilateral fibrous pepper root system (MWB)

3.3. Multi-whorled, multilateral fibrous root system (MWM, Fig. 3.)

No conductive-anchoring root is present but only fibrous roots with an admirably large absorptive surface. This type presumably enables proper water and nutrient uptake from the soil. We observed that this delicate, dense fibrous root system can also ensure similarly efficient protection as the thick, wiry roots of the Ancho.

The pinnate, multi-whorled bilateral root system was mostly the indicator of admirable resistance, in contrast with the entangled, bent roots. We observed some newly observed traits (*mcb* - micro cracking berry; *ccr* - chappy collar of root; *frx* - entire fragile plant) among the progenies in crossings of HD322, HD330, which indicate that deleterious characteristics may be linked to the genes of resistance.

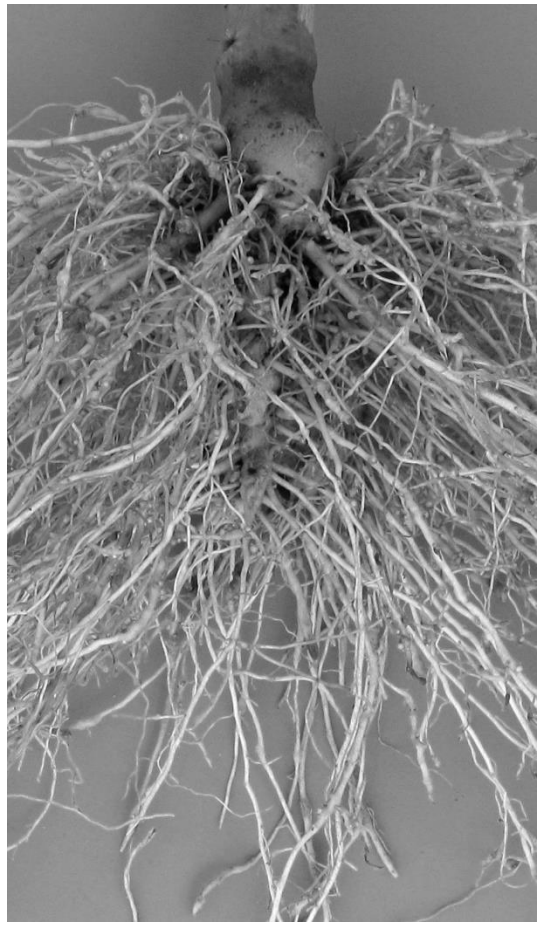

Figure 3:
Multi-whorled, multilateral fibrous pepper root system (MWM)

4. Conclusion

20-25 years ago, the greenhouse pepper production was almost exclusively made in soil. One possibility to protect the soil against harmful pathogens and pests was breeding of resistant varieties. The other option was the application of soil disinfectants. However the the use of most efficient metyl bromide have since banned because of its serious environmental impact. The third option was the use of artificial growing medium. The spread of this new technology has been hindered by high cost investment. Along with this the lack of effective chemicals like methyl bromide justifies the continuation of resistance breeding research against root pathogens and pests and also pepper roots types.

In addition to the high cost, the other disadvantage of artificial growing medium (Rockwool, cocopeat, perlite) usage is that the producing and also dispose of them entail environmental damage. Further problem is that significant amount of nutrient can release to the environment during production. We note the spread of soil-free cultivation technologies, but it need to reduce their harmful side effects, such as plant breeding methods. If the roots of varieties adapt to these

technologies and we can increase the efficiency of nutrient uptake, fewer nutrients will drain into the water and harmful emissions can reduce.

Based on 20 years of trial experience we can emphasize the importance of strong plant root growth in all circumstances even the presence of any resistance gene. The dense root system results more efficient nutrient uptake. Thick and thin tufts MWB pepper roots, such as cv. Carolina Cayenne, the cv. Hot Carolina can provide effective protection in the same way as the thick, wiry, OWM root system, such as cv. Ancho. Pepper "combed" MWM root system is usually an indication of resistance, in contrast to the tangled curved root systems.

We discovered heritable characteristics among the HD322, HD330 crossings successors which suggest that resistance can be closely linked to harmful genetic characters *(mcb; ccr; frx, see above)*. If we really want to study the above context relates to the pepper root "phenom matters it needs to consider the following general and methodological contexts:

- The different pepper root phenotypes should be always study in uninfected soil.
- The seeds should be sown directly into the soil because any educator pot, tray distorts the root. From the very beginning, the plant has to "feel" that this is the place which is available for it, hence water and nutrients can be uptaken from here. This is not easy, because the loose sandy soil is ideal for root washing.
- Pepper roots must be studied on rock wool. This is ideal from the aspect that after drying, the weight of original quilt and the quilt interlaced with roots can be easily measured. Even of the weight of the remaining ashes can be important information after combustion.
- It can be recommended to measure the root mass using voluntometry with visually determination.
- It can be explored the physiological background of different peppers root "Phenom's" by molecular biology tools.
- It can be targeted crossings between different types.
- It need to search molecular markers for the pepper root "phenotypization".

Our experiments confirmed that several type of root, "phenome" exist in the peppers. They may have different biological importance, as well. Along with this we think that the pepper can be an ideal model to study the genetic and molecular biological background of root "Phenom's" in the future.

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Aphid resistance in a *Capsicum* collection

Roeland E. Voorrips, Greet Steenhuis-Broers, Wendy van 't Westende, Ben Vosman

Wageningen UR – Plant Breeding, Wageningen, the Netherlands

Abstract

Aphids are a problem in pepper cultivation, causing direct damage as well as acting as vectors for viruses. Resistance to aphids is therefore a desired trait in pepper varieties, but resistant varieties are not available yet. Our research is focused on finding sources of aphid resistance and elucidation of the genetics and mechanism of resistance. In our poster we present some of our results: an efficient bioassay with *Myzus persicae* and a comparison of the performance of a collection of Capsicum accessions in this biotest.

1. Introduction

Several aphids can cause problems in pepper cultivation, including *Myzus persicae*, *Myzus nicotianae*, *Aulacorthum solani* and *Aphis gossypii*. These aphids produce several types of damage, such as chlorosis, necrosis, wilting, defoliation and fruit loss. They also produce honeydew, but the most important damage is caused indirectly by the viruses they transmit.

Biological control of aphids is used, but is quite expensive and not effective in all cases. Chemical control is also used, but increasing resistance of the aphids requires increasing dosage of these chemicals, which is unsustainable and undesirable from an environmental point of view. Host plant resistance to aphids would offer an alternative solution to chemical control. To develop resistant varieties, good sources of resistance are needed. Here we describe a method for testing *Capsicum* accessions for resistance to *M. persicae* and its results when applied to a collection of fifty gene bank accessions.

2. Materials and methods

Aphids (*Myzus persicae)* were reared on the susceptible *Capsicum annuum* cv Bruinsma Wonder. Plants were grown for six weeks before testing. Each plant received two clip cages with ten one-day-old nymphs (Figure 1). After 7 days each clip cage was observed and the numbers of living and dead aphids, of shed skins and of new-born nymphs were counted.

Two experiments were performed. In the first experiment, 50 accessions were tested, comprising 10 *C. annuum*, 13 *C. baccatum*, 14 *C. chinense* and 13 *C. frutescens*.Ten of these accessions were selected and re-tested in a second experiment. Both experiments used a randomized complete block design with one plant per accession in each block; the first experiment consisted of four blocks of 50 plants, and the second experiment of 10 blocks of 10 plants each.

Figure 1:
Two clip cages with aphids on pepper leaves.

3. Results and discussion

In the first experiment a wide variation in resistance was observed among the accessions for all measured parameters. The most resistant accessions, both for survival and reproduction, belonged to *C. chinense* and *C. baccatum*, but in each of these species we also observed accessions with a susceptibility comparable to that of the susceptible control (*C. annuum* cv Bruinsma Wonder). From the first experiment accessions with varying levels of reproduction were selected and re-tested with more replicates in the second experiment (Table 1).

Resistance was most clearly expressed in the reproduction rate (the number of new nymphs per adult), with the most resistant accession (2012022) showing no reproduction at all. In contrast, the survival was quite high (above 0.8) on all accessions except for accession 2012022, but even on that accession more than half of the adults survived.

Our present research continues with the study of the inheritance of resistance in a segregating population and with the extension of the resistance screening to other aphid species.

4. Acknowledgements

This project was funded by the Ministry of Economic Affairs of the Netherlands under the „Groene Veredeling" program (project nr. BO-12.03-017-019) and the seed companies Bayer Vegetable Seeds, Syngenta Seeds and RijkZwaan.

Table 1. Observation of aphid resistance traits among 10 Capsicum accessions.

Accession	**Species**	**Survival[1]**		**Skins[2]**		**Nymphs[2]**	
2012008	*annuum*	0.92	bc	2.73	d	1.24	f
2012022	*baccatum*	0.55	a	1.35	a	0.00	a
2012024	*baccatum*	0.92	bc	2.05	b	0.05	ab
2012033	*chinense*	0.91	bc	1.97	b	0.17	bc
2012035	*chinense*	0.81	b	2.30	bc	0.34	cd
2012036	*chinense*	0.94	c	2.11	b	0.24	cd
2012037	*chinense*	0.92	bc	2.18	b	0.30	cd
2012041	*chinense*	0.91	bc	2.12	b	0.53	de
2012045	*frutescens*	0.93	c	2.62	cd	0.78	ef
Bruinsma Wonder	*annuum*	0.93	c	2.35	bcd	0.47	de

*Survival was expressed as (living aphids) / (living+dead aphids) at the final observation; the number shed skins and new nymphs were divided by the estimated average number of living aphids present, calculated as (2*living aphids + dead aphids)/2.*

Means followed with the same letter were not significantly different (P≥ 0.05)

Symptoms caused by *Tomato spotted wilt virus* (TSWV) in pepper (*Capsicum* spp.) and marker assisted selection of TSWV resistant pepper lines for hybrid constructions

Pál Salamon, Judit Mitykó, Péter Kaló, Zoltán Szabó

NARIC Agricultural Biotechnology Research Institute, Gödöllő, Hungary

Abstract

In the past 20 years *Tomato spotted wilt virus* (TSWV) emerged as the most important pathogen of forced peppers (*Capsicum annuum* L.) and appeared also in open fields in Hungary. This paper gives an outline on the extreme variability of TSWV induced symptoms including the „fruit melanotic ringspot" (FMRS) observed in Hungarian TSWV resistant white cecei-type peppers. In Hungary, resistance breaking strain of TSWV has been isolated for the first time from pepper fruit showing FMRS. Considering that the infection of TSWV induces the development of high diversity of macroscopic symptoms and there are at least 7 other tospoviruses* known to induce symptoms similar to those of TSWV, we propose to term the disease caused by these viruses as „tospovirus disease of pepper". Incorporation of the *Tsw* resistance gene from *C. chinense* has been successfully used to breed resistant vegetable peppers. However, there are no TSWV resistant Hungarian cultivars among the spice peppers. Therefore we started to integrate the *Tsw* gene in spice pepper by crossing high valued susceptible lines („Kocséri", „PalF4" line) with a homozygous resistant male „TSR" bred in our laboratory. Using SCAR markers linked to the *Tsw* gene we selected heterozygous and homozygous resistant F1, F2 and BC1 plants of spice pepper habit which were used as parents to generate dihaploids (DH). At present ca. 30 DH lines are involved in agronomic evaluation and hybrid testing programs.

1. Introduction

Tomato spotted wilt virus (TSWV), the type species of the *Tospovirus* genus (family: Bunyaviridae) is a world-wide distributed polyphagous plant virus of high economic importance (1). It has a wide host range infecting more than 1000 plant species including a great number of vegetable, ornamental, industrial and medicinal plants (2). Like other tospoviruses it has spherical particles of ca. 80-90 nm, each of them encapsidate three segments of the genomic ssRNAs marked S, M and L. TSWV is transmitted by at least 9 thrips species, out of them the onion thrips (*Thrips tabaci*) and the western flower thrips (WFT, *Franckliniella occidentalis*) act as the major vectors in Europe. The virus is known to persist and replicate in the thrips and there are evidences that it influences the behaviour of the insect vectors (3).

Sporadic occurrence of TSWV was first presumed in 1972 on the bases of symptomatological surveys made in tobacco fields in Hungary (4), but effectively the virus was first identified in 1980 by isolation, pathological characterization and electron microscopical detection of virions (5). Outbreaks of TSWV, however, have been recognized after the western flower thrips was introduced to Hungary in 1989 (6). TSWV appeared shortly causing epidemics and heavy losses in protected pepper and tomato crops (7, 8). Additionally, it became a devastating pathogen of tobacco (9), a number of ornamental plants (10) and appeared in potato and pea crops (11, 12) as well as in weeds (13). Besides TSWV, out of the members of tospoviruses the only *Impatiens necrotic spot virus* (INSV) from ornamentals has been unambiguously demonstrated in Hungary (14). Pathological variability of TSWV isolates was

detected in pepper (15). One of these variants (TSWV-Hm) causing lethal necrosis in tobacco was the first Hungarian isolate characterized by partial sequencing and it was used also to transform tobacco and select transgenic TSWV resistant tobacco lines (16).

Because of their high scientific and economic impact, the diseases induced by TSWV in different crops, the virus itself, the virus-host interactions and virus-thrips relationships are continously in the focus of Hungarian plant virologist, entomologists, breeders and specialists of plant protection. Variability of symptoms caused by TSWV in pepper attracted our attention because during our work we observed unusual and rare symptoms associated with TSWV infection. In addition, we started to study the reactions of *Capsicum* lines to different virus pathotypes and to introgress the *Tsw* gene into spice peppers with the aim to select valuable lines for breeding pepper hybrids.

2. Materials and methods

Symptomatological surveys were carried out regularly in commercial pepper populations and in breeding lines grown in polythene tunnels at distant regions of the counrty (ie. Szegvár, Szeged, Hódmezővásárhely, Tömörkény, Csongrád, Tiszakécske, Kecskemét, Lajosmizse, Gödöllő (South-East and Middle regions), Hatvan, Boldog, Jászárokszállás (Jászság region,) as well as near Debrecen and Nyíregyháza (North-East Hungary). Symptomatic plants were collected and the presence of TSWV was demonstrated using diagnostic test plants (*Capsicum*, *Chenopodium*, *Nicotiana*, *Petunia*, *Solanum* spp.) and/or by RT-PCR assays applying TSWV specific primers (12). Genomic segments of selected isolates were cloned and sequenced (16). Resistance of *Capsicum* genotypes were evaluated following sap inoculation of young plants with Ca1 and Ca101C/RB isolates of TSWV (P0 and P1 pathotypes) respectively, as well as by molecular methods using SCAR markers linked to the *Tsw* gene (17). Thrips transmission experiments were carried out in cage as described before (18). For selection of TSWV resistant breeding lines, DH plants were prepared according to the method decribed by Mitykó and co-workers (19), grown in greenhouse for testing resistance and production of seeds.

3. Results and Discussion

Symptoms of TSWV infections and the tospovirus disease of pepper

Our 20 years experiences indicated that TSWV occur all over the country often causing catastrophic losses in forced peppers. The extreme variability of symptoms caused by TSWV could not be compared with other pepper pathogenic viruses. Besides the well known symptoms: yellowing, yellow concentric rings and necrotic spots on leaves (Fig.1A, B, C, E), stunting and top necrosis of the plants as well as yellow or fine necrotic rings, necrotic spotting, cracking and death of fruits (Fig. 1. D), we observed also rare and unusual symptoms (20). They were as follows:

a) whitish-yellow mosaic („calico") on the foliage very similar to those caused by alfalfa mosaic virus (AMV) or the calico strain of cucumber mosaic virus (CMV) (Fig 1. F)

b) in the case of late systemic infections symptoms usually appeared only in the fruits (22) either as unripe spots on the mature, colored skin (Fig 2. A) or as epidermal shock necrosis (Fig 2. C). The unripe spots on fruits sometimes formed bizarre triangular or semicircular pattern (Fig 2. B).

c) in cultivars having the Tsw resistance gene, thrips transmitted fruit infection with the resistance inducing (RI = P0) strain caused blackish-brown spots and/or ringspots (Fig 2. D., E) which we called fruit melanotic ringspot (FMRS) (22, 23). The causal relationship between FMRS and TSWV-P0 has been demonstrated by thrips transmission experiments (18). Although activation of the Tsw gene is associated with hypersensitive necrotic response (HR), localization of TSWV P0 in the melanotic fruit tissues of resistant cultivars seems often ineffective especially if young fruits are infected. The continuus propagation and local spread of TSWV in melanotic ring spots may enhance the possibility of selection of specific virus mutants.

It is worth to mention, that resistance-breaking (RB) strain of TSWV was isolated for the first time from the periphery of FMRS (24), and later disseminated in resistant pepper populations (25, 26, 27) in Hungary**.** With the aim to find resistance sources, a range of *C. annuum, C. baccatum, C. chachoense, C. chinense, C. eximium, C. frutescens, C. praetermissum* and *C. pubescens* derivatives were inoculated with TSWV-RB. We have not detected any resistant genotypes but the line of *C. eximium*, marked Cex1 (28). Plants of Cex1 responded with HR and leaf drop to TSWV-RB (Fig. 3 A), but sometimes the virus translocated to the top leaves and caused isolated necrotic spots.

Because of the great variability of visible symptoms associated with TSWV we propose to term the disease caused by TSWV as „pepper tospovirus disease". The „pepper tospovirus disease" can be characterized by each of the TSWV induced symptoms irrespective of the virus strain, pepper genotype and even of the tospovirus species known to be naturally infecting pepper* and causing similar macroscopic symptoms. Rapid global dissemination of plant materials and insect vectors greatly enhance the risk to introduce „exotic" tospoviruses (e.g. CaCV, TCSV) and pseudorecombinant viruses (29) to Europe. Consequently, reliable identification of the pathogens responsible for either of the tospovirus-like symptoms is required because the control of different tospoviruses and virus strains may need different strategies.

MAS assisted selection of spice pepper breeding lines carrying the Tsw gene

Inbreed lines of a landrace spice pepper „Kocséri" (pungent) and a selected PalF4 (sweet) were crossed with a TSWV resistant line „TSR" selected from a population of *C. annuum x C. chinense* P.I. 159236. F1, F2 and BC1 plants were inoculated with TSWV-P0 and the resistant individuals were evaluated using the linked codominant SCAR T2 marker. to *Tsw* (Fig. 3 B). Dihaploids were prepared both from F1 and BC1 (*Tsw* heterozygous) and F2 (*Tsw* homozygous) populations. The DH plants were analysed for the presence of the *Tsw* gene, characterized preliminary for habit, pungency and fruit shape and self pollinated in greenhouse. These lines have been used for hybrid breeding programm.

*Tospoviruses known naturally infecting pepper: CaCV = *Capsicum chlorosis virus*, GRSV = *Groundnut ring spot virus*, GBNV = *Groundnut bud necrosis virus*, INSV = *Impatiens necrotic spot virus*, PNSV = *Pepper necrotic spot virus*; TCSV = *Tomato chlorotic spot virus* TNRV = *Tomato necrotic ringspot virus*; TSWV = *Tomato spotted wilt virus*. These viruses cause similar symptoms in pepper. The only FMRS seems – at present – to be a specific disease symptom caused by thrips transmitted TSWV P0 strain in fruits of peppers carrying the *Tsw* gene.

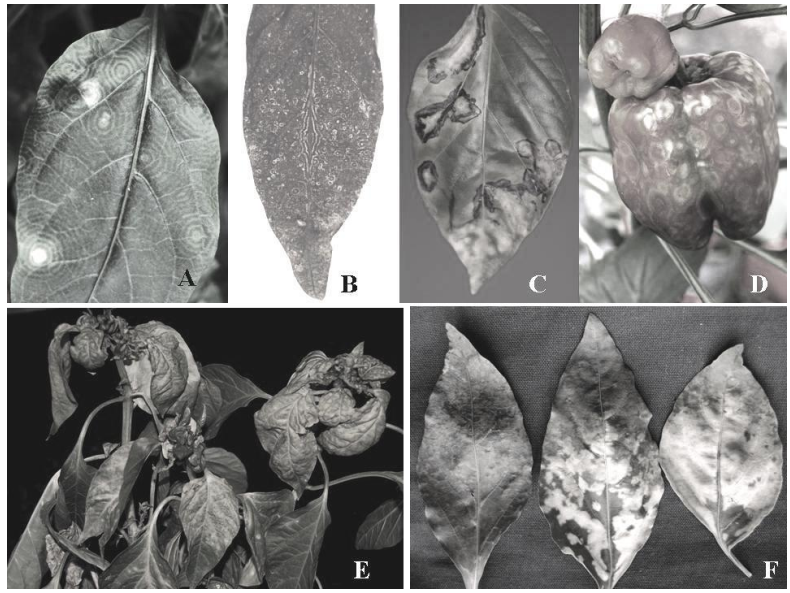

Figure 1:
Symptoms caused by TSWV in susceptible pepper (Capsicum annuum L.)
Chlorotic circular rings and patterns on leaves (A, B). Necrotic rings and patterns on leaves (C). Yellow rings on fruit (D). Top yellowing, leaf curl and necrosis in young plants (E). Calico mosaic on leaves (F)

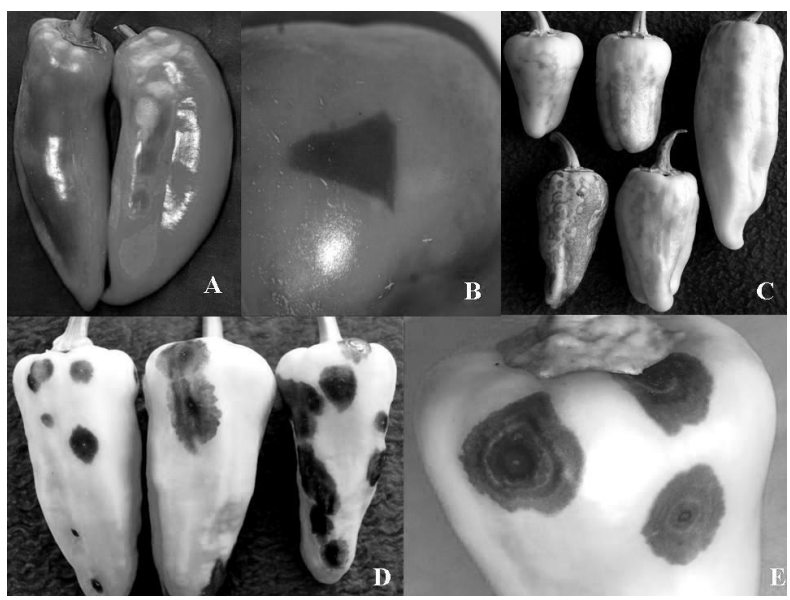

Figure 2:
Symptoms caused by TSWV in pepper (Capsicum annuum L.) fruits
Unripe (yellow and green) spots in mature (red) fruits (A). Green triangular spot in red tomato-shaped pepper (B). Epidermis shock necrosis in cecei type pepper (C). Fruit melanotic ringspot (FMRS) induced by TSWV-P0 in resistant peppers (D, E).

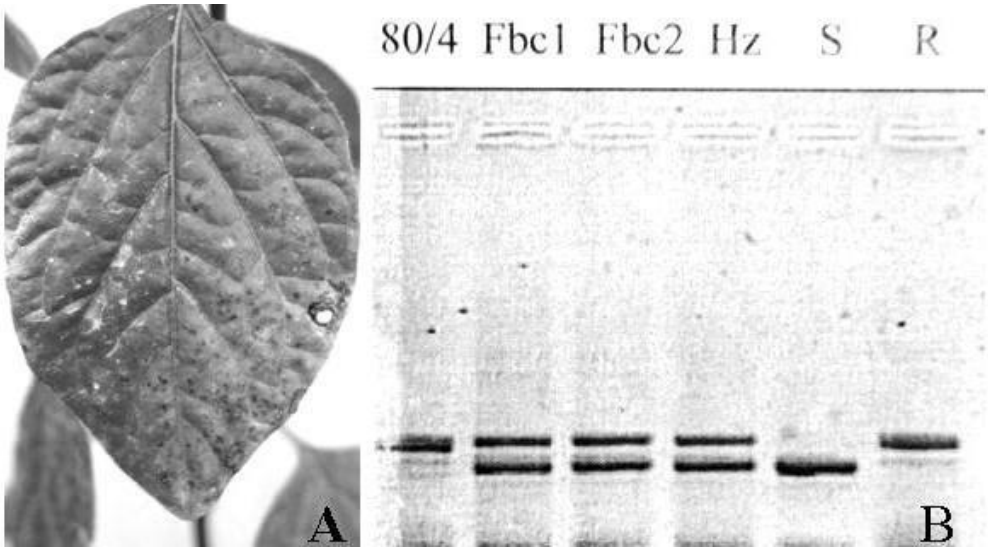

Figure 3:
Necrotic lesions on C. eximium caused by TSWV-RB (A). Detection of the introgressed region containing Tsw gene using a SCAR marker (B). Controls: R = homozygous resistant; S = susceptible; Hz = heterozygous resistant

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Evergreen question: Whether *Tobamoviruses* are transmitted via pepper seeds or not?

István Tóbiás[1], Gábor Csilléry[2], Katalin Nemes[1], Asztéria Almási[1], Katalin Salánki[1]

[1]*Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, H 1022 Herman O. Road 15, Budapest, Hungary*

[2]*Budakert Ltd., H-1114 Bartók B. Road 41, Budapest, Hungary*

e-mail: tobias.istvan@agrar.mta.hu

Abstract

It is generally accepted that for incidence of true seed transmission the virus must enter and survive in the embryo. According to the literature data *Tobamoviruses* unable to enter the embryo or albumen, but are found in maternal tissues such as seed coat or residual perisperm, or as contaminant on the seed surface (Broadbent 1965, Gonda et al 2005, Loebenstein and Lecoq 2012).

In late 70-s seed transmission tests were conducted with pepper infecting viruses (*Tobacco mosaic virus, Cucumber mosaic virus, Potato virus Y, potato virus X, Tomato aspermy virus, Broad bean wilt virus, Alfalfa mosaic virus*) on different papper varieties (Javított Cecei, Szarvasi 11, Soroksári and Budai édes konzerv). The seeds were originated from virus infected fruits (the viruses were isolated from them) and treated with 2% NaOH to exclude the external seed transmission of the virus. In case of Cucumber mosaic virus 2982 seeds, and from other viruses and pepper varieties 500 seeds each were tested, but virus seed transmission was not detected.

In the practice – especially after heavy virus infection, or selection of a new tobamovirus pathotype – always arise a question whether tobamoviruses can be seed transmitted, or not.

In 2015 pepper seed samples were tested for *tobamovirus* seed transmission. In the experiments untreated and NaOH treated seed lots were tested by RT-PCR and *Nicotiana tabacum* cv. Xanthi-*nc* plants were paralelly infected. The presence of tobamovirus was not detected by RT-PCR or test plants in either seed extracts. Seeds were germinated and the plantlets were verified for the presence of tobamoviruses. Tobamovirus was detected by RT-PCR method and test plant in the untreated seed lots but not in the treated ones.

Indirect tests such as ELISA or RT-PCR (detect the protein or nucleic acid of the target pathogen) are suitable for preliminary test but can not differentiate the viable and non-viable pathogen. For this reason the positive indirect tests should be followed always direct test to confirm the viability of the pathogen.

Introduction

Seed transmission plays a significant role in the survival of viruses from season to season and in long distance dissemination. In pepper virus transmission via seed was detected first time by McKinney (1952) in case of tobacco mosaic virus and by Sutic (1959) in case of alfalfa mosaic virus. Variable, but high seed transmission rate was demonstrated in seed originated from tobamovirus infected pepper plants (Avgelis 1986, McKinney 1952, Tosic 1980). Different treatment (80 ^{o}C heat, 10 % trisodium phosphate or 2 % NaOH) can prevent tobamovirus transmission via seed (Avgelis 1986, Svoboda et al 2006).

At the present time several conflicting data sheets or papers have been published on tobamovirus transmission via pepper seed. It is commonly assumed that virus seed transmission is depend on virus species, strain or pathotype, and plant species even on varieties. Since in the last 20-30 years several new tobamovirus pathotype appeared and in the same time new pepper varieties were produced it was reasonable to study again the seed transmission of tobamovirus in pepper.

As an important question virus seed transmission was studied in late 70-s and comprehensive experiments were conducted in our laboratory and greenhouses. Pepper infecting viruses (*Tobacco mosaic virus, Cucumber mosaic virus, Potato virus Y, potato virus X, Tomato aspermy virus, Broad bean wilt virus, Alfalfa mosaic virus*) isolated in Hungary were used in our experiments and inoculated different Hungarian papper varieties (Javított Cecei, Szarvasi 11, Soroksári and Budai édes konzerv). The seeds were collected from virus infected fruits (the viruses were isolated from pepper fruits) and treated with 2% NaOH to exclude the external virus seed transmission. 500 seeds from each varieties inoculated with different viruses were tested. Additionally in case of *Cucumber mosaic virus* 4140 seeds originated from naturaly infected pepper fruits - produced 2982 plantlets - were investigated. Plantlets from each treatment were monitored until flowering stage and each viruses were tested on adequate indicator plants. From this coprehensive study it was concluded that no virus seed transmission was detected in case of pepper infecting viruses on the tested 4 pepper varieties.

It is generally accepted that for occurence of true seed transmission the virus must enter and survive in the embryo. According to the literature data *Tobamoviruses* unable to enter the embryo or albumen, but are found in maternal tissues such as seed coat or residual perisperm, or as contaminant on the seed surface (Broadbent 1965, Gonda et al 2005, Loebenstein and Lecoq 2012).

In the practice it was found that several pepper seed lots showed the presence of tobamovirus by indirect method of ELISA, but no viable virus could be detected from the same sample by direct method of test plants. In the present study we report on seed transmission test of seed sample in which tobamovirus presence was detected by ELISA indirect method.

Materials and methods

Seed transmission test was conducted on two pepper seed samples: A100 and B100. From one of the sample tobamovirus was detected by ELISA method. 50 g seeds were divided into 3 parts and treated as follows:

1. A/K: 20 minutes in destilled water and washed with tap water (A/K)
2. A/T10: 10 minutes 2% NaOH treatment and washed with tap water (A/T10)
3. A/T20: 20 minutes 2% NaOH treatment and washed with tap water (A/T20)
4. B/K: 20 minutes in destilled water and washed with tap water (B/K)
5. B/T10: 10 minutes 2% NaOH treatment and washed with tap water (B/T10)
6. B/T20: 20 minutes 2% NaOH treatment and washed with tap (B/T20)

2.5 g seeds from each seed lots were homogenized with 6 ml Sörensen buffer pH 7.2 and inoculated onto *Nicotiana tabacum* cv Xanthi-nc plants.

2.5 g seeds from each seed lots were homogenized in liquid nitrogen and after nucleic acid extraction RT-PCR method was used for tobamovirus detection. Tobamovirus specific primer

pair for 5'-GATCGCGCGAGTCGTGATTCGTATTTAAATATG-3', rev 5'-TGGGCCGCCTACCGCGGCGG-3' amplified 700 nt long PCR product from every tobamovirus.

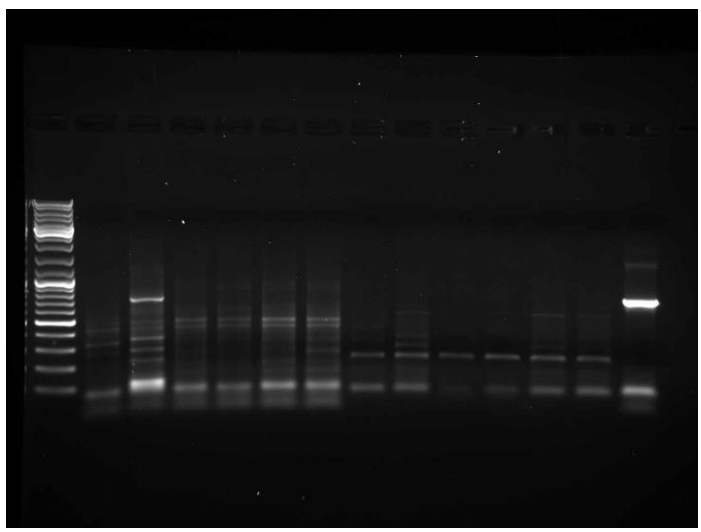
500 seeds from each seed lots were germinated on steril filter paper (50 seeds/petri dish).

Two weeks old plantlets were divided into two part and tested by indirect (RT-PCR) and direct (*Nicotiana tabacum* cv Xanthi-nc test plant) methods.

Results

2 % NaOH treatment did not influenced the germination of both pepper seed samples.

15 g pepper seeds were homogenized directly and 1500 seeds were germinated from A and B pepper seed samples. RT-PCR results show Figure 2



M 1 2 3 4 5 6 7 8 9 10 11 12 13 14

Figure 2. In lines 1-6 shows the plantlets PCR-products: 1- A/K, 2 - B/K, 3- A/T10, 4-AT20, 5- B/T10, 6- B/T20, lines 7-12 shows the seedlots results in the same order: 7- A/K, 8 - B/K, 9-A/T10, 10-AT20, 11- B/T10, 12- B/T20. In line 13 is a positive controll and line 14 negative controll.

In A samples no tobamovirus infection was detected by RT-PCR or test plant methods. In B samples control (water treated) plantlets showed tobamovirus infection and presence by test plants and RT-PCR methods.

No other cases were detected tobamovirus.

As a consequence the present seed transmission experiment it was concluded that external seed transmission was detected of a tobamovirus in B seed sample, proofed by indirect (RT-PCR) and direct (test plant) method. Since no other cases was detected tobamovirus, on basis of this experiment we conclude that external virus contamination was present and treatment with 2% NaOH prevent the tobamovirus tranmission via pepper seed.

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SESSION 2

Growing and seed production

Chairs: Zsuzsanna Füstös

Sweet pepper (*Capsicum annuum* L.) growing on a basis of thermal water with respect of protection the natural environment

Miklós Csikay[1], Katalin Ertsey Peregi[2]

[1] *Árpád Agrár Zrt Joint Stock Company Szentes Hungary*

[2] *Szent István University – Faculty of Horticultural Science, Department of Vegetable and Mushroom Growing, Budapest Hungary*

Abstract

In south part of Hungary Bulgarian gardeners started in year 1873 with pepper production under very favourable growing condition, which ones characterizes the area today. Duration of sunlight 2050 hours/year, yearly average temperature is 10-11 C, average rainfall 500-550 mm/year. Soil is composed mainly clay. Hungarian taste was involved in usage of different types of varieties continuously. On the pepper market there is a wide range of different colour, shape pepper type but the main consumption remained the traditional white and light green triangular shape and spicy pepper varieties and the types which are harvested in biologically matured stage kapia and tomato types.. The region became a significant growing area in the sixties and the production under foil equipment's increased 200 000 square metres in concentrated frameworks.

The exploration of the geothermal energy (1974) changed fundamentally the growing opportunities. The geothermal energy only renewable energy source like that today, which is cheaper than any of the fossil energies. The thermal water insures the energy to the, high tech" soilless technology of pepper production. Alone on Szentes administrative area 32 thermal wells can be found, with this Hungary 's largest the scene of thermal activity, and Europe's thickest geothermal field. The depth of the wells alternates between 1593 and 3250 metres, at all of them the temperature is above 60 C, the temperature of outflow water is above 30 C in case of 23 wells. Agricultural use is qualified as primary utilisation. *Szentesi Paprika brand after the successful EU process, on January 2014 reach the Protected Geographical Indication.*

Growing on a basis of thermal water is going on with respect of protection of the natural environment. The thermal water being used averagely in greenhouses heating 40-45C. The thermal water onto the end of the utilisation steadily under 30 C and so becomes suitable to replace into wet conditions where is possible the maintenance of aquatic lifestyles. The Szentes thermal lake is a considerable bird's aquatic world today, was set up in 1982. He has big significance in protection of bird races, 176 valuable bird races are observed here and it is nominated as NATURA 2000 areas.

Keywords: sweet pepper, growing, thermal water, protection of natural environment

1. Introduction

The pepper got into Hungary from the Balkans thorough Turkish and Bulgarian connections during 17. Century. At the beginning the spice pepper in small scales, produced by Franciscan mons as medicine and later Bulgarian gardeners started in year 1873 with sweet pepper production under very favourable growing condition, in South part of Hungary. Today the white sweet pepper defines the Hungarian taste and the Hungarian market.

The breeding of this types began with Angeli Lambert he selected first from till then only

spicy types, not spicy variants and he used at first the shortened internode (in upper part) in pepper breeding (Csilléry 2013). It is necessary to mention two other names on beginnings of professional improvement by breeding of sweet pepper. His follower Zatykó Lajos breeded the Fehérözön (*Figure 1*) which was market-leader in considerable volume for a long time, and Turi István, the first hybrids connected to his name and the HRF1 is in production till today. He developed the technology of hybrid pepper seed production procedure as well. (Csizmadia 2014)

Figure1
Fehérözön

The end of 20th century, the paprika „was considered one of Hungary's symbol worldwide. The production covers mostly all kind of variety types which are available and popular in Europe.

The Hungarian pepper and sweet pepper breeding is today successful, there are good programs, and several successful breeders. The National Variety List includes 191 Hungarian varieties 80 of them are sweet peppers and number of new registered varieties from the last 5 years (2010-2015) is 43 new registrations. (National Food Chain Safety Office 2015)

According the statistical dates (Hungarian Central Statistical Office 2014) the sweet pepper production, is a significant part of the Hungarian vegetable sector. The most considerable growing area is the Southern part of Hungary (Great Plain) *figure 2 figure 3.* part of this is the Szentes district where the growing is going on a basis of thermal water with respect of protection the natural environment.

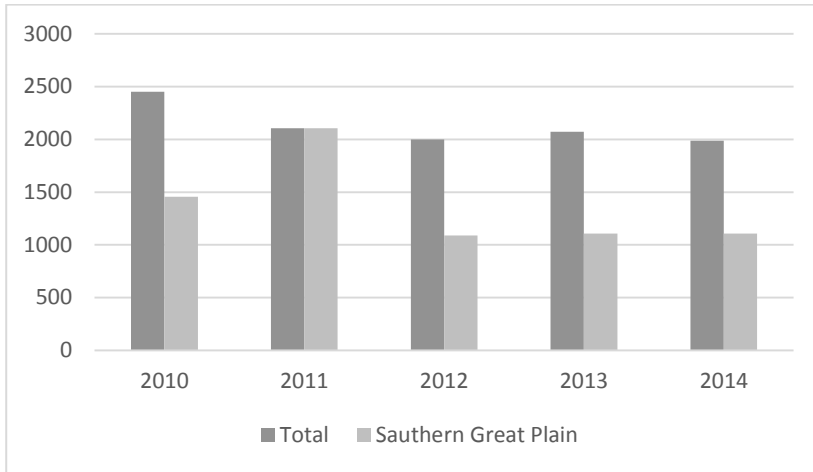

Figure 2
Sweet pepper production harvested area ha 2011-2014

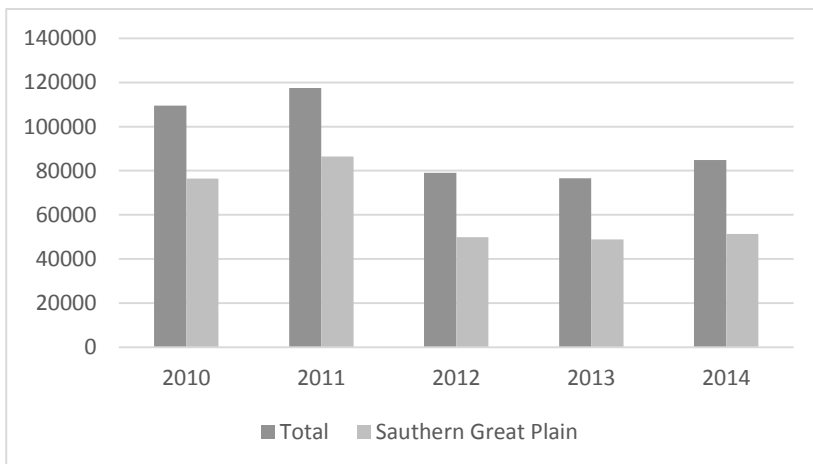

Figure 3
Sweet pepper production harvested tonnes 2011-2014

Thanks of this model the Szentesi Paprika brand after the successful EU process, on January 2014 reach the Protected Geographical Indication (Buleca2014).

2. Discussion

The growers of the Southern Great Plain Region achieved their excellent results with usage of thermal water. The horticultural utilisation of this system looks back at more decennial practice. The opportunity of this creates that after the Iceland Capital Reykjavik in surrounding of Szentes being founded Europe's second largest heating system based on geothermal energy. *Figure 4*. This countryside Hungary largest and Europe's thickest geothermal filed, with rich thermal water supply. On Szentes city's area altogether 32 thermal wells can be found its temperature is 85-90 Celsius. (Nagygál 2011)

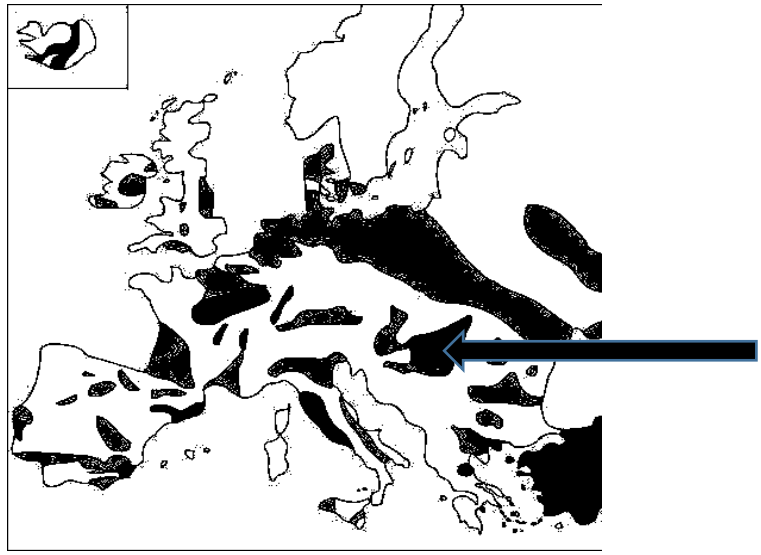

Figure 4.
Hungarian geothermal field

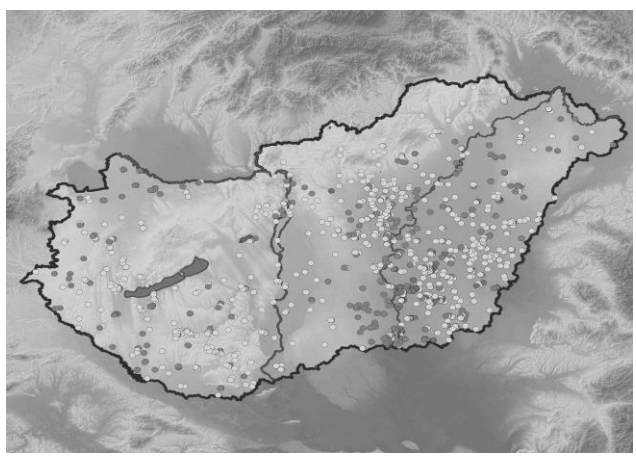

Figure 5
1300 Thermal wells (C>30)

2.1. Growing technologies under protected technologies

During the years the growing systems based on thermal water technology developed continuously, today the soilless system is preferred, but in this region there is an effective growing technology as the production in soil under foil and foil tunnels as well. The production in soil, based in all cases on strict soil analysis.

In case of soilless technology the cultivation substrate is Grodan (rock wool 60 % CaCo3 and basalt which make hot on 1600-1800 Celsius, then with a special procedure 0,04-0,05 mm thin threads are prepared and treated with resin), the result is a good absorbent substance with a

stable construction. Other useful substrate is (coconut fibre.) Both systems solve the water and the nutrient supply on one way.

With these technologies can be reached big crop with a distinguished quality. 12-15 kg/m2.

With big thermal water operated growing settlement is the Árpád Agrár Zrt Joint Stock Company Szentes but another the 5-6 thousand families deals there with pepper growing. Lot of them founded in 2002 the Gardener's Association Dél-Kertész for which one, the 485 members of today are who organize the marketing jointly (FruitVeb 2015)

2.2. Applied technology in harmony with the environment

At the beginning the water from the thermal wells after the heating of the greenhouses got into a drainage system promptly, from where the surrounding arable lands made use of it for irrigation. Later was necessary to stop it, in the interest of protection the environment. Then came in force a decision to construct a depot lake system which came true as a state investment in year 1982. The various substances which can be found in water was elutriated, and the water was cooled. The system is connected to the Kurca and Tisza-rivers. In year 2002 the dam system was renewed, his primary aim until today the water usage with agricultural aim.

The thermal lake system consists of two parts, one from 40 hectares is smaller the cooling lake with maximum capacity 1millio m3 and the bigger depot lake 100,7 hectare with maximum capacity 3 million m3. After the agricultural utilisation from the greenhouses the warm water gets into the smaller lake. The warm water became unnecessary; it does not freeze up on the hardest winters totally. In the lakes and in the surrounding area developed a special ecosystem. Well-known birdwatching place in Europe today. Special bird races nest here as the protected brown harrier, big heron and others. Ornithologists observed the regular appearance of 176 races. The lake belongs to Cserebökényi Pusta its classification is Natura 2000 and area of bird protection.

In spite of that the system written down above, works well, certain interpretations of European Water Framework consider it as environmental pollution. Accepting this interpretation, the Regulation the Hungarian Government number 147/2010. (IV. 29.) considered that it is necessary to change the present function of the thermal wells, until 2012 December 22. It would be necessary to retrieve the water in the sense of this into the bedrock. Deciding this was suspended after the weighing of the arguments and counter-arguments until 2025 according a government decision.

3. Conclusion

Oppositely and arose with scruples in the past years, this technology that does not push back the tired water into the bedrock is friendly for the environment. The Szentes thermal lake system with this technologies works well and does not protect only, the environment, but supports evaluation of valuable ecosystems Parallel with this, the sweet pepper production in greenhouses heated with thermal water yields serious economic profit. . Due for the well working vegetable companies of this area the Szentesi Paprika brand after the successful EU process, on January 2014 reach the Protected Geographical Indication

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Biology and management of bacterial spot of peppers in Oklahoma, United States

John Damicone[1], Felipe Cevellos[2], Claudia Diaz[1]

[1] *Oklahoma State University, Stillwater, Oklahoma, United States*

[2] *Universidad de las Fuerzas Armadas, Sangolqui, Equador*

Abstract

Chili and bell peppers (*Capsicum annum*) are produced in Oklahoma. Chili peppers are grown on a larger acreage for capsaicin oleoresin extraction. Bacterial spot, caused by *Xanthomonas* spp. is the primary foliar disease of peppers in the state. Bacterial spot in Oklahoma was previously reported to be caused by *X. campestris* pv. *vesicatoria* that mostly carried the avirulence allele (*Avr*) *AvrBs2* which interacts with the *Bs2* resistance gene to confer a hypersensitive or resistant response. The pathogen has since been reclassified into four species and the disease has been an increasing concern in chili production. The objectives were to identify the reclassified *Xanthomonas* spp. causing local outbreaks of bacterial spot, to determine the race structure (avirulence allele profile) of new and old strains of the pathogen, and to evaluate genetic resistance and copper sprays for disease management.

Isolates of *Xanthomonas* spp. from 2013 and 2014 were compared with isolates from 1999 to 2008 to determine if changes in species, avirulence alleles (*Avr*), or copper sensitivity have occurred. Polymerase chain reaction (PCR) assays with species-specific primers were performed on 31 representative isolates. Most isolates (n=20) were PCR positive for *X. euvesicatoria*, but none of the remaining isolates were PCR positive for *X. gardneri*, *X. vesicatoria*, or *X. perforans* even though 16S ribosomal sequences were at least 99% homologous with *Xanthomonas* spp. Bacteria were infiltrated into leaves of 'Early Cal Wonder' isolines containing no resistance genes or *Bs1*, *Bs2*, *Bs3*; and PI235047 containing *Bs4* and monitored for hypersensitive or compatible reactions. None of the *X. euvesicatoria* isolates had *AvrBs1*, 16 had *AvrBs2*, 5 had *AvrBs3*, and 14 had *AvrBs4*. Of the four isolates virulent on *Bs2*, two were from 2014 and one each was from 1999 and 2000. However, there was one isolate from 2014 virulent on all resistance genes. The cultivars 'Okala' and 'Jolox', grown locally for capsaicin production, were susceptible to all isolates. In a disc diffusion assay, 50% of the isolates were classified as censitive (growth inhibition at < 200 μg/ml), while the other 50% were tolerant (growth inhibition at 200 to 450 μg/ml or less metallic copper as copper sulfate. The effects of copper sprays on bell pepper cultivars with and without *Bs2* resistance were evaluated over a 3-year period. Both *Bs2* and copper effectively reduced (P=0.05) disease incidence by 75% and 50% respectively. However, only *Bs2* resistance resulted in a significant (P=0.05) yield response that averaged nearly 1000 kg/ha.

Bacterial spot in Oklahoma is caused by *X. euvesicatoria*. Most isolates carry *AvrBs2* and copper tolerance is a problem. *Bs2* resistance is more effective than copper sprays for disease management. Obvious changes in *Avr* alleles or copper sensitivity do not explain the recent severe outbreaks of bacterial spot. There is a need for bacterial spot resistance in locally grown chili cultivars.

Short evaluation of eggplant production and variety usage in Romania

András Kovács, Levente Szappanyos, Sándor György, Hajnalka Ledóné Darázsi

Agrosel SRL., Câmpia Turzii, Romania

Abstract

Eggplant (*Solanum melongena* L.) became wide-spread after the First World War in Romania. The most important growing areas of this plant are located in the Southern, South-Eastern and South-Western part of Romania and are usually cultivated on open fields, as well as in unheated greenhouses. In the past only Romanian OP varieties were grown. Over the past ten years requirements of eggplant varieties have increasingly shifted towards productivity, uniformity and high tolerance to stress factors, diseases and pests. Therefore, the cultivation of hybrids and the disappearance of Romanian OP cultivars have intensified. Due to monoculture practice the soil was attacked by pathogens in many areas. As a result, grafting became necessary to be put into practice. Consumption of eggplants is about 4,5 kg per person per year in Romania and are consumed in many different ways, such as baked, grilled or as a special cream.

Agrosel SRL has gained a significant role in supplying the Romanian vegetable seed market over the past twenty years and has started its own hybrid program to renew eggplant production in Romania.

The eggplant is a tropical perennial herbaceous plant often cultivated as a half-hardy annual in temperate climates. The species *S. melongena* has been cultivated and regarded as a native in Southern and Eastern Asia from ancient times. In Europe it was introduced throughout the Mediterranean area (first in Greece and Italy) by the Arabs in the early Middle Ages (around 1200) (Singh et al. 2004; Al-Awwam I., 1889).

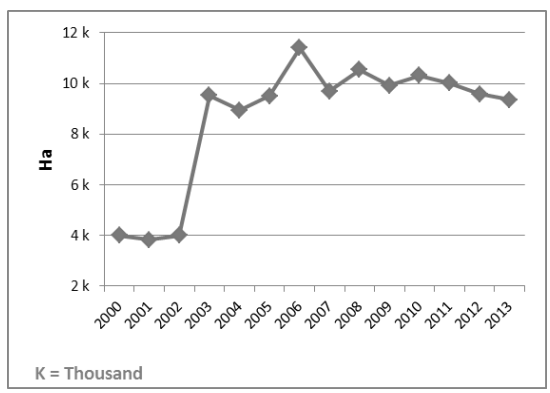

Figure 1:
Harvested eggplant area in ROMANIA
(FAO 2013)

In Romania it was propagated by Greek and Bulgarian gardeners in the 18thcentury and it became wide-spread after the First World War. In 1992 the cultivated areas of eggplant were 4500 ha with a 100 000 t yield in the country, which has significantly increased to almost 9500 ha with a 125 000 t yield to recent years (Echim I. et al. 1983; FAO 2013).

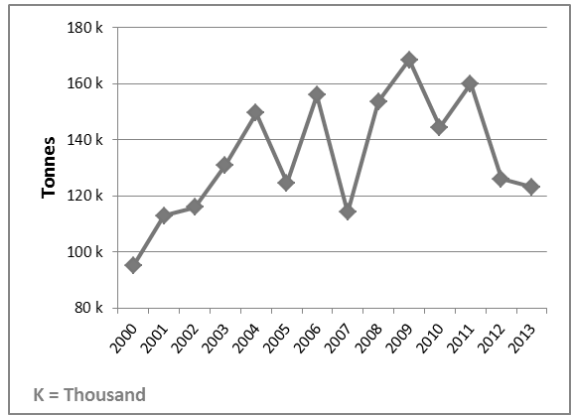

Figure 2:
Eggplant production quantity in ROMANIA
(FAO 2013)

The most important growing areas of eggplant are located in the Southern, South-Eastern and South-Western part of Romania: Matca-Tecuci, Galaţi; Coşereni, Ialomiţa; Izbiceni-Cilieni, Olt; Vidra, Giurgiu; and Seleuşi-Curtici, Arad.

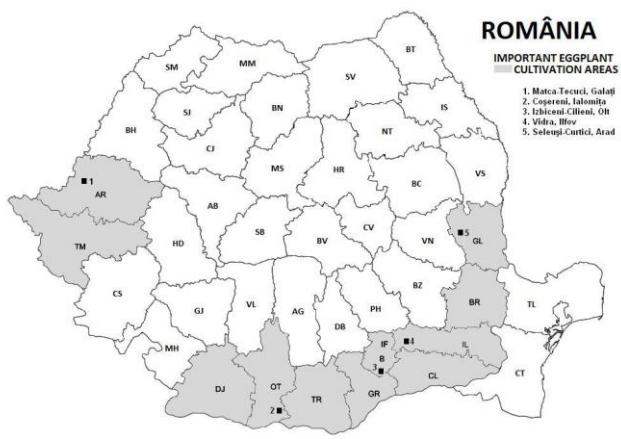

Figure 3:
Important eggplant cultivation areas
(AGROSEL)

Eggplants are usually cultivated on open fields, as well as in unheated greenhouses. For open field cultivation the nursing time begins with sowing at the end of February and the planting time starts when temperature of the soil reaches minimum 14 °C in 10-15 cm depth. The harvest begins when the fruit reaches market maturity and a 30-40 t/ha yield can be obtained. To achieve the market maturity earlier, plastic tunnels are being used to cover the plants.

Figure 4:
Open field cultivation (AGROSEL)

For unheated greenhouse cultivation the nursing time begins about three weeks earlier: the planting time begins when the air temperature remains stable at 15 °C outside the greenhouse. Eggplant production under heated greenhouses still does not exist (Ciofu R. et al. 2003).

Figure 5:
Unheated greenhouse cultivation (AGROSEL)

In the past only Romanian OP varieties were grown, namely **Danubiana** (55-65 cm plant height, light green colour; fruit weight 240-320 g, ovoid, dark violet colour),

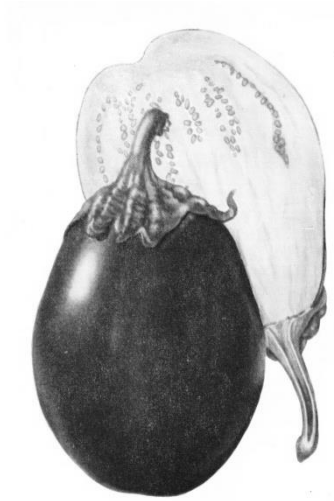

Figure 6:
Danubiana variety (Andronicescu D. et al. 1970)

Bucharest (70-80 cm plant height, grey-green colour; fruit weight 250-300 g, pear shaped, dark violet-black colour),

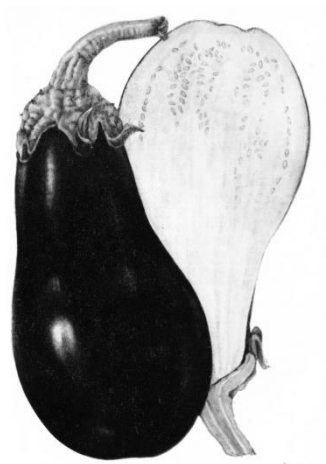

Figure 7:
Bucharest variety (Andronicescu D. et al. 1970)

Pana Corbului (75-85 cm plant height, green colour; fruit weight 240-300 g, pear shaped, glossy dark violet-black colour),

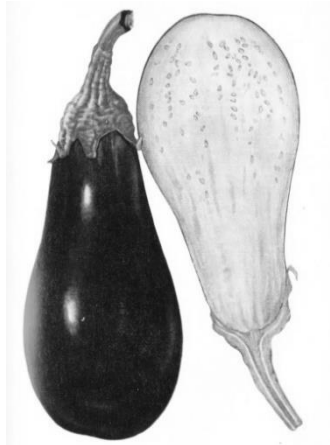

Figure 8:
Pana corbului variety (Andronicescu D. et al. 1970)

Lidia (60-65 cm plant height, fruit shape obovate, glossy dark violet-black colour, tolerant to *Verticillium dahlie* and *Phytophthora parasitica*), and **Narcisa**. In the 1960s a hybrid created in Ţigăneşti, Romania called **Delicia** (70-80 cm plant height, violet-green colour; fruit weight 300-350 g, obovate-pear shaped, glossy dark violet-black colour, tolerant to *Fusarium oxysporum* f. sp. *melongenae*) was mentioned (Gheorghe A. et al. 1997; Andronicescu D. et al. 1970).

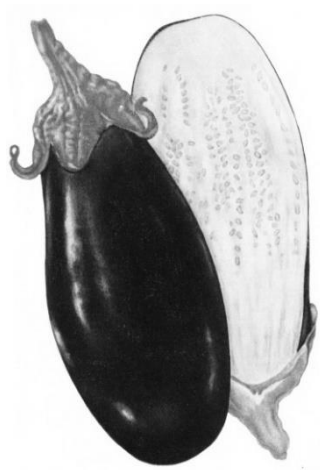

Figure 9:
Delicia hybrid (Andronicescu D. et al. 1970)

Over the past ten years requirements of eggplant varieties have increasingly shifted towards productivity, uniformity and high tolerance to stress factors, diseases and pests. Therefore, the cultivation of hybrids and the disappearance of Romanian OP cultivars have intensified. The most important international hybrids in Romania are: Aragon, Mirval, Classic, Mirabelle with more or less cylindrical shaped and dark purple-black fruits. White eggplant hybrid Bibo is also becoming accepted by consumers.

Due to monoculture practice the soil was attacked by soilborne pathogens and nematodes in many areas, which are very destructive in vegetable crops and can have an impact on the producers' income. Soil fumigation has been an essential component of greenhouse production since 1960s but it is a very expensive method. As a result, grafting (known for many years) has become necessary to be put into practice. Several trials have been carried out on grafted eggplants in Romania over the past years. The experiment in The Institute of Research and Development for Industrialization and Marketing of Horticultural Products Romania successfully proved the advantage of this method: there was a significant reduction of infested plants, the production increased up to 34% and the quality of the harvest improved by 19% (Bogoescu M. et al. 2014). While another research aimed to establish the technology for obtaining the eggplant grafted seedlings by manual or mechanical grafting with a semi-automatic machine. For manual grafting of 1000 eggplants 1,52 man were required per day, while when a semi-automatic machine was used only 0,16 man per day. The cost price reduction of grafting with semi-automatic machine was over 16% (Bogoescu M. et al. 2013).

Consumption of eggplants is about 4,5 kg per person per year in Romania. They are consumed in many different ways, such as baked, grilled, as a special cream, and they are also one of the main ingredients of the popular Balkanic food, Zakuszka.

Agrosel SRL has gained a significant role in supplying the Romanian vegetable seed market over the past twenty years. The company is one of the leaders in the small seed packages segment in Romania. Since 2004 the firm has started its own breeding program to widen the eggplant cultivars available in Romania.

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SESSION 3

Genetic resources

Chairs: Jaime Prohens, Marie-Christine Daunay

Eggplant resistance to bacterial wilt and to *Fusarium* wilt: Is there a link?

Marie-Christine Daunay[1], Jean Winoc Hennart[2], Sylvia Salgon[3], Jacques Dintinger[3]

[1] *INRA UR1052, Montfavet (84), France*

[2] *Vco, Ledenon (30), France*

[3] *CIRAD, Saint Pierre, Reunion Island (France)*

Abstract

INRA UR1052 maintains a germplasm collection of *S. melongena* and related species. The accessions of this collection that are described in the literature as resistant to *Ralstonia solanacearum*, agent responsible of bacterial wilt, were screened at plantlet stage against *Fusarium oxysporum* f.sp. *melongenae* on the basis of the number of leaves wilted and vessels browning. Results show that the panel of accessions tested display phenotypes ranging from fully resistant to fully susceptible to *Fusarium* wilt, with all intermediate resistance levels. Results are discussed on the basis of the complexity of resistance evaluation and of what is known in tomato about a link between resistances to both vascular diseases.

1. Introduction

Eggplant, *Solanum melongena* L., is susceptible to several vascular diseases of soil born origin, mainly *Verticillium dahliae* in cool temperate conditions, *Fusarium oxysporum* f.sp. *melongenae* in warm temperate conditions (sandy soils), and *Ralstonia solanacearum* in tropical and equatorial conditions. Resistances to each of these disease exist within the intraspecific and interspecific eggplant germplasm, although their genetic characteristics are variable. Fragmented experimental and literature information suggests the existence of some link(s) between resistances of eggplant, as well as of tomato, to these or other vascular diseases. By focusing on two pathosystems, eggplant on one side, *Fusarium* and bacterial wilt on the other side, our purpose is to investigate the possible relationship between eggplant resistances to both diseases. The first part is a literature based overview of the knowledge about the pathogens and eggplant resistances. Second part presents results obtained by testing with *F.o.* f. sp. *melongenae* a collection of eggplant genetic resources, chosen because described in the literature as resistant to bacterial wilt (BW). Results are then discussed in light of the genetic and functional information available for eggplant, and to a lesser extent, tomato.

2. Eggplant and wilts pathosystems

Eggplant - Fusarium wilt pathosystem

Resistance to *Fusarium* wilt is available in *S. melongena* germplasm (Abdullaeva & Shifman, 1988; Mandare & Patil, 1993; Sakata et al., 1996). A monogenic dominant control was identified in eggplant accessions -LS174, LS1934 and LS2436- [Sakata et al., 1996; Mochizuki et al., 1997; Mutlu et al., 2008; Boyaci et al., 2011). Miyatake et al. (2016)

positionned at the end of chromosome 2 the locus (*FM1*) two allelic forms of which were identified in LS174 ($Fm1^E$) and LS1934 ($Fm1^L$). Two closely linked SRAP and SRAP-RGA markers, mapped 1.2 cM from the LS2436 resistance gene were developed by Mutlu et al. (2008) for marker assisted selection. The locus responsible of the resistance of *S. melongena* LS2436 was mapped on the middle of eggplant chromosome 4, syntenic to tomato chr.4 (Miyatake et al., 2016)).

Resistance to *Fusarium* wilt was also found in several *Solanum* species related to eggplant such as *S. torvum, S. sisymbriifolium, S. aethiopicum, S. violaceum* and others (Yamakawa & Mochizuki, 1979; Cappelli et al;, 1995; Monma et al., 1996; Gousset et al., 2005; Boyaci et al., 2012). The genetic control of the resistance of two *S. aethiopicum* accessions belonging to cultigroups Aculeatum and Gilo, was shown as monogenic dominant, (Rizza et al., 2002; Toppino et al., 2008). Progenies derived from anther culture and interspecific somatic hybrids '*S. melongena* X *S. aethiopicum*' (Rotino et al., 2005) allowed Toppino et al. (2008) (i) to identify the locus *Rfo-sa1* which controls the resistance of *S. aethiopicum* to *Fusarium* wilt, as well as (ii) its two allelic forms, respectively specific to groups Aculeatum and Gilo. *Rfo-sa1* was positioned later on the eggplant Linkage Group 1 of Barchi et al. (2010). Miyatake et al. (2016) mapped *Rfo-sa1* very close to *FM1*.

All in all, three loci controlling *Fusarium* wilt resistance are identified today, two on *S. melongena* chromosome 2 (*FM1* and *Rfo-sa1*, with two allelic forms each) and one on chromosome 4 (Miyatake et al., 2016). So far, no interaction has been described between these loci and *Fusarium* strains, unlike monogenic resistances based on different mechanisms and controlling three *Fusarium* races in tomato (Gonzalez-Cendales, 2016). Further, in tomato polygenic tolerance has also been described (Crill et al., 1972).

Eggplant - bacterial wilt pathosystem

Resistance to BW, as in the case of *Fusarium* wilt, has been found in *S. melongena* germplasm. The resistances found in different accessions are described as dominant or recessive, monogenic or polygenic, depending on the accessions (compiled in Daunay, 2008). A first single dominant gene of resistance identified in the Chinese accession E-31, was marked with a SCAR marker distant from 3.33 cM (Cao et al., 2009). In this accession, the function of a putative BW resistance gene (the same ?), named *RE-bw*, was characterized, including its interaction with the bacterial *Popp2* avirulence factor (*Xiao Xi'ou* et al., 2015). Another major dominant gene, *Ers1*, probably positionned on chromosome 9 controls the resistance of the INRA accession AG91-25 (Lebeau et al., 2013); its interactions with *Ralstonia* type III effectors are under current research (Peeters et al., pers. comm.). *Ers-1* is escorted by a few QTLs, the efficiency of which depends on the bacterial strains used.

Resistances to BW have also been found in several *Solanum* species related to eggplant (Hébert, 1985; Clain et al., 2004; Gousset et al., 2005; Daunay, 2008), in particular in *S. torvum , S. aethiopicum, S. sisymbriifolium*, and *S. violaceum*, some of which are used as rootstocks for eggplant. This does not mean that all accessions of each of these species are resistant to all strains. For instance susceptibility or partial susceptibility of some accessions of *S. torvum* (Saito et al., 2010; Gousset et al., 2005) and *S. aethiopicum* (Hébert, 1985) is mentionned. Further, bacteria can be isolated from roots or lower stem of symptomless *S. torvum* (Clain et al., 2004; Gousset et al., 2005) which means that the resistance of this species, as in eggplant and tomato (Grimault & Prior, 1994), is an ability to limit the upward spread of the bacteria within xylem vessels.

The resistance of *S. aethiopicum* was transferred into *S. melongena* by sexual interspecific

crossing (Ano et al., 1991) as well as by protoplast fusion (Collonnier et al., 2001b). Somatic hybrids between *S. melongena* on one hand, and *S. torvum* (Collonnier et al., 2003b) or *S. sisymbriifolium* on the other (Collonnier et al., 2003a), as well as somatic hybrids between *S. integrifolium* (= *S. aethiopicum*) and *S. violaceum* (Tamura et al., 2002) are as resistant or less than their resistant parent.

The major trouble the breeders face for creating resistant material to BW is the unstability accross locations of the resistances they use, whatever the solanaceous crop they work with (Huet, 2014), because of biotic (complexity of the pathogenic process, interactions of the resistances with local bacterial strains, synergy with root knot nematodes), as well as abiotic reasons (influence of soil type and moisture, temperature and light intensity) (Hayward, 1991). In eggplant, as in tomato, the resistance is an ability to limit the spread of the bacteria within the stem xylem vessels (Grimault & Prior, 1994). This means that wilt is not a sufficient criteria for assessing resistance, and must be completed with a colonization index, as did Lebeau et al. (2011) who revealed the existence of compatible or incompatible interactions among a set of resistant accessions of both species. The wide genetic diversity of the thousands strains of *Ralstonia solanacearum* is another major impediment. The classification of the BW complex, initially based on host range (races), then on metabolic properties (biovars), has strongly evolved with the availability of molecular techniques, from which Sequevars, Clades, Phylotypes (Fegan & Prior, 2005) were defined. The numerous pathogenicity components of the bacteria have been reviewed by Peeters et al. (2013). New species have been defined recently within this complex (Prior et al., 2016). On the whole, all this means that research on resistance spectrum, genetic determinism and/or functional characterization needs intimate genetic knowledge of the bacterial strains used, as was shown in Lebeau et al. (2011). These authors exemplified the complexity of the interactions between a range of bacterial strains representative of the diversity of the bacteria, and a core collection of resistant genitors of three solanaceous crops (eggplant, tomato and pepper).

Resistance to several vascular diseases

Resistance to both *Fusarium* and bacterial wilts is mentionned in the literature for some eggplant accessions, such as LS1934 and LS 2436 (e.g. Sakata et al., 1996) as well as for several eggplant relatives such as *S. aethiopicum* Aculeatum and Gilo Groups, *S. torvum*, *S. sisymbriifolium* and *S. violaceum* (e.g. Narikawa et al., 1988; Collonnier et al., 2001a; Gousset et al., 2005; Daunay, 2008). However, the available data are limited in terms (i) of number of accessions tested for each *Solanum* species and (ii) fungal or bacterial strains used, although interactions between accessions and *Ralstonia solanacearum* strains exist. Such interactions could explain why some results even refute the link between both resistances, such as those of Monma et al. (1996) who found all their 53 *S. aethiopicum* accessions resistant to *Fusarium* wilt, being susceptible to BW.

Additional resistance to *Verticillium* wilt is described in the eggplant accession LS2436 (Sakata et al., 1996; Saito et al., 2010), as well as in some *S. torvum* accessions (Narikawa et al., 1988), which resist to the three vascular diseases.

Interestingly in tomato, association within single genotypes (such as the famous Hawaii 7996) of resistance to three vascular diseases, bacterial wilt, bacterial canker and *Fusarium* wilt race 2 (in the absence of gene I-2), has been noticed by Laterrot et al. (1978) and Laterrot & Kaan (1978).

Literature testifies that these « coincidences » (i.e. the resistance to two or three vascular diseases within a single gentotype) are not systematic, since they are found in a number of

accessions only, and not in all accessions resistant to the one or the other of these disease resistance examples.

The summing up presented above explains why, intrigued by these occasional (but frequent enough to stick curiosity) association of resistances to two or more vascular diseases, within single genotypes in eggplant, related *Solanum* species as well as tomato, we further tested the hypothesis of a putative link between the resistance to bacterial and *Fusarium* wilt, on a range of eggplant germplasm. The synteny between the genomes of eggplant and tomato,, and the growing knowledge of the genetic factors involved in the resistance to each of these diseases in both crops, provides a good background for unravelling in the future the possible genetic and functional similarities which could explain the presence in some accessions, of resistance to these two (or more) vascular diseases.

3. Screening of genetic resources

Material and methods

The INRA germplasm database was sorted out for identifying a sampling of *S. melongena* and related *Solanum* spp. accessions recorded as resistant to BW (**Table 1**). The behaviour « resistant » is a very « rough data », given bacterial wilt resistance is quantitative and depends on the bacterial strains tested. The information for resistance to BW originates either from INRA results obtained in the French West Indies, or from accessions donor, or from literature.

This material was tested to *Fusarium oxysporum* f. sp. *melongenae*. Susceptible controls were *S. melongena* Banaras Giant (MM 608) and Violette de Barbentane (LF3-24); Resistant controls were *S. aethiopicum* Aculeatum Group (MM 134) and *S. sisymbriifolium* (MM 284).The inoculation method is close to that used at INRA for tomato (Moretti & Laterrot, 1994). Inoculum was prepared by cultivating a Japanese *Fusarium* strain (TF 161), obtained from Takii France, on an agitated synthetic culture medium, 8 days at 10 hours day/14h night and 18/23°C. At the time of inoculation, the solution was grinded and filtered. Two dilutions were used, one $1/5^{th}$ in distilled water and $1/10^{th}$. Dilution $1/5^{th}$ matches 10^6 conidies/ml.

After sowing in pans, plantlets 18-21 days old were removed, roots are rinsed, partially cut, dipped into the inoculum for 5 mn and transplanted in new pans filled 2/3 compost, 1/3 sand (four accessions and one control -susceptible or resistant- per pan). Pans were then settled in a climatic chamber, regulated 12h/12h, 28°C constant. Twenty plants per genotype and per inoculum dilution were tested, given enough plantlets were available. Two weeks afer inoculation, symptoms were recorded on each plant as:

- resistant: no wilting, no vessels browing (or browning limited to hypocotyl),
- susceptible: wilting or no wilting, vessels browning present in the stem, further up cotyledons insertion.

Percentage of resistant plants was calculated as: 100 * [number of resistant plants / 20].

The test was repeated by Vilmorin in 2003, with two repeats of 7 seven plants per accession. The percentage of resistant plants was calculated on the basis of two measurements, foliar wilting and vessels browing (scales not shown).

Results

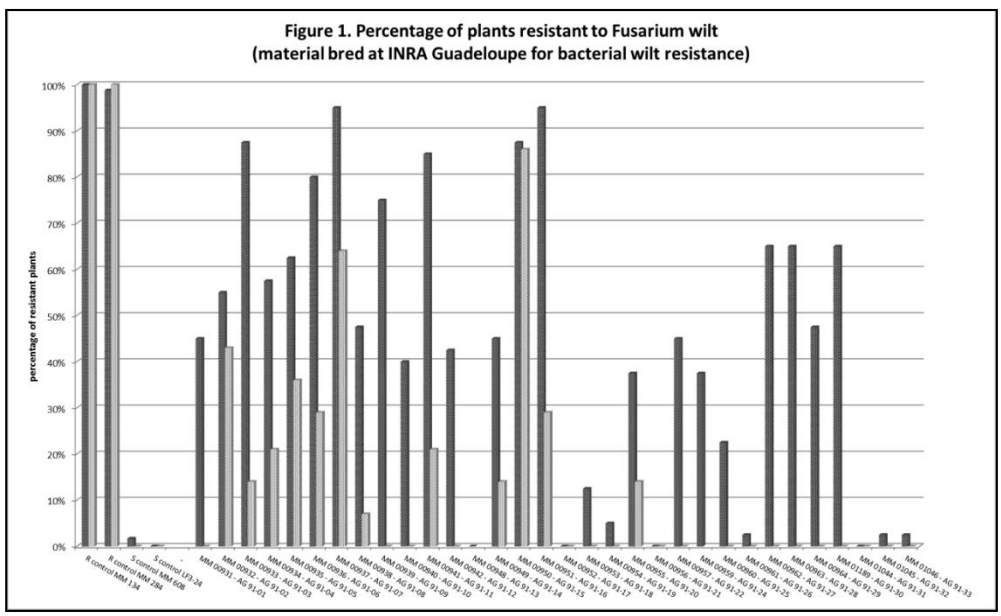
The inoculum dilution (1/5th or 1/10th), used in INRA screening test, did not affect the response of susceptible and resistant controls but some slight up or down variation of the proportion of susceptible and resistant plants was recorded for the accessions tested (data not shown). For the sake of clarity, we present the INRA results of both dilutions merged together. Further, as there was a close link between the two measurements used by Vilmorin - foliar wilting and vessels browing - (data not shown), we present only Vilmorin results on the basis of the percentage of resistant plants calculated from the vessels browning. Results are presented for the material subdivided into three categories. **Figure 1** is focused on AG-xx accessions, issued from BW breeding programs carried out at INRA Guadeloupe. **Figure 2** is focused on accessions of INRA *S. melongena* collection described as resistant to BW. **Figure 3** displays the results for a set of *Solanum* spp. also described as resistant to BW. All histograms display the results for both INRA and Vilmorin tests. For each accession tested, the left, dark grey, bar illustrates INRA results, and the right one, light grey, Vilmorin results.

Species	Accession number	Name or other number	Total number of plants tested (INRA)	Total number of plants tested (Vilmorin)
S. aethiopicum (Aculeatum group)	**R control MM 134**		60	41
S. sisymbriifolium	**R control MM 284**		80	56
S. melongena	**S control MM 608**	Giant of Banaras	60	63
S. melongena	**S control LF3-24**		70	68
S. melongena	MM 00931	AG 91-01	40	14
S. melongena	MM 00932	AG 91-02	40	14
S. melongena	MM 00933	AG 91-03	40	14
S. melongena	MM 00934	AG 91-04	40	14
S. melongena	MM 00935	AG 91-05	40	14
S. melongena	MM 00936	AG 91-06	40	14
S. melongena	MM 00937	AG 91-07	40	14
S. melongena	MM 00938	AG 91-08	40	14
S. melongena	MM 00939	AG 91-09	40	14
S. melongena	MM 00940	AG 91-10	40	14
S. melongena	MM 00941	AG 91-11	40	14
S. melongena	MM 00942	AG 91-12	40	14
S. melongena	MM 00948	AG 91-13	5	0
S. melongena	MM 00949	AG 91-14	40	14
S. melongena	MM 00950	AG 91-15	40	14
S. melongena	MM 00951	AG 91-16	40	14
S. melongena	MM 00952	AG 91-17	40	14
S. melongena	MM 00953	AG 91-18	40	14
S. melongena	MM 00954	AG 91-19	40	14
S. melongena	MM 00955	AG 91-20	40	14
S. melongena	MM 00956	AG 91-21	40	14
S. melongena	MM 00957	AG 91-22	40	14
S. melongena	MM 00959	AG 91-24	8	0
S. melongena	MM 00960	AG 91-25	40	14
S. melongena	MM 00961	AG 91-26	40	14
S. melongena	MM 00962	AG 91-27	40	14
S. melongena	MM 00963	AG 91-28	40	14
S. melongena	MM 00964	AG 91-29	40	14
S. melongena	MM 01189	AG 91-30	40	14
S. melongena	MM 01044	AG 91-31	40	14
S. melongena	MM 01045	AG 91-32	40	14
S. melongena	MM 01046	AG 91-33	40	14
S. melongena	MM 120	Nan Tan	40	0
S. melongena	MM 127	Turquie	40	14
S. melongena	MM 152	Ceylan SM 164	40	14
S. melongena	MM 165	Sinampiro	40	14
S. melongena	MM 412	Taiwan Naga	40	0
S. melongena	MM 413	n°1	40	14
S. melongena	MM 415	Mayon	40	14
S. melongena	MM 799	SM6	40	14
S. melongena	MM 853	Dingras Multiple Purple	40	14
S. aethiopicum (Aculeatum group)	MM 457		40	14
S. aethiopicum (Gilo group)	MM 196 TER		40	14
S. aethiopicum (Gilo group)	MM 229		40	14
S. aethiopicum (Gilo group)	MM 232 BIS		40	14
S. aethiopicum (Gilo group)	MM 283		40	14
S. aethiopicum (Gilo group)	MM 348		40	14
S. aethiopicum (Gilo group)	MM 854		40	14
S. aethiopicum (Gilo-anguivi group)	MM 236		40	14
S. aethiopicum (Kumba group)	MM 574		40	14
S. melongena gr E	MM 498		40	14
S. stramonifolium	MM 416		40	0
S. violaceum	MM 497		40	14
S. violaceum	MM 1027		40	14
S. virginianum	MM 265		40	14

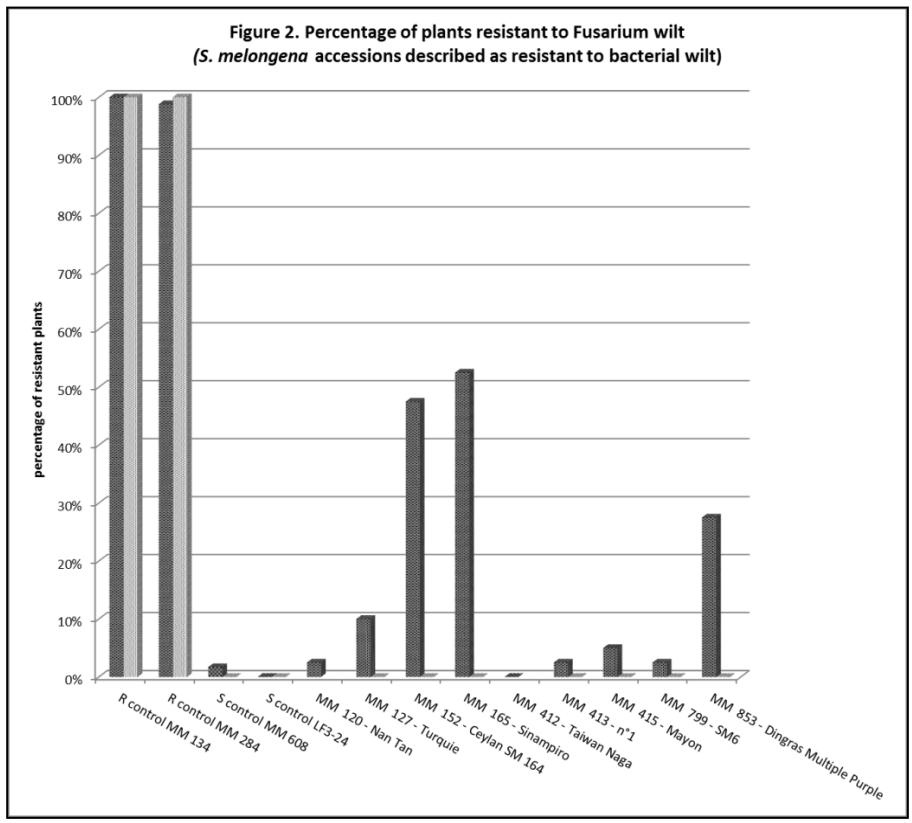
Table 1:
List of the eggplant germplasm resistant to bacterial wilt, tested for Fusarium wilt

Resistant and susceptible controls behaved as expected, with almost 100% of plants respectiveley healthy or dead, for both series of tests. However, Vilmorin test was more stringent than INRA test, since for all but a few accessions, the percentage of resistant plants is almost systematically lower (**Figures 1, 2, 3**).

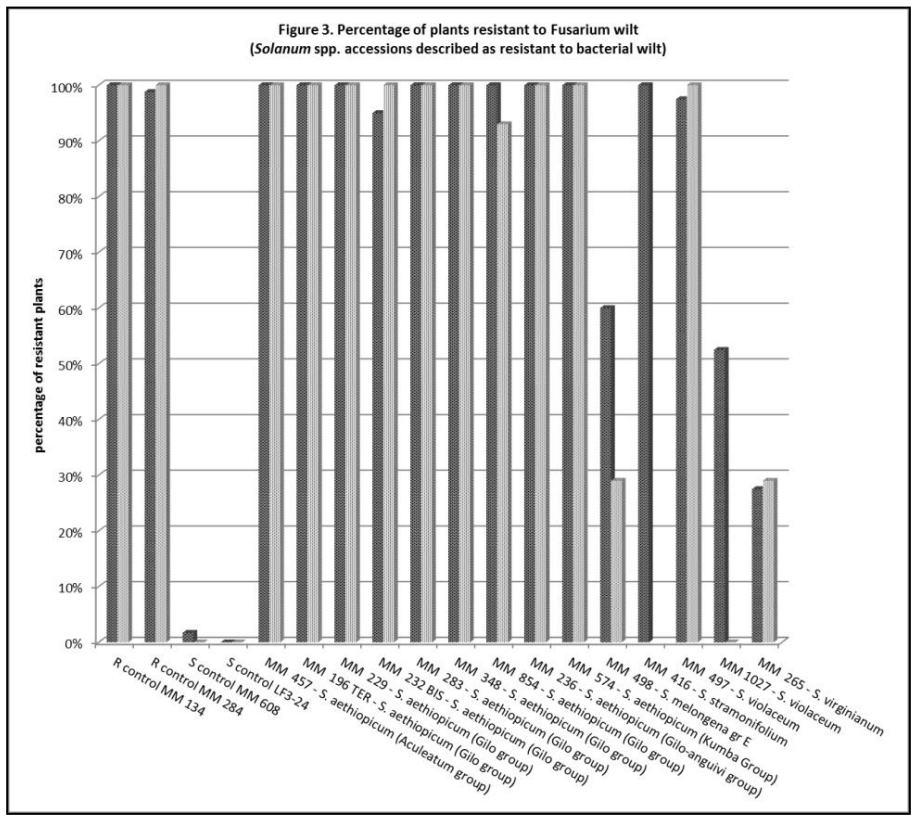
The material bred at INRA for BW resistance (**Figure 1**), displays a variable behaviour towards *Fusarium* wilt, from fully susceptible (e.g. AG91-17, AG91-21, AG91-31) to a high level of resistance in INRA test (AG91-07, AG91-16), with all intermediate levels of resistance between the extremes for the other accessions. For cases of resistance, resistance level is less than that of the resistant controls. For one accession only (AG91-15) the resistant behaviour is similar in both INRA and Vilmorin tests.



The set of *S. melongena* accessions described as resistant to BW, is overall susceptible to *Fusarium* wilt, although MM 152 and MM 165 are moderately susceptible with some 50% plants resistant (**Figure 2**).



Interestingly, all *S. aethiopicum* tested and as well as *S. stramonifolium* and one accession of *S. violaceum* (MM 497) are as resistant to *Fusarium* wilt as both resistant controls, *S. sisymbriifolium* and *S. aethiopicum* Aculeatum group. The few other accessions are moderately or slightly resistant (**Figure 3**).



4. Discussion and conclusion

Our experiments, carried out in 2002, suffer two limitations. First, they involve a material chosen on the basis of the (solely) rough/global information related to bacterial wilt resistance, although resistance to BW is complex in terms of (i) interactions between resistance progenitors and bacterial strains (Lebeau et al., 2011), (ii) of genetic controls (so far unravelled only for a very limited number of genotypes) as described in section 2, and (iii) of symptoms (unwilted plants can be severely colonized by the bacteria and environment changes can provoke their unexpected sudden wilting). Second, for the screening tests towards *Fusarium* wilt, we did not expect an heterogeneous behaviour within accessions, from one plant to another. This led to quantitative resistance levels (ranging between 0 and 100% resistant plants). Because of funding shortage, we could not check whether this heterogeneity was a matter of heterozygozity or of inoculum pressure.

Despite these limitations, our *Fusarium* wilt resistance screening tests of *S. melongena* accessions and *Solanum* spp. accessions bred for resistance to BW, or described as resistant, indicate, in some cases, the existence of a genotype-dependant association between the resistances to both wilts. The closer association is found in accessions of *S. aethiopicum* (**Figure 3**) as indicated by our results (9 accessions, described as resistant to BW, almost 100% resistant to *Fusarium* wilt) This association is found in the three cultigroups tested, Aculeatum (syn. *S. integrifolium*), Gilo and Kumba. However, these results contradict those of Monma et al. (1996) who found 100% of their 53 *S. aethiopicum* accessions resistant to *Fusarium* wilt, but susceptible to BW. These facts are clues that (i) resistance to *Fusarium* wilt is frequent in *S.*

aethiopicum and (ii) that the resistant or susceptible behaviour of this species to BW depends on the bacterial strains used. Further, the *Fusarium* wilt results of Cappelli et al. (1995), who found a 27 to 47% disease incidence in three accessions of *S. aethiopicum*, suggest that the resistance to *Fusarium* wilt of this species might be of a variable level, depending on the accessions. Interestingly, *S. anguivi*, the closest wild relative of *S. aethiopicum*, tested by Monma et al. (1996) (7 accessions), is also resistant to *Fusarium* wilt.

S. violaceum, described as resistant to BW, is also almost 100% resistant to *Fusarium* wilt, but for one accession only (MM 497) out of the two tested. For the wild eggplant (MM 498) and *S. virginianum* (MM 265), both described as resistant to BW, the percentage of *Fusarium* resistant plants varies from almost 30% up to 60%.

The incidence of *Fusarium* wilt on *S. melongena* accessions known as BW resistant (**Figure 2**) is important, since they are either susceptible (most accessions) or of limited resistance for the best one (50% of resistant plants in MM 165, Sinampiro). Unfortunately, but because of unavailability, we did not test *S. melongena* LS2436 which is the sole *S. melongena* accession described in the literature as resistant to both wilts. This suggests that simultaneous resistance to both diseases might be rare within *S. melongena* germplasm.

The INRA AG-xx accessions (**Figure 1**), issued from complex crosses involving many progenitors including, for most of them, *S. aethiopicum* Aculeatum Group (MM 134), display a variable percentage of *Fusarium* wilt resistant plants, from null to higher than 90%. The available data do not allow to speculate far further from the hypothesis that the breeding program carried out at INRA Guadeloupe for BW resistance, has somehow, and more or less, « diluted » the *Fusarium* wilt resistance(s) of *S. aethiopicum* and/or of the possible *Fusarium* wilt resistance of the other BW resistant progenitors.

Our results do not invalidate but do not validate either the starting hypothesis of this paper, about a possible link between resistances to *Fusarium* and bacterial wilts. This link, if existing, at least in some genotypes of eggplant and relatives as well as in tomato, could involve plant genes or QTLs involved in both resistances, or involve distinct genes inducing similar physiological reactions, or both. The existing synteny between both Solanaceous species, and the increasing knowledge of their genome provide tools for running cross search of the generalist ou pathogens-specific genes and mechanisms involved in controlling vascular diseases. Both diseases are vascular and induce the same kind of plant disorders, hence non specific plant defenses limiting these disorders probably exist. The root system and what happens at the cotyledons insertion point seem to play a key role in limiting the development and upward spread of the vascular pathogens, once entered within the roots xylem vessels. Hence, given (i) the developping knowledge of the genetic controls to both diseases, and of the structure and expression of *Fusarium* (e.g. Barbierato et al., 2016) and BW resistance genes (e.g. Reddy et al., 2015), as well as (ii) the ongoing better management of *Ralstonia solanacearum* strains (genotypes) used in resistance tests, promising studies are expected in a near future, aiming at a better understanding of the specific and/or common physiological and genetic mechanisms controlling the resistance to vascular wilts, including *Fusarium* and bacterial wilts.

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Study of morphological characteristics of eggplant (*Solanum melongena* L.) varieties

Marianna Fehér[1], Zsuzsanna Füstös[2]

[1] *National Food Chain Safety Office, Budapest, Hungary*

[2] *Szent István University, Budapest, Hungary*

Abstract

Eggplant varieties are very diverse in size, shape, colour and grown throughout the world. Eggplant market and consumption increased in the last decades. The number of registered varieties for the Hungarian National List doubled in the last years. We tested 34 varieties from the EU Common Catalogue from which 8 varieties are also on the Hungarian National List. We observed characteristics based on the CPVO Technical Protocol 117/1, trial place was in Tordas, Hungary. The characteristics were the following: anthocyanin coloration of hypocotyls and stem, intensity of purple colour of flower, main colour of skin at harvest maturity of fruit, anthocyanin coloration of calyx and colour of the fruit flesh. The measured characteristics were: height of stem, size of flower, size and weight of fruit. Tested varieties had mainly violet and some of them white colour. Hypocotyl of tested varieties shown mostly anthocyanin coloration. Intensity of anthocyanin coloration of hypocotyls predict the intensity of flower anthocyanin coloration but no correlation with the intensity of violet fruit main colour. Moreover, the white varieties have medium intensity of hypocotyls anthocyanin colour. The varieties with the darkest anthocyanin of stem has the darkest anthocyanin of hypocotyls, flower and calyx. It was significant correlation among the globular varieties. The medium and weak anthocyanin of stem was in correlation with weak anthocyanin of hypocotyl and no anthocyanin on calyx. The varieties which had medium and weak purple flower colour have weak anthocyanin of hypocotyls and absent of anthocyanin coloration of calyx but the colour of fruit skin was very dark. Relationship between fruit main colour and colour of flesh is significant. All white coloured varieties have white colour of flesh. The diversity between the weight and size of varieties was remarkable, 51-553 g. The results of our experiments not confirmed the published data correlation among the stem height, flower size and fruit size and weight, although the large–fruited varieties (above 400 g) have a large flower.

Keywords: eggplant varieties, plant, fruit, flower characteristics, anthocyanin coloration

1. Introduction

Wild eggplant is reported to be found in India the plant was spiny and the bitter. From India the domesticated non bitter fruited types spread eastward China in the fifth century BC and later were taken to Spain and Africa by traders (Yamaguchi, 1983). Eggplant (S*olanum melongena* L.) is an horticultural cultivation important crop and widely grown in Americas, Asia and Europe, characterized by great morphological diversity. Professional growers see the necessity of introducing new varieties, particularly hybrids, breeding for very high production in different climatic zone. The base of different Classification of eggplant the origin, shape and colour of varieties. (Sekara et al., 2007)

In Southern European countries are the most popular the growing and consumption of eggplant. The Italian varieties divided into eight groups by shape and colour (Masseria, 2013). Kowalska (2008) published depending on a variety, those may be: spherical, through oval, ovoid, piriform, to elongated and spiral. Black-purple fruits, in the skin of which anthocyanins

are present, dominate in production. There are also white-coloured varieties, the skin of which does not contain those pigments. Fruit's colour, sometimes its growth, depend on the flower's localization in an inflorescence. Side flowers characterized by slower growth rate and less intensive colour, set smaller and lighter fruits.

In Hungary eggplant is not a traditional vegetable crop therefore in the first part of the last century started the cultivation in the south part of the country with Bulgarian varieties (Géczi, 2010). We mainly use varieties from EU Common Catalogue because our breeding activity not very high, but we had Hungarian varieties, Lila bika, Kecskeméti lila.

The number of the eggplant varieties is high in the European Common Catalogue, 312 varieties and there are 25 protected varieties on the CPVO List, which has Plant Breeder Right. On the Hungarian National List are 12 eggplant varieties, mainly from EU countries.

The aim of the observation was to find correlation between different characteristics: plant height, fruit size, flower colour and size, anthocyanin coloration of hypocotyls, stem, skin and calyx to introduce varieties and give information for eggplant breeders.

2. Material and methods

The trial was carried out in the experimental place of NÉBIH (National Food-Chain Safety Office) in Tordas in 2015. The eggplants varieties were grown under plastic tunnel. The row and plant distance was: 90 x 50 cm. The sowing was on 27 of April in 5x5 cm pot and the planting on 30 of May.

The following 34 varieties were in the trial which were collected from the European Union:

globular: Birgah, Brillant, Egle, , Formosa, Gloria, Laura, Prosperosa, Purpura

ovoid: White Egg, White Imola

obovate: Angela, Basalto, Black King, Black Moon, Bonica, Clelia, Danka, Kamelia, Matrona, Top Bell, Top Gadir, Top Ora

pear shape: Giotto, Irene, Madonna, Nero

club shaped: Claudia, Lady root, Longo, Maiorca, Spany

cylindrical: Alabaster, Ideal

cylindrical *club shaped* *obovate*

pear shaped *ovoid* *globular*

Figure 2.Eggplant fruit shape by CPVO (TP/117/1)

The varieties mainly belongs to the varieties of purple skin colour, but Angela was a purple striped skin variety while Alabaster, White Egg and White Imola had white skin.

The observation based on the Technical Protocols of CPVO (TP/117/1) and the data analysis based on the regression analysis.

The observation of characteristics was visual but the plant height, fruit weight and flower size was measured.

state of expression 1: smallest size, lightest colour

state of expression 9: largest size, darkest colour

3. Results

The evaluation among the shape of eggplant varieties don't show linear relationship among the fruit weight, plant height and flower size (Table 1).

Table 1.
The result of regression analysis between fruit weight, plant height and flower size of different types of eggplant varieties

Characteristics	Correlation coefficient	Linear function
fruit weight - plant height	r= - 0,09	Y=-1.083x+432.405
fruit weight - flower size	r=0.53	Y=8.645x-152.868
flower size - plant height	r=0.16	Y=0.1178x+41.999

The evaluation shows that there is not correlation between the fruit weight and plant height, fruit weight and flower size and plant height and flower size. The slightest correlation is between fruit weight and flower size but it is not as strong as we accepted. The varieties which had a largest fruit over 400g had a large flower.

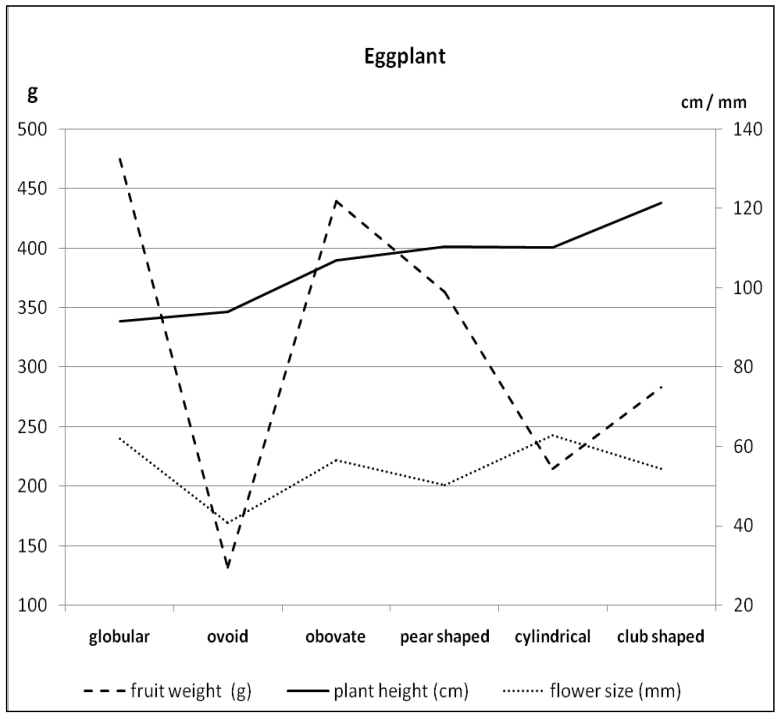

Figure1.
Average data of fruit weight, plant high and flower size at the different types of tested eggplant varieties

The evaluation of the eggplant shapes is on the base of the Figure 1.

Globular shape: the only slight correlation is visible by the variety of globular shape because they have the biggest fruit weight (552,7g) and a large flower size (71,5mm). The plant height of the globular varieties was the smallest (81,5cm).

Ovoid shape: the data of this type shows the opposite of the globular ones. The plant height of ovoid varieties was low (86,5cm) and the fruit weight (51,5g) and flower size (41,5mm) was also small, the smallest.

Club shaped: these varieties have the highest plants (127,8cm) but the fruit weight (269,3g) and flower size (57mm) shows medium data.

Cylindrical shape: the varieties which belongs to this type have a medium plant height (111cm), a small fruit size (152g) and a large flower size (66mm).

Obovate shape: the plant height of these varieties was medium (112,6cm). The weight of fruit (448,4g) was big but the flower size (55,4mm) was only medium.

Pear shape: these varieties had also medium plant height (115,8cm) and the medium fruit weight (340,8g) was accompanied with small flower size (49mm).

The measured data of flower size at globular type was the largest between 6-7 mm while at the other types between 4-7 mm.

Anthocyanin characteristics

The observation of these characteristics was in 2015.

The data of the observation of the anthocyanin coloration shows very different value among the eggplant shape. There was no connection between flower colour and intensity of anthocyanin coloration of hypocotyls, stem and calyx, except by globular shape. Varieties with dark flower colour had weak anthocyanin coloration of hypocotyl/stem (Irene) or medium coloration of hypocotyl/stem (Madonna).

Interesting result can be observed at the white varieties because they had a dark flower colour but the anthocyanin coloration of hypocotyl was absent by Alabaster and medium by White Egg and White Imola.

The globular varieties show only an unambiguous connection in the anthocyanin coloration among the different part of plants. (Table 2.) It can be determine if the hypocotyls has a dark anthocyanin coloration than the stem, the flower and the calyx will have also dark coloration. The only exception was the variety Formosa which has this year a medium skin colour.

Table 2.
Intensity of the anthocyanin coloration of hypocotyl, stem, flower, calyx and skin of different shape of eggplant

varieties	hypocotyl	stem	flower colour	calyx	skin
globular					
Birgah	7	7	7	7	6
Brillant	7	7	7	7	7
Eagle	7	9	7	7	7
Formosa	7	7	7	3	5
Gloria	7	7	7	7	7
Laura	7	7	7	9	8
Prosperosa	7	7	7	7	7
Purpura	7	7	5	9	7
ovoid					
White Egg	5	1	7	1	1
White Imola	5	3	7	1	1
obovate					
Basalto	3	1	7	1	9
Black King	3	5	5	1	9
Black Moon	3	1	4	1	9
Bonica	3	5	3	1	9
Clelia	3	5	5	1	9
Danka	3	4	4	1	9
Kamelia	3	1	5	1	9
Matrona	3	1	3	1	9
Top Bell	3	3	5	1	9
Top Gadir	3	3	5	1	9
Top Ora	3	5	4	1	9
pear shaped					
Angela	1	1	7	1	5
Giotto	3	3	5	1	9
Irene	3	3	7	1	9
Madonna	5	5	7	1	9
Nero	3	4	5	1	9
club shaped					
Claudia	3	3	5	1	9
Lady Root	3	7	5	1	9
Longo	3	5	5	1	9
Maiorca	3	5	5	1	9
Spany	3	4	5	1	9
cylindrical					
Alabaster	1	1	7	1	1
Ideal	3	5	5	1	9

State of expression 1 very weak *State of expression 9 very strong*

Specific description of the varieties essential not only for the registration and distinctness but for the genetic knowledge expansion and new breeding strategies.

4. Discussion

In the experiment 34 eggplant varieties were tested in 2015. The varieties belonged to 6 different shape on the base of CPVO TP/117/1. The evaluation of varieties did not show linear relationship among the fruit weight/plant height fruit weight/plant height, fruit weight/flower size and flower size/plant height.

The anthocyanin coloration was also observed and only at the globular varieties could be determined a connection. If the hypocotyl had a dark anthocyanin coloration than the other characteristics (stem, flower, calyx, skin) had also dark colour. The white varieties had dark flower colour but the anthocyanin coloration of hypocotyl, stem, calyx were different (absent, medium).

The results of the experiment confirmed that the earlier statements not valid in every cases, required continuous analytical study.

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Wild *Capsicum* in the area of the Amboró National Park in Bolivia

Claudio Dal Zovo[1], Leonardo Bruno[2]

[1] *Associazione Pepperfriends, Verona, Italy*

[2] *Associazione Pepperfriends, Roma, Italy*

Abstract

Bolivia is believed to be the source of the genus *Capsicum*; possibly *Capsicum chacoense* Hunz. is the species closer to the ancestor of all *Capsicum* species. About ten species of wild *Capsicum* grow in Bolivia: *Capsicum baccatum* L. var. *baccatum*, *Capsicum caballeroi* Nee, *Capsicum cardenasii* Heiser & Smith, *Capsicum ceratocalyx* Nee, *Capsicum chacoense* Hunz., *Capsicum coccineum* (Rusby) Hunz., *Capsicum eshbaughii* Barboza, *Capsicum eximium* Hunz., *Capsicum minutiflorum* (Rusby) Hunz. A couple of possible new species are under investigations. Many cultivated species are also grown and sometimes present in wild forms, especially *Capsicum pubescens* Ruiz & Pav., *Capsicum frutescens* L., *Capsicum baccatum* L. var. *pendulum* (Willd.) Eshbaugh. These species are preserved in herbaria and described in articles through drawings, but few or no images are available. We wished to produce a better documentation of live plants and their details; so we planned a trip to Bolivia starting in the area where most of the less known species are concentrated. We visited the area around the Amboró National Park, from Santa Cruz de la Sierra up to Samaipata, Mairana and Comarapa (South side of the Park) and the area near Buena Vista (North side of the Park). We found populations of *C.minutiflorum* (Rusby) Hunz., *C.caballeroi* Nee, *C.eximium* Hunz., *C.baccatum* L. var. b*accatum*, *C.coccineum* (Rusby) Hunz., fully described and documented them with many detailed images. These species are well differentiated and each of them has particular characteristics. The Bolivian wild *Capsicum* appear to be quite rare. The search for some relevant species was not successful. Some areas where *Capsicum* were found in the past seem no longer suitable for wild species, because of the increase of agriculture and grazing. Unlike the wild species of South-East Brazil, Bolivian wild species are known by the locals who use them, know their common names (ulupica, aji de monte, arivivi) and their characteristics, including flowering and fruiting time. The exploration and the search for wild species in the Amboró Park Area in the past were limited to the peripheral areas of the park; probably more populations and perhaps new species live inside the Park, in areas difficult to reach and unexplored so far.

Keywords: *Capsicum*, wild species, Bolivia, Amboró

1. Introduction

Bolivia is believed to be the source of the genus *Capsicum*; possibly *Capsicum chacoense* Hunz. is the species closer to the ancestor of all *Capsicum* species.[1]

The most recent point of view on the origin and differentiation of the species of *Capsicum*[2] confirms this hypothesis, on the basis or morphology, geographical distribution, crossing tests, citogenetical and biochemical analisys, genoma etc.

About ten species of wild *Capsicum* grow in Bolivia:

- *Capsicum baccatum* L. var. *baccatum*
- *Capsicum caballeroi* Nee
- *Capsicum cardenasii* Heiser & Smith
- *Capsicum ceratocalyx* Nee
- *Capsicum chacoense* Hunz.
- *Capsicum coccineum* (Rusby) Hunz.,
- *Capsicum eshbaughii* Barboza
- *Capsicum eximium* Hunz.
- *Capsicum minutiflorum* (Rusby) Hunz.

A couple of possible new species are under investigations; the main of these is the enigmatic *Capsicum pubescens* Ruiz & Pav. ssp. *arachnoideum* which could be the wild ancestor of *Capsicum pubescens* Ruiz & Pav (M.Nee, personal communication).

A possible new species was also recently collected in the province of Tomina, department of Chuquisaca.[3]

Many cultivated species are also grown and sometimes present in wild forms, especially *Capsicum pubescens*, *Capsicum frutescens* L., *Capsicum baccatum* L. var. *pendulum* (Willd.) Eshbaugh.

Botanists in the past did an enormous work of collection and documentation[4] and many of these species are preserved in herbaria and described in articles through drawings; however, few or no live images are available. In the last years many articles on wild *Capsicum* were published and an increased interest in the species of this genus can be noticed, especially in Bolivia[3]; nevertheless, images and detailed descriptions are scarce or totally absent.

We wished to produce a better documentation of live plants and their details; so we planned a trip to Bolivia on November-December 2015, starting in the area where most of the less known species are concentrated. Our goal was to document live plants and their habitat with good quality photographic images and to observe the characteristics of these species in the wild. Few images showing all the relevant details are better than many descriptions, even if, of course, the herbarium material is always irreplaceable. Therefore photos will be the most important part of our oral presentation.

Another important aim is to keep the interest on wild *Capsicum* species hoping to stimulate their study and conservation.

We are both members of the Italian Association "Pepperfriends" which has the statutory aim to deepen and spread the knowledge on *Capsicum* (especially wild ones) and to help preserving the biodiversity of this genus. Claudio Dal Zovo have previously completed four trips to South East Brazil (in 2011-2013) documenting about ten species endemic in that area.[5] Even at the beginning of this trip a day was spent in Brazil, visiting the Reserva Biológica at Paranapiacaba to clarify some points on *Capsicum dusenii* Bitter.

2. Material and Methods

We visited the area around the Amboró National Park, from Santa Cruz de la Sierra along the old road to Cochabamba, up to Samaipata, Mairana, Comarapa and El Empalme, on the South side of the Park, and the area near Buena Vista, in the North side of the Park.

The Amboró National Park covers an area of over 4,000 km² in the department of Santa

Cruz; together with the adjacent Carrasco National Park, it's one of the most important conservation unit in South America. The Park is surrounded by a protected area called ANMIA (Área Natural De Manejo Integrado Amboró) or IMNA (Integrated Management Natural Area).

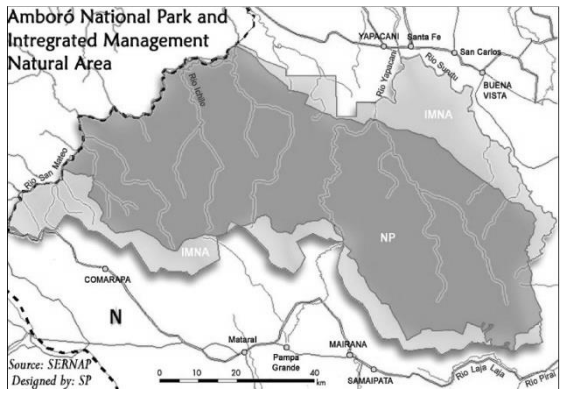

Fig. 1: Amboró National Park and IMNA

The Park contains many different habitats[6], being located at the confluence of several unique floristic regions: the tropical Amazon lowlands to the north and southeast, the subantarctic high Andes to the west and southwest, the subtropical Tucumán-Bolivian forests to the south, the semiarid inter-Andean valleys and the wet-tropical yungas forests that characterize the eastern slopes of the central Bolivian Andes.

The altitude varies from about 200-300 m asl in tropical evergreen rain forest along the northwestern border to over 3000 m in cool cloud forest on the highest peaks on the southwestern border.

The trip lasted two weeks, between late November and early December 2015. The park is the best place to start the exploration because endemic little known species not present elsewhere grow in it. The locations visited are in the provinces of Andrés Ibáñez, Florida, Manuel Maria Caballero, Vallegrande and Ichilo. The first stop was the wide Jardin Botanico of Santa Cruz de la Sierra, East of the city. Then we visited Quebrada Salada at Tarumá, the surroundings of the Rio Piraì at Bermejo, La Yunga de Mairana, Quebrada Seca at Mairana, the road from El Empalme to Khara Huasi, the high-altitude forest North of Comarapa (entering deeply in the park), Mataral, Vallegrande, the area South of Buena Vista along the Surutú river, the river bends towards El Carmen, the ANMIA area beyond the river up to La Chonta, the Park at the entrance of La Chonta.

To prepare the trip we studied almost all the literature available on the subject, including herbarium sheet labels. We received a lot of precious information by Prof. Michael Nee and Prof. Joshua Tewksbury. We planned a path to visit as much as possible of the sites where *Capsicum* species were collected in the past, compatibly with the available time. The dates of previous collections were also important to choose the best months to find both flowers and immature and ripe fruits. We have prepared a very accurate sheet to record the characteristics of the species without forgetting important details.

3. Results

Thanks to a precise planning, a well-established experience and a bit of luck, we found almost all the species that grow in the area. Also the trip period was the right one; we often

found plants bearing flowers and fruits in various stages of maturation.

We found populations of the following wild species:

- *C.minutiflorum* (Rusby) Hunz.
- *C.caballeroi* Nee
- *C.eximium* Hunz.
- *C.baccatum* L. var. b*accatum*
- *C.coccineum* (Rusby) Hunz.

Also individuals of the cultivated species *C.frutescens* were found in the wild.

These populations were fully described and documented with many detailed images.

The search for some other relevant species was not successful. We did not find *C.eshbaughii* and *C.pubescens* ssp. *arachnoideum*. Furthermore, we did not find a possible "new species" reported at La Yunga de Mairana, near the "bosque de helecios gigantes", at 2190 m (*Capsicum* sp, Serrano et al., 5482 NY).

Fig. 2: Capsicum caballeroi Nee

Capsicum caballeroi Nee (ají de monte, ulupica de yunga) grows in the "bosque nublado" (cloud forest) at 1900-2600m on South-West area of Amboró.[7]

We didn't find it in many of the sites where it was collected in the past; we only found it along the dirt road from El Empalme to Khara Huasi, West of Comarapa, at 2450 m asl.

In the site where our images were taken grow 2 very old plants and 1 young, small plant. The two adult plants do not seem healthy, bearing only few, small leaves; however they bear many flowers and fruits.

Plants are tall shrubs (up to 2.5 m). Leaves are lanceolate, light green, sometimes alternate along the branch in pairs of the same shape and size, coriaceous, almost glabrous or with sparse simple trichomes. Flowers are pendulous or intermediate, not geniculate, 1-2 per node, with long pedicel (3-5 cm). Calyx has 10 teeth, 5 long and 5 shorter (2-3 mm). Corolla is campanulate (15-20 mm long), bright yellow without spots; this is the main feature of this species. Anthers are yellow. Buds are tapered, bright yellow. Fruits are roundish, compressed in the apex, or irregular, with a diameter of 12-14 mm, pendulous, light green when immature, bright red when

ripe, soft, deciduous (but not easy to pick up; may dry on the plant), very hot. Seeds are straw, 10-25 per fruit, large (3 mm diameter), reniform, regular in shape and size. Chromosomes number is not documented.

Fig 3: Capsicum coccineum (Rusby) Hunz

Capsicum coccineum (Rusby) Hunz. (ají de monte, aribibi silvestre, tà yejti) grows in the tropical evergreen forest (300-400m) on East side of Amboró. [8] [9] [10]

We only found it inside the Park at the entrance of La Chonta.

Plants are herbaceous or slender climbing shrubs. Leaves are ovate, sometimes in pairs of the same shape and different size, almost glabrous. Flowers are erect, geniculate, with short pedicel, many per node. Calyx has up to 10 teeth, 5 long and 5 shorter alternating. Corolla is stellate, creamy yellow with purplish-brown spots; anthers are yellow, stylus violaceous. Fruits are roundish, 7-8 mm in diameter, erect, green when immature, bright red when ripe, soft, very hot. Seeds are straw, few per fruit. The chromosomes number is not documented. The petioles are bent just a few mm above the junction with the stem; when the leaf dehisces, it leaves this few mm as a stub, which could help the plant in climbing among other plants.

We are not sure that this species is the same growing in Perù and Brazil.

Fig. 4: Capsicum minutiflorum (Rusby) Hunz

Capsicum minutiflorum (Rusby) Hunz. grows in subtropical semi-deciduous forest (300-1000m) on South-East Amboró.[11] [10]

We found some isolated plants in the Jardin Botanico, four adults plants and other young plants along the Rio Piraì at Bermejo, other plants in the bends of the Surutú river near El Carmen. The plants prefer shady, moist places.

Plants are small trees (up to 3 m). Leaves are ovate, coriaceous, dark green, often in opposite pairs of the same shape and different size, almost glabrous with sparse unicellular trichomes. Flowers are erect, geniculate, with long pedicel (2-3 cm), 1-5 per node. Calyx has 5 teeth 2 mm long. Corolla is stellate (often quite closed), tiny (10-15 mm), creamy yellow with green marks; anthers are yellow. Buds are greenish. Fruits are roundish (sometimes with slightly pointed apex), 8-10 mm in diameter, erect or intermediate, green when immature, dark or bright red when ripe, soft; their heat is variable, sometimes almost absent. Seeds are straw or brownish, irregularly shaped (roughly triangular), 2x3 mm, many per fruit; they are straw in fresh fruits, but become brownish after short air exposition. Seeds in some fruits dried on a plant (prostrate and with the stem partially broken) were brownish. The chromosomes number is not documented.

Fig. 5: Capsicum eximium Hunz

Capsicum eximium Hunz. (ulupica) grows in subtropical deciduous dry forest at 1700-2100m on South-West area of Amboró and Vallegrande. [12]

This species is spread in a wide area, for example even in the mesothermic valleys of the Department of Chuquisaca.

We found four adult plants at Comarapa (1900 m), bearing flowers and fruits. At Vallegrande (2080 m) we found many young plants flowering, but with very few immature fruits, because of rains delay.

Plants are tall shrubs (up to 3 m). Leaves are small, ovate with acute apex. Pubescence is variable in all parts of plant. Flowers are erect, pendulous or intermediate, not geniculate, with short peduncle (1-2 cm), multiple (2-4) per node. Calyx has 5 long teeth. Corolla is stellate, whitish or purple with green spots; anthers are yellow/gray. Buds are whitish or violaceous. Fruits are roundish, 5-8 mm in diameter, erect or intermediate, green when immature, dark red when ripe, soft, very hot. Seeds are brownish, irregularly shaped, few per fruit (4-5), small (2 mm diameter). Genoma has 24 chromosomes.

Fig. 6: Capsicum baccatum L. var baccatum

Capsicum baccatum L. var. *baccatum* was found in many places around the Amboró limits and in the Jardin Botanico of Santa Cruz. [13] [14]

Plants are shrubs or small trees. Leaves are ovate with acute apex, sometimes slightly pubescent. Flowers are erect, geniculate, with long pedicel, 1-2 per node. Corolla is stellate, white with yellowish-greenish spots; anthers are yellow or grayish. Fruits are roundish with diameter of 8-10 mm, elliptic or elongated (up 20-30 mm), erect, green when immature, red when ripe, soft, quite hot. Calyx has 5 small teeth. Seeds are straw. Genoma has 24 chromosomes.

Fig. 7: Capsicum frutescens L.

Capsicum frutescens L. (aribibi, arivivi) was found along the dirt road to La Chonta (entrance to the East area of Amboró).

Plants are small trees. Leaves are ovate with acute apex, dark green, glabrous. Flowers are erect, strongly geniculate, with long pedicel, 1-2 per node. Calyx is toothless. Corolla is stellate, greenish without spots; anthers are purple. Fruits are elongated (20 mm long, 6-8 mm in diameter), erect, green when immature, red when ripe, soft, very hot. Seeds are straw, many per fruit. Genoma has 24 chromosomes.

4. Discussion

The Bolivian wild species of *Capsicum* of Amboró are well differentiated and present many particular characteristics.

The most intriguing feature is the campanulate corolla of *C.caballeroi*. Only few species of *Capsicum* have the corolla campanulate: the first exception to the usual stellate corolla was found precisely in Bolivia: *C.cardenasii*.[15] Afterwards, the astonishing campanulate-urceolate corolla, entirely lilac-fuchsia, of *Capsicum friburgense* Bianchetti & Barboza was found in South-East Brazil.

Perhaps even the little-known *Capsicum scolnikianum* Hunz., has a similar corolla, but not so closed as in these species.

The heat (pungency) of fruits is very variable.

It's unexpectedly high in *C.caballeroi*.

C.minutiflorum heat varies from medium to almost inappreciable. Some populations of this species lacking any pungency were mentioned in literature; however, in all the fruits that we tasted the heat was detectable. The presence of individuals with pungent and not pungent fruits within the same species and the same population is probably important to understand the evolution of the genus *Capsicum*. [16] [17]

The variability of morphological traits in *C.eximium* is very interesting. In this area most flowers have withish corolla with green spots, but some individuals show a more or less purplish corolla; also buds can be white or purple even in plants growing side to side. In other areas of Bolivia (for example in the North of the Department of Chuquisaca), *C.eximium* has an entirely purple corolla (with greenish spots inside). Around Vallegrande many plants had very small flowers, but anthers of normal size; as a result the flowers appeared strange, with anthers of abnormal size. The pubescence of this species varies from plant to plant; some plants are quite or very pubescent. However, in very pubescent individuals we never found calyx with 10 teeth, feature that, together with pubescence and type of trichomes, differentiates *C.eshaughii* from *C.eximium*.

The Bolivian wild *Capsicum* appear to be quite rare. When found, there are always few individuals, not large populations of tens or hundreds of individuals (which is often the case in South-East Brazil). In South-East Brazil some species grow in very narrow areas (in some cases, few hundreds m^2), but other species may form large populations spread on a wide area. In Bolivia the wild species seem always spread on a wide area, but always in single individuals or very small populations. Only *C.eximium* and *C.chacoense* (not described in this article because it grows elsewhere) form quite large populations spread on a wide area.

Some species were not found in sites where they were present in the past. We visited promising sites, but in many cases we didn't find plants of *Capsicum* (but always a large number of *Solanum* sp.)

Some areas where *Capsicum* were found in the past seem no longer suitable for wild species, because of the increase of agriculture and grazing. For example, at La Yunga de Mairana, near the "bosque de helecios gigantes", at an altitude of about 2200 m, sites where *C.caballeroi* and a possible new species were found are now cultivated and fenced. However, these species should not be at risk of extinction because there can rely on a wide, still intact and partially unexplored habitat.

We had no luck in finding *C.eshbaughii* Barboza. Very few known sites exist for this species; it was collected near Mairana many years ago and initially described as *C.chacoense* or *C.baccatum* var *baccatum*. Later it was classified as *Capsicum eximium* var *tomentosum* Eshbaugh & Smith [18]. Recently G.E.Barboza described it as species apart. [19]

We located Quebrada seca, one of the two original sites, the dry bed of a small river covered with dense vegetation. However, we didn't find this species nor other *Capsicum*. Perhaps the environment was too dry, the plants could have been in vegetative pause and start again after more rainfall. Further investigations will be necessary to verify if this species is still in the wild.

In the area around Mataral we didn't find *C.eximium* and especially *C.pubescens* ssp. *arachnoideum*. Locals told us that many plants of *C.eximium* grow in the area, but they were "palo seco" (only stem and branches, no leaves) because of scarce rainfall. The enigmatic "*arachnoideum*" was found sporadically, not in recent times; very few information are available on the places where it was found.

C.caballeroi, *C.minutiflorum* and *C.coccineum* don't appear to be close to any domesticated species.

C.baccatum var *baccatum* should be the wild ancestor of cultivated *C.baccatum*.[13]

C.eximium is related to *C.cardenasii* and probably *C.pubescens*; these species cross easily each other. Natural crosses between *C.pubescens* and *C.eximium* should be quite common and characterized by soft fruits larger than fruits of *C.eximium*. [20]

Plants of this type are commonly grown by people keen on Capsicum around the world and they are known with the name Rocopica (rocoto+ulupica). However, there is no information on this subject in literature. At the moment it is not clear if a true wild ancestor for *C.pubescens* exists.

Unlike the wild *Capsicum* of South-East Brazil, Bolivian wild species are well known by the locals who use them, know their common names (ulupica, aji de monte, arivivi) and their features, including flowering and fruiting time. Locals in Vallegrande, Comarapa and Mataral were able to give us precise indications on where to find plants and their vegetative stage. Locals in Buena Vista knew yellow-flowered *ulupicas* (probably *C.coccineum*) growing inside the Park. However, in towns that we visited, fruits of ulupicas were not sold in the markets; only locotos (*C.pubescens*) and Aji (*C.baccatum* var *pendulum*) were available.

The exploration and the search for wild species in the Amboró National Park Area in the past were limited to the peripheral areas of the park; probably more populations and perhaps new species live inside the Park, in areas difficult to reach and unexplored so far. So, there is still much to be explored.

5. Aknowledgements

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Solanum insanum L. (Solanaceae): Linnaean species or introgressed hybrid?

John Samuels

Novel Solanaceae Crops Project, Penzance, Cornwall, United Kingdom

jsamuels1716@gmail.com

Abstract

Wild and weedy forms of brinjal, *Solanum melongena* L., have historically been referred to as *S. insanum* L. Views on the precise nature of *S. insanum* have diverged and its relationship to other wild and weedy brinjal relatives, as well as to brinjal itself, has remained unclear. Opinions have varied as to whether *S. insanum* populations arise as a result of *S. melongena* becoming feral, or as a result of hybridisation which may become introgressive. It is suggested here that some *S. insanum* populations result from hybrid swarms based on reticulate webs of hybridisation and introgression amongst brinjal and sympatric wild relatives. In addition, the correct application of *S. insanum* L. has proven doubtful and confusing, particularly since the 1980s. As a consequence, consensus on an accepted species concept seems unlikely. Its taxonomic currency is therefore uncertain.

Keywords: brinjal eggplant, hybridisation, introgression, Solanum insanum, species concept, weedy, wild relatives

1. Introduction

Cultivated brinjal eggplant, or brinjal, (*Solanum melongena* L.) is economically important in many parts of Asia and elsewhere. Associated with the domesticated forms of this cultigen are wild and weedy forms of South and South-east Asia (sometimes called *S. insanum* L., *S. cumingii* Dunal, *S. undatum* Lam., etc.) and wild relatives in Africa, the Middle East and South Asia (*S. campylacanthum* Hochst. ex A. Rich., *S. lichtensteinii* Willd. and *S. incanum* L.). Together, these taxa are known as the brinjal eggplant complex [1]. *Solanum insanum* ("wild brinjal") and *S. incanum* are considered to be components of the primary genepool of *S. melongena*. Detailed knowledge of the taxonomic relationships within this group is crucial to our understanding of the origins and domestication of brinjal, and may help in the search for pre-domestication traits that could be useful in crop improvement programmes.

2. The Linnaean concept of S. insanum

In the Linnaean protologue *S. melongena* was originally described [2] as having a prickly calyx. There is no other reference to the presence of prickles elsewhere on the plant, but the stem is described as unarmed. In the *Systema Naturae*, published by Linnaeus [3] it was placed in the *Inermia* group, indicating that he subsequently considered it to be completely unarmed, whilst *S. insanum* was included as a closely related but new species in the *Aculeata* ("prickly") group. Also in 1767, the *Mantissa Plantarum* [4] provided the protologue for *S. insanum*, describing it as a sparsely prickly annual, morphologically similar to *S. melongena* by way of its "scarcely-branched stems, stellate tomentum, subsinuate leaves" and "solitary, single-flowered peduncles" producing "large fruits" (see Table 1). Confusingly, the specific epithet *insanum* was based on the pre-Linnaean name for brinjal: *mala insana*, the "mad apple."

Solanum insanum was clearly considered to be a close ally of brinjal, but to differ from it by the presence of prickles: "stem with sparse prickles; prickles on both sides of the leaves; calyx very prickly" [4]. The lectotype specimen (sheet 248.29 at LINN-http://linnean-online.org/2605/) designated by Hepper and Jaeger [5] conforms to Linnaeus' protologue in terms of branching, tomentum and leaf characters; however, the presence of an inflorescence bearing three flowers (rather than one, as described in the protologue) does not conform. Unfortunately, there are no fruits present on the specimen. Thus, the designated lectotype may be an unreliable representation of Linnaeus' concept of *S. insanum*. Armature characteristics are highly variable in members of the brinjal eggplant complex [6] and, although cultivated *S. melongena* may or may not be prickly [7, 8], Linnaeus' emphasis on prickles as a major diagnostic character seems to have contributed strongly to the taxonomic rationale of delimiting *S. insanum* from *S. melongena*.

Figure 1:
S. insanum: straggly form, Taiwan

3. Varying species concepts

The taxonomic proximity of *S. insanum* and *S. melongena* suggested by Linnaeus has been recognised and accepted ever since the eighteenth century, but views on the precise nature of *S. insanum* began to diverge from the 1980s onwards, when crop improvement research became focused on brinjal and its allies (see Table 1). For example, Deb [9] held the opinion that the wild species *S. incanum* and *S. insanum* were conspecific (Table 1). This was refuted by Lester and Hasan [10], who showed experimentally that the two were reproductively and morphologically distinct. Deb's views were supported only by observations in the field; weedy forms of *S. melongena* also seem to have been mistaken for populations of *S. incanum*, as the latter never produces the white fruits described by him. Later, Meyer et al. [11] designated the weedy forms from India as "Asian *S. incanum*" (Table 1), challenging the species concepts of both *S. incanum* and *S. insanum* [12, 13]. Recent circumscription of *S. incanum* [14] refers to characteristics such as inflorescences with up to 15 flowers, and confirms that it is clearly distinct from *S. insanum* L. *sensu stricto*, which has single-flowered inflorescences [4].

The concept of *S. insanum sensu* Lester and Hasan (=*S. melongena* group E) [7, 10, 15] revolved around the assumption that it was a purely Indian form of *S. melongena*, with a low-growing, straggly habit, dense armature, and 5-9 flowered inflorescences producing few, smallish, spherical fruits (Table 1). Prain's [16] view that *S. insanum* (as *S. melongena* var. *insana* [L.] Prain) was a feral, secondary weedy form of *S. melongena* was supported by Lester and Hasan. Their view of the application of *S. insanum* L. was adopted as a result of observations of a limited number of accessions collected from northern India, and they reserved judgement as to its correct taxonomic status and nomenclature. There is no indication of growth habit in Linnaeus' original description of *S. insanum*, although it is described as "herbaceous," meaning that plants may be prone to become straggly. The limited material of the lectotype specimen shows no indication of habit, although the single shoot appears fairly robust. Samuels [17] adopted a similar view to that of Lester & Hasan, but also included bitter, ovoid-fruited forms, and a wider distribution range, covering Pakistan, northern and central India and Sri Lanka (Table 1). Recently, purely feral forms have been referred to as *S. cumingii*/*S. melongena* subsp. *cumingii* (Dunal) J. Samuels [6, 18].

Table 1. Comparison of species concepts of *S. insanum*

Linnaeus [3, 4]	Deb [9]	Lester & Hasan [7, 10, 15]	Karihaloo and colleagues [19, 20, 21]	Meyer et al. [11, 32]	Knapp et al. [22]	Samuels [6, 17], present paper
S. insanum L.	*S. insanum* L.	*S. insanum* L./*S. melongena* group E	"*S. insanum sensu lato*."	Equivalent to: Indian/"Asian *S. incanum"*; *S. undatum*	*S. insanum* L.	*S. insanum* L. *nomen ambiguum*/unresolved name.
Sparsely prickly, herbaceous annual; scarcely-branched, prickly stems; ovate, stellately-tomentose, subsinuate leaves with prickles (rarely straight) on both sides; very prickly calyx; single-flowered inflorescence, producing large, single fruits.	Purple or white flowers; fruits white, purple or yellow, oblong or rotund.	Extremely prickly, shorter, low-growing, straggling annual or perennial; small leaves; 5-9 violet flowers per inflorescence, producing few, smallish, spherical fruits.	Prickly or unarmed, erect or decumbent; small to large leaves; mostly prickly calyx; up to 8 violet flowers per inflorescence, producing up to 3 fruits per cluster; fruits up to 5cm. long, green, white, pink, or purple, spherical to oblong, non-edible/bitter.	Some forms are indistinguishable from *S. incanum*	Prickly, erect or low-growing forms; ovate leaves; few prickles on calyx; up to 4 violet flowers per inflorescence, producing small fruits (up to 3 cm. diameter).	Strongly armed, sprawling shrubs; broadly lanceolate to ovate, tomentose leaves; up to 9 violet flowers per inflorescence, producing up to 3 fruits per cluster; fruits globose or slightly ovoid, up to 4 cm. diameter, bitter.
---	Includes feral forms.	Weedy, feral derivative of *S. melongena.*	Semi-wild, weedy.	Wild/feral	Feral reversions from cultivated forms.	Weeds, adventives & semi-wild forms of disturbed habitats.
India.	Southern India, possibly elsewhere.	India	India, Sri Lanka, Bangladesh.	India.	Madagascar, Mauritius and India to SE Asia & southern China.	Pakistan, northern and central India, Sri Lanka.
---	---	Found in fields and fallow as a weed of agriculture, and also as an adventive in disturbed habitats.	Found around villages.	---	Ruderal-many habitats.	Disturbed habitats
---	---	---	Gene flow between *S. melongena*, *S. insanum* and *S. incanum* has produced an interbreeding complex of progenitor and derived forms.	Possibly of hybrid origin: hybridisation among domesticates/between domesticates and progenitors; mixed wild and domesticated genetic background.	Introgression occurs between *S. insanum* & *S. melongena*.	Hybrid origin/local hybrid swarms resulting from hybridisation and introgression amonst brinjal and several wild relatives.
Distinct from *S. melongena* L. & *S. incanum* L.	Conspecific with *S. melongena* & *S. incanum.*	Belongs to same biological and taxonomic species as *S. melongena* and *S. cumingii* Dunal.	*S. insanum s.l.* is equivalent to *S. insanum* and *S. cumingii*, together. *S. melongena* & *S. insanum* are genetically conspecific.	*S. insanum* is synonymous with Indian/ "Asian *S. incanum*," *S. undatum* Lam., and *S. cumingii*.	*S. insanum* is synonymous with *S. undatum* and *S. cumingii*. *S. insanum* is the wild progenitor of brinjal.	Distinct from *S. incanum* & *S. cumingii*.

Other authors have suggested a broader species concept of *S. insanum*, and have applied the name to the low-growing forms of Lester and Hasan, as well as erect, non-prickly, larger-fruited forms, found across India, Sri Lanka and Bangladesh [19, 20; Table 1]. Alternative circumscriptions [21, 22] have included the straggly forms (see Figure 1), combined with erect forms (sometimes known as *S. melongena* group F [7]) with moderate armature and smaller, spherical fruits, that are found across South and South-east Asia, and south-eastern China (see Table 1). Madagascar and Mauritius are sometimes included in the distribution range of *S. insanum* [22].

4. Hybridisation

Wide morphological variability in *S. insanum* has been described [22, 23, 24] and may reflect the polymorphic nature, typical of many solanums, and its expression in feral brinjal forms. Considerable evidence suggests that *S. insanum* is of hybrid origin [e.g. 19, 20, 25] and this provides an additional explanation of such variation. Experimentally, *S. melongena* has been shown to hybridise with around ten of its "spiny *Solanum*" relatives, including *S. insanum*, found in India and elsewhere [17, 24, 26]. In addition, some authors assert that brinjal and its wild relatives may freely interchange genes in nature by natural hybridisation [11, 22, 27, 28]. This has been demonstrated by the kind of genetic pattern observed by Mutegi et al. [29]. Using SSR marker analysis, they found that there was no clear genetic separation between sympatric populations of *S. melongena* and *S. insanum* in southern India. They suggested that gene flow between the populations contributed towards the shared genetic structure and that this was evidence for conspecificity of the two taxa. Morphologically [22], the two appear to lie on the same spectrum of continuous variation, and reproductively [7, 24, 26] they are interfertile-these features also support conspecificity. Several authors have opted to maintain *S. insanum* as a synonym of *S. melongena* [e.g. 18].

Outcrossing in brinjal, facilitated by insects, may be as high as 52.5% [29]. Many of those visiting brinjal are not species-specific, and pollen transfer between brinjal and other *Solanum* species with comparable phenologies can freely occur; if there is cross-compatibility, viable hybrids will be formed. Thus, the hybrid origin of wild populations identified as *S. insanum* must be considered, and crosses with species that are extraneous to the primary genepool would account for some of the wider variation that is observed.

5. Introgression

According to Karihaloo and colleagues [19, 20] *S. insanum* in the broad sense is a complex assemblage of "progenitor and derived forms." The "*S. insanum sensu lato"* of Karihaloo and Rai [20] can thus exchange pollen reciprocally with taxa within the primary genepool, as well as with hybrids arising amongst these, or even with those involving other wild relatives. Continued backcrossing between hybrid offspring and parents will lead to introgression. It is suggested here that in localities suffering habitat disturbance (such as roadsides and village borders) this will be encouraged because of the availability of many diverse microniches and the adventive nature of spiny solanums. As a result, hybrid swarm populations, based on reticulate webs of hybridisation and introgression amongst brinjal and sympatric wild relatives, are likely to develop.

The taxonomic status and appropriate nomenclature of such populations present a challenge. In addition, a discrete "species" circumscription leading to delimitation based on morphological criteria would prove difficult because of the large number of intermediates and the wide range of morphological variation arising as a result of the re-combination of a broad range of characters. A similar situation involving complex morphological variation caused by

hybridisation and introgression has been reported in the *Solanum nigrum* complex [30, 31].

6. Summary

Solanum insanum was first described by Linnaeus around 250 years ago, seemingly as a result of the narrowing of the species concept of *S. melongena*. The information in Table 1 illustrates the varying species concepts of *S. insanum*, and the development of different viewpoints on the origins of *S. insanum* populations. The foregoing information shows that some populations described as *S. insanum* seem to be conspecific with *S. melongena*; others consist of hybrid forms of complex origin, and present taxonomic challenges, particularly for nomenclature.

Consensus on a species concept for *S. insanum* does not prevail, not least of all because the correct application of *S. insanum* L. has proven doubtful and confusing, particularly since the 1980s. In addition, the lectotype [5] does not seem to be fully representative of the Linnaean taxon. *S. insanum* has been described as a *nomen ambiguum* [6] and is an "unresolved name" (http://www.theplantlist.org/tpl/record/tro-29605613). Its taxonomic currency is therefore uncertain.

7. Acknowledgements

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Development of DNA-markers to fruit quality genes of sweet pepper (*Capsicum annuum* L.)

Olga G. Babak, Tatyana V. Nikitinskaya, Konstantsia K. Yatsevich, Natalia A. Nekrashevich, Alexander V. Kilchevsky

Institute of Genetics and Cytology at National Academy of Science of Belarus, Minsk, Belarus

Abstract

The research work was aimed at the development of DNA-markers to genes determining biosynthesis of pigments (*Ccs* and *cl*) and the fruit ripening process (*nor* and *rin*) for identification of their allelic composition in sweet pepper. There are 3 basic unlinked genes (*Y, c1* and *c2*) in pepper which regulate formation of ketocarotenoids and control accumulation of pigments in fruit chromoplasts. The locus *c2* encodes phytoene synthase enzyme (*Psy*). Its mutation often causes a serious decrease in the amount of carotenoids. DNA-typing of the sweet pepper collection (31 varieties) using the markers, known in the literature, has not detected genotypes with mutations in this locus. The gene *Y* encoding bifunctional capsanthin-capsorubin synthase enzyme (*Ccs*) determines synthesis of red pigments in pepper: capsanthin and capsorubin. The research was conducted for identifying the *Ccs* gene. The locus *Y* was detected in all the pepper varieties with red colour of the fruit (the presence of amplicon of 1470 bp in length), there was no this fragment in the varieties with orange and yellow colour. The research is under way for developing codominant STS-marker for identification of the heterozygous allelic state of the *Ccs* gene.

Mutation *cl* (*chlorophyll retainer*), blocking chlorophyll destruction in ripening, is described in pepper. Depending on the allelic combination of the *cl* and *Ccs* genes fruits become brown or green-yellow. Based on the literature findings the dCAPS marker to the *cl* mutation was modified. The application of the developed primers has detected this mutation in four sweet pepper varieties of the studied collection characterized by brown and green-yellow colour of the fruit.

Carotenoid biosynthesis depends also on the terms of ripening. Specific genes encoding cellulase and polygalacturonase enzymes activate in plants during fruit ripening. Mutations in their transcription factors *nor* (*non-ripening*) and *rin* (*ripening inhibitor*) lead to inhibiting ripening in tomato. Based on the earlier developed primers to mutations in the genes of the extended ripening period (*nor* and *rin*) in tomato, the research was conducted for developing primers to analogue genes for pepper. The STS-marker to the analogue of the *rin* mutation, specific to sweet pepper, was developed during the study. Analysis of the sweet pepper collection for this locus has shown heterogeneity of the studied accessions. In tomato, the mutation *nor* was caused by a short deletion (2 bp) leading to the translational frameshift and formation of nonfunctional protein. Sequencing of the fragment, formed by the amplification of the total DNA of pepper with tomato primers to the mutation *nor*, has shown the difference in 7 bp between sequenced alleles. The mutant allele *norc162* was identified in three accessions and *norc169* – in the other part of the sweet pepper collection.

1. Introduction

DNA typing techniques are one of the most important selection methods for crops which genomes are completely or partially sequenced. The use of marker-assisted selection is very effective both during the initial lines selection for hybridization and the subsequent analysis of the hybrid material and varieties. MAS methods in sweet pepper are widely used to develop cultivars with high biochemical composition, particularly carotenoids. The main sweet pepper's carotenoids are carotene, capsanthin and capsorubin. Ripe fruit of *Capsicum* can be classified

into eight classes by colors, ranging from white to deep red. Color variation seems to be determined by three independent gene loci *Y*, *c1*, and *c2* (Hurtado-Hernandez and Smith model). The gene capsanthin-capsorubin synthase (*Ccs*), which is involved in antheraxanthin to capsanthin and violaxanthin to capsorubin conversion, is considered the candidate gene for the *Y* locus, at the same time the *c2* locus corresponds to the *Psy* gene. The identity of the *c1* locus remains unknown. Loci *c2* and *c1* regulate the amount of carotenoids rather than their type. Mutation of *chlorophyll retainer* (*cl*) gene of pepper inhibits the ability to degrade chlorophyll during ripening. It leads to the production of ripe fruits characterized by both chlorophyll and carotenoids accumulation. Brown fruits color is determined by combination of allele Y^+ and *cl* mutation, and yellow-green fruit color is determined by combination of homozygous recessive allele Y^- and *cl* mutation. Currently, sweet pepper genome sequencing is carried out. Given that the tomato genome which is phylogenetically closest to the pepper species is completely sequenced, the DNA typing work on economically valuable traits of sweet pepper is widely carried out in various international research centers with the use of markers, developed both for pepper and tomato.

Previously, we have used our own methods for DNA identification of fruit quality genes *nor*, *rin*, nor^A (long shelf life), *t*, *Del*, *r*, *B* from *S. pennellii*, og^c, *hp-1*, $hp\text{-}2^{dg}$, *gf-3* (increased carotenoid content). Tomato hybrids, combining alleles of the genes under study, were developed as a result of a number of topcrosses. The forms with the targeted allele combinations were selected in the F_2 generation, by MAS methods: homo- and heterozygotes by the carotenoid content genes and the genes extending fruit shelf life; hybrid combinations with two carotenoid content genes and one long shelf life gene. Further work on the development of lines combining the given groups of genes (*B/rin/gf-3*, $B/rin/hp\text{-}2^{dg}$; *B/nor/gf-3*, $B/nor/hp\text{-}2^{dg}$; $og^c/rin/gf\text{-}3$, $og^c/rin/hp\text{-}2^{dg}$; $og^c/nor/gf\text{-}3$, $og^c/nor/hp\text{-}2^{dg}$) in a homozygous stay was conducted [1].

The next stage of research presented in this article. Based on the earlier developed primers to mutations in the genes of the extended ripening period (*nor* and *rin*) in a tomato, the research was conducted to develop primers of gomologous genes for a pepper. A comparative analysis of marked allele fragment sequences in a tomato and a pepper was performed. The STS-marker to the gomologous of the *rin* mutation, specific to sweet pepper, was developed during the investigation. Along with the indicated alleles, the study of polymorphism of genes determining pigments biosynthesis in a sweet pepper (*Y* and *cl*) was conducted by the markers, known in the literature. Similar to a tomato, the hybrids combining alleles of long shelf life genes (*rinc*, *norc*) and pigments composition (*Y* and *cl*) were developed by topcrosses and forms with complex allele combinations will be selected in the F_2 generation. The unique model of tomato and sweet pepper forms with different combinations of fruit quality genes are used to study peculiarities of pigments accumulation in fruit and develop valuable hybrids and varieties for agriculture.

2. Materials and methods

Plant material and DNA isolation. The aim of this study was to screen the collected gene pool of sweet pepper for favorable alleles for determining carotenoids content. Plant material (31 sweet pepper forms varying in shape and color) was obtained from All Russian Research Institute of Vegetable Breeding and Seed Production and from Institute of Genetics and Cytology at National Academy of Science of Belarus. The study of economically valuable traits in the accessions was carried out in Biological Research Station greenhouses at Institute of Genetics and Cytology.

DNA was extracted using the Genomic DNA Purification Kit (Thermo Scientific) of different pepper varieties seedlings according to the manufacturer's instructions.

Allele-specific PCR amplification. The reaction PCR mixture of 15 μl volume contained 60-100 ng of genomic DNA; 2,5 mM dNTP Mix (Thermo Scientific); 25 mM $MgCl_2$; and 1,4 U of Taq DNA-polymerase in incubation buffer (Thermo Scientific) and 5 pmol/μl of oligonucleotide primers to STS- / dCAPS-markers synthesized by "Praymteh" (Belarus). The PCR reaction was performed in the Thermocycler Biometra T Professional Basic (Germany).

The reaction products were separated by electrophoresis in a 1,5% agarose gel in the presence etidium bromide and documented using Bio-Rad GelDoc 2000 system (USA). Amplified fragment sizes were determined by using 100 bp Plus DNA ladder (Thermo Scientific) as a molecular weight marker.

Sequencing analyses. For sequencing, amplification fragments were separated in 1,0% agarose gel and then excised and purified using the Agarose Gel Extraction Kit (Jena Bioscience) according to the manufacturer's procedure. Sequencing reactions were performed using a set of Big Dye® Terminator v 3.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing reaction products were purified using columns Centri-Sep™ (Applied Biosystems), dried under vacuum, dissolved in 20 μl of formamide, denatured by heating at 95°C for 2 minutes and then performed capillary electrophoresis using a DNA sequencer ABI PRISM™ 3500 Genetic Analyzer (Applied Biosystems). Computer processing of the data obtained from sequencing was performed using Sequencing Analysis Software v 5.2 (Applied Biosystems). Analysis of nucleotide sequence homology of the *ripening-inhibitor* and *non-ripening* genes, synthesized with the help of the developed markers were performed using GenBank database using the BLAST parser US National Center for Biotechnology Information.

Development of STS- / dCAPS marker. Based on previously developed STS-markers for the tomato genes [1] determining the ripening period (*ripening-inhibitor* and *non-ripening*), a comparative analysis of homologous DNA regions sequences has been carried out and specific STS-markers are developed to study their polymorphism in sweet pepper. DCAPS marker for *cl* alleles DNA typing was adapted for genomic DNA on the based of known in the literature marker.

3. Results and Discussion

For sweet pepper collection typing for *Psy* gene primer pair was used, described in the article by Lang Y.Q. et al. [2]. PCR-products 2844 bp are synthesized during the amplification with primers for *Psy* gene. Mutations in the *Psy* locus has not detected using the marker in our collection.

Specific primers for gene *Ccs* amplified a fragment of 1470 bp in length [2] in red fruit sweet paper varieties (L-25311, Igrok, Pregol', Otello, L-510, Pfioletoviy Krasavec, Belosnezhka, L-208, L-255-11, Cherniy Krasavec, Mayak, Shokoladnaya Krasavica, L-24, ZongKao and L-160-10), that is consistent with the reported data and confirms the presence of an active form of capsanthin-capsorubin synthase (*Ccs*).

In their article Borovsky Y. et al. [3] described dCAPS marker (clF/clR_Fok1), which the authors used for the cDNA for typing pepper forms with *chlorophyll retainer* mutation. When this marker is used on genomic DNA, obtained amplicon contains 2 restriction sites for enzyme *Fok1* that complicates the identification of the mutation (Fig. 1). Based on the NCBI database (EU196733.1) and Sol Genomic Network (Pepper locus CA01g03620) the optimal primer pair was developed for the identification of these gene alleles: cl-1F dCAPS 5'-GATGAAGTGGTTGCAGGA-3', cl-1R dCAPS 5'-GAACACGGCCGAACGATAT-3' (Fig. 1). Selected pair of primers amplifies a product of about 200 bp. The wild type gene *cl* contains *Fok1* restriction site while *cl* mutant is insensitive for digestion, that allows to identify the allelic polymorphism.

```
   1  CTTCTCTTTC ACACACACAC AGTGATGACA CACAAATTAG TATAGTTAGT ACACGTACTT AATGCCAATC TTCAAAGTCA CAAAATCCTA AAAAGTAAAG
 101  CAAAAGCAAC ACAACTCCTC TTTAAGTTTC TACTCATTCT CTTTGCCAAC TTTGATCAAA AACACCTTAT AATAAATTGA TCAAGATTCA AGGAGTTTTG
 201  GGTACCCAAT TTCTTGATGA AATGGGGACT TTGACTGCTT CTCTAGTAGC TCCATCTAAG CTCAACCCTG AAAAGCATAG CTCTCTTTTT GTATACAAAA
 301  CTAGAAGAAA GTCCCACAAG AATCAATCCA TAGTCCCTGT AATTAACTTT CACTCTCTCT TTTTGCTTAA TATTCGTTAG GTTATATACG CATATATTAA
 401  ATTTTGAACG CTTCACGAGG GTAGGGTTAA GGGGTGTGTA TATTCTTACC CTTTCGAGAC TCCACTTTGT GAGACTACGG TGCATATGTT GTTGTAGATA
 501  TTGTTGGCTT GAATCTTGAT AGTTTATTTT TTTGTTGATG AATATATATA TATATANNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
                                        601-1600 – N
1601  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNATATA TATATATATA TATATATAGG
1701  TGGCAAGGCT ATTTGGACCA GCTATATTTG AAGCATCAAA GTTGAAGGTA CTTTTCTTGG GAGTTGATGA GAAAAAGCAC CCAGGAAAGT TGCCAAGAAC
1801  ATATACACTG ACTCATAGTG ATATTACTTC TAAACTCACT TTGGCAATCT CTCAAACCAT CAATAACTCT CAGGTAATCA AATTTTTTTC ATTAACTTTG
1901  ATGACACATA TTATTCTGTG TGATCATTCA CAAATAGAGC AGGGGAAGAA TTACTAGTTT GTGATATATT GATTCTTGTA ATAGCTTAAT AATGTAAATT
2001  TCGGGTTTAT TTATCCGATG GTAACTCAAT TAATAGTTAT TAGGGTATTT CAGAAAATCA CCTTTCTTCT TGATTATAAC TTTTCTTCGA CAAAGAAAGT
2101  GACGTCATGA GATAATAACT ATTAGTTGAG TGACAAATCA ACTCTGTGAT TTTGTGGCAA TAAGAATTTG GAGGTCCTTG ATTAGTCAAT TTCTTGATTT
2201  AGTCATCACT AATCACTGAC ACAGATTCAA GATTTGATCT TTTATGAGTT TTATATCTCA AGTACTAGTT ATTGGAATAT AAATTTAATT TTTGTTTGTA
2301  TTTAACGAAT CTCTTAACAT AGGATCTGGA CCCAGGACAC TTGTCAATAA CCCACATATC ACGTGCTATG CTATTACCAC TAGAATTGTT AGCCATATAC
2401  TTATTTAGAA AAGTTAGAAA ATATGGTACT CTTAATCTTG AATCTGCCTC TATTTCTCTT TTGTATTTAA TAGAATTTTT GTTGAGAGAA GTGGAAATTG
                                                  FokI
2501  GGTGTTTGAT TTGCAGTTGC AAGGTTGGTA TAATAGACTT CAAAGGGATG AAGTGGTTGC AGAATGGAAG AAAGTTAAAG GGAAGATGTC ACTCCATGTA
2601  CATTGCCACA TTAGTGGAGG CCATTTTATG TTAGACTTAT TTGCTAGACT CAGATACTAT ATCTTCTGCA AAGAACTCCC TGTGGTAAGT TCATAGAGTA
2701  ATATGTTGTT CGAACTTCTC AAAAATATCG TTCGGCCGTG TTCCTTCCTT CAAAAATGTA TTACTTTTGA AGCATTTGAC ACACGCCTGT CGATATTTTG
```

Picture 1:
The sequence of genomic DNA of the sweet pepper chlorophyll retainer gene and the primers location (cl-F/R (gray) are described in Borovsky Y. et al [3]; cl-1F/RdCAPS (bold, underline) – adapted). The nucleotide sequence from 567 to 1675 has not been studied

The results of the developed marker cl-1F/RdCAPS_Fok1 on the sweet pepper collection are presented in Figure 2.

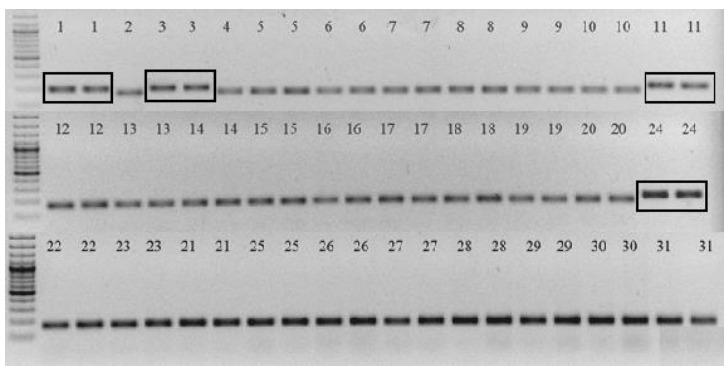

Figure 2:
PCR-profiles pepper genomic DNA with marker cl followed by restriction Fok1 (1 – L236/09, 2 – Isimo, 3 – Sladkiy Shokolad, 4 – L-25311, 5 – Igrok, 6 – L-160-10, 7 – Pregol', 8 – Otello, 9 – Zheltiy buket, 10 – L-510, 11 – L-45-11, 12 – Zheltoplodniy, 13 – L-277-11, 14 – Pfioletoviy Krasavec, 15 – Belosnezhka, 16 – Zlatozar, 17 – L-208, 18 – L-97, 19 – Sireneviy, 20 – L-255-11, 21 – Cherniy Krasavec, 22 – Oranzhevoe naslozhdenie, 23 – Mayak, 24 – Shokoladnaya Krasavica, 25 – L-24, 26 – Oranzhevoe tchudo, 27 – ZongKao, 28 – Ami, 29 – Nemezis F_1, 30 – Polskiy, 31 – Jalopeno)

DNA typing of the studied sweet pepper collection with a variety of colored fruits with marker cl-1F/RdCAPS_Fok1 identified mutant gene *cl* in four forms: L-236/09, Sladkiy Shokolad, L-45-11, Shokoladnaya Krasavica. Varieties of sweet pepper L-236/09, Sladkiy Shokolad and Shokoladnaya Krasavica on the stage of biological maturity are characterized by brown color of the fruit, and the L-45-11 at this stage has a greenish-yellow color, which corresponds to the literary description phenotypic manifestations [3] of this *cl* gene mutation. Thus, the modified marker can detect homo- and heterozygous state of *cl* allelic gene.

To determine allelic polymorphism delaying sweet pepper fruits ripening collection research were made using RinM/W to the tomato gene marker *LeMADS-RIN* (*Solanum lycopersicum* L.) [1]. In the tomato this marker identifies two fragments of 563 bp (wild allele – *Rin*) and 405 bp (mutant allele – *rin*) [1]. In amplification of the genomic DNA with a pepper marker RinM/W two fragments closest in length to tomato fragments –566 bp and 408 bp were obtained. To identify the structure of these fragments sequencing and comparison of the results with the data banks of genetic sequences were conducted (Fig. 3). Comparison of sequenced PCR-fragment 566 bp (wild allele – *Rinc* – *capsicum*) of pepper in the nucleotide sequences of *Rin* tomato allele (GenBank access number: AX074057, AR580674) showed the level of similarity of about 98%. Comparison of the nucleotide sequences of the pepper PCR-fragment 408 bp (*rinc*) with *rin* tomato allele data sequences showed the allele mutant character. Deletion in 1722 bp is detected for a given set of *rinc* allele as well as in the case of *rin* tomato allele. [4-5]. *Rinc* and *rin* alleles similar nucleotide composition was about 97%. Figure 3 shows an alignment block of received diagnostic gene sequences *Rinc* and *rinc* with corresponding sequences from tomato genomic DNA database GenBank (*Rin* allele), and *rin* allele sequenced by us.

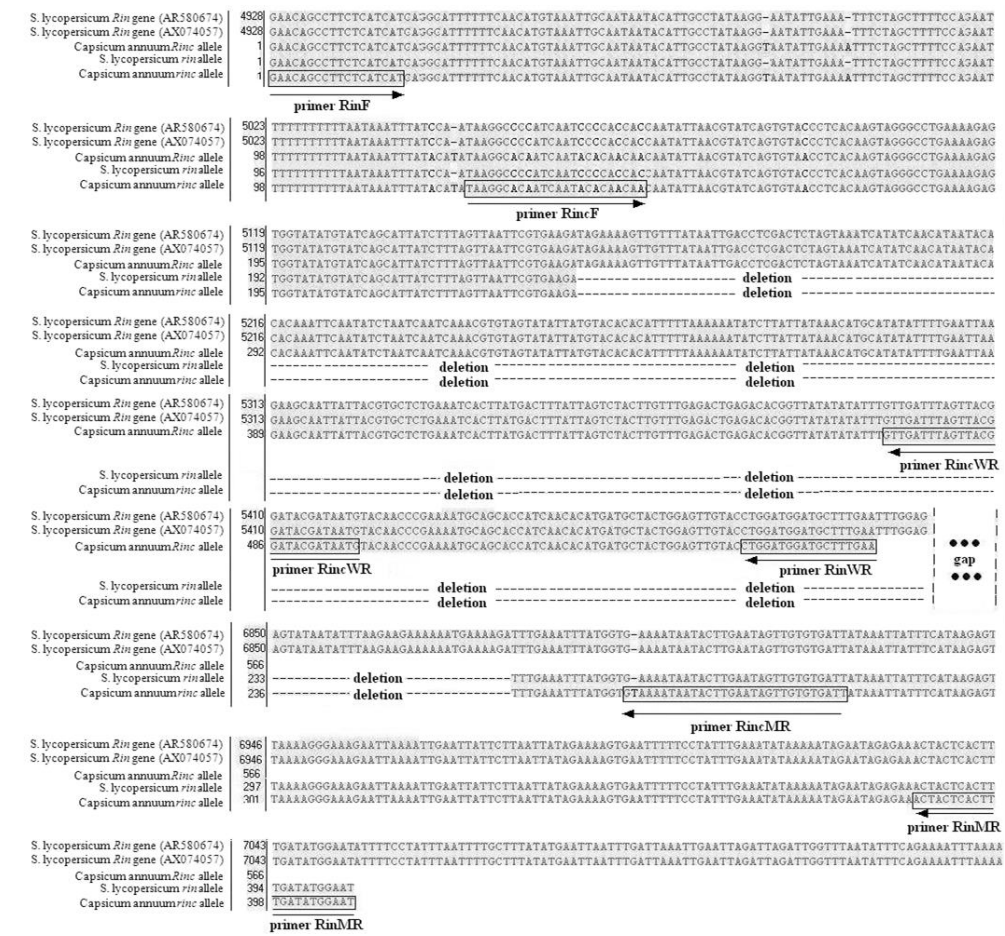

Figure 3:
Sequences alignment homolog sweet pepper gene alleles LeMADS-RIN with corresponding sequences of tomato genomic DNA

This indicates that the RinF/RinMR primer pair previously developed for identifying mutants *rin* gene in tomato plants, and is also suitable for the identification of the pepper mutation due to studied gene high homology in tomato (*Solanum lycopersicum* L.) and sweet pepper (*Capsicum annuum* L.).

In addition, on the basis of the comparative analysis specific marker RincM/W to a *LeMADS-RIN* gene homolog for pepper genotypes has been developed with the following primers:

RincF – 5'–TAAGGCACAATCAATACACAACAA–3'
RincWR – 5'–CATTATCGTATCCGTAACTAAATCAAC–3'
RincMR – 5'–AATCACACAACTATTCAAGTATTATTTTAC–3'

Their localization is shown in Figure 3. For the developed primers RincF / RincWR / RincMR alleged wild allele should have a size of 373 bp, and mutant allele – 156 bp. The studied collection testing results of sweet pepper with these primers are shown in Figure 4.

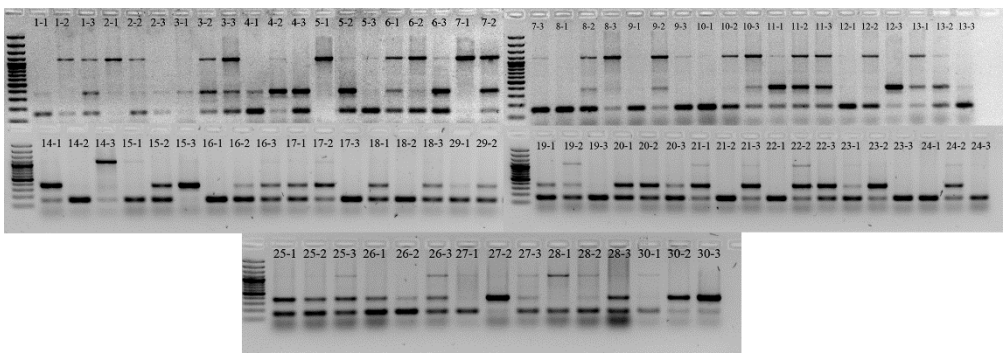

Figure 4:
DNA amplification products sweet pepper collection with primers RincF / RincWR / RincMR, (1-30 – see Figure 2)

Analysis of sweet pepper collection on the locus revealed a polymorphism between the samples and the heterogeneity of the studied material in the samples. The results showed that the mutant allele *rinc* occurs in sweet pepper significantly more frequently than tomato *rin* allele it might be explained by the longer ripening period of the first species.

Another fruit shelf life tomato mutation is also associated with a mutation in the *LeNAC-NOR* transcription factor. In accordance with the literature [5], mutation is caused by a short deletion, leading to a shift in the reading frame, and the formation of non-functional protein. Based on the tomato gene primers to *LeNAC-NOR*, studies the sweet pepper collection have been carried out. In tomato fragments' length is 159 bp for the mutant allele (*nor*), and 161 bp – for the wild allele (*Nor*). Analysis of the sweet pepper collection showed 27 samples with a fragment of 169 bp and 4 samples with a length of 162 bp (Fig. 5).

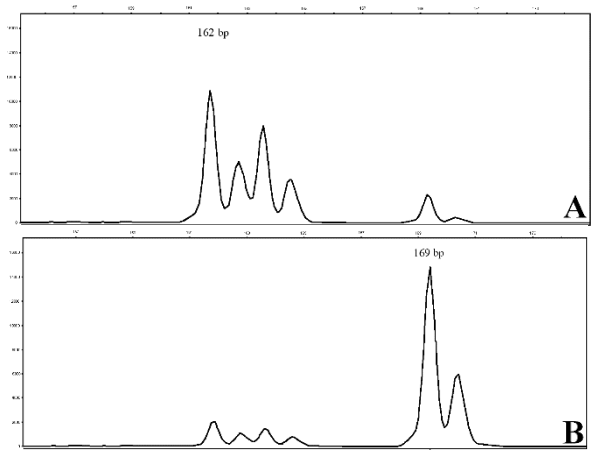

Figure 5:
The fragment analysis of sweet pepper collection with markers for LeNAC-NOR tomato gene (A – L-160-10; B – Long Kao)

For sequencing amplicons formed using *NorF/R* marker sweet pepper genomic DNA samples with L-160-10 and Long Kao were selected. We identified the marked alleles sequences *LeNAC-NOR* with length of 169 bp of Long Kao (denoted by *norc169*) and 162 bp of L-160-10 (denoted by *norc162*). Comparison of the nucleotide sequence of the tomato *Nor* allele marked region (AY573803) with *norc169* sequence showed similarity of about 75%. Comparison of the PCR-fragment sequence of the tomato *nor* gene sequence [5] with *norc162* showed their similarity level of about 98%. Deletion of a 2 bp revealed between *Nor* and *nor* tomato alleles have not been identified in pepper. Pepper *norc162* allele and tomato *Nor* allele differ in one nucleotide (Fig. 6).

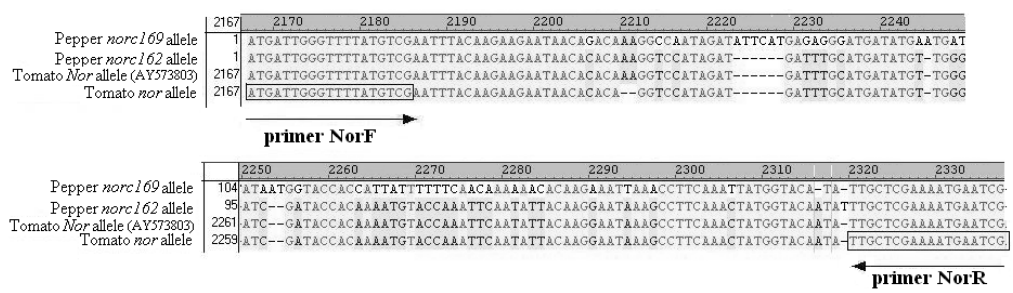

Figure 6:
Sequences alignment of the sweet pepper homolog gene wild and mutant alleles LeNAC-NOR with sequences of tomato genomic DNA

Comparison of the pepper *norc169* and *norc162* alleles nucleotide sequences showed differences in the length of 7 bp fragments, yet nucleotide composition differences were significantly higher (43 nucleotides). The use of tomato markers allows numerically determine the size of the marked fragment, but not to identify the nucleotide polymorphism. Currently phenotypic expression of the identified *norc* polymorphism is under study.

Accessions with different alleles of each studied genes were selected by developed markers. Hybrids combining alleles of long shelf life genes (*rinc, norc*) and pigments composition (*Y* and *cl)* were developed by topcrosses. Currently, forms with complex allele combinations in the F_2 generation are selected.

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A breeding program for resistance to anthracnose in sweet and chili pepper

Rosana Rodrigues[1], Cláudia Pombo Sudré, Cíntia dos Santos Bento, Ingrid Gaspar da Costa Geronimo

[1]*Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, RJ, Brazil*

Abstract

Many pathogens can cause diseases in *Capsicum* plants, including *Colletotrichum* spp., the causal agent of anthracnose. Typical anthracnose symptoms on sweet and chili pepper fruit include sunken necrotic tissues, with concentric rings of acervuli. Fruits showing symptoms have reduced marketability. Although the management and control of anthracnose are still being extensively researched, commercial genotypes of sweet and chili pepper that are resistant to the *Colletotrichum* spp. have not yet been developed. Pathogen variability is one of the major constraints in establishing a resistance breeding program and there is insufficient information concerning the interactions between the complexes of species involved in chilli anthracnose. This work describes preliminary results of a breeding program focused in developing anthracnose resistance commercial genotypes in Brazilian conditions. Fruits of thirty seven *Capsicum* spp. accessions were evaluated at two stages (unripe and ripe) in two different environments (fruits inoculated in the plant and detached fruit inoculated under laboratory conditions). For inoculum preparation, the isolate was grown on Potato-Dextrose-Agar (PDA) medium, and incubated in the dark at 25 ºC until the formation of colonies. The spore suspension was prepared at a concentration of 1 x 10^6 conidia/mL. Twenty fruit of each accession were inoculated at two developmental stages (unripe and ripe). The unripe and ripe fruit were inoculated in two different environments, which resulted in four combinations: unripe fruit inoculated in the plant (UFP); unripe fruit detached and inoculated under laboratory conditions (UFL); ripe fruit inoculated in the plant (RFP); and ripe fruit detached and inoculated under laboratory conditions (RFL).The absence of symptoms was only recorded for two accessions, both *C. baccatum* var. *pendulum*. One *C. annuum* accession was also identified as resistant. To investigate pathogenic variability in field commercial areas, samples of 49 fruits with typical anthracnose symptoms were collected in the local markets and county fair of Campos dos Goytacazes, RJ, Brazil. Fruits were individually collected, placed in plastic bags to avoid cross contamination, and transported to the Plant Breeding Department of the Universidade Estadual do Norte Fluminense where the isolation was performed to obtain fungus cultures. Fungi were isolated by aseptically transfer the spores from the fruits to the Petri dishes containing PDA with addition of streptomycin in concentration 1:10 (v:v). The Petri dishes were kept in a BOD at 27ºC until mycelial growth, for approximately 15 days. After that, mycelium disks were stored in sterile distilled water. The PDA medium favors *Colletotrichum* mycelial growth, when compared to oat-agar medium, which can be observed with the lowest number of conidia produced in PDA. This fungi collection was characterized using morphological traits and molecular approaches. Inheritance of resistance is being investigated in crosses between a susceptible commercial genotype and UENF 1381, a resistant *C. annuum* accession.

Introduction

Brazil has become the largest consumer of the world's pesticides in 2008, using more than 700 tons of products [8], surpassing countries like the United States of America. About 27% of small farms (0-10 hectares) and 80% of large farms (greater than 100 hectares) use of pesticides to combat diseases in their crops [1]. The use of pesticides, as well as the mixture of products with different chemical characteristics are risks to human health and the environment. These

practices are concerning mainly concentrated in agricultural production sites in smallholdings where vegetable production is the main activity.

The genus *Capsicum*, Solanaceae, produces the fruits leading the product ranking most contaminated by pesticides in Brazil, as sweet pepper. The species of this genus also have great versatility of use. Besides adding color and flavor to many fresh and processed food, *Capsicum* fruits are used in the pharmaceutical and cosmetic industries. Also, plants can be used for ornamental purposes, especially chili peppers [13].

Chili and sweet peppers are affected by many diseases, especially the ones occasioned by fungi, including anthracnose, caused by a complex of *Colletotrichum* species [4]. Four species occur more frequently in the *Capsicum* genus, which are *C. gloeosporioides*, *C. acutatum*, *C. coccodes* and *C. capsici*. To control the plant diseases, genetic resistance is considered the most appropriate strategy and one of the integrated disease management pillars [13].

Anthracnose is a disease of pre and post harvest, occurring mainly in tropical and subtropical regions during the hot and rainy season [9]. Typical anthracnose symptoms on sweet and chili pepper fruit include sunken necrotic tissues, with concentric rings of acervuli. Fruits showing symptoms have reduced marketability. This disease is highly destructive and can achieve losses of up to 100% [2].

Although the management and control of anthracnose are still being extensively researched, commercial genotypes of sweet and chili pepper that are resistant to the *Colletotrichum* spp. have not yet been developed. However, sources of resistance identification studies have detected potential genotypes as the PBC 80 and PBC 81 (*Capsicum baccatum*) resistant to *C. acutatum* and PBC 932 (*Capsicum chinense*) resistant to *C. capsici* [7; 5;6].

Pathogen variability is one of the major constraints in establishing a resistance breeding program and there is insufficient information concerning the interactions between the complexes of species involved in peppers anthracnose. The characterization of different isolates and their interaction with different genotypes could contribute to the achievement of more consistent results.This work describes preliminary results of a breeding program focused in developing anthracnose resistance commercial genotypes in Brazilian conditions.

Identifying sources of resistance

The *Capsicum* breeding program for resistance to anthracnose at UENF started with Silva [10] and Silva *et al.* (2014) [11]. The authors evaluated 37 *Capsicum* spp. accessions at two fruit stages (unripe and ripe) in two different environments (fruits inoculated in the plant and detached fruit inoculated under laboratory conditions). For inoculum preparation, one *C. gloeosporioides* isolate was grown on Potato-Dextrose-Agar (PDA) medium, and incubated in the dark at 25 ºC until the formation of colonies. The spore suspension was prepared at 1 x 10^6 conidia/mL concentration. Twenty fruit of each accession were inoculated at two developmental stages (unripe and ripe). The unripe and ripe fruit were inoculated in two different environments, which resulted in four combinations: unripe fruit inoculated in the plant (UFP); unripe fruit detached and inoculated under laboratory conditions (UFL); ripe fruit inoculated in the plant (RFP); and ripe fruit detached and inoculated under laboratory conditions (RFL). The evaluation was performed daily, for seven days, using grade scale proposed by Montri et al. (2009) [7]. We also evaluated the incubation period and the latent period. After obtaining the data, the area under the disease progress curve (AUDPC) was calculated: $\text{AUDPC}= \sum_{i}^{n-1}\left(\frac{x_i+x_{i+1}}{2}\right)(t_{i+1}-t_i)$, where: n = number of observations; Yi = the severity of the disease in the "i" th observation; Ti = time in days on the "i" th observation. All analyzes

were performed using the GENES software [3].

From 37 *Capsicum* accessions, anthracnose resistance was only recorded in three accessions, two *C. baccatum* var. *pendulum* (UENF 1797 and UENF 1718) [11] and one *C. annuum* (UENF1381) (Figure 1).

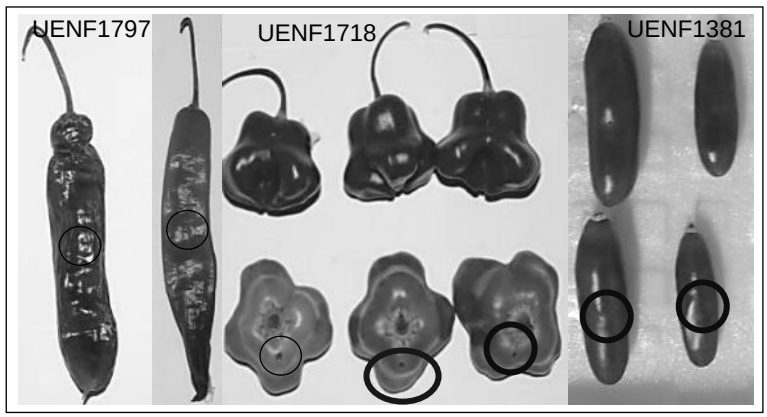

Figure 1:
Fruits of resistant C. baccatum var. pendulum accessions (UENF 1797 and UENF 1718), and C. annuum (UENF 1381), inoculated with C. gloeosporioides. Cicles show inoculated areas.

Pathogen Variability

Since one of the major constrain for developing new resistant cultivars is the pathogen variability, a breeding program should test more than one isolate in order to promote a better understanding of plant-pathogen interaction. To obtain *Colletotrichum* spp. isolates naturally occurring in the region where the *Capsicum* breeding program is inserted, samples of 49 fruits with typical anthracnose symptoms were collected in the local markets and county fair in Rio de Janeiro, São Paulo and Espírito Santo States, Brazil, to investigate pathogenic variability in field commercial areas. Fruits were individually collected, placed in plastic bags to avoid cross contamination, and transported to the Plant Breeding Department of the UENF, where the isolation was performed to obtain fungus cultures. Fungi were isolated by aseptically transfer the spores from the fruits to the Petri dishes containing PDA with addition of streptomycin in concentration 1:10 (v:v). The Petri dishes were kept in a BOD at 27ºC until mycelial growth, for approximately 15 days. Monosporic cultures were obtained and used for stock and characterization of isolates. Spore suspensions were prepared in a test tube containing 2 mL of sterile water with 1 to 10 spores per microscopic field when analyzed at 10x objective. An aliquot of 20 µL of suspension is deposited on the agar-water. Small fragments medium containing germinated spores were removed with the aid of a stereoscopic microscope, and transferred to PDA medium.

The cultural characterization of isolates was done, according to the mycelial growth rate and the colonies morphological characteristics observation of the isolated cultured in PDA medium. In order to compare, we used previously characterized isolates of *C. acutatum* and *C. gloeosporioides*. Evaluations were carried out daily with measurements of orthogonal diameters of the colony with the aid of calipers. The mycelial growth was computed in mm/day and measured for seven days. At the end, it was also observed color of each colony and the presence of microsclerotia. The experiment was carried out in a completely randomized design with four replications, and each repetition consists of a Petri dish.

Morphological characterization was done by determining the size and shape of conidia produced by the isolates. Initial cultures were prepared from each isolate in Petri dishes on PDA medium at 25° C and after five days three discs of spore area were removed and plated on PDA medium and incubated under the same conditions for seven days. Three semipermanent laminas of each isolate were made and were chosen randomly for 50 conidia measurements. Regarding to format, the conidia were classified in four groups, as proposed by Sutton (1992) [12]: 1) straight, fusiform with tapered tip; 2) straight, oblong, with rounded apex; 3) straight, clavate, tapered at one end; 4) straight, with constriction.

The storage of isolates was done on filter paper soaked in skim milk solution 10% and spores. The filter paper discs are soaked in the solution and stored in microtubes with silica gel and hydrophobic cotton. These isolates are being used in testing accessions from our genebank seeking to identify multiple sources for resistance to anthracnose.

The 49 fruits collected in three Brazilian states resulted in 32 isolates with characteristics compatible to *Colletotrichum* spp. The morphological features used to characterize the isolates were based on the size and shape of the conidia, since they are consistent to the identification of species of the genus *Colletotrichum*. Morphological characterization based on the size of the conidia differentiate isolates studied in two groups. The first group was formed by the isolates identified as 01, 02, 03, 06, 09, 15, 17, 36, 50, 53, 71 and 72, which showed the sizes of conidia between 8.5 to 16.5 mM of length and 2.5 to 4 mm in width, which values are characteristic for identification of *C. acutatum*. Isolates 13, 16, 22, 26, 27, 28, 37, 42 and 61 were characterized by the measures proposed for *C. gloeosporioides*, ranging between 12 and 17 mm in length is 3.5 to 6 microns wide. The isolated 8.1 was used as control of *C. gloeosporioides*. Isolates 29, 41 and 45 have dimensions that do not match the description for any of the species of *Colletotrichum* [12]. The predominant format of the conidia was straight, oblong with rounded ends, cited as typical of *C. gloeosporioides*. Also, straight conidia, fusiform and apexes tapered, characteristic of *C. acutatum* were observed (Figure 2).

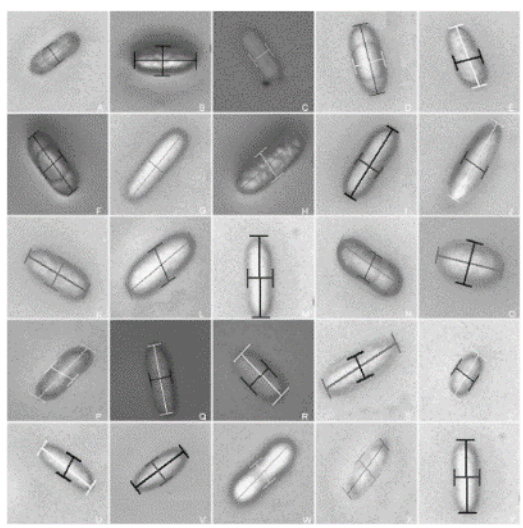

Figure 2
Samples of Colletotrichum conidia isolated from fruit with anthracnose in three different Brazilian states. A) Isolate 8.1 - Standard C. gloeosporioides; B) Isolate 1; C) Isolate 2; D) Isolate 3; E) Isolate 6; F) Isolate 9; G) Isolate 13; H) Isolate 15; I) Isolate 16; J) Isolate 17; K) Isolate 22; L) Isolate 26; M)

Isolate 27; N) Isolate 28; O) Isolate 29; P) Isolate 36; Q) Isolate 37; R) Isolate 41; S) Isolate 42; T) Isolate 45; U) Isolate 50; V) Isolate 53; W) Isolate 61; X) Isolate 71; Y) Isolate 72.

Among the cultural characteristics evaluated, the mycelial growth rate and the color of the colony were considered, since they are useful for distinguishing between some species of the genus.

There was a significant difference to the characteristic speed average of mycelium growth, which ranged from 8.72 to 5.58 mm/day. The isolates were divided into four groups by Scott-Knott test. The mycelial growth rate was 7.3 mm/day, which according to the literature is the corresponding growth rate *C. acutatum*. Moreover, it was also observed color of the colony, with the possible observation of phenotypically similar samples with the exception of isolate 2, which produced white mycelium, and isolated 13:45, which have colonies with orange center and white edges (Figure 3).

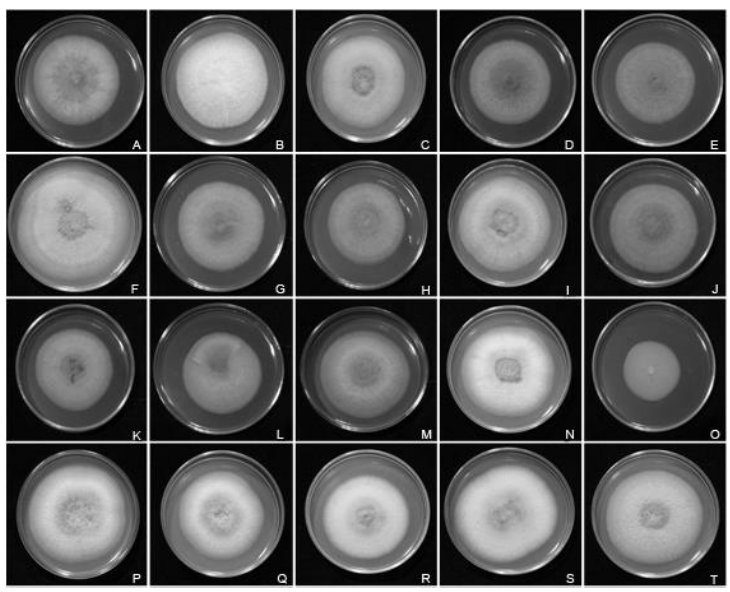

Figure 3
Colonies of Colletotrichum isolates grown for seven days on PDA. A) Isolate 8.1 - Standard C. gloeosporioides; B) Isolate 1; C) Isolate 2; D) Isolate 3; E) Isolate 6; F) Isolate 9; G) Isolate 13; H) Isolate 15; I) Isolate 16; J) Isolate 17; K) Isolate 22; L) Isolate 26; M) Isolate 27; N) Isolate 28; O) Isolate 29; P) Isolate 36; Q) Isolate 37; R) Isolate 41; S) Isolate 42; T) Isolate 45; U) Isolate 50; V) Isolate 53; W) Isolate 61; X) Isolate 71; Y) Isolate 72.

Preliminary results obtained from the morphological characteristics indicate that isolates represent two species: *C. gloeosporioides* and *C. acutatum*. The study of genetic diversity among the isolates using molecular markers is in progress.

Inheritance of anthracnose resistance

Inheritance of resistance is being investigated in crosses between a susceptible and one resistant (UENF 1381) accessions to anthracnose from our germplasm bank, both *C. annuum* genotypes. For the inheritance study, seeds from the generations P_1, P_2, F_1, F_2, BC_1 and BC_2 were obtained in a greenhouse. Generations P_1, P_2 and F_2 were obtained by controlled selfing

pollinization using paper bags to protect the flower buds in the pre-anthesis. Artificial hybridization was promoted to produce F_1, BC_1 and BC_2 seeds, making emasculation and pollen deposition in previously targeted stigma (Figure 4).

Figure 4.
Generating studying population with artificial hybridization: pre anthesis collection of buds, pollen removal and emasculation of C. annuum flower buds.

Approximately 300 seeds of generations P_1 and P_2, 350 seeds from F_1, 200 F_2 and 250 of each backcross were obtained and plants are being phenotyped (Figure 5) and genotyped for anthracnose resistance. Phenotyping these populations for resistance to anthracnose, the development of a linkage map and the identification of QTLs that explain the resistance to anthracnose are the next steps to take that will contribute to the breeding program for anthracnose resistance.

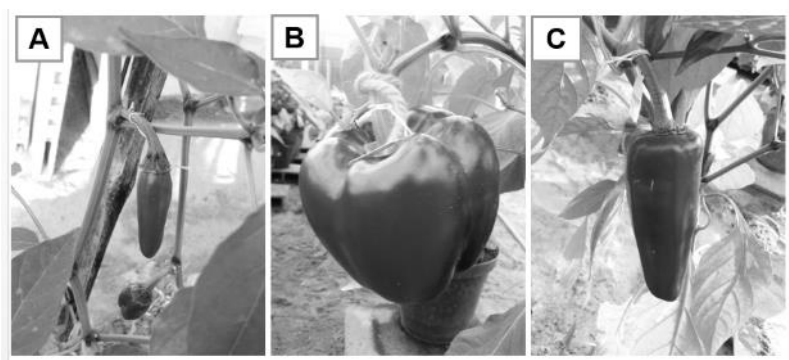

Figure 5.
(A; B) Parentals used in crosses to study genetic resistance to anthracnose (A=resistant; B=susceptible);
(C) Hybrid used in the study of inheritance of anthracnose resistance in Capsicum annuum.

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Screening of solanaceous wild relatives for graft affinity with eggplant (*Solanum melongena* L.)

François Villeneuve[1], François Latour[1], Théophile Théry[1], Patricia Erard[2], Christine Fournier[2], Marie-Christine Daunay[3]

[1] *Ctifl, Centre de Lanxade, Prigonrieux, France*

[2] *Ctifl, Centre de Balandran, Bellegarde, France*

[3] *INRA, UR1052, Génétique et Amélioration des Fruits et Légumes, Montfavet cedex, France*

Abstract

Eggplant (*Solanum melongena* L.), an important solanaceous crop in the world, is susceptible to soilborne bio-agressors, the main of which, in temperate areas, being Verticillium wilt (*Verticillium* sp.) and root knot nematodes (*Meloidogyne* spp.). Grafting on resistant rootstock is an alternative method to soil disinfection. In France, the generalization of grafting spread over the 2000's. After several years of utilization of resistant tomato rootstocks for eggplant production, and to a lesser extent, of *Solanum torvum* rootstocks, damaging wilt-like-symptoms are often observed by growers. Hence it is necessary to identify alternative rootstocks, able to sustain the soil pathogenic complex by a combination of vigor and genetic resistance. However, graft affinity between potential rootstocks and eggplant (scion) is the first step to investigate. Solanaceae family offers a wide choice of candidate rootstock species. The objective of this research was to identify solanaceous species having a good graft affinity with eggplant. Screening of a wide set of genetic resources was carried out at the Centre Technique Interprofessionnel des Fruits et Légumes -Ctifl- (Lanxade and Balandran center) in collaboration with INRA. Experiments were carried out during a five years period (2011-2015). Several *Solanum* species displayed a good graft affinity with eggplant and are candidates for further agronomic evaluation.

1. Introduction

Eggplant (*Solanum melongena* L.) is an important solanaceous crop in the world and represents an important source of income for growers. The species is susceptible to several soilborne bio-agressors. Verticillium wilt (*Verticillium* sp.) and root knot nematodes (*Meloidogyne* spp.) are the major limiting factors for eggplant production in temperate conditions, worldwide. Up to a recent past, both diseases were controlled by soil fumigation with methyl bromide prior to planting. However, since this chemical is no more authorized for this use in European countries since 2005, no alternative chemicals have provided sufficient control of soilborne diseases. Grafting on resistant rootstock is an alternative method to soil disinfection. In France, the research on grafting for vegetables production started in the 1950's, and grafting onto Verticillium resistant tomato rootstocks started in the 1960's for commercial production (Messiaen and *al.,* 1967; Beyries, 1974). The generalization of grafting spread over the 2000's and nowadays 63% of French eggplant surfaces are grafted (Torres and Brand, 2015). After several years of utilization of resistant tomato rootstocks for eggplant production, and to a lesser extent, of *Solanum torvum* rootstocks, damaging wilt-like-symptoms are often observed by growers. A survey by Villeneuve *and al.* (2016) in two main French production areas indicated that *Verticillium*, as well as at least two additional fungi, were responsible of the wilt symptoms observed on grafted plants, thus revealing the development of new pathogenic

complexes in intensively cultivated soils. Hence it is necessary to identify alternative rootstocks, able to sustain the new soil pathogenic complexes by a combination of vigor and genetic resistance. Graft affinity between potential rootstocks and eggplant (scion) is the first step to investigate. S*olanum* genus, and more largely, Solanaceae family, offer a wide choice of candidate rootstock species. The objectives of this research were to identify solanaceous species having a good graft affinity with eggplant and yielding good agronomic results. Screening of a wide set of genetic resources was carried out at the Centre Technique Interprofessionnel des Fruits et Légumes -Ctifl- (Lanxade and Balandran center) in collaboration with INRA.

2. Materiels and method

Graft affinity between eggplant and candidate rootstocks was investigated in a two steps process, (i) screening of rootstocks on young plants in semi-controlled conditions, and (ii) for the best rootstock-scion combinations, evaluation of agronomic results in greenhouse conditions.

Plant materials

Seventy five accessions of several genera and species belonging to the *Solanaceae* family, as well as five interspecific hybrids between eggplant and related *Solanum* species, were included in this study (table 1). Seeds were obtained from INRA (Vegetables Genetic Resources Centre, CRB-Leg, GAFL, Montfavet, and Ploudaniel) and also from the French institute of Tabaco (Bergerac, France), the Universitat Politècnica de València (Spain) and the Radboud University (Nijmegen, The Netherlands). The interspecific hybrids (F1) were created at INRA GAFL. Germination was secured with a 500 ppm gibberellic acid (GA_3) treatment applied to the seeds 24 hours before sowing in a substrate containing 67% of compost and 33% of quartz. Two leaf stage plantlets were transplanted in pots containing the same substrate (1.5l).

Species	Accession number	Seed source	Accessions used in confirmation trials
Capsicum annuum	RV6	Inra GAFL	
C. baccatum	PM 1034	Inra GAFL	
Cestrum parqui	874750007	Nijmegen	
Cyphomandra betacea	894750221	Nijmegen	2012-13
Hyoscyamus niger	88475005	Nijmegen	
Iochroma australe	904750118	Nijmegen	
Lycianthes rantonetii	814750064	Nijmegen	
Lycium barbarum	MM 1378	Inra GAFL	
Nicandra physaloides	884750065	Nijmegen	2013
Nicotiana tabacum n°1	MS 270	Institut du tabac	
N. tabacum n°2	MS 33518	Institut du tabac	
Physalis edulis	MM 1321	Inra GAFL	
P. peruviana	MM 1358	Inra GAFL	
Solanum acanthoideum	MM 12296	Inra GAFL	2013
S. aculeastrum	MM 1425	Inra GAFL	2012-13
S. aculeatissimum	MM 369	Inra GAFL	
S. aethiopicum group Gilo	MM 232 bis	Inra GAFL	2013-15
S. aethiopicum group *Aculeatum*	MM 134	Inra GAFL	2013-14-15
S. anguivi agg.	MM 1689	Inra GAFL	2013-14-15
S. arundo	MM 1369	Inra GAFL	2015
S. atropurpureum		Inra GAFL	2012-13
S. burchellii		COMAV	
S. canense		COMAV	

S. caripense	MM 1526	Inra GAFL	2015
S. catombelense	MM 987	Inra GAFL	
S. cerasiferum	UPV 23386	Inra Ploudaniel	
S. chacoense	UPV 23372	Inra GAFL	
S. citrullifolium	MM 1218	Inra GAFL	2015
S. coccineum	MM 866	Inra GAFL	2014
S. cyaneo-purpureum		Inra GAFL	
S. dasyphyllum	MM 1174	Inra GAFL	
S. dennekense	MM 992	Inra GAFL	
S. dinteri	MM 994	Inra GAFL	2015
S. elaeagnifolium	MM 1137	Inra GAFL	
S. erianthum (verbascifolium)	MM 1312	Inra GAFL	2015
S. glaucophyllum	MM 1221	Inra Ploudaniel	
S. hastifolium	MM 1534	Inra GAFL	2012-13-15
S. hougasii	MM 1326	Inra GAFL	2013-15
S. incanum group A	MM 1793	Inra GAFL	2013-15
S. incanum group B	MM 1349 bis	Inra GAFL	
S. incanum group C		Inra GAFL	2013-15
S. incanum group C	MM 716	Inra GAFL	
S. incanum group D	MM 1428	Inra GAFL	
S. jatrophifolium	MM 664	Inra GAFL	2013
S. kurzii (sanitwongsei)	MM 684	Inra GAFL	
S. laciniatum	MM 1248	Inra GAFL	2013
S. lidii	MM 1529	Inra GAFL	2013-15
S. linnaeanum	MM 1003	Inra GAFL	
S. macrocarpon	MM 370	Inra GAFL	
S. *mammosum*	MM 1005	Inra GAFL	2012-2013
S. marginatum	MM 195	Inra GAFL	
S. mauritianum	MM 1136	Inra GAFL	
S. melanospermum	MM 1715	Inra GAFL	
S. muricatum	MM 824	Inra GAFL	2012-13-14
S. palinacanthum	MM 573	Inra GAFL	
S. pyracanthos	MM 1350	Inra GAFL	2013
S. renschii	MM 1821	Inra GAFL	
S. richardii	MM 1762	Inra GAFL	
S. rigescens	MM 1014	Inra GAFL	
S. rigescentoides	MM 1015	Inra GAFL	2014
S. rostratum	MM 1753	Inra GAFL	2015
S. rubetorum (rigescens auct. non Jacq)	MM 1224	Inra GAFL	
S. scabrum	MM 1226	Inra GAFL	
S. *schimperianum*	MM 1190	Inra Ploudaniel	
S. sisymbriifolium	MM 1018	Inra GAFL	
S. stoloniferum	MM 831	Inra GAFL	
S. stramonifolium	MM 12192	Inra GAFL	
S. supinum	MM 284	Inra GAFL	
S. tomentosum		Inra GAFL	
S. torvum	MM 416	Vilmorin	2012-13-15
S. trachycarpum	MM 1022	COMAV	2012-13
S. trilobatum		Inra GAFL	
S. viarum (without spines)		Inra GAFL	2012-13-14
S. violaceum		Inra GAFL	2012-13-14
S. virginianum		Inra GAFL	
Withania somnifera		Inra GAFL	

	MM 1024 STT3 UPV 23392 MM 1025 MM 1602 MM 497 MM 511 MM 1262		
F1 (S.*aethiopicum* Gilo X *S. melongena*)	MM 232 x LF3	Inra GAFL	2013-14-15
F1 (*S. linnaeanum* X *S. melongena*)	MM 195 x LF3	Inra GAFL	2013-14-15
F1 (*S. melongena* X *S. incanum* gr C)	LF3 x MM 664	Inra GAFL	2014-15
F1 (*S. melongena* X *S.incanum* gr D)	LF3 x MM 1248	Inra GAFL	2013-15

Table 1: Plant material used as experimental rootstocks

Early screening for graft affinity

The experiments were carried out from 2011 to 2014 in greenhouse at Lanxade center of Ctifl located in southwestern France (lat.: 44.86, long.: 0.40). Controls were (i) the non-grafted 'Monarca F1' eggplant variety (Rijk Zwaan, Aramon, France), (ii) the self-grafted 'Monarca F1', and (iii) the commercial tomato rootstock 'Maxifort' (Monsanto, France) which is an interspecific hybrid *S. lycopersicum* X *S. habrochaites*.

Plants of the 'Monarca F1' scion at the 2-4 true leaves stages (20-50 days old) were grafted onto rootstock plants having 3-4 true leaves (40-50 days old) using the cleft grafting method. To be sure that scions and rootstocks were to have similar stem diameter at grafting time, sowing was made three times for the variety, at one week interval. After grafting, plantlets were kept for 5 days within a closed plastic shelter in a greenhouse with a day/night thermoperiode maintained between 25° and 18°C. Later on, grafted plantlets were progressively acclimatized by perforating the plastic. After acclimatization, grafted plants were placed in greenhouse under natural lighting for 150 days. Grafting combinations were randomized in a complete block design, with three replications of 5 plants per treatment.

Success of graft union was recorded as well as, 100 days after grafting, plant height and fresh weight of aerial part. Each graft union was longitudinally cut in order to observe the presence of browning (data not shown).

Agronomic trials

The experiments were carried out in 2012 to 2015 in a greenhouse at Balandran center of Ctifl in southeastern region of France (lat.: 43°75', long.: 4°.45'N). Seeds were sown at INRA and grafting (tongue approach) was realized by a professional nursery. Experimental design and controls were the same as for the early screening assays.

Mortality throughout the cultivation period, plant height at the end of trial (150 days after transplanting) -data not shown- were recorded. Early and total yield (kg/m²) were measured for each individual plant. Early yield was calculated over the first three weeks of harvest.

3. Results and discussion

Early screening for graft affinity

The best percentages of successful grafting are displayed in table 2. One hundred to 90% success rate was observed for 26 graft combinations; ■ 90-70% for 22 combinations; ■ 70-50% for 7; and ■ less than 50% for 19.

Overall, the species phylogenetically distant from *S. melongena* expressed a bad graft affinity, such as *Nicotinia* spp, *Physalis* spp, *Capsicum* spp., and *S. canense*. Our results for *S. sisymbriifolium*, a species valued for its resistance to *V. dahliae* (Bletsos *et al.*, 1998), don't confirm the good grafting affinity observed by Bletsos *et al.* (2003). We recorded only 13% of success grafting rate whereas these authors observed a rate over 70 %. Our results are consistent with those of Rahman *and al.* (2002) who concluded that *S. sisymbriifolium* is not a promising rootstock for eggplant.

Conversely, some rootstocks, in particular the interspecific hybrids, exhibit a high rate of graft success, a good quality graft union and allow a good development of the scion. It should be noted that the grafting technique used in these early trials is not adapted to the commercial control rootstock 'Maxifort', since we obtained a random rate of grafting success (figure 1).

100% of grafting success	**Grafting success between 90 and 99,9%**
Cyphomandra betacea	Hyoscyamus niger
S. aethiopicum group aculeatum	S. aethiopicum Gilo X
S. coccineum	S. anguivi agg.
S. dasyphyllum	S. arundo
S. dennekense	S. atropurpureum
S. erianthum	S. dinteri
S. glaucophyllum	S. kurzii
S. incanum group A	S. marginatum
S. incanum group B	S. rigescens
S. incanum group C (MM 664)	S. viarum (without spines)
S. lidii	S. virginianum
S. pyracanthos	
S. rigescentoides	
S. aethiopicum Gilo X S. melongena	
S. melongena X S. incanum gr C	
S. melongena X S.incanum gr D	

Table 2:
The best accessions for grafting success in compatibility trials

The successful graft combinations induce however a wide growth range of the Monarca scion, from about 80% dwarfing to about 80% vigor boosting, when compared to the control Monarca auto-grafted (figure 1). The rootstocks inducing the highest scion vigor are *S. lidii, S. rubetorum*, S. *virginianum* and *S. rostratum*. The rootstocks depressing growth don't present a great interest.

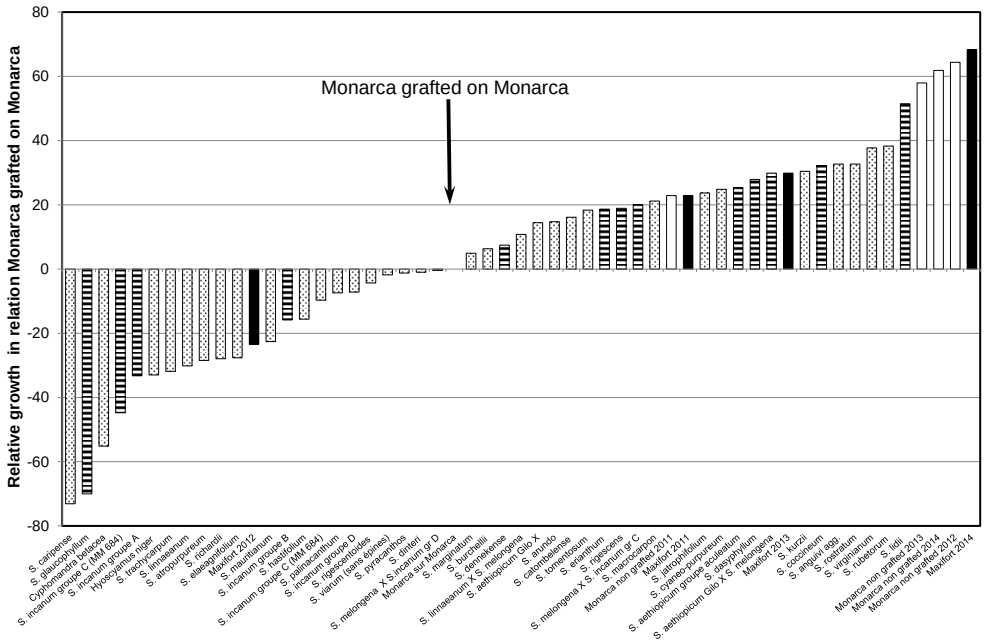

Figure 1:
Scion growth for the rootstocks displaying up to 80% graft success, expressed on the basis of the control Monarca auto-grafted, taken as a growth reference of zero,
rootstocks with 100% rate of grafting success; rootstocks with 80-99% rate of grafting success; Monarca F1, not grafted (control); rootstock Maxifort (commercial control).

Different *Solanum* species are spiny, but as the spines are soft at an early plant development stage, they are not problematic.

Agronomic trials

We recorded some mortality shortly after plantation for *Cyphomandra betacea* and *Nicandra physaloides* and later (after the third harvest) for *S. atropurpureum* (75% of plants) and *S. coccineum* (53%). Mortality was less for *S. mauritianum*, *S. trachycarpum* and *S. viarum*.

Some Solanum species produce suckers, very strongly for S. acanthoideum, S. trachycarpum and S. linnaeanum and at a lower level for S. aculeastrum, S. atropurpureum, S. pyracanthos and the interspecific hybrid S. linnaeanum x S. melongena. When the suckers have dense and sharp spines, like S. pyracanthos, this may be a problem in cultivation.

Early production is an important aspect for growers. *Nicandra physaloides* and *S. rostratum* are part of the earliest rootstocks, with also good total commercial yield, unlike *S. trachycarpum* and *S. atropurpureum* which present also a good early production but a low total yield By contrast, some botanical species used as rootstock induce a delayed production like *S. hastifolium*, *S. erianthum* and *S. coccineum* (figure 2).

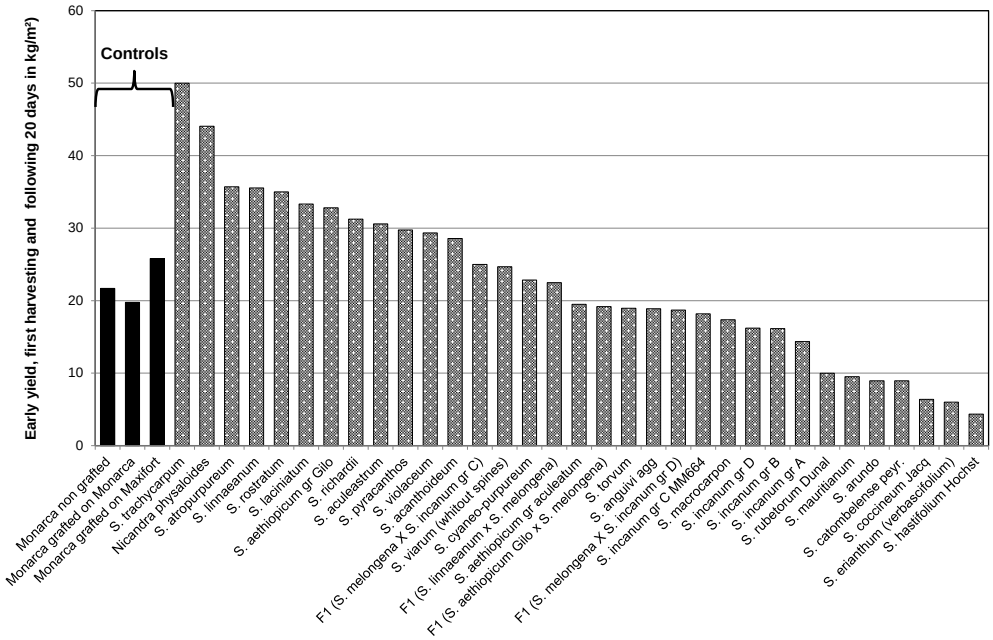

Figure 2:
Effect of rootstock on early production (in kg/m^2) for the three first weeks of production

For the total commercial yield, the interspecific hybrid (*S. melongena* X *S. incanum* group C) gives much better results than the commercial rootstock controls 'Maxifort' and *S. torvum* STT3 (figure 3). Yield of the two interspecific hybrids F_1 (S. *aethiopicum* Gilo X *S. melongena*) and F_1 (*S. linnaeanum* x *S. melongena*), as well as *S. aethiopicum* gr Gilo, *S. anguivi*, *S. incanum* group A and D, S. *macrocarpon*, *S. pyracanthos*, *S. rostratum*, and *S. violaceum* is similar to the yield of the two controls (figure 3). On the contrary, several rootstocks provide low to very low yields like S. *trachycarpum*, *S. mauritianum* and *S. hastifolium*. Globally the interspecific hybrids have comparable or better results than their botanical parent.

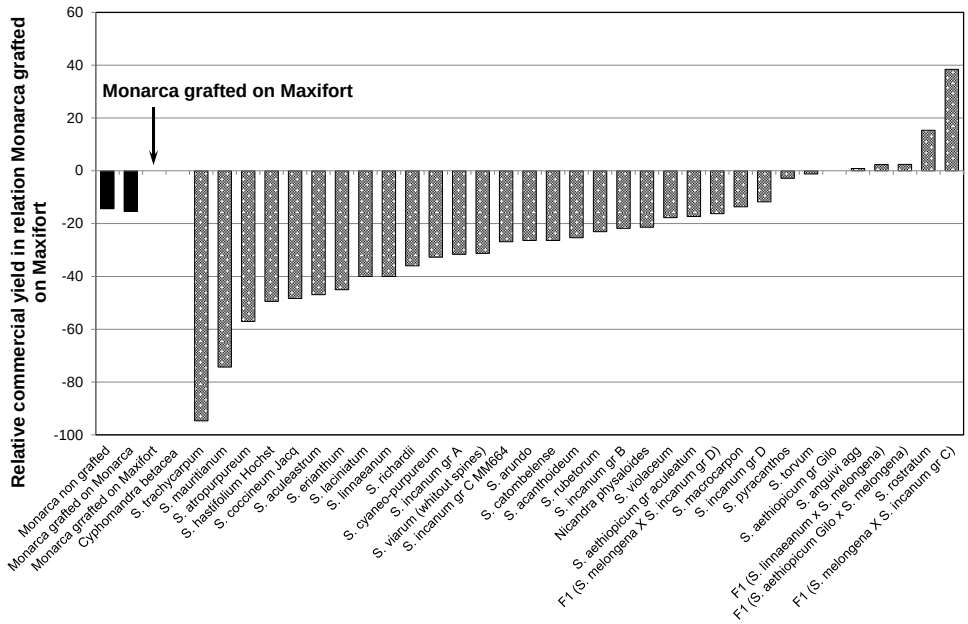

Figure 3:
Relative total commercial yields 60 days after planting for the different botanical species tested as rootstock grafted onto the variety Monarca. The yield of the control Monarca grafted on Maxifort is taken as the reference of zero.

The data obtained during this prospective research on diverse parameters measuring graft affinity between eggplant (scion) and numerous Solanaceous species, from graft success to commercial yield, reveals the potentialities as rootstocks of several *Solanum* species, in particular S. *aethiopicum* gr. *aculeatum*, *S. aethiopicum* group Gilo, *S. anguivi*, *S. incanum* (group A, B, C, D) and *S. macrocarpon*, as well as interspecific hybrids (F_1 *S. aethiopicum* group Gilo x *S. melongena*, F_1 *S. melongena* x *S. incanum* group C and D and F_1 *S. linnaeanum* x *S. melongena*) present also interest. Similar promising results, although obtained with less rootstocks germplasm, were obtained by Gisbert *and al.* (2011).

Further research is still needed before developping commercial new rootstocks. Indeed the agronomic performances of the best rootstocks identified has to be retested in different production conditions. Further, it is necessary to estimate their root vigor as well as their level of resistance to the major elements of the soil pathogenic complex, in particular *Verticillium dahliae*, *Colletotrichum coccodes* and *Meloidogyne* species. Furthermore, the alkaloid content of the eggplant fruits produced on these rootstocks has also to be looked at carefully.

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Antixenosis and antibiosis based resistance of chilli pepper to melon aphid

Ady Daryanto[1], Muhamad Syukur[1], Purnama Hidayat[2], Awang Maharijaya[1]

[1] *Department of Agronomy and Agriculture, Faculty of Agriculture, Bogor Agricultural University (IPB), Jl. Meranti Kampus IPB Darmaga, Bogor, Indonesia*

[2] *Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University (IPB), Jl. Kamper, Kampus IPB Darmaga, Bogor, Indonesia*

Abstract

The melon aphid or cotton aphid (*Aphis gossypii* Glover) is one of the major pests of pepper. Aphid colonies can cause significant yield loss by damaging leaf and stem tissues. They can also act as vector of many kind of viruses. Chemical based crop protection is the major way to control aphid until now. In fact, melon aphid has been resistant to many kind of insecticides. Insecticides can also damage beneficial insects, predators, parasitoids, and pollinators. Therefore the use of insecticides should be limited.

The use of resistant varieties may help to reduce the use of insecticides, together with Integrated Pest Management. The objective of this research was to identify the antixenosis and antibiosis based resistance of melon aphids in several pepper genotypes that may be explored as sources of resistance in aphid resistance breeding program of pepper. We used choice and no-choice test, and detached leaf based experiments. Antixenosis based resistance was detected as shown by significant number of aphid per leaf, total aphid per plant, and total winged aphid per plant. Antibiosis based resistance was also detected as shown by significant difference in longevity time, reproduction time, number of aphid progeny per day, and the fecundity of the melon aphid among genotypes.

1. Introduction

Pepper production is constrained by *Aphis gossypii* Glover (Hemiptera: Aphididae) or melon-cotton aphids, one of the important insect pests in pepper, especially in low altitude and humid areas when no control measures are taken [1]. The aphid management and control practices include chemical treatments, biological controls and cultural practices. Up to now, chemical based crop protection is the major way to control aphids. However, insecticides might also killed beneficial insects, predators, parasitoids, and pollinators. Besides that, large scale application of chemical pesticides can lead to serious health and environmental problems. Melon aphids have also been resistant to many insecticides such as organophosphate and pyrethroid [2].

The use of host-plant resistance is one of the best management strategy against insect pests. Incorporation of resistant varieties may be a valuable addition to the IPM system. Resistant varieties can be used together with cultural practices (e.g. field sanitary and crop-rotation measures) to prevent infestation. Resistant varieties may also increase the suppression of the pest development in combination with biological control [3]. Toward breeding for resistance against aphids, it is important to identify the resistance of several pepper genotypes to aphids.

There are three mechanisms of plant defense against pests i.e. antixenosis, antibiosis, and tolerance [4, 5]. Antixenosis or non-preference is a defense mechanism in form of morphology,

phenology, and odor from the plant to reject the presence of pests. Antixenosis can be evaluated through the reduction number of colonies of pests [6]. Antibiosis is the ability of plants to limit and reduce the proliferation of pathogens after contacting with the plant. Antibiosis on insect are reflected in high mortality, low breeding rate of the neonate, and decrease reproductive ability of pests [7]. Tolerance is the difference in the ability of plants to respond to pests and limiting damage to the broader per unit where these pests [4]. However these different mechanisms are not always easy to separate [3]. The objective of this research was to identify the antixenosis and antibiosis based resistance of melon aphids in several pepper genotypes that may be explored as sources of resistance in aphid resistance breeding program of pepper.

2. Materials and methods

2.1. Plant materials

Twenty one genotypes of peppers (*Capsicum annuum* L.) from Bogor Agricultural University and AVRDC collections were used for this study. The plants were grown from seeds in plastic tray with 50 holes and placed in insect-tight box. Firstly, two seeds were sowed on each holes of plastic tray containing a mix of growing medium (soil: coco peat: green manure; 1:1:1 v) and separated to be one each hole after two weeks. No insecticide was used during this experiment to avoid insecticide effects on the treatment.

2.2. Aphid population

Melon aphids were collected from pepper cultivation at Unifarm of Bogor Agricultural University, Indonesia following by the identification of the species to ensure that the aphid colonies were *A. gossypii* Glover. The identification was based on the identification key guides of Blackman and Eastop [8]. The specific identification keys for Aphid *gossypii* were the black color of cornicles, the pale color of cauda (cauda lighter than cornicle), and the antennal tubercles that were weakly developed (not exceeding height of medial part of frons). Adult aphids (imago) were cultured on susceptible pepper plants and propagated in insect-tight box (temperature of 28 ± 2 0 C; RH 65 ± 10%). Routine maintenance by moving the adult aphids to fresh susceptible pepper plants were done when the aphid population had already seen crowded or diseased plants.

2.3. Choice tests

Screening of the twenty one genotypes was conducted during the seedling phase of pepper (4-6 leaves or 5 weeks after sowing), in an insect box. Two adult wingless-aphids (apterous) were transferred with a soft brush to the leaves of the seedlings. Aphids were allowed to migrate, feed, and reproduce freely (choice-test). The experiment was designed in a randomized complete block design with pepper genotypes as treatment with three replications. Observation was done at 12 day after infestation by counting the number of aphids per leaf on each genotype. Further, the genotypes were categorized as follow: 8-21 aphids per leaf = very low infestation, 22-35 aphids per leaf = low infestation, 36-49 aphids per leaf = lower-medium infestation, 64-77 aphids per leaf = high-medium infestation, 78-91 aphids per leaf = high infestation, 92-105 aphids per leaf = very high infestation. Six genotypes were selected using above criteria. Those selected genotypes were used for further the antixenosis and antibiosis based resistance tests.

Further antixenosis based resistance test were done in a choice test setup as in previous screening test. Aphids were allowed to migrate, feed, and reproduce. Six genotypes selected

based on the result of the first screening test i.e: IPB C5, IPB C12, IPB C20, IPB C145, and IPB C313 were used. Observation was done at 12 day after infestation by counting the number of aphids per leaf and per plant on each genotypes.

2.4. No-choice test

Antibiosis based resistance test was done in a no-choice setup using detached leaf system. Leaves of pepper, the third or fourth fully opened leaves from the top, of each genotypes were used in this experiment. Each leaf was placed in a single container (6.3 cm x 5 cm) with addition of wet cotton to keep the leaves fresh. Each container was covered by muslin (50 meshes) for ventilation. Environmental conditions were kept at 28 ± 2°C and 65 ± 10% RH based on Satar et al. [9]. Observations were carried out every day until all aphids died. Initial infestation placed one apterous adult for 24 hours and after that got first newborn nymph. Nymphs, 3-5 nymphs, were maintained until be imago for testing the nymph survival and development time. Furthermore we selected one imago from nymphs that had become imago to be tested fecundity, longevity, and reproduction time. All newborn nymphs were counted and removed daily.

Nymph survival was the number of living nymphs of first birth to be imago while the life cycle was the time interval from first instar to first instar back. Longevity time was calculated from the first newborn nymph to death selected imago. Fecundity was the total number of nymphs (progenies) produced by an aphid during its lifetime. The experiment was designed in a randomized complete block design with pepper genotypes as treatment with three replications.

2.5. Statistical analysis

Normality test and Bartlett's test at 5% level of significance were done to meet the assumption $\varepsilon ij \sim N(0, \sigma 2)$; error normal spread, the mean μ, and variance homogeneous. Furthermore, the data were tested by ANOVA (F-test), when the treatments significantly difference, followed by Honestly Significant Difference (HSD) test. Correlation (Pearson) was performed on leaves character against aphid infestation. The statistical analysis were done using Microsoft Excel 2013, IRRI's STAR, and Minitab 15.

3. Results and discussions

3.1. Screening of pepper genotypes against melon aphids

There were significantly differences ($P < 0.05$) in respond to the number of aphids infestation per leaf among genotypes of pepper used in this study (Table 1). The range of aphid infestation was 22.9 - 95.8 nymphs per leaf. IPB C5 had the lowest number aphid per leaf with an average of 22.9 nymphs. However, it was not significantly different with IPB C145, IPB C325, IPB C324, IPB C120, IPB C313, IPB C140, IPB C4 and IPB C20, while genotype IPB C3 had the highest aphid per leaf by 95.8 nymphs and was not significantly different with IPB C19, IPB C10, IPB C142, IPB C51, IPB C15, and IPB C12. This kind of differences was also found by Frantz et al. [10] in peppers against *Myzus persicae* infestation with a range of 15.5 - 115.4 nymphs per leaf. This indicates that there is a clear differences among pepper genotypes for their suitability or resistance as host for aphids which might be explored as natural resistance sources in pepper. Since *C. annuum* is the major cultivated pepper species [11], the finding of resistance sources among *C. annuum* is very important considering their compatibility to transfer the resistance into commercial varieties of pepper through conventional crossing and selection.

No	Genotypes	Aphid per leaf*	Classification**
1	IPB C5	22.9 h	Low infest
2	IPB C145	23.3 h	Low infest
3	IPB C325	25.4 gh	Low infest
4	IPB C324	28.4 fgh	Low infest
5	IPB C120	28.4 fgh	Low infest
6	IPB C313	29.4 fgh	Low infest
7	IPB C140	36.8 efgh	Medium-low infest
8	IPB C4	36.9 efgh	Medium-low infest
9	IPB C20	45.7 efgh	Medium-low infest
10	IPB C9	51.5 defg	Medium infest
11	IPB C159	54.4 def	Medium infest
12	IPB C323	58.5 cde	Medium infest
13	IPB C111	59.9 bcde	Medium infest
14	IPB C322	59.9 bcde	Medium infest
15	IPB C19	72.3 abcd	Medium-high infest
16	IPB C10	76.5 abcd	Medium-high infest
17	IPB C142	81.5 abc	High infest
18	IPB C51	82.3 abc	High infest
19	IPB C15	86.1 ab	High infest
20	IPB C12	93.4 a	Very high infest
21	IPB C3	95.8 a	Very high infest

**Numbers followed with same letter are not statistically different; Duncan test with α=0.05*
*** 8-21 aphids per leaf = very low infestation, 22-35 aphids per leaf = low infestation, 36-49 aphids per leaf = lower-medium infestation, 64-77 aphids per leaf = high-medium infestation, 78-91 aphids per leaf = high infestation, 92-105 aphids per leaf = very high infestation*

Table 1:
Number of aphids per leaf 12 days after aphid infestation in twenty one genotype of pepper

3.2. Antixenosis based resistance

Difference was found in the number of aphid per leaf among the same genotypes in the first screening (Table 1) compare to the second choice test (Table 2). This might indicates the detection of antixenosis based resistance in pepper. Genotype IPB C20, consistently, had the lowest aphids per plant compared to other genotypes (Table 2). Difference of the number of winged aphid as shown on Table 2 stressed the presence of antixenosis effect in pepper to aphids. In certain condition, such as non preference condition, adult aphid can be equipped with a pair of wings as a mechanism of dispersal colonies [12]. Antixenosis was suggested to be the defense mechanism active in *C. pubescence* against *Myzuz persicae* [13]. The dense of hairiness of *C. pubescence* leaves may be impregnable to aphid feeding, or at least not preferred by aphids. This is not the case in our study since we did not detect hairiness in *C. annuum* leaves. Therefore the antixenosis based resistance in our study must be caused by other factors.

Antixenosis based resistance on pepper cultivation may strongly protects the plant from the infestation of aphid, especialy in a mix cultivation system of pepper varieties. A strong antixenosis could reduce direct damage, virus acquistion and transmission [14]. However, incomplete antixenosis can enhance the spread of the viruses within a pepper crop or to other crops since it can increase insect probing and movement [15].

Genotypes	Total aphid per plant	Aphid per leaf	Winged aphid
IPB C5	213.9 [ab]	40.5 [ab]	6.6 [ab]
IPB C12	207.5 [ab]	51.1 [a]	6.6 [ab]
IPB C15	191.1 [ab]	46.6 [ab]	4.6 [b]
IPB C20	101.1 [b]	21.2 [b]	1.7 [b]
IPB C145	195.9 [ab]	40.4 [ab]	6.5 [ab]
IPB C313	271.7 [a]	51.2 [a]	13.1 [a]

[Z] *Numbers followed with same letter are not statistically different; Tukey test with α=0.05*

Table 2:
The average number of aphid infestation on six genotypes choice test method

3.3. Antibiosis based resistance

Antibiosis based resistance in pepper against aphids was identified. In the no-choice test, all of the biological characters of aphid were affected by genotype except the life cycle. Life cycle was 4-5 days and did not difference significantly among the genotypes. This result is similar with previous finding on cucumbers [16] and *Colocasia esculenta* var. esculenta [17].

Reproductive time and longevity of melon aphid on six genotypes in the range of 7-12 days and 13-18 days (Table 3). Genotype IPB C20 made shortest longevity and reproduction time of melon aphid compared others the genotypes tested, each 13 days and 7 days, whereas genotype IPB C313 caused longer longevity and reproductive time for melon aphid among the six genotypes tested, 18 days and 12 days. Short longevity and reproduction time in natural conditions will suppress the development of aphid colonies [18].

There were differences in the number of progeny per day and total nymph (fecundity) during the period of reproduction among the six genotypes. Range number of newborn aphids (progeny) per day was 3-5 nymphs, while fecundity was 23.4 – 54.5 nymphs (Table 4). IPB C20 genotype demonstrated the ability to suppress the progeny aphids per day and fecundity compared with IPB C313. These data supported previous experimental data on antixenosis resistance test where IPB C20 was a genotype with low aphid preference. Antibiosis influence also found in soybean against *A. glyciness* by reducing fecundity on genotype resistant or tolerant [19, 20].

Host plant quality is one of important factor that influence the antibiotic resistance of plants [21]. The ability of melon aphid to reproduce and to survive are influenced by amino acids and secondary metabolites of host plant. For example, the fecundity and survival of *A. gossypii* on *Chrysanthemum iindicum* plants positively correlated with the levels of amino acids or nitrogen in it leaves [22].

Genotypes	Life cycle	Longevity time	Reproduction time
	(------day------)		
IPB C12	4.5	15.9 [b]	8.4 [bc]
IPB C145	4.9	13.8 [cd]	7.9 [bc]
IPB C15	4.4	16.1 [b]	9.6 [b]
IPB C20	4.6	13.0 [d]	7.2 [c]
IPB C5	4.6	14.4 [c]	8.3 [bc]
IPB C313	4.6	17.9 [a]	11.8 [a]

[z] *Numbers followed with same letter are not statistically different; Tukey test with α=0.05*

Table 3:
Effect of six selected genotypes to biological aspect of aphid infestation by non-choice test method

Wild relatives are already well known as good and reliable sources of resistance traits for plant genetic improvement including resistance to insect pests [23, 24]. However, the use of wild relatives as source of resistance is constrained by biological constraints such as hybrid sterility and low cross-ability, retention of undesirable traits [23]. Fortunately, IPB C20 is *C. annuum*, the largest cultivated of chili pepper which farmer already has planted. Therefore the introgression of resistance factors can be done through conventional crossings.

Genotypes	Nymph Survival (%)	Progeny per day (nymph day^{-1})	Fecundity (nymph aphid^{-1})
IPB C12	91	4.3 [ab]	36.0 [b]
IPB C145	62	3.7 [abc]	29.7 [bc]
IPB C15	81	3.6 [bc]	33.5 [bc]
IPB C20	70	3.4 [bc]	23.4 [c]
IPB C5	73	3.3 [c]	26.8 [bc]
IPB C313	91	4.6 [a]	53.5 [a]

[z] *Numbers followed with same letter are not statistically different; Tukey test with α=0.05*

Table 4:
Nymph survival, Number progeny per day, and fecundity on six selected genotypes

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Genetic diversity of Thai native chili using diversity arrays technology

Orarat Mongkolporn[1,3], Wassana Kethom[2,3]

[1] *Tropical Vegetable Research Center, Department of Horticulture, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand*

[2] *Center for Agricultural Biotechnology, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand*

[3] *Center of Excellence on Agricultural Biotechnology: (AG-BIO/PERDO-CHE), Bangkok, Thailand*

Abstract

Thai native chili (*Capsicum* spp.) is pungent with unique flavors, and has potential to be commercialised. Thai chili is quite diversed, over 200 germplasm accessions have been collected and are being evaluated for their performances and resistances to pests. Genotypic data of these native chili germplasm are required for efficient germplasm management and also future breeding programs. Total genomic DNA of the chili germplasm will be assessed by the Diversity Arrays Technology or DArT, combining with the next generation sequences (DArTseq). DArTseq, a high throughput in a microarray platform, enables us to discover tens of thousands of markers in a single experiment. Genetic diversity of the Thai native chili is being investigated, and the association of the markers with some important traits will be analysed.

Keywords: *Capsicum*, DArT, association analysis

Quantitative trait loci in pepper genome control the effective population size of two RNA viruses at inoculation

Lucie Tamisier[1,2], Elsa Rousseau[2,3,4], Sébastien Barraillé[2], Ghislaine Nemouchi[1], Marion Szadkowski[1], Ludovic Mailleret[3,4], Frédéric Grognard[3], Frédéric Fabre[5], Benoît Moury[2], Alain Palloix[1]

[1] *INRA, UR1052 GAFL, Unité de Génétique et Amélioration des Fruits et Légumes, Montfavet Cedex, France*

[2] *INRA, UR407 PV, Unité de Pathologie Végétale, Montfavet Cedex, France*

[3] *Inria, Biocore Team, Sophia Antipolis, France*

[4] *INRA, Univ. Nice Sophia Antipolis, CNRS, UMR 1355-7254 Institut Sophia Agrobiotech, Sophia Antipolis, France*

[5] *INRA, UMR 1065 Santé et Agroécologie du Vignoble, Villenave d'Ornon cedex, France*

Abstract

Infection of plants by viruses is a complex process that involves several steps: inoculation into plant cells, replication in inoculated cells, cell-to-cell movement during leaf colonization and long-distance movement during systemic infection. The success of the different steps is conditioned by the effective viral population size (*Ne*) defined as the number of individuals that pass their genes to the *next generation*. During the infection cycle, the virus population will endure several bottlenecks leading to drastic reductions in *Ne* and to the random loss of some virus variants. If strong enough, these bottlenecks could act against selection by eliminating the fittest variants. Therefore, a better understanding of how plant affects *Ne* may contribute to the development of durable virus-resistant cultivars. We aimed to (i) identify plant genetic factors that control *Ne* at the inoculation step, (ii) understand the mechanisms used by the plant to control *Ne* and (iii) compare these genetic factors with other genes controlling virus life cycle and plant resistance durability.

The virus effective population size was measured in a segregating population of 152 doubled-haploid lines of *Capsicum annuum*. Plants were inoculated mechanically either with a *Potato virus Y* (PVY) construct expressing the green fluorescent protein (GFP), or a necrotic variant of *Cucumber mosaic virus* (CMV), the CMV-N strain of Fulton. *Ne* was assessed by counting the number of primary infection foci observed on inoculated cotyledons under UV light for PVY-GFP or the number of necrotic local lesions observed on inoculated leaves for CMV-N.

The numbers of primary infection foci and local lesions were correlated among the doubled-haploid lines (r=0.57) and showed a high heritability (h^2=0.93 and 0.98 for PVY and CMV, respectively). The effective population size of the two viruses was shown to be controlled by both common quantitative trait loci (QTLs) and virus-specific QTLs, indicating the contribution of both general and specific mechanisms. The PVY-specific QTL colocalizes with a QTL that had previously been shown to be involved in PVY accumulation and capacity to break a major-effect resistance gene down.

1. Introduction

During the plant infection process, RNA viruses are generally able to quickly evolve and adapt to their host thanks to their high mutation rate and short generation time [1]. As a result, breakdown of plant resistance and emergence of new virus variants may occur and cause important losses for agricultural production [2,3]. Better understanding of evolutionary processes that shape viral populations and of the extent to which we can control them are therefore required for a sustainable management of crop disease [4].

In the plant, two well-known evolutionary forces act on the frequencies of the different variants composing virus populations: natural selection and genetic drift. Natural selection is a deterministic force that increases the frequency of the fittest variants at each generation. In contrast, genetic drift is a stochastic force that randomly changes the frequencies of the virus variants from generation to generation. The two forces act jointly on the viral populations and can have opposite effects on its adaptation. Indeed, if genetic drift is stronger than selection, deleterious mutations may randomly be fixed or advantageous ones may be lost. The strength of genetic drift depends on a key parameter of virus evolution: the effective population size (*Ne*). *Ne* is defined as the number of individuals that pass their genes to the next generation [5] and the strength of the genetic drift is inversely proportional to *Ne*. Through the infection process, the viral population will endure several genetic bottlenecks that will strongly reduce *Ne*, and so, increase the genetic drift [6,7]. Bottlenecks can occur during all the infection steps like vector transmission, virus inoculation into plant cells, replication in infected cells, cell-to-cell or long-distance movements. Although estimation of bottleneck size and their effects on the genetic diversity of the viral population are well documented [8,9], the plant genetic determinants controlling bottleneck size are still unknown. However, studying how plant genetic factors affect *Ne* may contribute to the development of cultivars with durable virus resistance.

In this study, we focus on *Ne* during the inoculation step. We inoculated *Capsicum annuum* plants with two RNA viruses, a *Potato virus Y* (PVY) variant tagged with the green fluorescent protein (GFP) reporter gene and a necrotic variant of *Cucumber mosaic virus* (CMV), the CMV-N strain of Fulton. *Ne* was estimated by visualizing the number of primary infection foci under UV light and counting them for PVY-GFP and by counting the number of necrotic local lesions observed on inoculated leaves for CMV-N, which are two robust approaches to evaluate *Ne* [10,11].We aimed to (i) identify plant quantitative trait loci (QTLs) that control *Ne* at the inoculation step, (ii) understand the mechanisms used by the plant to control *Ne* and (iii) compare these genetic factors with other genes controlling virus life cycle and plant resistance durability.

2. Materials & Methods

2.1. Plant and virus material

A doubled-haploid (DH) population of *C. annuum* was obtained from the F_1 hybrid between Yolo Wonder, a line susceptible to PVY isolates, and Perennial, a cultivar carrying the PVY resistance allele *pvr2*^{3}. A genetic map comprising 190 molecular markers was previously built for this progeny [12]. From this population, we phenotyped 152 *DH lines* carrying *pvr2*^{3} and differing in their genetic background.

The DH lines were mechanically inoculated with two different viruses. The first one was a variant of the *Potato virus Y* (PVY; genus *Potyvirus*, family *Potyviridae*) isolate SON41p carrying the 115K substitution in the VPg cistron, allowing it to overcome the *pvr2*^{3} resistance.

The virus was also tagged with a green fluorescent marker, the green fluorescent protein (GFP) reporter gene. The PVY-GFP was constructed by duplicating the NIa protease cleavage site at the C-terminus of the NIb cistron and inserting the GFP gene between the two sites, allowing the NIa to cleave the GFP. The second one was the CMV-N strain of Fulton, a necrotic variant of *Cucumber mosaic virus* (CMV; genus *Cucumovirus*, family *Bromoviridae*). Finally, for the purpose of a control experiment, the same PVY infectious clone carrying the mCherry reporter gene (expressing a red fluorescent marker) instead of the GFP gene was used.

2.2. Counting the primary infection foci and local lesion numbers for PVY and CMV

The effective population size (*Ne*) of PVY and CMV during the inoculation step was estimated by counting the number of primary infection foci on the inoculated cotyledons or leaves. For the PVY variant, we determined this number thanks to the fluorescence of the GFP under UV light. For CMV, an intrinsic property of the Fulton strain is to cause necrotic local lesions on the leaves which correspond to primary infection foci.

The PVY-GFP cDNA clone was first inoculated in *Nicotiana clevelandii* plants by DNA-coated tungsten particle bombardments. In order to obtain the inoculum, extracts of these plants were then used to propagate the virus in *Nicotiana tabacum cv.* Xanthi plants. Finally, ten pepper plants per DH line were mechanically inoculated on their two cotyledons three weeks after sowing. At six days post inoculation (dpi), the number of primary infection foci on each inoculated cotyledon was counted under UV light (450-490 nm) (Figure 1A). All the plants were grown under greenhouse conditions.

The CMV-N strain of Fulton was propagated on *Vinca rosea* plants. From extracts of these plants, ten pepper plants per DH line were mechanically inoculated on their two first leaves three weeks after sowing. At five dpi, the number of necrotic local lesions per inoculated leaf was counted (Figure 1B). The experiment was realized in a climate-controlled room (20–22 °C, 12-h light/day).

We realized an additional experiment to study the link between the number of foci and *Ne*. A 1:1 mixture of PVY-GFP and PVY-mCherry was inoculated in the first leaf of plants belonging to 16 DH lines. The number of foci showing green and/or red fluorescence was then estimated.

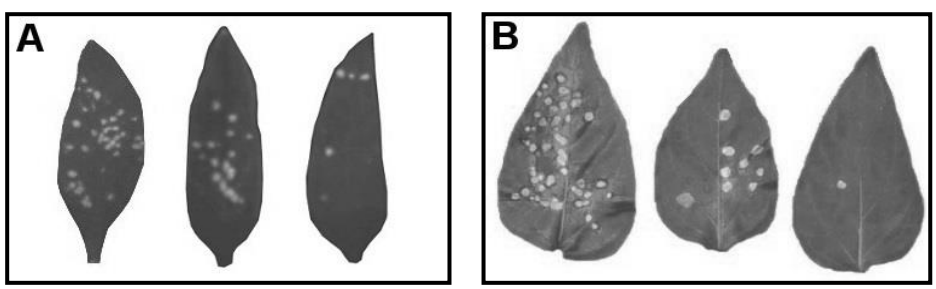

Figure 1:
Illustration of the symptoms obtained after inoculation.
A: Foci of primary infection due to PVY-GFP. B: Local lesions due to CMV-N.

2.3. Statistical analyses

The statistical analyses were performed using the R software (http://www.r-project.org/). For the two phenotypic traits, narrow-sense heritability was estimated using the formula $h^2 = \sigma^2_G / (\sigma^2_G + \sigma^2_E/n)$, where σ^2_G corresponds to the genotypic variance, σ^2_E to the environment variance and n to the number of replicates (n=10). An ln(x+1) transformation was applied to the two traits to approximate a normal distribution.

2.4. QTL analysis

Quantitative trait loci (QTLs) detection was performed with the R/qtl software package [13]. A preliminary analysis was realized by using a standard interval mapping approach. In addition, a two-dimensional genome scan was done to identify potential interactions between QTLs. Multiple QTL mapping (MQM) was then performed, using the markers previously identified as the initial set of cofactors. Finally, the positions and the effects of the QTLs were refined in the context of a multiple QTL model. The significance LOD threshold was calculated by performing a permutation test with 10000 replicates. The LOD threshold was set at 3.79 for the foci induced by PVY and 3.18 for the lesions induced by CMV (P=0.05). The confidence intervals for the location of each QTL were determined by using a 1-LOD and 2-LOD drop-off method. *The graphical* representation of the QTLs was generated using MAPCHART version 2.3 [14].

3. Results

3.1. Measure of the primary infection foci and local lesion numbers

Two populations of 152 DH lines of *C. annuum* were inoculated with two different RNA viruses: the PVY-GFP and the CMV-N strain of Fulton. The effective population size at the inoculation step was assessed by quantifying the number of primary infection foci and local lesions on cotyledons or leaves respectively inoculated by PVY and CMV. After applying log transformation to the data, the number of primary infection foci ranged from 0.77 to 3.8 with a mean number of 2.64±0.67 (mean±sd) (Figure 2A). The number of local lesions varied from 0.14 to 4.54 with a mean number of 2.61±1.14 (Figure 2B). The two variables were well correlated among the doubled-haploid lines (Pearson r=0.57, *p-value* < 2.2e-16). They also both shown a high heritability with h^2=0.93 for the foci induced by PVY and h^2=0.98 for the lesions induced by CMV.

To evaluate the link between the number of foci and *Ne*, we co-inoculated DH lines with two PVY variants tagged with different fluorescent markers. In 59.7% of the inoculated leaves, no infection foci with dual fluroescences were observed. The mean frequency of foci showing both red and green fluorescences was of 0.9%, with a maximum frequency at 5.3%.

3.2. Detection of QTLs controlling the number of primary infection foci and local lesions for PVY and CMV

MQM was performed and three QTLs were detected for each virus (Table 1, Figure 3). They were named PVY-6, PVY-7, PVY-12 and CMV-6, CMV-7, CMV-12 according to the virus used for the inoculation and the chromosome location. The QTLs PVY-6, PVY-7 and PVY-12 explained respectively 6.28%, 34.73% and 26.22% of the variation of the primary infection foci numbers for PVY. Similarly, QTLs CMV-6, CMV-7 and CMV-12 explained respectively 11.18%, 31.53% and 21.67% of the variation of the local lesion numbers for CMV. For both viruses, the analyses revealed a significant epistatic interaction between the QTLs on

chromosomes 7 and 12. The Perennial allele decreased the trait value for all QTLs, except CMV-6. Finally, the model combining the additive and epistatic effects of the three QTLs explained 57.82% and 50.88% of the trait variation for PVY and CMV, respectively.

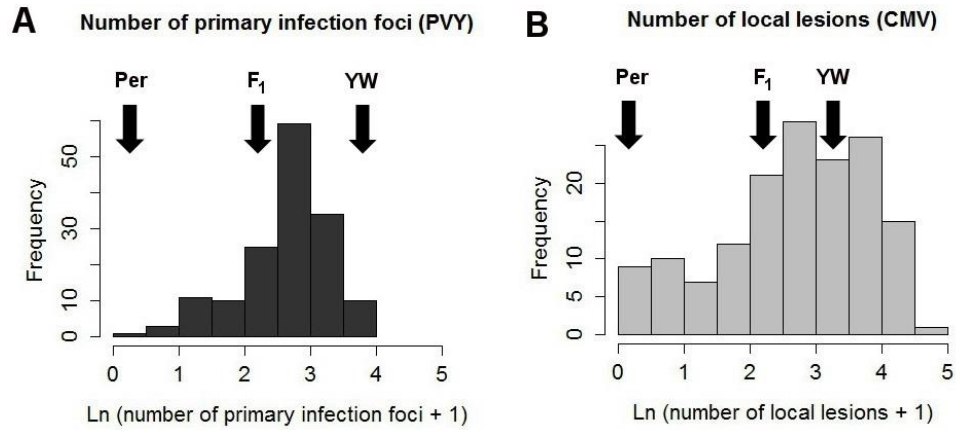

Figure 2:
Histograms of the frequency distribution of the DH lines for (A) the number of primary infection foci caused by PVY-GFP and (B) the number of local lesions caused by CMV-N. The position of the parents and the F_1 are indicated. Per: Perennial and YW: Yolo Wonder.

QTL	Chr	Position (cM)	Closest marker	LOD score	*2-LOD* support interval	Variation explained (%)		Estimated effect of Perennial QTL allele	h^2
						QTL	Model		
PVY-7	7	45.6	HpmsE114	20.71	45.1-49.3	34.73	57.82	-0.803	0.93
PVY-12	12	139.1	SNP11168	15.30	125.7-144.8	26.22		-0.652	
PVY-6	6	123.8	Epms_376	4.93	73.2-161.9	6.28		-0.320	
PVY-7 x PVY-12	7/12	-	HpmsE114/ SNP11168	8.48	-	11.46		-0.906	
CMV-7	7	48.4	HpmsE114	16.36	44.9-53.7	31.53	50.88	-1.200	0.98
CMV-12	12	125.7	HpmsE128	12.06	119.5-140.7	21.67		-1.091	
CMV-6	6	31.5	C2_At2g39690	6.77	1.8-45.2	11.18		0.712	
CMV-7 x CMV-12	7/12	-	HpmsE114/ HpmsE128	5.77	-	9.38		-1.397	

Table 1:
Description of QTLs detected for the effective population size of PVY and CMV at inoculation.

4. Discussion

The breakdown of plant resistance genes is a major threat to the genetic control of crop diseases. Since the durability of the resistance relies on the evolution of the pathogen, a better

understanding of the evolutionary constraints imposed by the plant to the pathogen and of the underlying genetic factors can improve our management of crop diseases. In this study, we identified and mapped pepper QTLs controlling a key parameter of virus evolution, the effective population size (*Ne*) at inoculation, for PVY and CMV. We also found that one of these QTLs colocates with a QTL previously shown to be implicated in pepper resistance against PVY.

4.1. Link between the number of primary infection foci or local lesions and the effective population size

The effective population size (*Ne*) of viruses corresponds to the number of virus individuals that pass their genes to the next generations. The inoculation step is a particularly narrow bottleneck for viruses [8,15]: only few individuals from the inoculum source succeed in initiating infection of new plants. The numbers of infection foci (for PVY) or local lesions (for CMV) are minimum value for *Ne* at the inoculation step, since they are initiated by at least one virus particle. They would correspond to exact *Ne* values if and only if each infection focus/local lesion is initiated by exactly one virus particle. Zwart et al. [11] proposed an experimental test of this hypothesis by inoculated a plant leaf with a mixture of viruses tagged with two different fluorescent proteins, GFP and mCherry, that can be visualized by green and red fluorescence, respectively. Infection foci showing both a green and a red fluorescence were initiated by the two virus variants.

We performed a similar experiment with our pathosystem by co-inoculating PVY-GFP and PVY-mCherry in plants corresponding to 16 DH lines. We found that the frequency of foci showing both green and red fluorescence was very low, with a mean number of 0.9%. Thus, we could conclude that the huge majority of foci were initiated by a single virus particle and that the number of primary infection foci was a highly precise estimation of *Ne*. The same approach could not be undertaken for CMV. However, the timing and development of CMV local lesions are similar to those of PVY infection foci and certainly correspond to the same processes of infection initiation followed by cell-to-cell movement. The only difference is the elicitation of plant defenses by CMV leading to necrosis of the infection foci. This suggests that the number of local lesions is also a precise estimation of *Ne*.

4.2. Common and virus-specific QTLs control the effective population size of PVY and CMV at inoculation

For each virus, we identified three QTLs involved in *Ne* at inoculation and localized on chromosomes 6, 7 and 12 (Table 1, Figure 3). The QTLs on chromosome 7 (PVY-7 and CMV-7) were detected at the same location on the genome (marker HpmsE114), and the QTLs on chromosome 12 (PVY-12 and CMV-12) were identified at very close positions (139.1 and 125.7 cM). On each chromosome, the confidence intervals of the 2 QTLs overlap largely. Moreover, the phenotypic variation explained by the QTLs was similar, with PVY-7 and CMV-7 explaining 34.73% and 31.53% of the trait variation and PVY-12 and CMV-12 explaining 22.26% and 21.67% of the trait variation. We also found that, for both viruses, there was epistasis between the QTLs on chromosomes 7 and 12. Therefore, the same QTLs on chromosomes 7 and 12 control *Ne* for PVY and CMV and the same genetic factor may be responsible for this dual effect. In contrast, the two QTLs detected on chromosome 6 (PVY-6 and CMV-6) differed according to the virus. PVY-6 was localized at 123.8 cM and associated with the marker Epms_376 whereas CMV-6 was positioned at 31.5 cM and associated with the marker C2_At2g39690. Besides, QTL effects shown opposite directions since Perennial allele decreased the value of the trait for PVY-6 and increased its value for CMV-6.

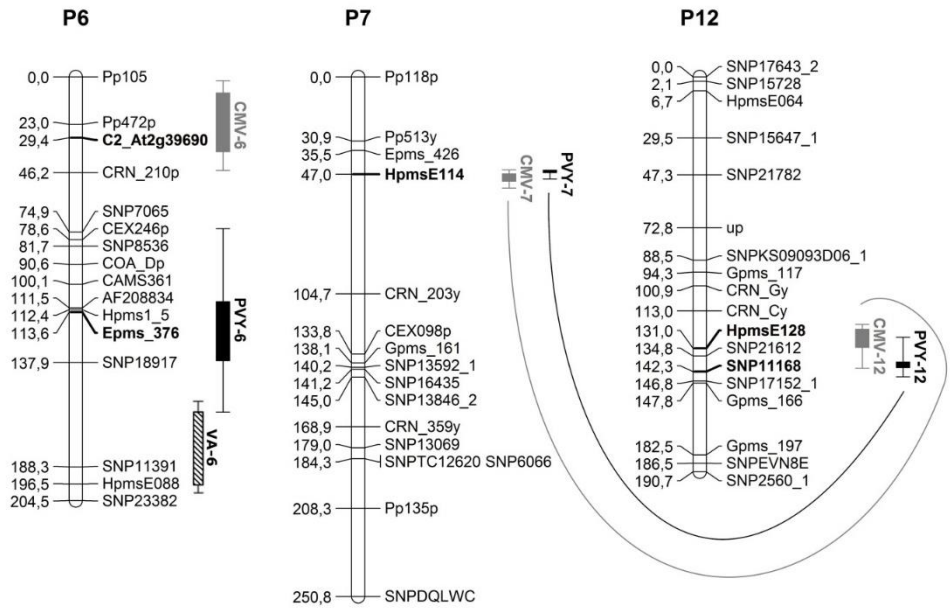

Figure 3

QTL map for the effective population size at the inoculation step for PVY (black) and CMV (gray). A previously detected QTL (VA-6) controlling PVY accumulation is also mapped (hatched). For each QTL, confidence intervals obtained using the 1-LOD drop and 2-LOD drop methods are indicated. The lines represent epistatic effects between loci.

Even if the two viruses are quite different, our study highlights that two common QTLs control the effective population size of both viruses, indicating that general mechanisms are under this trait. However, we also found one specific QTL for each virus, demonstrating that virus-specific mechanisms also act.

4.3. Relationship between QTLs of effective population size and the plant resistance

With the same DH population as the one we used, Quenouille et al. [12] identified QTLs controlling the durability of the major PVY resistance gene *pvr2*^{3} and PVY accumulation. They notably mapped a QTL on chromosome 6, named VA-6, which affects the PVY accumulation (Figure 3). The confidence interval of VA-6 includes a part of the confidence interval of PVY-6, although the two QTLs are not exactly localized at the same position. Interestingly, VA-6 shown epistatic interaction with RB-3, a QTL controlling the virus capacity to break the *pvr2*^{3} resistance down. We could make the hypothesis that VA-6 and PVY-6 are linked or belong to the same locus, and so that PVY-6 might contribute to increase the resistance durability. This hypothesis could be valid because the Perennial allele at QTL PVY-6 decreases *Ne* while at QTL VA-6 it increases resistance durability. Indeed, by reducing the effective population size, the allele could help to lose well-adaptated virus variants by genetic drift at inoculation, therefore increasing resistance durability.

Our study demonstrated the existence of plant genetic factors capable of controlling pathogen evolution and which could slow down their adaptation. From an agricultural point of

view, the use of these factors could be even more beneficial because our results suggest that they could induce general mechanisms and therefore act against multiple pathogens.

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Germplasm of pepper in the Czech Republic

Helena Stavělíková, Sandra Benicka

Crop Research Institute, Olomouc, Czech Republic

Abstract

The pepper is very popular, old, annual and widespread in the world vegetable.

182 varieties of the pepper are grown on 333 ha in the Czech Republic. Crop Research Institute, Department of Genetic Resources of Vegetables and Specialty Crops, Olomouc is holder the Czech collection of the pepper genetic resources. This collection consists of 528 accessions. The oldest accessions have been in collection from 1952. The collection presents materials from 28 countries. New accessions are obtained from the seeds companies. The management of the germplasm collection comprises conservation, evaluation, multiplication and description. 40 plants have used for multiplication. All accessions have been described by Descriptors for Capsicum (*Capsisum* spp.) to use 29 characters. We have the photo documentation of the pepper accessions. We have tasted the pepper pungency by sensory test. Important quality attribute of the pepper is pungency. Capsaicinoids are derivate from the secondary metabolism of the alkaloids and they are responsible for the pungency and hotness of the *Capsicum* fruits. The pepper produces high amounts of vitamin C, provitamin A, E, P, B 1, B 2 and B 3.

We analysed 21 accessions by 10 samples. Samples for analysis were prepared by lyophilization of the whole fruit peppers. 50 mg homogenized pepper in the centrifuge tubes were added 10 ml acetonitrile, the samples of the pepper were analysed by RP-UHPLC. The quantification was done by external standardization with standards of capsaicin and dihydrocapsaicin. Contents of capsaicin, dihydrocapsaicin and dry matter lie in wide ranges: 25 – 1947 mg/kg DW, 24 - 3371 mg/kg DW and 5 – 2%.

1. Introduction

The pepper is very popular, old, annual and widespread in the world vegetable. Primarily, pepper fruit is consumed as a fresh vegetable or dehydrated for use as spice. Red pepper is pungent and non-pungent, represent one of the most important spice commodities in the world [1].

The pepper has been grown very long time. The oldest known records of pepper come from the desert valley of Tehuacan, in Southern Mexico. It is known that the indigenes were eating peppers as early 7000 B.C. Now we know that peppers were among the first plants to be domesticated in the Americas [2].

The pepper was unknown in Europe, Asia and Africa prior to Christopher Columbus landing in the Americas. Columbus was given the credit for introducing the pepper to Europe, and subsequently to Africa and Asia. Columbus erroneously named the *Capsicum* spice "pepper". The plant was not black pepper, but a heretofore unknown plant that was later classified as Capsicum.

After Columbus returned to Europe with the pepper seed, the extensive trading routes of the Spanish and Portuguese helped spread peppers around the globe [1]. At the beginning the pepper was planted as the ornamental and medicinal plant in the Spain and Portugal and later in Italy. In the 16th century the pepper was brought by Turks to Bulgaria. The Bulgarian gardeners expanded the pepper to other Europe countries [3]. The pepper was known as spice plant in 16th century in Bohemia [4]. The intensive growing of pepper started after the First World War in the Czechoslovakia [3].

Now the harvest area is 333 ha in the Czech Republic (Tab.1). The pepper is grown in green houses, on an average 5 ha per year, and on the field. Annual consumption of the pepper is 5,5 kg per person [5]. In this time 182 varieties (62 hybrid and 120 open pollinated, 24 chilli) of the pepper are in National List of Varieties listed in the State Variety Book [6].

	2012	2013	2014	2015
Harvest area (ha)	233	214	233	333*
Production (t)	9 320	8 566	9 320	13 320*
Import (t)	48 689	47 921	50 535	33 487*
Export (t)	6 304	6 587	6 559	4 663*

**estimation of Czech and Moravian Vegetable Union*

Table 1:
The harvest area, production, import and export of pepper in the Czech Republic

Important quality attribute of pepper is pungency. Pungency is one of five main taste senses, along with bitter, sweet, sour and salt [1]. Capsaicinoids are derivate from the secondary metabolism of the alkaloids [7] and they are responsible for the pungency and hotness of the *Capsicum* fruits [8]. The pepper pungency is established as a mixture of seven or more homologous branded – chain alkyl vanillylamides, named capsaicinoids [9]. The capsaicinoids are unique to the *Capsicum* genus [1]. In the most represented capsaicinoids belong capsaicin (N-[(4-hydroxy-3-methoxyphenyl) methyl]-8-methyl-E-6-nonenamide) and dihydrocapsaicin (N-[(4-hydroxy-3-methoxyphenyl) methyl]-8-methyl-6-nonanamide). Capsaicin is the main pungent and irritating constituent of hot peppers. It has antimicrobial, pharmaceutical and antioxidant properties and it is used as food additives [7].

Peppers are good sources of many essential nutrients. The pepper produces high amounts of vitamin C, provitamin A, E, P (citrin), B 1 (thiamine), B 2 (riboflavin) and B 3 (niacin). Peppers also include trace elements (for example Fe, Mn, Cd, Ca, Co, Cu, Mg, P, K, Na, Zn) and carotenoids. The peppers are also among the richest known plant sources of vitamin C (ascorbic acid). A pepper fruit can contain six times more vitamin C than the orange [1]. The fresh fruits contain from 90 to 400 mg vitamin C/100 g of fruit. One pepper pot provides for recommended daily allowance (RDA) of vitamin C. Contain of vitamin C depends on the variety, not depend on contain of capsaicin [3]. Vitamin C was first purified from the pepper in 1928 by Hungarian biologist Albert Szent – Györgyi, who later got a Nobel Prize in physiology and medicine for his work with vitamin C [1].

25 species have been ascribed to the genus *Capsicum* [10].

2. Germplasm of pepper (Capsicum annuum L.) in the Czech Republic

Crop Research Institute, Department of Genetic Resources of Vegetables and Specialty Crops, Olomouc is holder the Czech collection of the pepper genetic resources. The germplasm collection of the pepper has very long and rich tradition in Olomouc. The basis of the germplasm collections were originated in the course of the Research Institute of Vegetables Growing and Breeding in Olomouc (RIVGB in Olomouc). RIVGB in Olomouc was established in 1951 and in 1994 RIVGB in Olomouc concluded its work. The collection of the pepper consists of 528 accessions in this time. The oldest accessions have been in collection from 1952. *Capsicum annuum L.* present 96, 4 % of whole collection, others are *Capsicum fructescens* L. The

collection presents materials from 28 countries. Main part of this collection presents the open pollinated varieties from Hungary, Czechoslovakia and Czech Republic, former Soviet Union, Bulgaria and USA (Tab.2). New accessions are obtained from the seeds companies. The management of the germplasm collection comprises conservation, evaluation, multiplication and description.

Country of origin	**No. of accessions**
Hungary	129
former Czechoslovakia	67
Czech Republic	27
former Soviet Union	58
USA	46
Bulgaria	44
Romania	32
Poland	22
Germany	13
Other	90
Total	**528**

Table 2
Structure of the pepper collection according to country of origin

2.1. Pepper germplasm multiplication

In past 20 years we have used two system of regeneration of the pepper. From 1995 to 1999 the pepper was grown in the plastic greenhouse. The plants were isolated by special bags by non-woven fabrics to avoid the cross pollination. After the fruit setting the bags were removed, the fruits sets were labelled by the cotton. We took seeds only from labelling fruits. This system had one big advantage - it was possible to multiply 50 and more than accessions per year but it had disadvantages – high time and human consuming. From year 2000 the pepper has been grown in the isolation cages. On an average 20 - 25 accessions are regenerated every year. We have used 40 plants for multiplication.

2.2. Characterization

During multiplication all accessions have been described. Descriptors for Capsicum (*Capsisum* spp.) [11] is used for description of pepper germplasm. We use for characterization of the pepper 29 characters (ch.):

Plant (3ch.) - stem pubescence, height and habitus

Leaf (2ch.) - pubescence, length

Inflorescence (9ch.) - number of flowers per axil, flower position, corolla colour, corolla

spot colour, anther colour, filament colour, stigma axsertion, calyx margin, calyx annular constriction

Fruit (13ch.) – anthocyanin spots or stripes, colour at intermediate stage, position, set, colour at mature stage, shape, length, width, shape at pedicel attachment, neck at base of fruit, shape at blosoom end, cross-sectional corrugation and surface.

Seed (2ch.) – colour, 1000-seed weight

We have the photo documentation of the pepper accessions, too. We have taken photos of all pepper accessions three times by vegetation period - in phase of flowering, in phase of ripening and fruits - sideways look, top point of view and horizontal cross section.

Also we have tasted pepper pungency by sensory test. Currently 483 accessions are completed characterized.

2.3. Evaluation

All multiplication accessions have been tested for dry matter contents. Currently we know the dry matter contents in 409 accessions (Tab. 3).

Dry matter content	**Number of accessions**
Low > 4 %	7
Medium 4 % - 8 %	277
High 9 % - 11 %	87
Very high < 12 %	39
Suma	**409**

Table 3:
Dry matter content in pepper germplasm collection

In 2006 we did chemical analysis for capsaicin and ascorbic acid in the set of 71 accessions, disposably [12], [13]. In 2015 we started the regular chemical analysis for capsaicin and dihydrocapsaicin.

3. Chemical analysis of pepper for capsaicin and dihydrocapsaicin

3.1. Material

We analysed 21accessions (Tab.4) from pepper germplasm collection. For chemical analysis we chose the accessions were hot in the sensory evaluation because the accessions were sweet in the sensory evaluation to have very low content of capsaicin and dihydrocapsaicin, its concentration was below the limit of quantification (LOQ).

Accession number	Name	Country of origin
09H3100015	Koral	CSK
09H3100035	Ozdobná	CSK
09H3100041	Polévková žlutá	CSK
09H3100049	Nitranská krajová	CSK
09H3100200	Třešňová červená	CSK
09H3100205	Tatar	CSK
09H3100206	Pfefferonka	CSK
09H3100318	Kocu	CSK
09H3100530	Feferonka třešňová	CSK
09H3100567	Beros	CZK
09H3100577	Drakula	CZK
09H3100135	Sipka No. 1067	BGR
09H3100143	Kajenskij A 35	SUN
09H3100174	Sarga Mammut	HUN
09H3100176	Ardei Iute	ROM
09H3100345	Coral Gem	USA
09H3100356	Zierpfeffer Market Gar 1330	DEU
09H3100399	Buckettstandiger	HUN
09H3100436	Nagayatsubusa	JPN
09H3100456	Druznyj 401	SUN
09H3100475	Dekorativnyj	SUN

Table 4:
List of accessions for chemical analysis

We analysed 10 samples for every acc. One sample represents one plant. The weight of fresh pepper sample was 80 g minimally. The number of fruit pepper in the sample was dependent on weight of fruit pepper.

3.2. Plant preparation

Pepper seeds were drilled to the small pots with pearlite on 20th March. These were put on Jacobsen´s germination apparatus for ten days. Tree days, the temperature was 35^{o} C and seven days the temperature was 25^{o} C. The period of light was 12 hour light and 12 hours dark. After 10 days the seedlings were transplanted to plastic pots by two plants per pot with growing medium. Six weeks the pots were in glasshouse. The monitoring of state of health was done. Thereafter the pepper plantings were planted to the isolation cages.

Planting distance was 25 x 30 cm. During all growing period the pepper growth was watered twice per week. The monitoring of state of health were done whole vegetative period. The fruits

were harvested at intermediate stage for chemical analysis, in period from 19th August to 15th September. In mature stage the pepper was harvested for seed. The seeds were taken out from fruits, cleaned and sent to the Czech Gene bank for long storage.

3.3. Samples preparation

Lyophilization - samples for analysis were prepared by lyophilization of the whole fruit peppers. Lyophilization was done on the device CHRIST BETA 1-8 LD plus. After lyophilization fruits without seeds were homogenized in the liquidizer and finally samples were sieved through the sieve (max. diameter 1mm).

Extraction - 50 mg homogenized pepper in the centrifuge tubes were added 10 ml acetonitrile, the centrifuge tubes were put in the ultrasonic bath to 30 minutes at 55ºC. Thereafter the tubes were centrifuged by 15 minutes with 4500 rpm/min. We used the centrifuge HERMLE Z 300. After the centrifugation, samples were decanted to storage vials. Before HPLC analysis the samples were filtered by disk microfilters (0,22 μm) to the vials.

The samples were stored in the vials, in fridge at ±5ºC.

3.4. UHPLC analysis

The samples of the pepper were analysed by RP-UHPLC (UltiMate 3000, Thermo Scientific) with UV detection at 222 nm (reference wavelength 500 nm), the column was C18 (EC 100/2 NUCLEODUR C18 Gravity, 1,8 μm with the precolumn EC 4/2 NUCLEODUR C18 Gravity, 1,8 μm). The temperature of the column was 25ºC. The mobile phase consisted of 40% acetonitrile and 60% H_2O + 0,1 % HCOOH. The analyses were done by isocratic elution. The flow was 0,150 ml/min. Time of analysis was 20 minutes and injection volume was 0,5 μl.

The quantification was done by external standardization with standards of capsaicin and dihydrocapsaicin (Aldrich). Calibration solutions of capsaicin and dihydrocapsaicin were prepared in concentrations: 0,004 mg/5 ml ACN, 0,2 mg/5 ml ACN, 1 mg/5 ml ACN and 2 mg/5 ml ACN.

3.5. Results

Readings of capsaicin, dihydrocapsaicin and dry matter content are presented in Table 5. The contents of capsaicin, dihydrocapsaicin and dry matter lie in wide ranges: 25 – 1947 mg/kg DW, 24 - 3371 mg/kg DW and 5 – 2%. The most high content both capsaicin and dihydrocapsaicin was in acc. 09H3100041, 09H3100345 and 09H3100135. More low content both capsaicin and dihydrocapsaicin was in acc. 09H3100174, 09H3100577, 09H3100456 and 09H3100567. The chemical analysis confirmed the sensory evaluation. The acc. with the low content both capsaicin and dihydrocapsaicin had soft pungency and with the high content had strong pungency. The descriptive statistics – median was used for evaluation analysis.

accession	capsaicin (mg/kg DW)	dihydrocapsaicin (mg/kg DW)	dry matter content (%)
09H3100015	604	1219	20
09H3100035	1013	787	14
09H3100041	1232	1694	14
09H3100049	132	124	13
09H3100135	1947	3371	13
09H3100143	449	410	9
09H3100174	25	24	12
09H3100176	223	315	5
09H3100200	367	232	20
09H3100205	587	1265	14
09H3100206	730	1450	15
09H3100318	123	124	9
09H3100345	1887	2703	10
09H3100356	791	473	17
09H3100399	380	216	11
09H3100436	448	493	16
09H3100456	41	46	10
09H3100475	1295	1193	11
09H3100530	362	351	13
09H3100567	102	87	11
09H3100577	36	42	10

DW – dry weight

Table 5: The contents of capsaicin, dihydrocapsaicin and dry matter

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SESSION 4

Physiology and nutritional values

Chair: Lajos Helyes

The nutrition value and storage of eggplant (*Solanum melongena* L.) varieties

Füstös Zsuzsanna[1], Fehér Marianna[2]

[1] *Szent István University, Budapest, Hungary*

[2] *National Food Chain Safety Office, Budapest, Hungary*

Abstract

Eggplant is very rich in minerals, nutrient, vitamins. Eggplant is a warm season crop, growing temperature 22-30 ^{0}C. Mature eggplant fruits can be stored at optimal temperature (10-15 ^{0} C). Storing below, 10 ^{0} C causes chilling injury, decay, dark spots. Optimal harvest stage when the eggplant fruit firm and glossy but before seeds begin to hard and turn brown. We tested different eggplant varieties in two places Tordas (Plant variety trial station of NFCSO) under plastic and Monorierdő in open field (Post control station of NFCSO). The harvest time was in all variety in optimal consumption stage.

In the trial in Tordas were 18 varieties from that varieties 8 were replaced in Monorierdő. The fruit colour of varieties were violet and white and the fruit shape were globular, obovate, pear shaped, cylindrical and ovoid.

We tested nutrition value of fresh fruits the water soluble solids and dry matter content, N (Nitrogen) P (Phosphorus) K (Potassium) content , antioxidant capacity and polyphenol content and stored under two different circumstances. The first one was a cool room where the temperature was between 6,3 -8,4^{0}C and the RH 62-72%, The second was under room temperature between 22,8-24,6 ^{0}C and RH 58-65%.

The differences among the water soluble solids (3.9 -4,6 %) very close but the dry matter content of varieties (4,9-7.3 %) are varies widely. In open field we measured higher value.

There was no significant difference in phosphorus and potassium value in Tordas among 18 varieties but the globular varieties shown the lowest data. The antioxidant capacity and polyphenol content were close correlation with the colour of fruit. The dark violet skin colour varieties shown the highest data.

For storage were chosen 6 typical violet globular, obovate and pear shape varieties

During 2 weeks storage we measured the weight loss and observed deterioration. The tested varieties in the weight loss were not significant differences but the globular shape variety was the highest weight loss. In the room temperature the weight loss was higher than in cool room but we experienced softening and brown spots in a cool temperature too.

Keywords: eggplant, nutrition value, antioxidant capacity, polyphenol, storage

1. Introduction

Eggplant is very rich in minerals and vitamins has a low energy contains protein (1,3g) and carbohydrates (4,8 g), fat (0,2 g), dietars fiber (2,58 g) ,Potassium (145 mg) , Posphorus (21 mg)

In 100g Fresh weight (FW) by the Hungarian nutritional table (Rodler, 2005). The data are similar to the USDA database. Arivalagan M.et al. (2012) published Development of mineral-rich varieties of eggplant is a breeding objective, increasingly important to achieve eradication of malnutrition. Significant differences in the mineral composition among the genotypes were detected. Germplasm accessions were found to be rich in mineral composition as compared to

commercial varieties. Dry matter content ranged from 6.03 to 9.14 g/100g FW.

Raigo M. et al (2008) analyzed several proximate composition traits (dry matter and protein content), content in phenol, and eight minerals (P, K, Ca, Mg, Na, Fe, Cu, and Zn) in 31 varieties of eggplant from three different varietal groups (commercial varieties, landraces, and hybrids between the landraces). They identified several landraces with high nutritional quality.

Antioxidants, like oxidative injury causing pro-oxidants, have a profound role in health and diseases in humans. Akanitapichat P et al. (2010) studied five varieties of eggplant (purple coloured moderate size, white-green coloured moderate size, long green, green striped moderate size and pale-green coloured small size, respectively, for total phenolic and flavonoid content, antioxidant activity. Purple and white-green colour varieties contained high total phenolic and flavonoid had better antioxidant activities than the other varieties.

The antioxidant capacities and total phenolic contents of lipophilic and hydrophilic extracts of 56 commonly consumed vegetables were studied. The different vegetables had diverse antioxidant capacities. The highest antioxidant capacities and phenolic contents were found for example cowpea, caraway, lotus root, sweet potato leaf, soy bean (green), chives, and broccoli, marrow squash and eggplant (1,89-8.66 mimol/g) were lower.(Gui-Fang Deng,2013)

Solo M. 2014 et al. had the aim of their study was to evaluate the antioxidant capacity of the edible portion of 44 fruits and vegetables grown in Andalusia and commonly consumed by both Spanish and other European consumers. The antioxidant capacity of eggplant sample from different growing season analyzed by FRAP methods were 3.52-10,58 mimol/g

The influence of organic and conventional farming practices on the phenolic content in eggplant samples belonging to two cultivars, Blackbell (American eggplant) and Millionaire (Japanese eggplant) grown under similar environmental conditions was evaluated. The results presented in this study clearly indicate that the LDL antioxidant activity was correlated with the phenolic content of two eggplant cultivars grown under organic and Blackbell cultivar eggplant showed marginally higher (Singh A.P., 2009).

Eun-Ju Jung et. al, (2011) This study shows the calyx part had strong antioxidant activity.

Eggplant is a warm season crop, growing temperature 22-30 ^{0}C. Mature eggplant fruits can be stored at optimal temperature (10-15^0 C). Storing below 10 ^{0} C causes chilling injury, decay, dark spots. Optimal harvest stage when the eggplant fruit firm and glossy but before seeds begin to hard and turn brown. Yung, immature fruits hard, pale colour, over-mature fruits lose the glossiness and the skin colour turn to greenish bronze. (Yamaguchi, 1983)

Aubergine (eggplant) is classified as a non-climacteric fruit and will freeze at about –1.0 to 0.7°C

Chilling injury was reported after at 7.2°C can result in surface scald, browning, pitting and excessive decay that may not be apparent until the fruit are removed from storage. Their shelf-life a simulated room temperature of 20°C and 60% RH was shown to be only

3–4 days. Refrigerated storage recommendations are as follows: 10–12.8°C and 92% RH. for 2–3 weeks with 9.6% weight loss ,8.3–10°C and 85–90% RH for 4 weeks with 17.7% loss 10–13°C and 90–95% RH for 10–14 days with weight loss of about 10% (Thompson A.K. 2003.) Füstös et al.(2005) measured 14% weight loss storage result at room temperature after one week.

2. Material and methods

The trial was made in two experiment central place of National Food-Chain Safety Office in 2015. Tordas: the 18 eggplants varieties was grown under plastic tunnel. The row and plant distance was 90 x 50 cm. The sowing was on 27 of April in 5x5 cm pot and the planting on 30 of May. Monorierdő: where were replication of the 8 eggplants varieties was grown in open field. The row and plant distance was 100 x 50 cm. The sowing was on 29 of April in 5x5 cm pot and the planting on 4 of June.

We selected varieties from EU Common Catalogue .The fruit shape were *globular*: Birgah, Brillant, Egle, Formosa, Laura, Purpura,ovoid: White Imola, *obovate:* Angela, Bonica, Clelia, Kamelia, Top Gadir, *pear shape:* Giotto,Madonna, *club shape:* Lady root, Longo, Maiorca, *cylindrical:* Alabaster, Ideal.The varieties skin colour mainly purple, but Angela purple striped, Alabaster and White Imola white.

The harvest stage was a market maturity when the eggplant fruit firm and glossy but before seeds begin to hard and turn brown.

The measuring of water soluble solids and dry matter content was from fresh fruit, the N, P, K from dried fruit and the antioxidant and polyphenol content from frozen sample.

The measuring of water soluble solids performed from 3 fruits by HANNA portable digital refractometer which report the sugar content of aqueous solutions as % Brix.

The loss of weight of this sample was determined after drying to constant weight for 3h at 80°C and then 105 °C.

Nitrogen content was obtained through the Kjeldahl method.

Phosphorus was analysed by the molibdo-vanadate method using a Jenway 6100 spectrophotometer.

Potassium was analysed by flame photometry using an Alpha 4 flamephotometer

The total phenolic content (TPC) was determined using Folin–Ciocalteu reagent and expressed as gallic acid equivalents - GAE (mg 100 g−1 of eggplant). (SingletonV.L.and Rossi AJ., 1965). Absorbance was then measured at 765 nm of Jenway 6100 spectrophotometer.

The method of measuring antioxidant was FRAP (Ferric Reducing Ability of Plasma). The FRAP method described by Benzie I.F.F. and Strain J.J. (1999).

The trial for storage 5 eggplant varieties 5fruits/ variety made in room temperature

(22.8-24.6 °C, 58-65 RH) and 6 varieties cold chamber (6.3-8.4 ^{0}C, 62-72 RH). The duration of weight measuring in cold chamber was 2 weeks, in room temperature was 1 week. We published the weight loss.

Linear regression analysis between the different variables measured was carried out with the SPSS 19.0 statistical package for Windows software (SPSS, Chicago, IL, USA); correlation between variables was considered to be statistically significant when $P < 0.05$.

3. Results

The results of measuring water soluble solids gave the very similar data from 3,9 till 4,6%. The standard deviation of the data is high among dry matter content, the highest 7,3 %, the lowest 4,9% (Figure 1.)

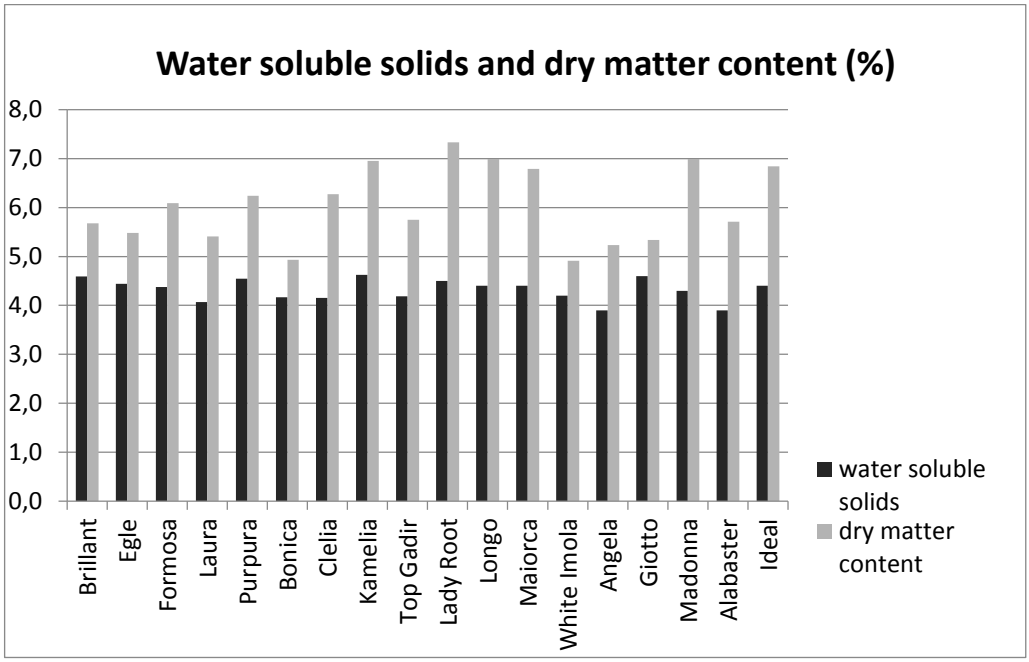

Figure1.
Water soluble solids and dry matter content of different eggplant varieties under plastic tunnel

We compered the inner contents of 8 eggplant varieties grown in protected place and open field . We found that the dray matter content of the eggplant fruits harvested from open field mainly were higher than the fruit from plastic tunnel (Figure 2.) The highest dry matter content was over 8%.

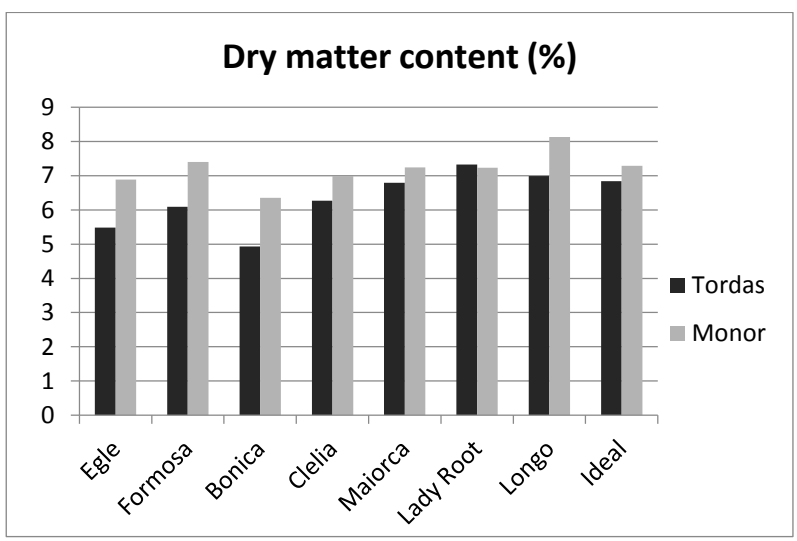

Figure 2.
Dry matter content of 8 eggplant under plastic tunnel (Tordas) and in open field (Monorierdő)

In Nitrogen content was not significant differences. The Potassium content typical characteristic of the varieties, shown correlation with Phosphorus content (r=0,81)

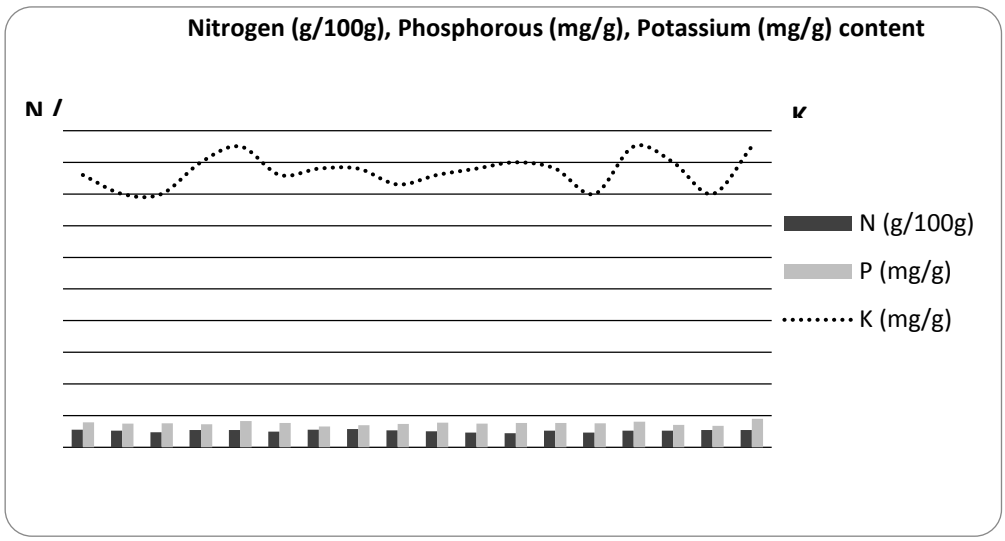

Figure 3.
Nitrogen, phosphorous and potassium content in different eggplant varieties under plastic tunnel

Diversity of antioxidant and polyphenol content of tested variety is very high.

The variability of the antioxidant content among the tested varieties from 4,32 till 18,18 ((μg/g), of the polyphenol content from 34,02 till 88,76.mg/g. The correlation between antioxidant and polyphenol content is very close (r = 0,91).

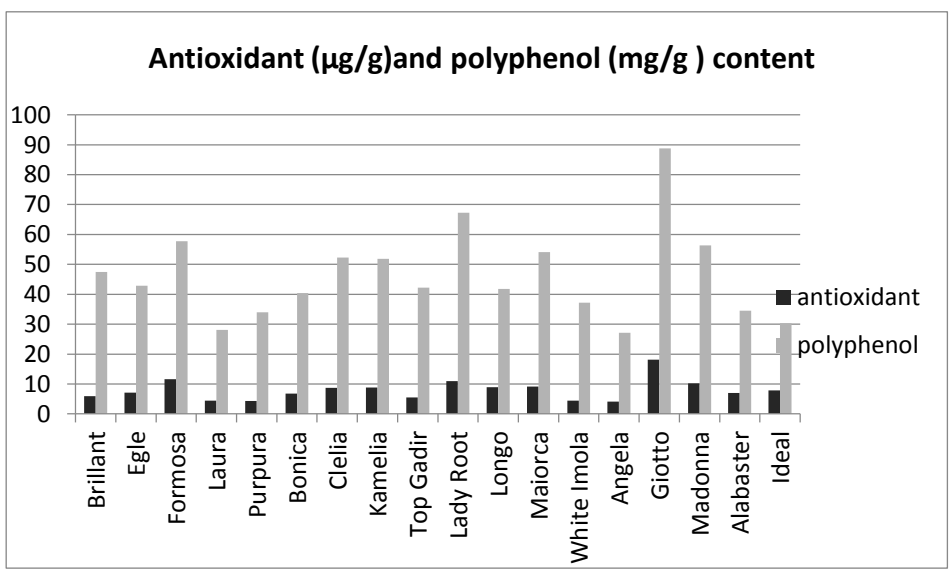

Figure 4.
Antioxidant and polyphenol content of different eggplant varieties in Tordas

The storage in the cool room was during 2 weeks, after this time was the weight loss was among 7,8-10,8 %. , almost double than after 1 week. After 2 weeks we experienced 65 % soft and brown spots eggplant fruits. We compared the results of two storage method. After one week in the cold room the weight loss and fruit quality were marketable, the fruits stored in room temperature average of weight loss 11,8%, after 4 days started to became soft and wrinkle.

Brillant globular shape variety had a highest weight loss.

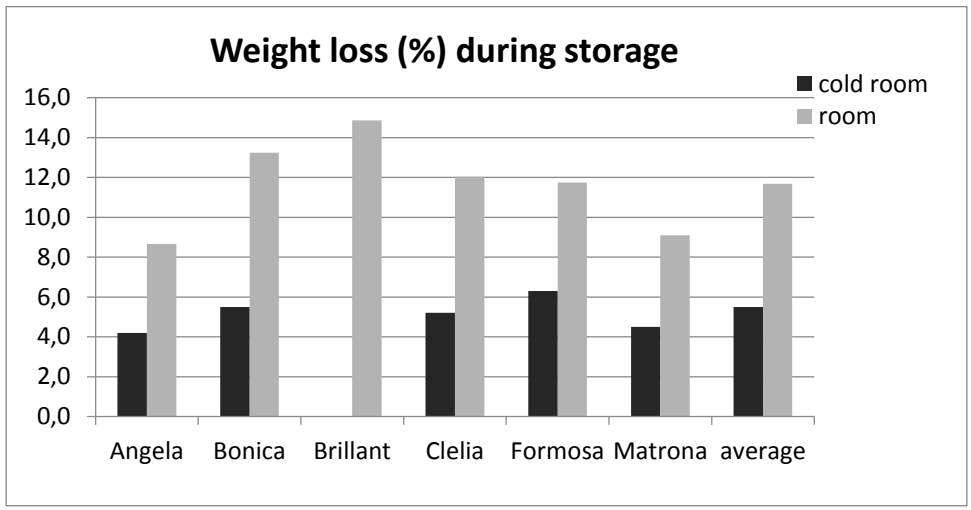

Figure 5.
Weight loss after 1 week in cool room and room temperature of eggplant varieties and their average

4. Discussion

The results of measuring water soluble solids had no significant differences. The standard deviation of the dray matter content is high among dry matter content, the highest 7,3 %, the lowest 4,9% .We found that the dray matter content of the eggplant fruits harvested from open field mainly were higher than the fruit from plastic tunnel, the highest measured data was over 8%.which is close to the maximum value of literature published.

In Nitrogen content were not found variety typical data however the Potassium content typical characteristic of the varieties the highest value 47,5 mg/g (Purpura, Giotto and Ideal purple varieties) , shown correlation with Phosphorus content (r=0,81).

The high polyphenol content correlated with a high antioxidant capacity. The dark purple varieties had a highest polyphenol content (over 54 mg/g) and antioxidant capacity (over 10 µg/g**)** Giotto, Lady Root, Formosa, Madonna.

The best to store Angela purple striped variety. The biggest deviation from the average of weight loss showed the Brillant globular variety.

We suggest to store the tested eggplant varieties 4 days in room temperature, 7 days in cold room.

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Effects of mychorriza on pepper plant growth and nutrients under salinity stress

Ozlem Altuntas[1], H. Yildiz Dasgan[2], I. Kutalmis Kutsal[1]

[1] *Inonu University Faculty of Agriculture Department of Horticulture, Malatya, Turkey*

[2] *Cukurova University Faculty of Agriculture Department of Horticulture, Adana, Turkey*

Abstract

This study was conducted in the greenhouses of Cukurova University Karaisali Vocational School in order to determine the effects of mycorrhiza on root length, stem diameter, plant height, shoot and root fresh and dry weight and nutrient elements (N, P, K, Mg, Ca, Na, Fe, Zn, Cl) content in local pepper varieties (cv. Karaisali, Demre and Maras). The plant growth parameters were measured every week in total five times and nutrient element were analyzed three times. The experiment was carried out with three replicates in pots. The salt doses of 0, 25, 50, 10, 150 mM were applied. The mycorrhiza application was done during the transplanting of seedlings into the pots (for per plant 1000 mycorrhizal spores). The results showed that mycorrhizae application had a positive effects on plant growth of plants under saline stress conditions. Also, the application increased the plant growth parameters approximately at the rate of %15-20. Additionally, in mycorrhiza-treated plants, chlorophyll contents have been measured more than the non-mycorrhiza plants. According to nutrient element analysis, it was determined that mycorrhiza application had significantly increased the nutrient element uptake

1. Introduction

Pepper (*Capsicum annuum* L.) is one of the most important vegetables for Mediterranean region also all over the World, because of its consumer preference and economic success. Late studies have informed varied responses of pepper to salinity stress. The pepper plants, salinity doses ranging from 0 to 2 dS/m are acceptable. However, increasing salinity doses might occur linear decrease ranging between %8 to 15 in yield [1][2]. Soils in the arid and semiarid regions have excessive concentrations of soluble salts, which adversely affect plant growth [3]. Yet, one of the most effective ways for coping with salinity stress is growing genetically-tolerant plant species and cultivars to saline conditions[4]. However, some other factors that are enable to makesensitive plants more tolerated to salt stress might improve production under saline conditions [5], [6]. One of these factor is using mycorrhiza fungi. According to [7]Arbuscular mycorrhizal fungi (AMF) commonly exist in the soils which are salt-affected. Recent studies revealed that AMF can increase plant growth, nutrient elements uptake, and decrease yield losses in pepper and tomato under saline conditions [8], [9]. AMF's root colonization contains a range of morpho-physiological and biochemical incidents which caused by the mutual effects of plant and fungus and also by the environmental factors. For this reason, it is quite important to understand the relationship between plants and the fungi for the successful AMF usage under specific conditions [10]. The mechanism of how AMF enhance salt stress tolerance for plants is not fully understood. However, some researchers stated that increasing nutrient elements uptake on AMF-infected plants, especially of immobile soil nutrients such as P, Cu and Zn is one of the major factors responsible for improving plant growth under saline conditions [8], [11].AM fungi can also benefit plants by stimulating growth regulating substances, increasing

photosynthesis, improving osmotic adjustment under drought and salinity stresses and increasing resistance topest [12].

In this study, we report the effects of mycorrhizal inoculation on three different pepper cultivars growing under four different saline dosages.

2. Materials and Methods

2.1. Material

In the study, 3 local pepper varieties of Karaisali, Demre and Maras were used as plant materials. The mycorrhizae specie used in the study is *Glomus clarum* which we determined that it is well-colonized with pepper plant in the former studies carried out by us. Salt dosages in the study are 0, 25, 50, 100 and 150 mM.

2.2. Methods

The study was conducted in the 200 m^2 plastic greenhouse belongs to Cukurova University, Karaisali Vocational School, as pot trial. Pepper seeds were sown into the viols filled with peat + pearlite in2:1 ratio on the 27th of April. Afterwards, the seedlings were transplanted into the 2 liter pots on the 31stof May. Mycorrhizae inoculation were done into the pots by means of 1000 spores for each pot [13], nutrition solution was used for the irrigation. Salt treatments were started one-week after the transplantation on the 6th of June. Following the salt treatments four weeks later, 28 days in saline stress, plant growth parameters were measured. The parameters were investigated were the plant height, stem diameter, root dry weight, shoot dry weight were measured. Besides, plant nutrient elements of K, P, Ca, Na, Zn analyzes were also done. Plant nutritional elements analyzes were done by the method of dry-ashing. The samples were dried and grinded, after then, they were burned at 550 ^{0}C, following, they were soluted in %3.3 (v/v) HCl solution and the elements of K, Ca, Na and Zn readings were done by the atomic absorption spectrophotometer [14],[15],[16]. The P contents of samples on the other hand, were determined according to Barton method [16]. Lastly, mycorrhizae infection in the roots were done according to [17]. In the roots that were painted with Trypan Blue, the infection was made firm by the help of in and out hypes and vesicular and arbuscular structures.

3. Results

The salinity levels and the use of mycorrhiza on plant growth parameters have been found to be statistically significant.

Plant Height: The mycorrhiza in 50mM salt in Demre pepper plants has positive effect, and it is in the same group as the control and 25 mM salinity levels (Table 1). In 150 mM salt, which is the highest salt, the heights of the plants with mycorrhiza have been found to be higher than that of without mycorriza. In the 50, 100 and 150 mM salt treated Karaisalı pepper plants, the heights of the plants with mycorrhiza are higher than those without mychorriza (Table 2). In Maras pepper, at 50 and 100 mM salinity levels the mychorriza has been found to be effective, however no clear effects were determined in 150 mM mycorrhiza (Table 3). The plant height increase were at a rate of 21% in Demre in 150 mM salt, 21% in Karaisali in 100 mM and 22% in Karaisali in 150 mM salt, 17% in Marasin 100 mM salt.

Stem Diameter: It has been determined that the mycorrhiza use has been effective on stem diameter in Demre pepper. This effect was clearly visible even in 25 mM salinity level. The stem diameter results of the plants in 150 mM salt with mycorrhiza were close to the plants

without mycorrhiza in the controls of 25 and 50mM salt (Table 1). The stem diameters of the 150 mM plants in Karisali pepper which did not receive mycorrhiza were measured as the lowest (Table 2). The use of mycorrhiza increased the stem diameter in Demre in 100 mM salt at 19%, in 150 mM at 17%, in Karaisali in 50 mM at 96% and in 150 mM at 28%, and no increase was observed in Maras.

Varieties x Mycorrhizae	Salt Dosages	Plant Height	Stem Diameter	Root Dry Weight	Shoot dry weight
Demre (M+)	0	42,33 a	5,45 a	1,40 a	4,70 a
	25	39,50 a	5,31 b	1,18 bc	3,88 b
	50	39,16 a	4,81 c	1,10 cd	3,34 c
	100	31,33 b	4,81 c	1,05 efg	2,23 e
	150	26,50 c	4,37 de	0,97 ef	1,02 g
Demre (M-)	0	40,16 a	4,43 d	1,27 b	3,03 cd
	25	39,33 a	4,35 e	1,09 cde	2,83 d
	50	32,83 b	4,31 e	0,95 f	2,34 e
	100	29,83 b	4,04 f	0,76 g	1,83 f
	150	21,83 d	3,75 g	0,60 h	0,93 g

Table 1.
Effects of salinity levels and mychorriza on Demre pepper plant growth

Varieties x Mycorrhizae	Salt Dosages	Plant Height	Stem Diameter	Root Dry Weight	Shoot dry weight
Karaisali (M+)	0	44,16 a	5,43 a	2,25 a	3,35 a
	25	42,16 ab	5,07 b	1,97 b	3,34 a
	50	39,16 bc	4,33 c	1,57 c	3,26 a
	100	38 c	4 cde	0,98 de	2,81 b
	150	33,66 d	3,87 de	0,74 f	1,18 f
Karaisali (M-)	0	41,33 abc	4,86 b	2,05 a	3,36 a
	25	37,33 cd	4,27 c	1,04 b	2,23 c
	50	33,33 f	4,19 cd	0,80 ef	1,82 d
	100	31,33 f	3,77 e	0,77 ef	1,51 e
	150	29,66 f	3,18 f	0,58 f	1,21 f

Table 2.
Effects of salinity levels and mychorriza on Karaisali pepper plant growth

Dry Root Weight: In Demre pepper, the dry root weight has been found to be the highest in the control plants which did not receive salt, and the mycorrhiza application increased the root development, which is the expected result (Table 1). While there were similar results in the Karaisali pepper, the plant roots at 150 mM saltwith mycorrhiza gave results that were close to the plant roots which did not receive mycorrhiza at 100 mM (Table 2). The Control plants were in the same group, and the effect of mycorrhiza at 150 mM salt was not as clear as in the other two peppers (Table 3). The mycorrhiza increased the root dry weight in 3 pepper cultivar in the order of Karaisali, Demre and Maras (Tables 1, 2, 3). The mycorrhiza application increased the dry root weight in Demre pepper in 150 mM at 62%, in Karaisali pepper in 25 mM at 89% and in 50 mM at 96%, and in 150 mM in 28%, in Maras in 25 mM at 49%, in 150 mM at 8%.

Varieties x Mycorrhizae	Salt Dosages	Plant Height	Stem Diameter	Root Dry Weight	Shoot dry weight
Maras (M+)	0	53,33 a	4,37 ab	1,8 b	5,69 a
	25	49,66 bcd	4,29 bc	2,02 a	4,62 b
	50	47,66 cd	4,19 cd	1,66 b	3,64 c
	100	46,33 de	4,19 cd	1,06 d	3,24 d
	150	38,5 f	3,53 f	0,96 de	2,06 ef
Maras (M-)	0	54,16 a	4,47 a	1,67 b	3,4 cd
	25	51 abc	4,24 c	1,36 c	2,15 e
	50	42,5 ef	4,11 d	1,34 c	2,29 e
	100	39,66 f	4,09 d	1,25 c	1,98 ef
	150	38,5 f	3,94 e	0,89 e	1,76 f

Table 3.
Effects of salinity levels and mychorriza on Maras pepper plant growth

Dry Weight of Shoot (stem+leaves): The effect of mycorrhiza was observed in shoot growth, and this effects was observed at a clearer level in the control plants which did not receive salt. The highest value was observed in the Demre and Maras peppers in the control plants which received mycorrhiza (Table 1 and 3). The mycorrhiza use increased the shoot dry weight in 3 pepper cultivar in the order of Maras, Karaisali, Demre (Tables 1, 2, 3). The mycorrhiza application provided an increase in Demre pepper in 50 mM at 43%, in 150 mM at 10%, in Karaisali pepper in 25 mM at 50%, in 50 mM at 79%, in 100 mM at 86%, in Maras type in 25 mM at 115%, in 50 mM at 59%, in 100 mM at 63%.

Plant Nutrients

Potassium: The effect of mycorrhiza has been observed as being better in the high saline levels. Mycorrhiza application increased the potassium uptake especially in higher salt concentrations (50, 100 and 150 mM NaCl) in Demre and Karaisali peppers (Tables 4, 5). Increasing salt decreased the potassium uptake, and the potassium level was found to be at a statistically significant in the plants which received 100 and 150 mM mycorrhiza (Tables 4, 5). Mycorrhiza use increased the K content in Demre in 50 mM at 22%, in 150 mM at 20%, in Karaisali pepper in 100 mM at 11%, in 150 mM at 23%, in Maras peper in 100 mM at 11% and in 150 mM at 6%.

Varieties x Mycorrhizae	Salt Dosages	K	P	Ca	Na	Zn
Demre (M+)	0	4,17 a	0,39 a	1,35 ab	0,27 j	46 a
	25	4,03 ab	0,38 a	1,32 ab	0,56 h	43 ab
	50	3,94 b	0,36 a	1,32 ab	1,05 f	42 ab
	100	3,16 c	0,3 bcd	1,27 ab	1,39 d	34 cd
	150	2,65 e	0,25 de	1 c	2,14 b	29 de
Demre (M-)	0	3,98 b	0,38 a	1,36 a	0,35 i	42 ab
	25	3,99 b	0,35 ab	1,34 ab	0,8 g	41 abc
	50	3,24 c	0,34 abc	1,3 ab	1,27 e	38 bc
	100	2,87 d	0,29 cd	1,25 b	1,86 c	29 de
	150	2,21 f	0,22 e	1 c	2,68 a	23 e

Table 4.
Effects of salinity levels and mychorriza on Demre pepper nutrients

Phosphor: The phosphor levels have been increased in 25 and 50mM NaCl in Demre and Maras, however P increase in Karaisalı were lesser in the same salt dosages (Tables 4, 5, 6). The Karaisali pepper with the mychorriza showed the higher P increases in 100 mM and 150mM NaCl (Table 5).The Demre with the mychorriza showed its highest P uptake in 150mM NaCI (Table 4). Mycorrhiza application increased the P content in Demre in 150 mM at 14%, in Karaisali in 100 mM at 10%, in 150 mM at 8%, in Maras in 25 mM at 11%, in 50 mM at 9% and in 150 mM at 8%.

Calcium: In saline conditions, the Ca content in Demre was not significantly increased by the mychoorriza, only in the case of the 100mm and 150mM NaCl there were slight increases (Table 4). Mycorrhiza was not found to be very influential in calcium uptake (Tables 4, 5, 6). Calcium uptake increased with mycorrhiza application in 150 mM in Karaisali type at 8%, and in Maras type at 6%. AMF root colonization had little effect on shoot Ca content in pepper plants in comparison to K, P and Zn.

Varieties x Mycorrhizae	Salt Dosages	K	P	Ca	Na	Zn
Karaisali (M+)	0	4,02 a	0,43 a	1,53 a	0,31 h	50 a
	25	3,91 ab	0,41 ab	1,5 ab	0,44 g	49 a
	50	3,89 ab	0,37 bc	1,42 abc	1,01 e	48 a
	100	2,97 c	0,33 cd	1,36 cde	1,31 d	35 c
	150	2,88 c	0,26 ef	1,28 ef	2 b	33 c
Karaisali (M-)	0	3,97 a	0,43 a	1,49 ab	0,29 h	50 a
	25	3,83 b	0,39 ab	1,51 a	0,73 f	46 ab
	50	3,81 b	0,36 bc	1,4 bcd	1,08 e	41 b
	100	2,68 d	0,3 de	1,31 de	1,52 c	34 c
	150	2,35 f	0,24 f	1,19 f	2,10 a	31 c

Table 5.
Effects of salinity levels and mychorriza on Karaisali pepper nutrients

Zinc: The mycorrhiza was really effective for Zn uptake in Demre the highest Zn was recorded in 150mM salt (Table 4). In Karaisali pepper, in the 50mM NaCl there was remarkable Zn increase (Table 5). Mycorrhiza application increased the zink uptake in Demre in 100 mM at 17%, in 150 mM at 26% in Karaisali in 50 mM at 17% in Maras in 150 mM at 7%.

Varieties x Mycorrhizae	Salt Dosages	K	P	Ca	Na	Zn
Maras (M+)	0	4,11 a	0,41 a	1,66 a	0,28 e	52 a
	25	3,96 b	0,41 a	1,57 ab	0,3 e	48,33 a
	50	3,92 b	0,38 ab	1,57 ab	0,97 c	48 a
	100	3,33 c	0,32 bcd	1,37 c	1,3 b	36,33 b
	150	3,02 d	0,28 cd	1,34 e	1,99 a	32 b
Maras (M-)	0	4,08 a	0,4 ab	1,64 ab	0,28 a	50 a
	25	4 ab	0,37 ab	1,6 ab	0,54 b	48 a
	50	3,93 b	0,35 abc	1,55 b	1,02 c	46 a
	100	2,99 d	0,33 abcd	1,39 c	1,35 b	35 b
	150	2,86 f	0,26 d	1,26 e	2,03 a	30 b

Table 6.
Effects of salinity levels and mychorriza on Maras pepper nutrients

Sodium: The sodium increase in non-mychorrizal plants is expected. The sodium level was the lowest in the control plants, and the highest was determined in 150 mM salt. The Na decreases of the mychorriza in salt in comparison to their controls are highest in Demre among the 3 pepper cultivars (Tables 4, 5, 6. In mychorriza inoculated plants the 25mM NaCl was the most Na decreased level in all pepper cultivars. Mycorrhiza application decreased sodium uptake in Demre in 25 mM at 30%, in 150 mM at 20%; in Karaisali in 25 mM at 40%, in 100 mM at 14%, in 150 mM at 8%, in Maras in 25 mM at 44% and in 100 mM at 5%.

Mycorrhiza Infection: The colonization of the mycorrhiza spores in the roots decreased with the increasing saline dosages (Table 1), indicating that salinity suppressed the growth of AM. It has been determined that the Karaisali and Demre peppers were infected better with mycorrhiza spores when compared with Maras. [11] reported that the salt-tolerant tomato cultivar showed higher mycorrhizal colonization than the salt sensitive cultivar.

	Salt Dosages	**Demre**	**Karaisali**	**Maras**
Mycorrhizae infection (%)	0	77	65	60
	25	59	55	54
	50	55	49	43
	100	31	28	18
	150	20	17	11

Table 7.
Effects of salinity levels on the mychorriza inoculation in 3 different pepper cultivars

4. Discussion

The present study showed a profound effect of AM fungal inoculation on growth of pepper cultivars in salt stres conditions.Salinity stress significantly reduced the plant shoot and root growth, howevermycorrhizal plants maintained greater root and shoot biomass at all salinity levels compared to nonmycorrhizal plants[12],[8],[18]).Enhanced growth of mycorrhizal plants grownin saline environments has been related to many benefits for the host plant during its symbiotic life. As it has been reported by many authors in previous studies, salt stress increases the coping skill of the plant [19],[20].

The present investigation showed that AM symbiosis plays a important role in improving the essential inorganic nutrients such as K, P, Zn, Ca nutrition of the pepper plants depending on the cultivar under salt stress conditions [21].

Sodium content in leaves was significantly reduced by the mhcorriza. Sodium concentration was lowered in shoot tissues of mycorrhizal plants, which increased non mycorrhizal plants as salinity increased. High concentration of Na creates various osmotic and metabolic problems (e.g., reduced photosynthesis, protein synthesis) for plants [22].Mycorrhizal inoculation of pepper plants prevented Na translocation to shoot tissues.The reduction in shoot Na may be significant in helping mycorrhizal plants to survive in saline conditions [21].

The leaf K^+ concentration was lowered in pepepr plants by increasing NaCl concentration, however the mycorrhizal pepper plants had a higher concentration of K at all salinity levels.Higher K accumulation and lower uptake of Na by mycorrhizal plants in saline conditions could be beneficial by maintaining a high K/Na ratio and by influencing the ionic balance of the cytoplasm or Na efflux from plants [23]; [21] The improved K/Na ratios in shoot tissues of mycorrhizal plants may help in protecting disruption of K-mediated enzymatic processes under salt stress conditions [24].

It is known that salt-stress induces P deficiency in plants by reducing P uptake or translocation, however in this study the mycorrhiza increases the phosphor uptake [14] and facilitates the endurance against salt stress [25]. About the salt stress endurance, its increasing the antioxidative enzyme activity is influential in the endurance of the plant against stress conditions because it decreases the oxidative damage [26].Mycorrhizal inoculation improves P nutrition of plants under salinity stress and reduces the negative effects of Na^+ by maintaining vacuolar membrane integrity [27].

Mychorriza increases the uptake of the mineral nutritious elements with the help of its hyphae [25], protect the ion balance [11] facilitates the activities of the enzymes [26] and water uptake. In our study, high saline dosages prevented the colonization of the mycorrhiza; and the hyphae being less as a result of the decrease of the mycorrhiza colonization may decrease the mycorrhiza benefit in nutrient uptake [19], [27], [28].

5. Conclusion

According to the results of our study, it has been determined that mycorrhiza has positive effects on plant growth and development under the saline conditions. The mycorrhizal fungi protected the host plant against the detrimental effect of salinity.Shoot and root dry matter and plant height and stem diameter were higher in mycorrhizal than nonmycorrhizal plants of 3 pepper cultivars. However, among the 3 pepper cultivars, about the plant growthKaraisali was the first, Demre second and Maras was the last for the positive responses. The nutrient uptakes K, P, Ca and Zn were increased and Na was reduced under saline conditions by the mychorriza inoculation. Among the 3 pepper genotypes Demre was the best, Karaisali was the second and the Maras was the last for the nutrient uptakes in salinity by the mychooriza inoculation.

The results suggest that Demre and Karaisali benefited more from AMF colonization than Maras under saline conditions, due toboth cultivars roots were higher infected with the AMF than Maras.Our results indicate that mycorrhizal fungus alleviates deleterious effects of salinityon plant growth that could be primarily related to improved P, K, Zn, Canutrition and reduced Na. This is further confirmation that mycorrhizal symbiosis is especially beneficial for pepper growth under saline conditions.

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Does anthracnose resistance associate with cuticle characteristics and spore attachment?

Kietsuda Luengwilai[1], Kamonwan Sangsoi[1], Thanarat Salahong[3], Orarat Mongkolporn[1,2]

[1] Tropical Vegetable Research Center, Department of Horticulture, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand

[2] Center for Agricultural Biotechnology, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand

[3] Princess Chulaporn's College Chon Buri, Thailand

Abstract

Anthracnose (*Colletotrichum* spp.) causes fruit rot in chili (*Capsicum* spp.). Resistances to anthracnose are mostly found at the fruit surface level, while the systemic (hypersensitive response) is rare. *Capsicum annuum* L. is the most popular species grown worldwide, lacks the systemic but contains the surface resistance. Surface resistance is the outermost defense mechanism found in the plant cuticle. The cuticle thickness is believed to relate to the level of resistance, whereby the chili with thick cuticle often shows high resistance, which is commonly found in the *Capsicum baccatum* species. Among the *Capsicum annuum* varieties, variations of the cuticle thickness are limited, although degrees of anthracnose infection range from 0 – 100% with spray inoculation. Therefore the cuticle thickness may not be the only factor involving the cuticle resistance. Ability of the fungal spores to attach on the fruit surface should hence be investigated, which could be influenced by physical cuticle properties including epicuticular wax composition and arrangement, quantity of wax and cutin. Selected chili varieties with various levels of anthracnose cuticle resistance are being investigated the cuticle thickness and other cuticle characteristics as mentioned, in order to find the association of the resistance and those cuticle properties.

Keywords: Capsicum, Colletotrichum

Effects of myhorriza on alleviating salt stress of *Capsicum annuum* L. by ion regulation

Ozlem Altuntas[1], H. Yildiz Dasgan[2], Yelderem Akhondnejad[2]

[1]*Inonu University, Agricultural Faculty, Horticultural Department 44210, Malatya, Turkey*

[2]*Cukurova University, Agricultural Faculty, Horticultural Department 01330, Adana,Turkey*

Abstract

Salinization of soil is a serious problem and is increasing steadily in many parts of the world, in particular in arid and semi-arid areas. Pepper is one of the sensitive species to salinity stress Yield and quality reductions are the consequences of the irreversible salt damage to pepper plants. Salinity-induced reductions in yield are generally caused by ion toxicity, osmotic stress, and nutritional imbalance. The aim of this study was to investigate the role of arbuscular mycorrhizal fungi (AMF) in alleviating salt stress by the regulation of K, Ca, Mg, Na, Zn and Cl ion uptake of pepper plants under salt stress. In the study, two pepper genotypes, one is resistant (Karaisali) and other is sensitive (Demre), and two NaCl concentrations 75mM and 150mM, and *Glomus clarum* that is well colonized AMF to pepper were used. The experiment was carried out in growth chamber. Pepper plants were grown in 2 L capacity pots in vermiculite and irrigated modified Hoagland nutrient solution. Potassium, P, Ca, Mg, Na and Cl analysis in shoot and root tissues were done. Nutrient uptake by the AMF were generally increased in salt conditions, however it change depending on the pepper genotype. Potassium concentrations in shoot of the resistant genotype were increased by 22.3% and 24.5% in 75mM and 150mM NaCl, respectively by the AMF. However K in sensitive genotype was increased by 2.02% and 18.5%. Calcium concentrations in resistant genotype were increased by 14.6% and 11.3% by AMF. In sensitive genotype Ca increases by the mycorrhiza were 21.3% and 20.8% in 75mM and 150mM NaCl, respectively. Sodium concentrations in resistant genotype were decreased by 10.4% and 1.0% in 75mM and 150mM NaCl. However, Na in shoot of the sensitive pepper genotype was increased by 28.7% and 20.4% in 75mM and 150mM NaCl, respectively.

1. Introduction

Salinization of soil is a serious problem and is increasing steadily in many parts of the world, in particular in arid and semiarid areas [1],[2]. Saline soils are one of the major abiotic stresses that bring reduced plant growth, yield and quality of crops. The saline soils constitute approximately 7% of the global land surface [3]. The direct effects of salt on plant growth may involve: (a) reduction in the osmotic potential of the soil solution that reduces the amount of water available to the plant causing physiological drought [4], [5] toxicity of excessive $Na^+$and Cl^- ions towards the cell the toxic effects include disruption to the structure of enzymes and other macromolecules, damage to cell organelles and plasmamembrane, disruption of photosynthesis, respiration and proteinsynthesis [6], [4] nutrient imbalance in the plant caused by nutrient uptake and/or transport to the shoot leading to ion deficiencies [7], [8].

Salt stress reduces plant growth, leaf expansion and induces mineral deficiency, destabilizes cell membranes, alters selective permeability (leakage of cell solutes), fluidity, microviscosity, and affects the solubility of many essential substrates and ions [9]. According to [6] arbuscular mycorrhizal fungi (AMF) commonly exist in the soils which are salt-affected and form

symbiotic associations with roots, in most plant species. This association makes plants able to uptake more nutrient elements and water. It also increases the absorption rate of immobile nutritional elements such as phosphorus (P), ensures resistance to some soil pathogens and drought stress, and enhances water use efficiency [10],[11]. The AMF symbiosis increases leaf area, delays senescence [12] and increases salinity resistance in several host plants and one of these plants is pepper [13]. Recent studies showed that AMF is able to enhance the ability of plants to deal with salt stress by followings; improving nutrient element uptake [14] keeping ion balance [15] maintaining enzyme activities, and facilitating water uptake [16].

In agriculture, horticulture is one the most promising area for practical utilization of AMF due to its high income from per unit area. Pepper *(Capsicum annuum* L.) is one of the most important crops in the World and especially in Mediterranean region, however pepper is quite sensitive to salt stress. Salinity in pepper production areas induced reductions in yield and crop quality. Since, mycorrhizae make associations with pepper roots, mycorrhiza infected plants can be able to uptake more nutrients and water. Under these circumstances, mycorrhizae treatments might keep the ion regulation which causes to yield loss under salt stress conditions. The aim of this study was to investigate the role of arbuscular mycorrhizal fungi (AMF) in alleviating salt stress by the regulation of K, Ca, Mg, and Na ion uptake of pepper plants under salt stress.

2. Materials and Methods

This study was carried out in the growth chamber of Cukurova University, Faculty of Agriculture, Department of Horticulture in order to alleviate salt stress with mycorrhizae on pepper plant by ion regulation.

2.1. Material

Two local pepper varieties of Karaisali (tolerant to salt stress) and Demre (sensitive to salt stress) were used. The plants were treated with 3 salt dosages of 0 (control), 75mM and 150 mM and *Glomus clarum* was used as mycorrhizae species which is previously tested and found more convenient mycorrhizae species for the pepper plant.

2.2. Methods

Two liter pots filled with vermiculite was used as growth medium. Three pepper seedlings were planted to each pot on 8th November 2013 and the design of the study was randomized block design with 3 replicates. 1000 mycorrhizae spores inoculated to every plant during the plantation. Hoagland nutrition solution was used with the half strength for the plant nutrition in order to enhance mycorrhizae's effectiveness. NaCI added with 25 mM and 50mM increments on every day and in 3 days final 75mM and 150 mM concentrations were reached. When the reaching of the final salt concentrations, the day after, 75mM and 150mM salt dosages were ordinarily applied into the pots. After 30 days under salt stress, on day 31, plants were harvested into the root and leaves for the mineral analysis. Whole root systems and several leaves from the medium part of the plants were used for the mineral analysis. Plant nutritional elements analyzes were done by the method of dry-ashing. The samples were dried and grinded, after then, they were burned at 550 ^{0}C, following, they were soluted in %3.3 (v/v) HCl solution and the elements of K, Ca, Na and Zn readings were done by the atomic absorption spectrophotometer [17], [18]. The P contents of samples on the other hand, were determined according to Barton method [19]. Lastly, mycorrhizae infection in the roots were done according to the Trypan Blue method by [20]. Tissue Cl concentration was determined by the Mohr method [21].

The data were subjected to factorial analysis of variance using Statistical Package for Social Sciences (SPSS version 16) and means were separated by Duncan's Post Hoc Tests at the 5% level *($P < 0.05$).*

3. Results And Discussion

The positive influence of AM inoculation on nutrient uptake exhibits that root colonization by mychooriza can alleviate the adverse effects of salt stress.The extent of AM response on root colonization varied with pepper cultivars, and with the level of salinity.The colonization of the mycorrhiza spores in the roots decreased with the increasing saline dosages (Table 1), indicating that salinity suppressed the growth of AM. NaCl prevented the infection of the mycorrhiza spores, and also, according to the previous studies, prevented the development and spread of the hyphae. As it has been anticipated, the colonization was observed to be at the lowest level in the highest saline dosage. It has been determined that Demre were infected better with mycorrhiza spores when compared with Karaisali (Table 1).

Salt Doses / Variety	**Demre**	**Karaisali**
0 (control)	80	68
75 mM	53	37
150 mM	22	16

Table 1: Mycorrhizae Infection (%

Salt stress lead to a decrease of all the nutritional elements (except for Na and Cl) in green parts. The saline medium the plants are placed have negative effects like low osmotic potential, ion toxicity, and nutritional imbalance. Mycorrhiza plays an important role in increasing the skills of the plants in coping with these abiotic stress factors [1].

The nutritious element intake has increased in the plants inoculated with mycorrhiza at two saline levels when compared with the plants that were not inoculated; and especially the Na intake decreased, which is one of the ions with toxic effects.

In Demre cultivar, which was used in the study, and which is sensitive to salt, the benefit of the mycorrhiza is clearer. As it is observed in Table 2, the effect of mycorrhiza appeared more clearly especially at 150 mM saline level. According to the nutrition elements results that were analyzed in green parts (shoot), it has been determined that the element intake increased at a significant level in plants inoculated with mycorrhiza. The nutrient intake of the shoot which received mycorrhiza application at 150 mM saline level in salt-sensitive Demre (Table 2), 19% increase was observed in K; 29% increase was observed in P; 23% increase was observed in Mg; 21% increase was observed in Ca ; 18% increase was observed in Zn. In addition, the Na intake, which has toxic effects, decreased at 20%, and the Cl intake decreased at 3%. The nutrient intake of the root which received mycorrhiza application at 150 mM saline level in salt-sensitive Demre (Table 2), 39% increase was observed in K; 12% increase was observed in Ca. Na concentration in the root of Demre, which has toxic effects, decreased at 1%, and the Cl intake decreased at 10% in 150mM NaCl.

In Karaisali, which was used in the study, and which is tolerant to salt, the benefit of the mycorrhiza for nutrient uptake and regulation is less than the sensitive cultivar due to Karaisali may be had less colonization of the mychorriza. The nutrient intake of the shoot which received mycorrhiza application at 150 mM saline level in salt-tolerant Karaisali (Table 3), 24% increase was observed in K; 16% increase was observed in P; 12% increase was observed in Mg; 11% increase

was observed in Ca; 9% increase was observed in Zn. In addition, the Na concentration in shoot, which has toxic effects, decreased at 1%, and the Cl intake decreased at 7%.

The nutrient intake of the root which received mycorrhiza application at 150 mM saline level in salt-tolerant Karaisali (Table 3), 22% increase was observed in K; 8% increase was observed in Ca. Na concentration in the root of Karaisali, which has toxic effects, decreased at 17%, and the Cl intake decreased at 17% in 150 mM NaCl.

High Na concentration in tissues lead to various osmotic and metabolic problems (like the decrease in photosynthesis, transpiration and protein synthesis) [22]. Although Sodium is an element carried both in xylem and in phloem, it is mostly carried in a single way, and generally accumulated in the body and old leaves of the plant. When we examine the Na levels in the roots of the plants which receive mycorrhiza application, it has been determined that they were more when compared with the ones that did not receive mycorrhiza application. In this situation, the plants with mycorrhiza held the Na ions in the roots and partly prevented from being carried upwards thus decreasing the harmful effects of salt. In addition, the Na ions are held and prevented from being carried upwards in the plants with mycorrhiza, and the hyph of mycorrhiza enter the roots in symbiotic life (receives the photosynthesis products via its hyph in the roots). Sodium ion is held in the roots of the plants with mycorrhiza, and also held in intra-radical hyph and prevented from being carried upwards [14].

The increase of the Na ions in the medium leads to the decrease of the K ions. The K ions are necessary and important for various functions in the cell, and Na ions may compete with the K ions, and migrate to the binding area of the K ions in the cell. However, the KI element plays an important role in plant metabolism, in the stoma movements, in protein synthesis, and in the binding of tRNAs to ribosome. It is also responsible for the activation of a series of enzymes. When Na ions take the place of the K in the cell, they cannot perform this role [23], [24]. For this reason, high Na content or low K/Na rates disrupt the ion balance in the cytoplasm, and eventually destroy various metabolic processes [19]. In the results of our study, the K content of the green parts of both pepper types, which received mycorrhiza application, has been found to be high, which is similar to the results reported in previously conducted studies. Depending on this, the K/Na rate in the plants with mycorrhiza has been observed to be higher in the roots and green parts. The Na intake being lower in the plants with mycorrhiza makes us consider that mycorrhiza leads to a buffer effect in Na ion intake [25]. This shows that it also affects to the running of a regulatory mechanism in the plants which contain Na ions. Mycorrhiza inoculation prevented the Na from being carried in the green parts, and increased the K intake [26], [27], [15], [28], [29]. Root cells may receive Cl ions by means of anion channels in saline conditions. They reach the root xylem firstly, and then to the green parts via these channels. The chlorine accumulation increases in a great deal in saline conditions. The accumulation of salt in tissues at a high rate may show toxic effect and limit the agriculture in saline media [30]. According to the results of the study and to the reports of the previous studies, the Cl intake may be decreased to a certain extent with mycorrhiza application [29], [31]. The Cl ions may be separated into divisions in vacuole membranes, and therefore it can be prevented from being an obstacle for metabolic ways [14].

Calcium is another important element in salt stress. According to the results of our study, the Ca content was found to be higher in the plants with mycorrhiza. [14] showed that the Ca rate increased in lettuce and [32] in banana, which are similar to the results of our study. Contrary to these, [1]. Reported that the calcium rates did not change in the acacia plants in the tissues with and without mycorrhiza. When the movements of the nutritious elements in plant roots are compared, the data obtained so far make us consider that the mycorrhiza is not important in the flow via diffusion according to the flow via mass. High Ca concentration is a beneficial element on the toxic effect of the NaCl [33], [34].

		In leaves							In roots			
Varieties x Mycorrhiza	**Salt Dosages**	**K**	**P**	**Mg**	**Ca**	**Na**	**Zn**	**Cl**	**K**	**Ca**	**Na**	**Cl**
Demre (M+)	0	3,98 a	0,34 a	0,36 a	1,18 a	0,30 d	45 a	0,2 c	1,73 a	0,95 a	0,55 c	0,70 d
	75	3,03 c	0,29 b	0,32 ab	1,08 a	1,54 c	38 b	2,71 b	1,20 b	0,64 b	1,00 b	1,69 c
	150	2,11 d	0,18 d	0,27 bc	0,93 b	2,14 b	26 c	2,98 ab	1,03 b	0,55 bc	1,50 a	1,81 b
Demre (M-)	0	3,51 b	0,33 ab	0,34 a	1,14 a	0,34 d	44 a	0,24 c	1,75 a	0,92 a	0,61 c	0,81 d
	75	3,03 c	0,24 c	0,29 b	0,89 bc	2,16 b	34 b	2,86 ab	1,11 b	0,57 bc	1,11 b	1,78 bc
	150	1,78 e	0,14 d	0,22 c	0,77 c	2,69 a	22 c	3,07 a	0,74 c	0,49 c	1,52 a	2,02 a

Table 2.
Leaf and root ions content of salinity-sensitive Demre pepper cultivar

		In leaves							In roots			
Varieties x Mycorrhiza	**Salt Dosages**	**K**	**P**	**Mg**	**Ca**	**Na**	**Zn**	**Cl**	**K**	**Ca**	**Na**	**Cl**
Karaisali (M+)	0	3,84 a	0,38 a	0,38 a	1,29 a	0,29 d	58 a	0,19 d	1,58 bc	0,84 a	0,48 c	0,67 c
	75	3,62 b	0,35 a	0,35 a	1,16 b	1,28 c	50 bc	2,02 c	1,98 a	0,60 b	0,97 b	1,53 b
	150	2,53 d	0,22 c	0,29 c	0,98 c	1,97 a	47 bc	2,06 bc	1,42 cd	0,52 b	1,09 b	1,6 b
Karaisali (M-)	0	3,67 b	0,37 a	0,38 a	1,27 a	0,30 d	52 ab	0,20 d	1,87 ab	0,76 a	0,50 c	0,72 c
	75	2,96 c	0,26 b	0,33 ab	1,01 c	1,43 b	48 bc	2,17 ab	1,64 bc	0,53 b	1,06 b	1,65 b
	150	2,04 e	0,19 c	0,26 c	0,88 d	1,99 a	43 c	2,21 a	1,16 d	0,48 b	1,31 a	2 a

Table 3.
Leaf and root ions content of salinity-sensitive Karaisali pepper cultivar

4. Conclusion

The genotypes that have developed tissue tolerance against salt stress keep the Na ions away or use them in osmo-regulation in the cell and cope with stress. On the other hand, the genotypes like Demre, which is sensitive to salt and that has not developed tissue tolerance, it is possible to claim that the inoculation of mycorrhiza is an easy and applicable method. Although the Karaisali type has a tolerance against salt, mycorrhiza application has increased the nutrition element intake in this types. Using genotypes that are resistant in saline soils and benefiting from an environmental-friendly application like mycorrhiza, which increases the endurance against salt, will be an economic and rational solution.

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Correlation between carotenoid components of chili pepper fruits and VIS/NIR reflectance

Péter Szuvandzsiev[1], Zoltán Pék[1], Zsuzsa Nagy[1], Daood Hussein[2], Dinara Seidazimova[3], András Neményi[1], Lajos Helyes[1]

[1] *Institute of Horticulture, Faculty of Agriculture and Environmental Sciences, Szent István University, Gödöllő, Hungary*
Helyes.Lajos@mkk.szie.hu

[2] *Regional Knowledge Centre, Szent István University, Gödöllő, Hungary*

[3] *Faculty Forest, Land Resources and Horticulture, Kazakh National Agrarian University, Almaty, Kazakhstan*

Abstract

Carotenoids of chili peppers are important components of their antioxidant profiles. Analytical quantification of carotenoid components is time and labour consuming. That is why researchers try to develop non-destructive and rapid methods to assess the quality parameters. The present study reports the suitability of a portable handheld visible near infrared (VIS/NIR) spectrometer to predict yellow and red carotenoid components of chili peppers. Spectral ranges of 325-1075 nm were directly acquired on fresh fruits in three ripening stages of three different chili varieties using a FieldSpec HandHeld 2™ Portable Spectroradiometer. Immediately after spectral measurement, each fruit sample was analysed to determine total red and yellow carotenoid components in fresh weight. Partial least square regressions (PLSR) were carried out to perform models of prediction between spectral data and the values obtained from the analytical results. The accuracy of the predictions was discussed according to the coefficient of determination value (R^2), the root mean square error of calibration/cross-validation (RMSEC/CV). Such analyses resulted in calibration equations with R^2=0.88 and 0.63; root mean square error of calibration were 11.4, 17.6; and RMSEC/CV results were 17.1 (yellow) and 21.4 mg/100g (red) respectively.

Keywords: carotenoids, chili, VIS/NIR reflectance

1. Introduction

Peppers are used as spices, and are significant in the Hungarian diet (Koncsek et al, 2014). Chili peppers belong to this group and they are a good source of nutritionally important phytochemicals, such as carotenoids tocopherols, ascorbic acid and phenolic compounds (Gómez-Garcia and Ochoa-Alejo, 2013; Nagy et al., 2015). Carotenoids of chili peppers are important components of their antioxidant profiles, which have nutritional benefits (Daood et al., 2015), due to their positive influence in the prevention of cancer in the human body (Maoka et al., 2001).

Ripened fruits' colour is due to carotenoids, mainly capsanthin and capsorubin, which are red, and both exclusively occurring in peppers. The red pigments are accompanied by other xanthophylls and carotenes such as zeaxanthin, β-cryptoxanthin, violaxanthin, antheraxanthin and β-carotene. The carotenoid pattern and the pigment concentrations vary widely depending on cultivars and ripening stage (Deli et al., 2001; Daood et al., 2014; Gómez-Garcia and Ochoa-Alejo, 2013)

The main carotenoid of red-fruited peppers is capsanthin (the predominant pigment) which represented 35% (Curl, 1962) of total carotenoid content. Concentration of thirty-five different known carotenoids varied depending on the fruit developmental stage. Lutein was the most abundant at the immature stage, then decreased in further ripening stages and was absent in the red or deep red stages. Capsanthin levels were low in the early stages and increased gradually until mature stage (Deli et al., 2001). The carotenoid content and composition is quite different in yellow-fruited peppers as compared to the red fruits; Violaxanthin (34%), antheraxanthin (10.5%), lutein (9.2) and zeaxanthin (8.5) were the most abundant carotenoids (Gómez-Garcia and Ochoa-Alejo, 2013).

Nevertheless, analytical quantification of these components is destructive, time and labour consuming. That is why researchers try to develop rapid and non-destructive methods to assess carotenoids. In recent decades, the application of visible near infrared (VIS) and near infrared (NIR) spectrometry to vegetables and fruits has become more popular for quality and composition studies. Among the non-destructive methods applied in agriculture, near infrared spectroscopy is probably the most studied and accomplished one. Numbers of studies have investigated the potential of VIS/NIR technology for assessing quality parameters of vegetable products including carrot (Belie et al. 2003), *Brassica oleracea* varieties (Szigedi et al., 2012) and tomatoes (Szuvandzsiev et al., 2014; Deák et al., 2015).

Chillies and chilli based-products are important sources of carotenoids for humans, so modern diet needs to develop rapid and cheap techniques for carotenoid measurement. Therefore, the main aim of the present study was to evaluate the ability of NIRS to predict the carotenoid content of chilli pepper samples.

2. Material and Methods

2.1. Plant material

The study was conducted in 2014 at the experimental field of the Institute of Horticulture, Szent István University, in Gödöllő, Hungary (lat. 47°61' N, long. 19°32' E). The soil of the experimental field is sandy loam classified as Cambisol with 1.8-2% humus content and pH value around 7. Beibeihong 695 F1 (Beibeihong) which belongs to *Capsicum frutescens* and Lolo 736 F1 (Lolo) and Chili 3735 F1 (C3735), which belong to *Capsicum annuum* were all purchased from East-West Seeds Company, from Thailand. The pods of Beibeihong, Lolo, C3735, are red when fully ripe.

Seeds were sown in a heated plastic house in the beginning of April and transplanted outdoor in May. All plants received the same irrigation and fertilization. Fruits of chilli pepper plants were harvested at ripened stage on the 5th of September. One sample consisted of three replicates, where each replicate indicates one individual plant.

2.2. Analytical measurements

Spectral and analytical measurements were performed with chilli samples right after harvesting. The fruits were washed, cut and mixed and 42 puree samples were used for analysis. Spectral ranges of 325-1075 nm were directly acquired on five different tomato varieties using a FieldSpec HandHeld 2™ (Analytical Spectral Devices. Inc., Co. USA) Portable Spectroradiometer. A black teflon plate (diameter 75 mm) was filled with 26±1 mm of samples. Spectral measurements were taken with the instrument positioned 20 mm above samples, with Hi-Brite Contact Probe. The instrument has a spectral resolution of <3.0 nm at 700 nm and a wavelength accuracy of ±1 nm.

Carotenoid content of fruits was determined in freshly homogenised samples using procedures described previously by Biacs and Daood (1994). Peak identification was based on comparison of retention and spectral characteristics of each sample peak with those of available standards such as β-carotene, zeaxanthin (Sigma Aldrich, Budapest) and capsanthin (authentic standard). Detection of the different compounds was carried out at the maximum wavelength. The yellow compounds were quantified as β-carotene equivalent, the red compounds as capsanthin equivalent, and the results were given μg/g in fresh weight (FW).

2.3. Statistical analyses

Partial least square regression (PLSR) with non-linear iterative partial least squares algorithm was applied to the chilli sample spectra. The carotenoid calibration models validation were executed therefore, first of all Savitzky-Golay filter was applied with polynomial degree 1, on 11 data points. After the smoothing, adequate signal-to-noise ratio was determined between 400 and 1075 nm. The Standard Normal Variate (SNV) and Multiplicative Scatter Correction (MSC) pre-processing techniques were carried out on the smoothed spectra. For the 1st derivative, Savitzky-Golay filter was applied, the spectra was constricted to 400-1075 nm.

The best models were selected by the lowest root mean squared errors of and cross-validation (RMSECV), the numbers of factors were selected by the lowest predicted residual sum of squares (PRESS) values. After the assessment of the carotenoid calibration models, the 1st derivative of the spectra proved to be the best for each calibration model.

3. Results and Discussion

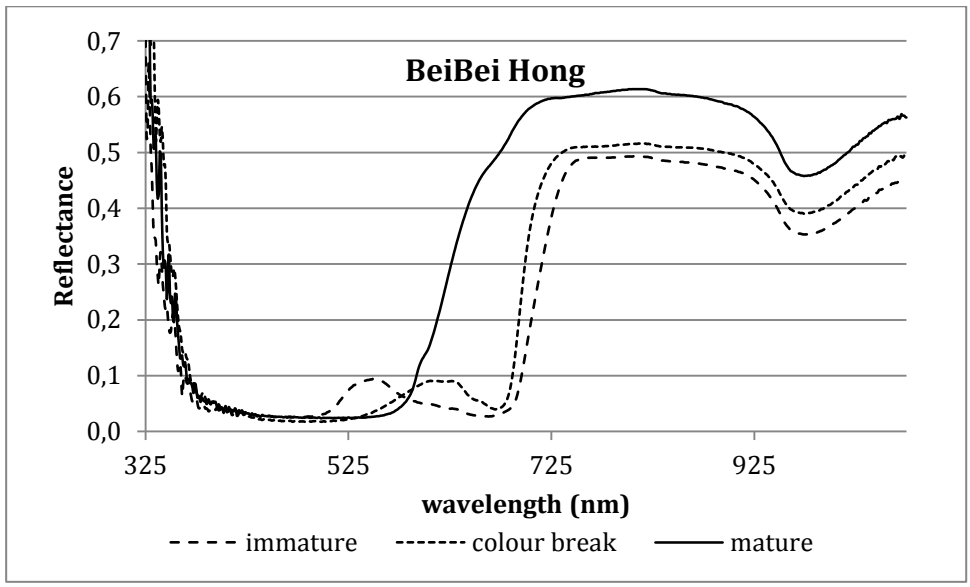
Both carotenoid component of pepper fruits continuously increased during the ripening process, and significantly differed in the three ripening stages in all the three varieties (Table 1).

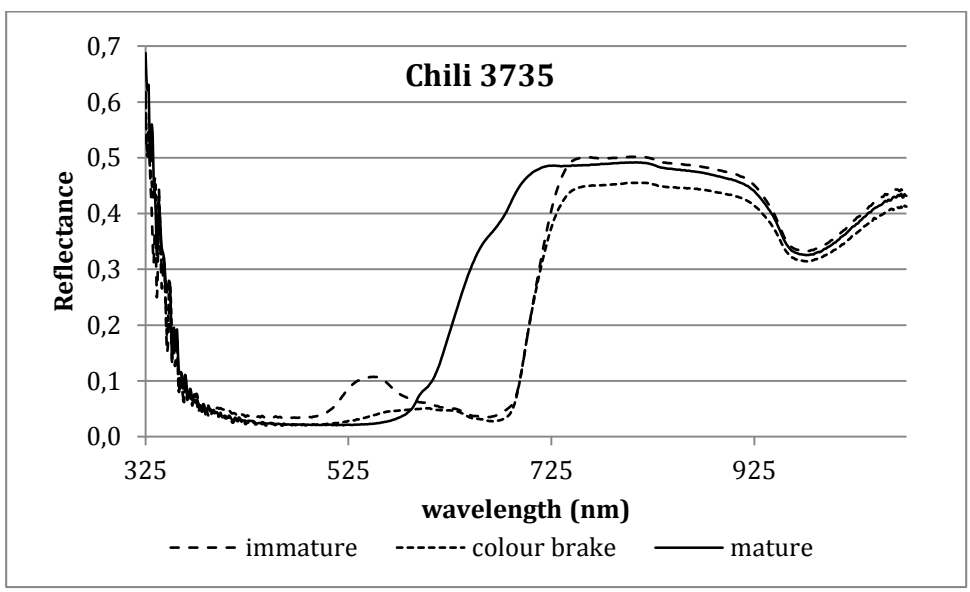
Table 1.
Total yellow (460 nm) and red (470 nm) carotenoid content (μg/g in FW) of chilli peppers (mean±SD; n=3)

Cultivars	Ripening stage		
yellow	inmature	colour break	mature
BeiBei Hong	9,18±1,49	24,05±2,02	65,08±0,98
Chilli735	7,90±1,74	16,31±6,13	97,35±12,51
Lolo	5,82±0,53	20,99±5,45	68,70±6,85
red	inmature	colour break	mature
BeiBei Hong	10,80±0,07	26,36±2,04	67,09±1,49
Chilli735	8,68±2,24	20,35±7,37	111,2±10,57
Lolo	6,25±0,53	23,23±7,87	67,78±8,76

Figure 1 shows the average reflectance spectra of chilli puree from different ripening stage combinations. Above 560 nm, reflectance values became higher, the maximum reflectance was measured between 700 and 900 nm, depending on sample. In the near infrared region there was a local absorption maximum at around 960 nm like in tomato (Szuvandzsiev et al., 2014). Treatments could be discriminated by the different average values of reflectance spectra. The

reflectance values decreased in different order in the three cultivars. Pepper cultivars differed significantly in reflectance spectra, which is in agreement with Ledóné and co-workers (2013). There are reflective peaks at 540 nm, which cause the green colour of immature fruits in all of the three cultivars. The red edge is moving into visible spectra.





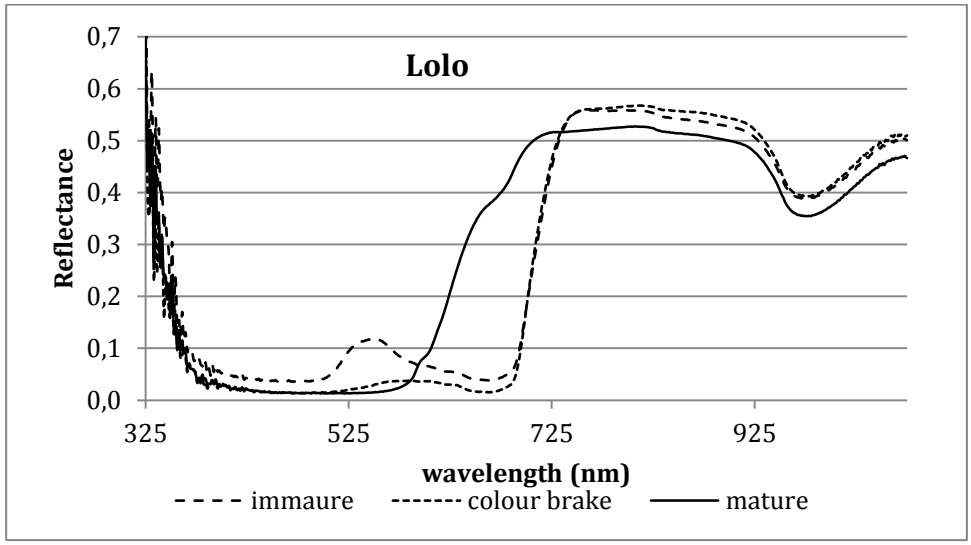


Figure 1.
Average VIS/NIR (325-1075 nm) raw reflectance spectra of chilli pepper samples from different cultivar/ripening stage combinations (n=3).

Partial least square regression (PLSR) with non-linear iterative partial least squares algorithm was applied to the fruit sample spectra.

The coefficient of determination for the calibration was R^2=0.94, for the CV was R^2=0.88. The RMSECV of the CV was 11,4 µg/g β-carotene equivalent total yellow compound.

In the red carotenoid model, the coefficient of determination for the calibration was R^2=0.75, for the CV was R^2=0.63. The RMSECV of the CV was 17.6 µg/g capsanthin equivalents in FW (Table 2).

Table 2.
Predictive capability of calibration models for total yellow and red carotenoid content after S-G 1st derivative

	Calibration		Cross-validation			
Quality parameter	R^2	RMSEC	R^2	RMSECV	RMSECV%	Factors
yellow	0.94	7.4	0.88	11.4	14,8%	6
red	0.75	12.6	0.63	17.6	22.1%	7

4. Conclusion

Our results suggest that different cultivars and ripening stages affect the important carotenoid components, which are detectable and predictable in the VIS-NIR range.

5. Acknowledgement

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SESSION 5

Molecular genetics and biotechnologies

Chairs: Sergio Lanteri, Anikó Gémes Juhász

A high quality eggplant (*Solanum melongena* L.) genome sequence

E. Portis[3], M. Delledonne[1], S. Lanteri[3], A. Dal Molin[1], A. Minio[1], A. Ferrarini[1], L. Venturini, C. Avanzato[1], L. Toppino[2], T. Sala[2], L. Bassolino[2], C. Comino[3], A. Acquadro[3], L. Barchi[3], R. Rinaldi[3], D. Scaglione[3], G. Francese[4], A. D'Alessandro[4], G. Mennella[4], D. Perrone[4], N. Acciarri[5], M. Pietrella[6], G. Aprea[6], M. Sulli[6], G. Giuliano[6], G. L. Rotino[2]

[1]*University of Verona, Italy*
[2]*CREA-ORL, Montanaso Lombardo, Italy*
[3]*DISAFA, University of Torino, Italy*
[4]*CREA-ORT, Pontecagnano, Italy*
[5]*CREA-ORA, Monsampolo del Tronto, Italy*
[6]*ENEA, Casaccia Research Center, Roma, Italy*

Abstract

Eggplant (*Solanum melongena* L. 2n = 2x = 24, projected genome size 1..3Gbp) is the third most important Solanaceous crop after potato and tomato, with a worldwide production of about 49.5 Mt in 2013 (http://faostat.fao.org), being Italy the first European producer. In addition to *S. melongena*, two other Old World *Solanum* species are commonly cultivated: *S. aethiopicum* and *S. macrocarpon* L., both native to Africa.

In the Solanaceae family, the genome sequences of potato and tomato were firstly released, and so far, eggplant genome organization was investigated merely by inspecting reduced complexity libraries. Recently a first draft genome sequence was made available (Hirakawa et al. 2014) followed by a high quality sequence we recently developed.

Unlike tomato and potato, which belong to the subgenus Potatoe, eggplant belongs to the subgenus Leptostemonum and thus at present it represents a unique member for comparative genomic analyses within the genus *Solanum.*

A reference genome and transcriptome were produced by sequencing the inbred eggplant line '67/3', which is the male parent of a RIL mapping population of 157 F6 RILs. Size-selected libraries with different insert sizes (from 270bp to 10kb) were sequenced using Illumina technology, producing approximately a 155 X coverage. The genomic sequences were also combined with Bionano Genomics optical mapping data. The obtained hybrid assembly covered ~1.2 Gb, with an L50 of >3 Mb. Of these, over 900 Mb were covered by Illumina contigs. Thanks to the sequencing of both the line 305E40 (female parent, 35X) and the RIL mapping population (1X), the hybrid assembly was finally assigned to pseudomolecules, by combinaning linkage and optical mapping information. RNA-Seq assisted annotation using Maker resulted in ~40k protein-coding genes. The genome assembly, annotation and RNASeq data are being used for comparative analyses with other Solanaceae genomes. The RIL population has been extensively phenotyped for key horticultural traits, and examples of QTL mapping will be presented.

Keywords: eggplant, Solanaceae, genome, QTL

Ultrastructural study of *in vitro* and *in situ* pepper embryo development

Zoltán Kristóf[1], Pál Vági[1], Bence Temesvári[1], Csaba Lantos[2], János Pauk[2], Anikó Gémesné Juhász[3]

[1] *Department of Plant Anatomy, Eötvös Loránd University, Budapest, Hungary*

[2] *Department of Biotechnology, Cereal Research Non-Profit Ltd., Szeged, Hungary*

[3] *Medimat Ltd, Budapest, Hungary*

kristofz@ludens.elte.hu

Abstract

DH plant regeneration from microspore is the most important biotechnological method in pepper breeding. The efficiency however is quite low. To understand the *in vitro* plant regeneration it is essential to compare it to the *in situ* embryogenesis. The structures developed from microspores in anther or microspore cultures were similar, but not identical to zygotic embryos. Plant regeneration was a kind of mixture of embryogenesis and organogenesis verifying that plants have a great plasticity, and there are different solutions of plant regeneration between pure/real embryogenesis and organogenesis.

Keywords: pepper, capsicum, microspore, embryo, androgenesis, embryogenesis, zygotic embryo, endosperm

1. Introduction

Pepper (*Capsicum annuum* L.) is one of our most important cultivated plant species, used as spice, food, medicine, dye and vitamin source. According to its importance there are huge efforts to produce better cultivars. Biotechnological methods mainly focus on the doubled haploid (DH) induction from microspore to produce homogenous genetic material for hybrid production. Applying different stress procedures like heat, cold, starvation, darkness or a combination of these tortures, researchers try to change the original gametophytic program of the microspores and force them to change their original differentiation way into a sporophytic development, namely to produce embryos instead of pollen grains. Anther and microspore cultures have different, but basically low DH plant production efficiency in pepper. It is not surprising as we have to change a genetic program without really knowing it and redirect to an embryogenic developmental pathway to another unknown direction. What is also not clear, what intensity of stress is actually needed to elicit a stress response in microspores or pollen grains to stimulate androgenesis.

After the first published success of pepper anther culture by three different laboratories (George and Narayanaswamy 1973; Kuo et al. 1973; Wang et al. 1973) the method was gradually improved (Sibi et al. 1979; Dumas de Vaulx et al. 1981; Kristiansen and Andersen 1993; Mitykó et al. 1995; Dolcet-Sanjuan et al. 1997; Kim et al. 2004; Gémes Juhász et al. 2010). Anther culture has already been applied in breeding programs (Thomas et al. 2003; Gémes Juhász et al. 2006; Mitykó and Gémes Juhász 2006, Gyulai et al. 2000). The most critical elements of the efficiency of DH production include the genotype and growth conditions of

donor plants, collection of donor buds in the optimal developmental stages of microspores, and stress treatments, as well as the composition of medium and culture conditions (Irikova et al. 2011). Although anther culture is a prospective method for the production of DH plants, an efficient protocol for a genotype-independent system is yet to be developed (Irikova et al. 2011).

Another problem with the anther culture is the presence of uncontrolled sporophytic tissues. However the haploid level of embrioids or regenerants in the culture verify their gametophytic origin to distinguish DH plants from plant of sporopytic origins is quite difficult.

The problems with anther culture, like tapetal secretion, presence of uncontrolled sporophytic cells, possibility of somatic regenerants and their genotype dependency prompted further efforts to improve anther culture and other alternative methods for DH plant production (Seguí-Simarro et al. 2011), like shed microspore culture (Supena et al. 2006) and isolated microspore culture (Kim et al. 2008).

Supena et al. (2006a) published the first microspore culture-derived haploids in Indonesian hot pepper. Isolated microspore culture was established and developed for a hot pepper genotype, 'Milyang-jare' (Kim et al. 2008). Isolated microspore cultures of Hungarian and Spanish spice pepper genotypes were improved by using the wheat ovary co-culture method (Lantos et al. 2009). Experiments conducted with sweet pepper (Capsicum annuum L.) aimed at improving the efficiency of microspore cultures (Gémesné Juhász et al. 2009; Parra-Vega et al. 2010; Seguí-Simarro et al. 2011; Pauk et al 2010; Lantos et al. 2012).

As microspore derived embryogenesis involves reprogramming of the gametophytic cell or cells towards embryogenesis, it is essential to use functional genomic tools that allow the identification of genes associated with microspore embryogenesis. As genetic approaches to identify genes associated with induction of androgenesis have been unsuccessful for a while it lead to the hypothesis that downregulation of gametophytic genes is more important than induction of sporophytic genes (Harada et al. 1986). By now however more than a dosen of genes in wheat, barley and rapeseed were found to be involved in microspore embriogenesis (Joosen et al. 2007; Maliket al. 2007; Maraschin et al. 2006; Muñoz-Amatriaín et al. 2006), such studies still missing in pepper. The genes associated with different stages of microspore embryogenesis are upregulated in good embryogenic lines compared to the low activity, or even downregulation in recalcitrant genomic lines (Żur et al. 2014), while some of the genes related to gametophytic development downregulated. The reprogramming by stress was marked by the upregulation of transcripts involved in sugar and starch hydrolysis, proteolysis, stress response, inhibition of programmed cell death, and signaling and downregulation of genes related to lipid biosynthesis, cell division.

Besides molecular biology approaches ultrastructural studies can help us to understand the developmental changes during androgenesis. To describe the embryogenesis in anther or microspore culture in detail it is very useful to compare it to the *in situ* embryo development of pepper. Unfortunately there are only sporadic light- and electron microscopy studies on *in situ* pepper embryo development. On the basis of the division pattern of zygote and proembryo, *Capsicum annuum L*. belongs to the *Onagrad* group, similar to *Arabidopsis* and *Capsella bursa-pastoris* (Johri et al. 1992). The further development is better known mainly because of the embryo rescue studies. (Manzur et al. 2013). Even less is known about the early stages of endosperm development however it has a great importance in embryo nutrition.

We carried out light and electron microscopy studies on pepper embyogenesis both *in situ* and *in vitro*. In these studies we focused on to structural similarities or differences in development, to learn more about microspore derived plant regeneration.

2. Materials and methods

2.1. Plant material for in situ and anther culture

Twelve sweet pepper genotypes, F1 breeding materials (B1; B2; B3; B4; B5; B6) were used to carry out the experiments.

B1 F1 (Turkish sivri type, B2 F1 (Kapya type), B3 F1 (Charliston type), B4 F1 (dark green blocky type), B5 F1 (light green blocky type), B6 F1 (lamuyo red type)

2.2. Donor plant growth conditions

The germinating and growing of the donor genotypes was conducted in the greenhouse under a 16 h photoperiod with a temperature of 25-30°C in the daylight and 15-19°C during the dark. After pollination the plants were grown in 16h photoperiod illuminated with 400 µmól foton/ m^2/s in almost constant 20-22 °C temperature.

2.3. Anther culture

In vitro anther culture is routinely used to produce homozygous pepper plants. For anther culture we use the protocol described by Dumas de Vaulx et al.,1981 with modification of Mityko and Gémes Juhász, 2006. This protocol proved to be efficient and results in high amount of haploid and spontaneous DH pepper plants.

2.4. Microspore culture

Plant material and culture techniques has already published in Lantos at al 2012.

2.5. Microscopy and microtechnique

Samples were fixed in a 4% paraformaldehyde solution buffered to pH 7.2 and infiltrated under a slight vacuum. Developing pepper seeds testa was partially removed with razor blade under dissecting microscope to improve penetration. Fixed samples were washed in PBS buffer, and for transmission electron microscopy postfixed in 1% osmium-tetroxid solution. Samples were dehydrated in graded ethanol series. After dehydration samples were embedded in Durcupan (Fluka) resin or for light microscopy in JB4 resin. For light microscopy 4 µm-thick sections were cut with a Microm HM 360 (Zeiss) microtome from both resin. The sections were heat-dried on slides. For routine microscopy sections were stained in an aequous solution of 0.5% toluidine-blue and 0.1% Na_2CO_3 for 5 min. Photographs were taken with Olympus BH2 microscope. For transmission electron microscopy 70 nm sections were cut with diamond knife (Diatome) with Ultracut (Reichert-Young) ultramicrotome. Sections were stained with lead citrate and uranyl acetate and visualized by Hitachi 7100 TEM.

Correlative light and electron microscopy, namely the resectioning of light microscopy sections for electron microscopy was carried out according to the protocol developed by Kristóf Z. 1997. For scanning electron microscopy the dehydrated samples were critical point dried (CPD 7541, Polaron) and vacuum evaporated with gold. Samples were visualized by Hitachi NSEM 3600.

3. Results and Discussion

In situ zygotic pepper embryo development starts quite slow, the embryo reach the globular stage in two weeks -17 days after pollination (DAP) and the heart stage in 20 DAP. It can vary 1-2 days depending on genotypes. The fertilized central cell starts to divide first, so the proembryo grows into the developing endosperm tissue (fig 1). Endosperm has a well-defined outer cell layer. Parallel to the volume increase of endosperm the nucellar tissue has to shrink. An interface layer can be seen between the endosperm surface and the intact nucellar cells. This layer contained mainly cell wall remnants of degenerated nucellar cells (fig. 2). This layer has a weak affinity to toluidine blue O, but with Nomarsky interference contrast or phase contrast microscopy the presence of wall remnants can be visualized. As the whole nucellus disappears by the time of seed ripening, the wall material has to be dissolved as well. Similar situation can be observed at the border of developing embryo and endosperm tissue (fig. 2D). The inner part of the endosperm has to disappear as the developing embryo requires more room and nutrients. At heart stage the suspensor degenerates and the embryo is fed directly by the endosperm that is still developing. Endosperm is a heterogeneous tissue, continuously dividing, and increasing in volume. Pepper seed has no perisperm, so the nucellus has to dissolve completely. The cytoplasm and cell wall materials transported to the endosperm and partially to the embryo itself.

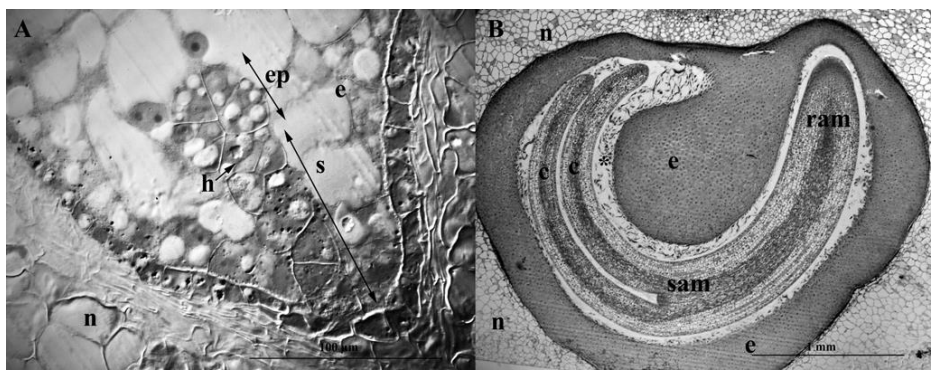

Figure 1.
In situ zygotic embryogenesis. A. Proembryo development 13 DAP. B. "Walking-stick" developmental stage. c-cotyledon, e-endosperm, ep-embryo proper, h-hypophysis, n-nucellus, ram-root apical meristem, s-suspensor, sam-shoot apical meristem

Anther culture is the most frequently used technique of pepper DH production. Scanning electron microscopy proved that not only microspores are activated in the culture. It is most obvious in the connectivum (fig 3). The connectivum proliferated and elongated producing a callus-like surface. The anther wall layers remained relatively unchanged during culture. In spite of the high activity of connective tissue the developing plants were all haploids.

In microspore culture, dividing microspores could follow either the original gametophytic way or turn into a sporophytic development. The most obvious difference between them was the mode of cell division (fig.4). In the case of "gametophytic division", there was no cell wall formation after cytokinesis and the division itself was asymmetric. The sporopytic divison was symmetric and followed by cell wall synthesis. Dividing cells remained inside the microspore wall for a while than the microspore wall broke and cells were released. Those cells that were unable to get out from microspore wall produced smaller and smaller daughter cells with extremely wavy cell walls and finally died. Surviving cell groups can develop into embryo like structures or callus. The latter structures have large vacuolated cells from the very beginning,

so they can be distinguished from embryoids at the time of release from microspore wall. Embryo like structures never developed suspensor. The first divisions were different from the division pattern of zygotic proembryo. The first two subsequent divisions were oblique similar to the divisions of the apical cell during in situ embryo development (fig.4).

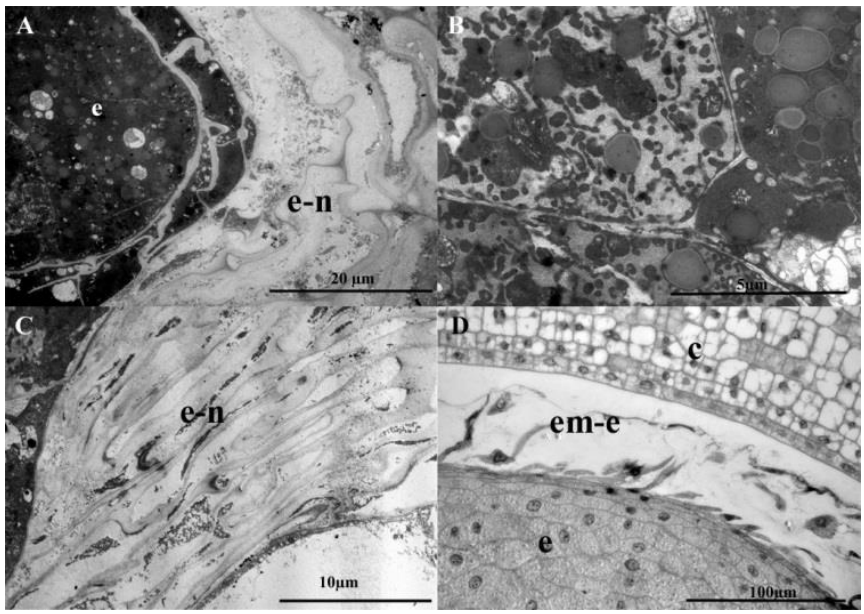

Figure 2.
Endosperm development. A. dens endosperm and nucellus wall remnants. B. Endosperm cells with dilated ER. C. Degraded nucellus. D. Embryo- nucellus border. c-cotyledon, e-endosperm, em-e embryo-endosperm interface, e-n endosperm-nucellus interface

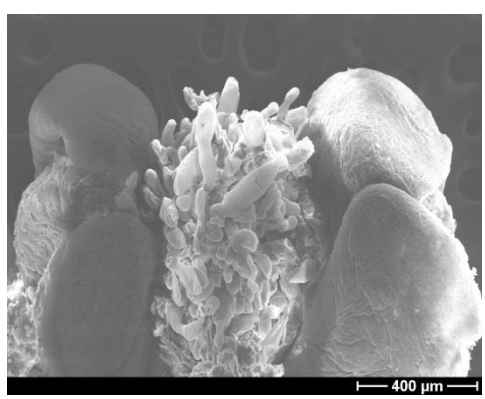

Figure 3.
SEM image of cultured anther, with active connectivum cells in the middle.

During microspore derived embryogenesis the embryo like structures resembled the stages of zygotic embryo development, but these structures were bigger, and contained much more cells (fig.5). There were histological differences as well. An *in vitro* globular or heart shape structure already had well defined central cylinder with xylem elements and root hairs on the

surface. These structures could develop directly to plantlets, but in many cases not only one shoot apical meristem has organized. This type of development was a mixture of embryogenesis and organogenesis. As in our system pure embryogenesis has never occurred we prefer the term embryoid or embryo-like structure.

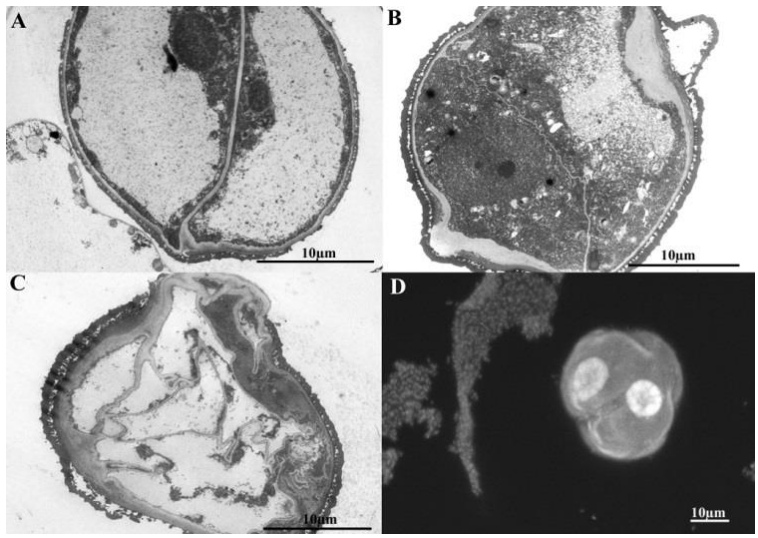

Figure 4.
Dividing microspores A-C TEM image, D fluorescence image A. Equal division with prominent wall formation. B. Tiny wall separates the daughter cells. C. Multiple division inside the microspore wall. The cells degenerated. D Equal division, DAPI staining.

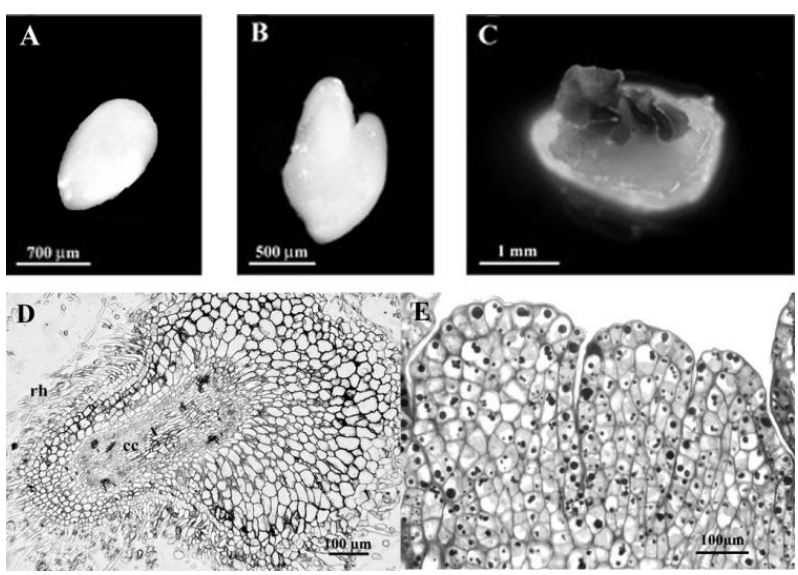

Figure 5.
Embryo like structures in microspore culture. A "globular" stage. B. "Heart shape" stage. C. Multiple shoot organization. D. Longitudinal section of an embryo like structure similar to picture A. E. Multiple meristem formation. cc-central cylinder, rh-root hairs, x-xylem elements

4. Acknowledgements

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MicroRNA156/7-mediated control of anthocyanin pigment accumulation in eggplant fruit skin

L. Chappell-Maor[1], S. Meir[1], I. Rogachev[1], T. Sala[2], L. Toppino[2], G. L. Rotino[2] and A. Aharoni[1]

[1] *Weizmann Institute of Science, Department of Plant & Environmental Sciences, Rehovot, Israel*

[2] *CREA-ORL, Research Unit for Vegetable Crops, Montanaso Lombardo, Italy*

Abstract

MicroRNAs (miRNAs) are known to play key roles in many developmental processes and stand at the core of regulatory networks. MiRNAs have been sequenced and studied in some aspects of Solanaceae growth and development, but very little is known regarding their role in fleshy fruit. We have taken miRNA156/7 as an example of a well-conserved plant miRNA, this miRNA is known to directly target a family of Squamosa Promoter binding-Like (SPL) transcription factors through sequence-specific recognition, and has been shown to play a profound effect on the timing of vegetative phase change and plant architecture in a wide variety of plant species.

Overexpression of miRNA156/7 in eggplant has a distinct negative effect on the production of anthocyanin pigments at the early stages of fruit development, whilst not influencing the accumulation of the yellow pigment naringenin chalcone at later stages of ripening. The point of action of miRNA156/7 within the phenylpropanoid pathway is therefore very specific. In order to elucidate this, we have performed detailed transcriptomic and metabolomic analyses at several stages of ripening in miRNA156/7 overexpression lines. This has enabled us to identify several direct *SPL* gene targets of the miRNA, plus putative downstream targets of the SPLs which may represent the point of regulation within the phenylpropanoid pathway. Validation of these targets is currently ongoing. The study of eggplant fruit displaying a unique pattern of flavonoid pigment accumulation in the skin has provided us with insights of how a well conserved miRNA controls metabolic processes in a species-specific manner.

Keywords: Gene expression**,** Transcription factor, Ripening, *Solanum melongena*

Interactions between *Bell pepper endornavirus*, bell pepper, and acute plant viruses

Cesar Escalante[1], Noa Sela[2], Aviv Dombrovsky[2] and Rodrigo Valverde[1]

[1] *Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, USA*

[2] *Department of Plant Pathology, Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel*

Abstract

Persistent plant viruses do not cause detectable symptoms in their plant hosts. In contrast, acute viruses cause symptoms and, in most cases, disease. Most bell pepper (*Capsicum annuum*) cultivars are infected with the persistent virus *Bell pepper endornavirus* (BPEV). To study possible interactions between endornaviruses, the host, and acute viruses, we developed two near isogenic lines of the bell pepper cultivar Marengo: one BPEV-infected and the other BPEV-free. Some agronomic characteristics of the two lines were evaluated under greenhouse condition. The two lines were mechanically inoculated with *Pepper mild mottle virus* (PMMoV), *Potato virus Y, Tomato spotted wild virus* (TSWV), and *Cucumber mosaic virus*. Symptoms were recorded and relative amounts of PMMoV and TSWV were determined by ELISA. Preliminary studies on differential gene expression were conducted using RNAseq data. Overall, there were no significant differences in fruit yield and plant phenotype. After acute virus inoculations, there were no differential symptoms between the two lines. Results of the ELISA test showed that the BPEV-infected line yielded consistently less PMMoV than the BPEV-free line; however, the differences were not statistically significant. Results obtained in this investigation suggest that at the symptom level, limited interactions take place between endornaviruses and acute viruses. However, at the molecular level, interactions were obtained.

Keywords: Capsicum, persistent viruses, acute viruses, RNAseq

1. Introduction

Peppers are cultivated throughout the world for their nutritional and cooking condiment value (DeWitt and Bosland, 1996). *Capsicum annuum* is the species most widely cultivated. Diseases caused by acute viruses such as *Cucumber mosaic virus* (CMV), *Pepper mild mottle virus* (PMMoV), *Potato virus Y* (PVY), *Tobacco etch virus, Tomato spotted wild virus* (TSWV), and several begomoviruses are a limiting factor in pepper production in many parts of the world (Black *et al*., 1991).

The family *Endornaviridae* includes linear RNA viruses that infect plants, fungi, and oomycetes. Their genome ranges from 10-17.6 kb, lack coat protein, and in plants do not cause detectable symptoms (Fukuhara and Moriyama 2008). Plant endornaviruses (*Endornaviridae*) are persistent RNA viruses that do not cause detectable symptoms. In contrast, acute RNA viruses cause symptoms and in most cases disease. Endornaviruses have been reported to infect economically important crops including avocado (Villanueva *et al*., 2012), barley (Candresse *et al*., 2016), bell pepper (Okada *et al*., 2011), common bean (Okada et al., 2013) fava bean (Pfeiffer, 1998), melon (Sabanadzovic *et al*., 2016), and rice (Fukuhara and Moriyama, 2008).

Most bell peppers (*C. annuum*) cultivars and other capsicum species are found infected with

the well characterized endornavirus: *Bell pepper endornavirus* (BPEV) and related strains (Okada *et a*l., 2011; Jo *et al*., 2016). *Bell pepper endornavirus* (BPEV), a persistent virus of pepper, has a linear genome of 14,727 bp and contains a single, long ORF encoding a 4815 aa protein (Okada *et al*., 2011). The virus was detected in all bell pepper cultivars tested and transmitted through seed but not by graft inoculations. RT-PCR using degenerate primers revealed variants of BPEV, or closely related species, infecting other *C. annuum* genotypes and three other *Capsicum* species (*C. baccatum, C. chinense* and *C. frutescens*) (Okada *et al*., 2011).

Little is known about the effects that endornaviruses have on plants. The results of testing for the presence of endornaviruses in various crop cultivars suggest that endornaviruses have been introduced into most cultivars of melon, and pepper (Okada *et al*., 2011, 2013; Sabanadzovic *et al*., 2011). In the case of endornavirus infecting melon and bell pepper, all tested cultivars of these two crops were infected (Okada *et al*., 2013; Sabanadzovic *et al*., 2016). Therefore, it appears that during the development of bell pepper and melon cultivars, plant breeders, and possibly people involved in earlier domestication of these crops, unaware of the existence of endornaviruses in the germplasm, selected endornavirus-infected genotypes. This could be an indication that the presence of endornaviruses in these crops is beneficial.

Interactions between endornaviruses and plant pathogens such as acute viruses, fungi or bacteria, have not been studied. It is possible that like acute viruses, endornaviruses could affect the host response to infection by any of these pathogens or other biotic agents.

We have conducted preliminary studies on the interactions between BPEV, bell pepper, and selected acute viruses. The objectives of this investigation were: to evaluate the phenotype and fruit yield of endornavirus-free and endornavirus-infected Marengo bell pepper near-isogenic lines under greenhouse conditions; study the interactions of four acute viruses, CMV, PVY, TSWV, and PMMoV with BPEV in BPEV-infected and BPEV-free Marengo bell pepper isogenic lines; and to conduct Next-Generation Sequencing (NGS) of RNA from the two bell pepper lines infected with selected acute viruses.

2. Materials and methods

Plant materials and viruses. Using the backcross breeding method, we developed two near isogenic lines of the bell pepper cultivar Marengo: one BPEV-infected and the other BPEV-free. These lines were used in all comparative studies. Virus isolates used in this study consisted of local isolates stored as desiccated tissue in the laboratory.

BPEV detection. DsRNA from BPEV-infected plants and from plants inoculated with PMMoV was extracted using the method reported by Valverde *et al.* (1990). Purified dsRNA was analyzed in agarose gel electrophoresis. Alternatively BPEV ssRNA was detected by RT-PCR as described previously (Okada *et al*., 2011).

Planting. Seeds were planted in steam sterilized soil mix. One month after planting, seedlings were transplanted into 6 L plastic pots. Twenty pots (10 for each line), each containing one plant were placed randomly in the greenhouse. Osmocote© (19-6-3) was incorporated during soil mix preparation. The phenotype of the plants, including time of flowering and fruit setting, were evaluated throughout all developmental stages. Mature, fruits from each line were harvested, counted, measured, weighed, and ANOVA performed to determine fruit yield variations.

Virus inoculations. The two near-isogenic lines were mechanically inoculated with PMMov, CMV, TSWV, and PVY to study the host reaction. Viruses were mechanically inoculated using crude sap diluted in phosphate buffer pH 7.2. Based on preliminary inoculation results, together with available information on the dilution end point for these two viruses, a 1:20 dilution of sap

extracted from systemically infected leaves (2-week-old infection) of Tabasco pepper was used. Negative controls consisted of mock inoculated plants. At least eight 4-week-old plants of each line were inoculated with each acute virus by rubbing diluted crude sap onto carborundum-dusted primary leaves. Plants were kept in a greenhouse and symptoms recorded during the first 6 weeks. One and two weeks after inoculation, 1.0 g samples were taken for ELISA testing. RNA was extracted two weeks after inoculation using Qiagen's RNeasy Plant Mini Kit. RNA was quantified, quality assessed and used for RT-PCR testing and NGS. RNAseq libraries were prepared with Truseq protocol and sequenced as single-end with Illumina 2000 at the Technion-Institute of Technology, Israel. Obtained RNA sequences were analyzed using bowtie2 (Langmead and Salzberg, 2012) for RNAseq mapping, RSEM (Li and Dewey, 2011 for transcription quntification and egdeR package (Robinson et al., 2010) for statistical analysis of the data.

3. Results and discussion

The phenotype of the two near isogenic lines of bell pepper Marengo was similar (Figure 1).

Figure 1.
Marengo bell pepper lines, one infected with BPEV (BPEV+) and the other BPEV-free (BPE-)

The plant height, total fresh weight, fruits per plant and total fruit weight was higher for BPEV- than for BPEV+, although, not significant in the case of total fruit weight (Figures 2 and 3).

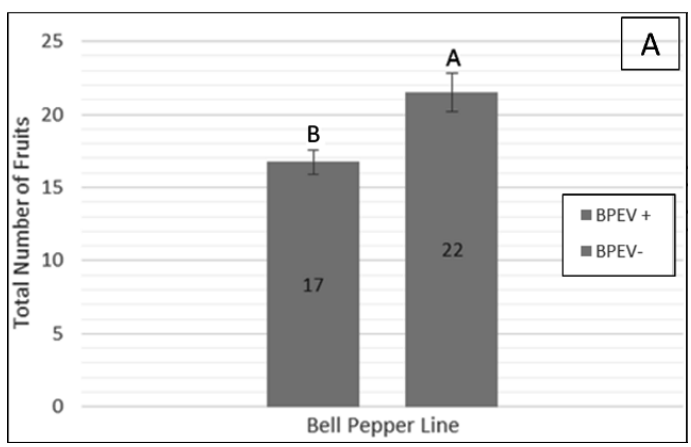

Figure 2.
Total number of fruits obtained from two near-isogenic bell pepper lines, one infected with BPEV (BPEV+) and the other BPEV-free (BPE-).

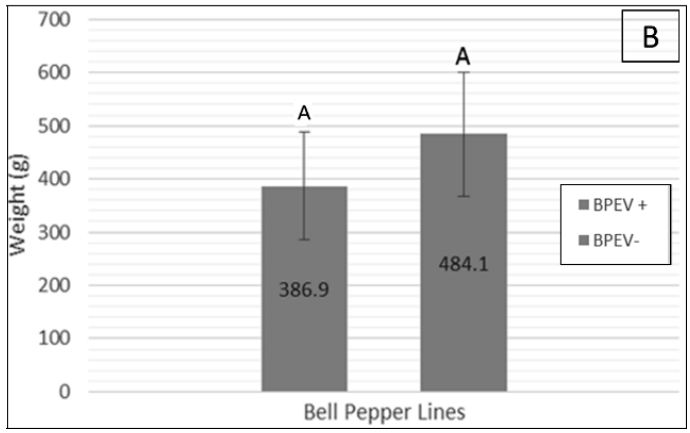

Figure 3.
Total fruit weight obtained from two near-isogenic bell pepper lines, one infected with BPEV (BPEV+) and the other BPEV-free (BPEV-).

Biological interactions between CMV, PVY, TSWV, and BPEV were limited and in most cases symptoms caused by these viruses on the two Marengo bell pepper lines were undistinguishable (Figure 4).

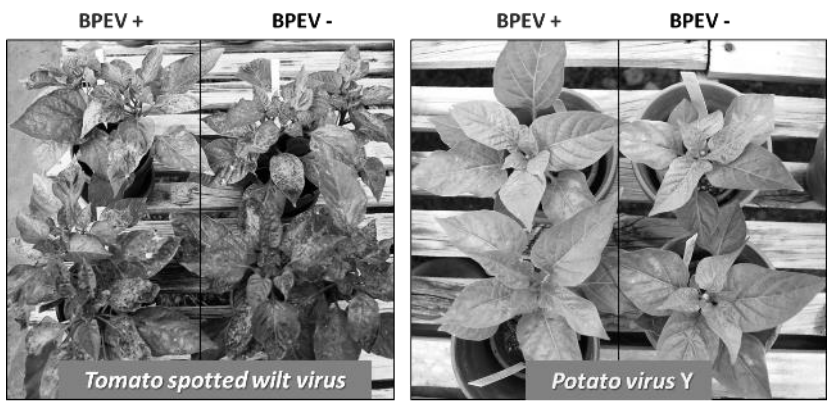

Figure 4.
Symptoms on two bell pepper near-isogenic lines, one infected with BPEV (BPEV+) and the other BPEV-free (BPE-) after inoculation with TSWV and PVY.

ELISA results for PMMoV infections showed a difference (Figure 5). The BPEV + line yielded lower readings than the BPEV- lines. Nevertheless, the difference was not significant. DsRNA yields of both, BPEV and PMMoV were not affected.

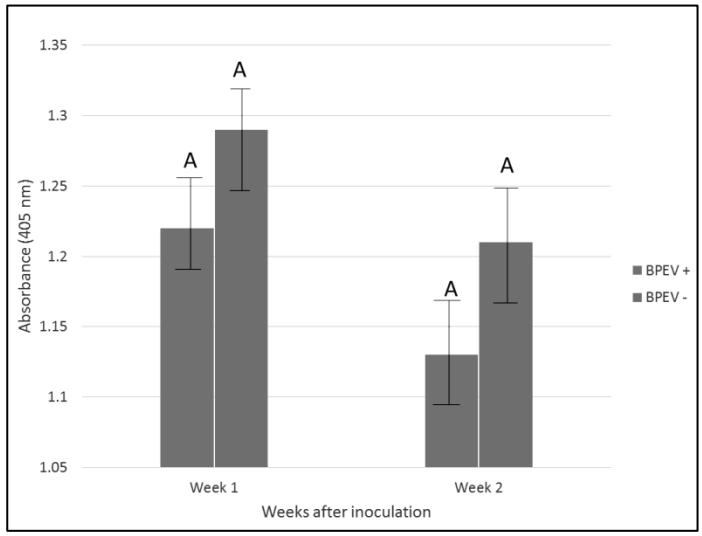

Figure 5.
Results of ELISA testing of two bell pepper near-isogenic lines, one infected with BPEV (BPEV+) and the other BPEV-free (BPE-) after inoculation with PMMoV.

Preliminary results of the RNA sequence analyses revealed variations in gene expression between the two bell pepper lines suggesting molecular interactions between BPEV and the host. Transcriptome analysis of peppers infected with PVY, TSWV, or CMV with or without the presence of BPEV revealed various levels of differential gene expression and that the plant response to infection is virus specific (Figures 6 and 7). We plan to further characterize the mechanism underlying the difference in the response of the two bell pepper lines to infection by these viruses.

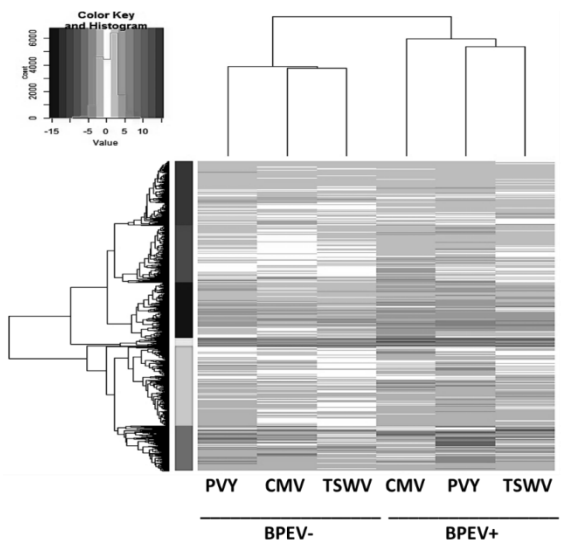

Figure 6.
Heat map showing differential gene expression of the various sample treatments.

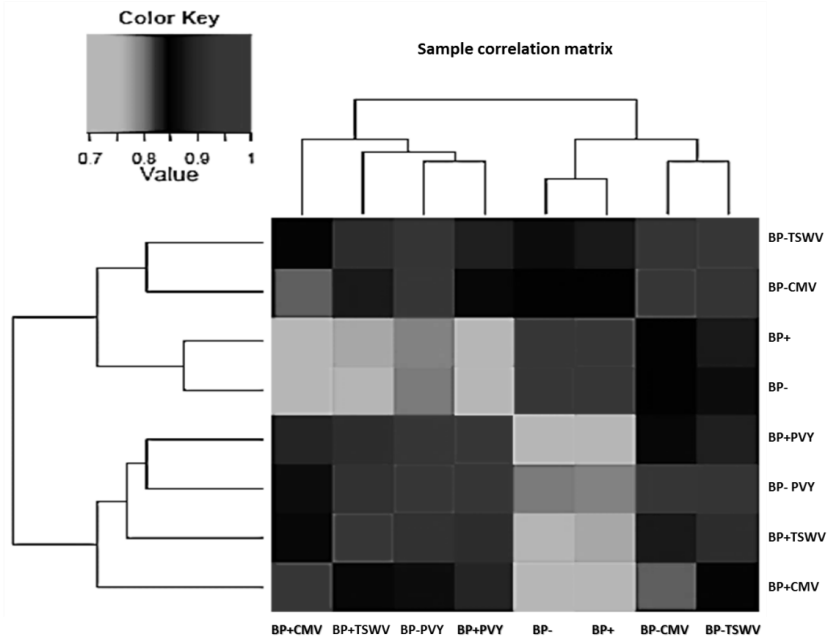

Figure 7. *Clustering using a correlation matrix resulting from the comparison of the transcript expression values. BP-=BPEV free line; BP+=BPEV infected line.*

4. Acknowledgments

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The transcriptomes of *Solanum incanum* and *S. aethiopicum* provide information of relevance for common eggplant breeding

Pietro Gramazio, José Blanca, Peio Ziarsolo, Francisco J. Herraiz, Mariola Plazas, Jaime Prohens and Santiago Vilanova

Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, València, Spain

Abstract

Common eggplant (*Solanum melongena*) is a staple food for millions of people. Unfortunately, part of production is lost due to pest and disease attacks and to abiotic stresses. In other crops, like tomato, some of these problems have been addressed by exploiting the great genetic diversity present in related species. In order to obtain information of relevance for common eggplant breeding through the use of diversity present in other species, the sequencing of transcriptomes of related species can be of interest. Here we present the de novo transcriptome assembly, comprehensive analysis and molecular-marker discovery of *Solanum incanum* and *S. aethiopicum*, two species belonging to the common eggplant genepool. In order to obtain the transcriptomes, we pooled RNA of young leaf, floral bud and young fruit tissues in order to perform RNA-Seq. This generated over one hundred millions raw reads per species. The assembly of the transcriptomes resulted in more than 80.000 unigenes, which were annotated. Detection of SNVs and SSRs was performed by mapping *S. incanum* and *S. aethiopicum* reads against the eggplant genome. In order to identify genes of potential interest for breeding, detection of potential resistance genes, transcription factors and candidate genes for stress resistance was performed. The genomic information, molecular markers generated and genes identified in these two non-model species will be extremely useful in the eggplant breeding programs.

1. Introduction

Eggplant (*Solanum melongena* L.), also referred to as common eggplant, production has almost doubled in the last 15 years [1], and the trend is expected to continue in the coming years. However, up to now, and contrarily to what occurred in tomato, eggplant relatives have been barely used in eggplant breeding [2]. Also, genomics studies in eggplant and its relatives have been limited when compared with other important crops. In this respect, several intraspecific and interspecific genetic maps, collections of molecular markers, a transcriptome assembly and a draft genome sequence are available [3-5].

Given that eggplant relatives are known to be resistant or tolerant to biotic or abiotic stresses [2,6,7], these materials are of great interest for eggplant breeding in particular for adaptation to climate change challenge.

Among the cultivated eggplants the scarlet eggplant (*S. aethiopicum* L.) is an important vegetable crop in Africa and can be hybridized with *S. melongena* [8]. Scarlet eggplant presents some resistances and tolerances to diseases [9], and being a cultivated eggplant it has the advantage that it does not display undesirable traits commonly present in wild species. The interspecific hybrids between *S. melongena* and *S. aethiopicum* are very vigorous and are used as rootstocks for commercial production of eggplant in several countries [10].

Another interesting species related to common eggplant is the wild *S. incanum* L., which grows in desertic and semi-desertic areas of African and the Middle East [11]. This species is highly tolerant to drought and also presents some resistances to diseases [12]. Interestingly, it also presents high levels of bioactive phenolics [13]. Furthermore, the hybrids of *S. incanum* with *S. melongena* are fully fertile [12].

Here we present the results of the sequencing of the transcriptomes from *S. incanum* and *S. aethiopicum*, including their assembly, annotation, detection of genes of potential interest for resistance and tolerance to stresses, and the discovery of molecular markers. The results obtained will provide information of relevance for the breeding of common eggplant, and in the case of the transcriptome of *S. aethiopicum* also for scarlet eggplant breeding.

2. Material and methods

2.1. Plant material and RNA extraction

We used an accession of *S. aethiopicum* (BBS135), which belongs to the Gilo group, which is the most economically important cultivar group of scarlet eggplant [7]. In the case of the wild *S. incanum*, we used accession MM577, which was used for the construction of an interspecific genetic map of eggplant [3]. The plants of both accessions were cultivated in a greenhouse and tissue and from one plant of each accession we collected young leaf, floral bud and young fruit. Total RNA was extracted from 100 mg of tissue using the TRI Reagent® Protocol (Sigma-Aldrich, St. Louis, USA). After confirming RNA integrity and quantification, equal amounts of total RNA from each tissue were pooled for each accession.

2.2. Sequencing and de novo assembly

The RNA samples were sent for sequencing to Macrogen Korea (Seoul, South Korea). After the construction of paired-end library (insert size of 300 bp), RNA-Seq was performed in HiSeq 2000 sequencer (Illumina, San Diego, USA). The quality of the reads was checked using the FastQC program [14]. The raw reads were pre-processed and trimmed using in-house developed software, NGS_CRUMBS [15]. The trimmed reads were finally assembled into transcripts using Trinity [16]. The most expressed transcripts of each Trinity transcript cluster were selected to create a set of unigenes for each species.

2.3. Annotation and mapping against eggplant genome

The assembled transcripts were compared using BlastX (cut-off value of 1e-20) against Swiss-Prot, ITAG2.4, Arabidopsis and UniRef90 public protein databases and subsequently, a functional annotation was performed using the Blast2GO software [17]. Blast2GO was used also to obtain the KEGGs pathways from KEGG database. The high-quality clean reads were aligned against the eggplant genome using the Top Hat program. After that, the reads were realigned using the GATK software [18]. The FASTA sequence of the draft eggplant genome was downloaded from the Eggplant Genome Database [19].

2.4. Detection of stress genes and transcription factors

Unigenes were compared using Blastn (cut-off value of 1e-20) against the *plant resistance gene database (PRG) selecting Solanaceae species [20]*. Unigenes were also compared using Blastn (cut-off value of 1e-40) against *Solanum lycopersicum* transcription factors database

located in Plant Transcription Factor Database (PlantTFDB) [21]. Finally, the unigenes were compared using Blastn (cut-off value of 1e-40) *to candidate genes related to salt stress and drought in S. pennellii [22]*.

2.5. Molecular markers discovery

Single nucleotide variations (SNVs), consisting of SNPs and INDELs were detected using the FreeBayes software [23]. Different filters have been applied to VCF file (Variant Call Format) in order to maximize the polymorphism validation. The annotation of EST-SSRs was carried out with Sputnik software [24], selecting the sequences containing ≥ 9 di-, ≥ 6 tri, or ≥ 4 tetranucleotide motifs. The sequences of unigenes which contain EST-SSR were blasted against the eggplant genome database in order to know their physical position while their region in the transcripts (ORFs, 3'-UTR and 5'-UTR) were detected using the Bedtools utilities.

3. Results and Discussion

3.1. Illumina paired-end sequencing and EST assembly

A total of 105,625,594 and 114,162,500 raw reads were obtained from *S. incanum* and *S. aethiopicum* respectively (Table 1). After the filtering and trimming process more than 90 million high-quality sequences were obtained for each of *S. incanum* and *S. aethiopicum*.

	S. inc.	*S. aeth.*
Raw reads	105,625,594	114,162,500
Filtered reads	91,579,142	99,012,712
Transcript	108,322	106,66
Unigenes	83,905	87,084
Max lenght	12,181	12,159
Av. lenght	696	722
Residues	58,447,674	62,899,378

Table 1
Statistics of S. incanum and S. aethiopicum assembled transcripts and unigenes, using Trinity software.

The trimmed reads were assembled into transcriptomes generating more than 100,000 transcripts for both species. In order to obtain a set of single-copy gene locus (unigene), only the most expressed transcript from the isoforms of each locus were chosen. A total of 83,905 unigenes were identified in *S. incanum* and 87,084 in *S. aethiopicum* (Table 1).

3.2. Annotation of S. incanum and S. aethiopicum transcriptomes

A total of 30,630 (36.5%) *S. incanum* and 34,231 (39.3%) scarlet eggplant unigenes have shown at least one hit in the protein databases. The total number of unigenes is consistent with the number of genes described in tomato (33,837 genes) and in previous works in other plant species [25,26]. A total of 136,904 and 109,044 GO terms were assigned to 25,650 (30.5%) and 25,169 (28.9%) unigenes in *S. incanum* and scarlet eggplant, respectively. The majority of GO

terms (44.6% for *S. incanum* and 47.4% for *S. aethiopicum*) were related to biological processes. The annotated unigenes were blasted against the KEGG pathway database. A total of 794 *S. incanum* unique unigenes were assigned to 146 KEGG biological pathways. Regarding scarlet eggplant, 891 scarlet eggplant unique unigenes were ascribed to 147 KEGG pathways (Figure 1).

A best reciprocal Blast hits was performed with the tomato reference genome (version SL2.50). A total of 16,388 (19.5%) and 17,630 (20.2%) unigenes have presented orthologs with tomato reference genome in *S. incanum* and scarlet eggplant, respectively, while 46,498 orthologs were found when considering the two transcriptomes. Regarding structural annotation, 35,943 (42.8%) ORFs were predicted in *S. incanum* unigenes while 40,353 (46.3%) were predicted in *S. aethiopicum*.

3.3. Detection of potential resistance genes, transcription factors and candidate genes

Solanum incanum presents resistance at some fungal diseases, like *Fusarium oxysporum* and *Phomopsis vexans* [27,28]. Also, scarlet eggplant shows resistance to fungi (*Fusarium oxysporum*, *F. solani*, *Pythium vexans*, *Phytophthora parasitica*), bacteria (*Ralstonia solanacearum*), insect (*Leucinodes orbonalis*) as well as root-knot nematodes (*Meloidogyne incognita*) [9]. *To elucidate the potential genes responsible of these resistances, we performed a BLAST of the transcripts against plant resistance genes of tomato presents in PRG database. We found 101 transcripts in S. incanum versus 401 found in S. aethiopicum (Figure 1). The four-fold increase in the number of transcripts involved in resistance is consistent with the data found in the literature [6,9], where more resistance genes have been described in S. aethiopicum. These genes will help to increase the resistance of eggplant to different diseases.*

Transcription factors are able to activate or silence several important pathways that can modify complex traits in crop plants [29]. For instance, it is known that MYB1 factor is involved in the regulation of both anthocyanin and polyphenol biosynthetic pathways [30].

For this reason it is important to detect the different transcription factors that have been expressed in different species with different important traits. The search against S. lycopersicum transcription factors database resulted in *1474 transcripts belonging to 54 different types of transcription factors in S. incanum.*

Similar results were obtained in S. aethiopicum, where 1542 transcripts matched with 55 different types of Transcription factors (Figure 1).

Resistance and tolerance to abiotic stresses have been described in common eggplant relatives [2-6]. To reveal potential genes involved in these stresses, we compare our unigenes against candidate genes for stress tolerance detected in *S. pennellii* [22]. We found 490 and 544 unigenes in *S.incanum* and *S. aethiopicum* respectively *(Figure 1)*. These unigenes could be the starting point of new investigation that could, for instance, allow the transference of resistance to drought or salinity into eggplant genetic background.

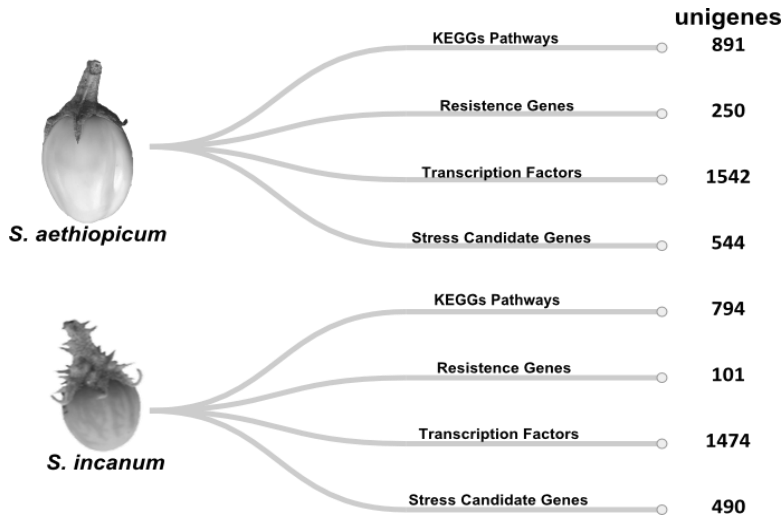

Figure 1
Summary of the unigenes assigned to KEGGs pathways, resistance genes, transcription factors and stress resistance genes in S. incanum and S. aethiopicum

3.4. Molecular markers discovery

Hundreds of thousand of molecular markers have been discovered, which will be very useful for breeding programs as well as for diversity studies. *Solanum aethiopicum* presented the highest value of intraspecific SNVs (SNPs and INDELs), with 159,571 SNPs and 4,556 INDELs. Many less intraspecific variations (12,396, of which 11,861 were SNPs and 535 INDELs) were identified in *S. incanum*. This indicates that the scarlet eggplant accession used presented a much larger degree of heterozygosity than the *S. incanum* accession (Table 2).

The interspecific polymorphisms were detected in the comparisons between two species or three species at the same time. The comparison of *Solanum melongena* with *S. aethiopicum* detected 75,451 SNVs and 105,777 with *S. incanum*. The lowest variation was found between *S. incanum* and *S. aethiopicum* (15,162 SNVs) and the interspecific SNVs were substantially less abundant when three species were compared. Subsequently, all intraspecific and interspecific SNVs detected were filtered in order to create subsets of the most suitable and effective variations for genotyping assays, both manually and with high throughput platforms.

	INDELs	SNPs	SNVs
S. aeth.	4,556	159,571	164,127
S. inc.	535	11,861	12,396
S. inc. and *S. aeth.*	586	14,576	15,162
S. inc. and *S. mel.*	3,673	102,104	105,777
S. aeth. and *S. mel.*	2,392	73,059	75,451
S. aeth., *inc.* and *mel.*	108	41	149

Table 2
Single nucleotide variations statistics for the S. incanum and S. aethiopicum transcriptomes.

Regarding EST-SSRs, a set of 976 EST-SSRs were identified in 954 unigenes (1.1%) of *S. incanum*, while 1,708 EST-SSRs were detected in 1628 unigenes (1.8%) of scarlet eggplant. The microsatellites identified are summarized in Figure 2. The analysis of localization revealed that most of EST-SSRs were located in ORFs (33.5% for *S. incanum* and 32.7% *for S. aethiopicum*) and much less in the UTRs. The EST-SSRs discovered in this study will provide a valuable set of molecular markers to evaluate the intraspecific and interspecific variability across the eggplant genepool.

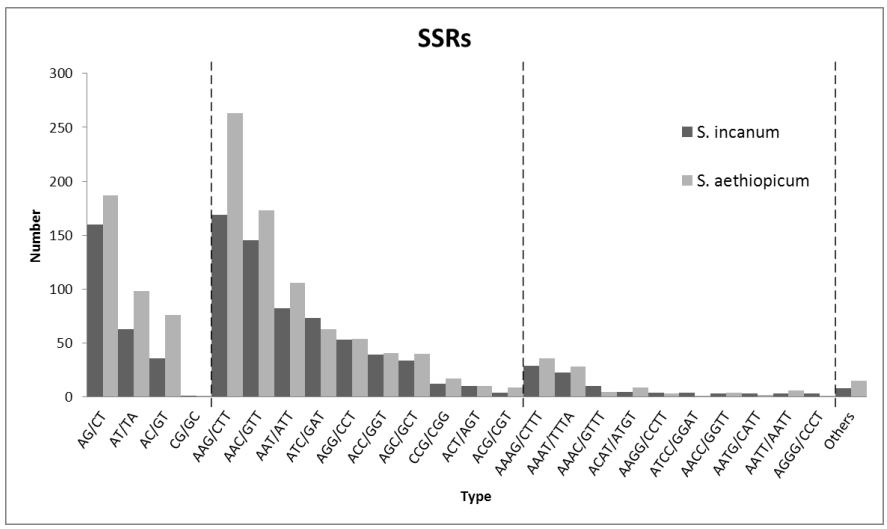

Figure 2
SSRs statistics corresponding to the S. incanum and S. aethiopicum transcriptomes.

4. Conclusions

The data and analysis presented in this study provide a global picture of gene expression in two species where the lack of genomic information slows down their use in eggplant plant breeding. In fact, the candidate genes identified are the starting point for understanding the genetics of important agricultural and economical traits that can be introduced and transferred among the relatives. Furthermore the huge amount of molecular markers discovered will be a powerful tool, not only in marker-assisted selection but also for genotyping large collections of plant materials through high throughput platforms or arrays, increasing the efficiency of the programs and allows the discovery of new interesting allele combinations.

5. Acknowledgements

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Genetic mapping of broad-spectrum QTLs and strain-specific major QTL for resistance to *Ralstonia solanacearum* in eggplant using GBS

Sylvia Salgon[1,2,3], Cyril Jourda[1], Christopher Sauvage[4], Marie-Christine Daunay[4], Emmanuel Wicker[1], Jacques Dintinger[1]

[1] *CIRAD, UMR PVBMT, Saint-Pierre, La Réunion (France)*

[2] *ARMEFLHOR, Saint-Pierre, La Réunion (France)*

[3] *Université de la Réunion, Saint-Denis, La Réunion (France)*

[4] *INRA UR1052, Montfavet, France*

Abstract

The Solanaceae family includes major agricultural crops cultivated worldwide such as tomato, potato, pepper and eggplant. Production of these crops in tropical and subtropical areas is limited by Ralstonia solanacearum, the causal agent of the bacterial wilt disease. This pathogen represents a threat for crops due to its large host range, its persistence in fields and its extensive genetic diversity. The species complex of R. solanacearum is divided into four phylotypes, which all are able to wilt eggplant.

Seeking for efficient and stable resistance to bacterial wilt, an intraspecific population of 178 eggplant recombinant inbred lines (RILs) was evaluated for resistance to R. solanacearum strains belonging to phylotypes I, IIA, IIB, and III. In addition of 162 AFLP and SSR markers, genotyping-by-sequencing (GBS) of the RILs provided 661 SNPs used to construct a dense genetic map anchored to the physical map of tomato. Quantitative Trait Loci (QTL) analysis showed the presence of a major QTL (ERs1) specifically associated with resistance to phylotype I strains on the chromosome 9 of eggplant. Two broad-spectrum QTLs were also identified on chromosome 2 and chromosome 5. Although less efficient than ERs1, these QTLs were found to partially control strains belonging to phylotype I, IIA and III.

Molecular markers linked to QTLs will be very useful for breeding resistance to Ralstonia solanacearum in eggplant. The anchored map will also help to identify candidate genes underlying the so far identified resistance factors.

Keywords: Eggplant, *Ralstonia solanacearum*, resistance, breeding

Multiple mutated putative aminotransferase alleles contribute to low pungency and capsinoid biosynthesis in *Capsicum chinense*

Yoshiyuki Tanaka, Yuji Muraga, Shiho Fukuta, Tanjuro Goto, Yuichi Yoshida, Ken-ichiro Yasuba

Graduate School of Environmental and Life Science, Okayama University, Okayama, Japan

Abstract

Capsicum chinense is a domesticated hot pepper species in the *Capsicum* genus that originated in the Amazon and is consumed in USA, the Caribbean and South America. Although a characteristic of this species is high pungency, some non-pungent or low- pungent strains, called ‘‘Aji Dulce’’, exist in the Caribbean region. In the present study, low-pungent *C. chinense* accessions were analyzed in order to elucidate the genetic mechanisms responsible for low pungency. All low-pungent *C. chinense* accessions in this study carried mutation alleles of putative aminotransferase (pAMT), which catalyzes the formation of vanillylamine from vanillin in the capsaicinoid biosynthetic pathway. These low-pungent accessions produced capsinoids, low-pungent capsaicinoid analogs. The *pamt* mutation in each strain was characterized using allele-specific markers, and several novel *pamt* alleles were identified. The some of *pamt* alleles had a hAT family transposon insertion in exon or intron region, which change pAMT expression. A phylogenetic analysis of *pamt* alleles was performed to examine their relationships. Combined with structural variations of *pamt* alleles, the Tcc family transposon insertion and its excision were involved in the generation of various *pamt* alleles in *C. chinense.* A phylogenetic analysis of *pamt* alleles showed that at least five occurred within *C. chinense* after speciation of the *Capsicum* genus. In conclusion, the results of the present study identified *pamt* as the main and most frequent gene controlling low pungency in *C. chinense*. Allelic variations in loss-of function *pamt* and their wide distribution demonstrated the potential of *C. chinense* bioresources for genetic improvements to pungency and metabolic profiles in hot pepper breeding programs.

Keywords: Hot pepper, *Capsicum chinense*, Capsaicinoid, Capsinoid, Low pungency

QTLs mapping for *Fusarium oxysporum* and *Verticillium dahliae* resistance in eggplant (*Solanum melongena* L.)

Laura Toppino[1], Lorenzo Barchi[2], Danila Valentino[2], Ezio Portis[2], Sergio Lanteri[2], Giuseppe Leonardo Rotino[1]

[1] *CREA-ORL, Unità di ricerca per l'Orticoltura, Montanaso Lombardo, Italy*

[2] *DISAFA Plant Genetics and Breeding, University of Torino, Grugliasco, Torino, Italy*

Abstract

Fusarium and *Verticillium* are widespread soil pathogens responsible for vascular wilts, which cause heavy yield losses worldwide in eggplant as well as many other crops. Genetic resources (wild and allied relatives, progenitors, and landraces) represent a key source of resistance for genetic improvement of eggplant. We previously developed eggplant breeding lines resistant to *Fusarium oxysporum* f. sp. *melongenae* by introgressing the *Rfo-sa1* locus from the allied species *S. aethiopicum*.

Here we report on the identification of QTLs affecting the resistance/tolerance to *Fusarium* and *Verticillium* in an F_2 intraspecific population of 156 individuals, bred from the cross '305E40' x '67/3' and previously characterized for key agronomic and biochemical traits. The female parent ('305E40') is an androgenetic di-haploid line resistant to *Fusarium*, which was obtained through anther culture of an introgression line carrying the locus *Rfo-Sa1*. The male parent ('67/3') is an F8 selection from the eggplant intra-specific cross cv. 'Purpura' x cv 'CIN2' which, unexpectedly, showed a tolerance to *Fusarium* not previously characterized.

The degree of resistance of the F2 population was assessed using $F_{2:3}$ progenies obtained by selfing each F2 individual which were either artificially inoculated with *Fusarium* and *Verticillium* in greenhouse condition or grown in a naturally *Verticillium* infested field. As expected, a major QTL (PVE ≥ 10%) for the resistance to *Fusarium* and lying in the genomic region of the *Rfo-Sa1* locus was identified, but other major/minor QTL influencing the response to *Fusarium* and *Verticillium* were also spotted and putative orthologies with tomato identified. The markers linked to these QTL may find application in the context of marker-assisted breeding.

1. Introduction

Eggplant (*Solanum melongena* L.) is the third most economically important Solanaceous crop after potato and tomato (Rotino et al., 2014), and it is cultivated worldwide. More than 90% of worldwide harvested area and total yield is concentrated in Asia, followed by Africa and the Mediterranean Basin (http://faostat3.fao.org/browse/Q/QC/), being Italy the leader European producer. Despite its economic value, genomics studies on this species have been only recently carried out. Inter-specific genetic maps (Wu et al., 2009; Gramazio et al., 2014) were developed and employed to spot QTL underpinning several morphological traits (Frary et al., 2014) and genes involved in the chlorogenic acid biosynthetic pathway (Gramazio et al., 2014). Further genetic maps were also obtained from intra-specific crosses (Barchi et al., 2012; Fukuoka et al., 2012) The intra-specific mapping population developed by Fukuoka was the basis for mapping two QTLs underpinning parthenocarpy (Miyatake et al., 2012). The linkage map developed by Barchi et al. (2012) and based on RAD-tag derived markers (Barchi et al., 2011), allowed at first to locate QTLs affecting anthocyanin content and then to spot QTLs for

key horticultural traits (Portis et al., 2014) and biochemical compounds (Toppino et al., 2016). Furthermore, through a GWAS approach a number of new marker/trait associations were confirmed and further on detected (Portis et al., 2015).

Eggplant is susceptible to numerous diseases and pests. Soil-borne diseases (e.g. bacterial and fungal wilts, nematodes) and insects are the most serious causes of yield losses both in greenhouse and in open field cultivations (Sihachakr et al., 1994). Indeed, fungal wilts caused by *Verticillium dahliae* (*Vd*) Kleb. (Fradin & Thomma, 2006) and *Fusarium oxysporum* f. sp. *melongenae* (*Fom*) (Cappelli et al., 1995) are two of the main diseases in eggplant. Partial resistance to several pathogens was found within the cultivated eggplant gene pool, but often too slight for effective utilization in breeding programs (Rotino et al., 2014). On the contrary, *S. melongena* allied and wild relatives are generally resistant to the pathogens and represent exploitable genetic resources in breeding programs (Rotino et al., 2014).

Advanced breeding lines carrying improved partial resistance to *Vd* have been obtained by introgressing useful traits from *S. linnaeanum* (syn. *S. sodomaeum*) into the *S. melongena* gene pool through a sexual cross between these two species, followed by several cycles of backcrosses with recurrent eggplant lines (Liu et al., 2013, Acciarri et al., 2004). Resistance to *Fusarium oxysporum* was introgressed in eggplant from the allied species *S. aethiopicum* and *S. integrifolium* through somatic hybridization. The tetraploid somatic hybrids were subjected to anther culture for obtaining dihaploid plants, which were backcrossed with different genotypes (typologies) of recurrent eggplants (Rotino et al., 2014). Advanced Introgression Lines (ILs) were developed through 6-8 cycles of backcross and selection, followed by selfing and/or anther culture. The molecular characterization of the ILs highlighted that the introgressed resistance trait is controlled by a single dominant locus (named *Rfo-sa1*, *Resistance to Fusarium oxysporum* f. sp. *melongenae* from *Solanum aethiopicum 1*) and made it possible to identify markers associated to the resistant and/or the susceptible phenotype (Toppino et al., 2008). Sources of partial resistance to *Fom* and associated markers were also detected in Asian landraces and introgressed in European eggplant genotypes (Mutlu et al., 2008).

To date a few studies aimed at identifying QTL affecting resistance to biotic stresses have been conducted in eggplant. Lebeau et al. (2013) identified a QTL associated to a major resistance gene to *Ralstonia solanacearum* in an intraspecific F2 mapping population. Recently two *Fusarium* semi-dominant inherited resistance loci were mapped on chromosomes 2 and 4 in three F_2 eggplant populations, and the one on E2 was found to be orthologs to *Rfo-sa1* (Miyatake et al., 2015).

The goal of the present work was to identify genomic regions affecting resistance to *Fusarium oxysporum* f. sp. *melongenae* and *Verticillium dahliae* using the RAD-tag based intraspecific linkage map (Barchi et al., 2012), as the female parent bears the *Rfo-sa1* locus conferring resistance to *Fusarium* and displays an improved response to *Verticillium* compared to the male parent. QTLs for the considered traits were identified, located in the genetic map, and syntenic relationships with other Solanaceae species highlighted.

2. Material and methods

2.1. Plant materials

The 156 individuals of a F_2 mapping population obtained by crossing the breeding lines '305E40' (female parent) and '67/3' were selfed in order to obtain as many $F_{2:3}$ progenies.

The female parent, '305E40', is a double-haploid introgression line derived from a somatic hybrid between eggplant and *S. aethiopicum* gr. *gilo* (Rizza et al., 2002), which was repeatedly backcrossed with the recurrent lines DR2 and Tal1/1 prior to be selfed and anther cultured. The breeding line displays resistance to *Fusarium oxysporum* f. sp. *melongenae* coded by the locus *Rfo-sa1* (Toppino et al., 2008) and shows to be partially resistant to *Verticillium*.

The highly homozygous '67/3' is an F_8 selection from the intra-specific cross between the eggplant accessions Purpura and Chinese (CIN2). Although it lacks the locus *Rfo-sa1*, it revealed an unexpected partial resistance to *Fusarium*, while full sensitivity to *Verticillium.*

2.2. Plant phenotyping

2.2.1. Fusarium oxysporum f. sp. melongenae resistance evaluation

Fusarium inoculation of each $F_{2:3}$ progeny, as well as of the parental lines and the F1 hybrid, was performed at Montanaso Lombardo (45°20′12″N 9°28′11″E, Italy) according to the dip-root method reported by Cappelli et al., (1995). A set of 48 seed-derived plantlets of each $F_{2:3}$ progeny as well as of the parental lines and F1 was grown under greenhouse conditions randomly arranged in two randomized blocks with 24 replicate plants per entry per block and then artificially inoculated at the 2-3th true leaf stage using a conidial suspension of $1.5X10^6$ conidia/ml for 15 minutes. At 30 Days After Inoculation (DAI) each single plant was phenotypically assessed and the degree of symptoms was classified according to a scale ranging from 0 to 5 as follows: 0-0.5, "fully resistant" (healthy plants with no symptoms); 0.6-2.5 "partially resistant"; 2.6-4.5 "highly sensitive"; 4.6-5 "fully sensitive" (dead plants). Resistance ratio was calculated as (n° of fully resistant plants + (n° of partially resistant plants *score) + (n° of highly sensitive plants *score))/ total n° of inoculated plants)*100.

2.2.2. Verticillium spp. resistance evaluation

Verticillium inoculation of the $F_{2:3}$ progeny as well as the parental lines and the F1 hybrid was performed according to a root-dip method at Carmagnola (44°53'N; 7°41'E, Italy) as well as in an open field infested with *Verticillium dahliae* in 2012. For greenhouse test, four weeks old plants (3 true leaves) were arranged as a set of two randomized complete blocks (two growth chambers) with 20 replicate plants per entry per block and inoculated with a *Verticillium dahliae* isolate at concentration of $5x10^5$ conidia/ml for 15 minutes. Inoculated plants were kept in a growth chamber (25 ± 2C day, 20 ± 2C night , 50 lEm-2 S-1) with a 12-h photoperiod. Disease outcomes were evaluated at leaves level at 20 and 40 days after inoculation (DAI) using a scale ranging from 0 (leaves with no symptoms) to 5 (dead leaves).

For open field test, four weeks old plants (3 true leaves) of a subset of $F_{2:3}$ lines (90 genotypes) were arranged as a set of two randomized complete blocks with 6 replicate plants per entry per block. Disease outcomes were evaluated at leaves level at 20 and 40 days from transplanting in the infested soil using a scale ranging from 0 (leaves with no symptoms) to 5 (fully withered leaves).

The disease data were treated as adjusted accession means (best linear unbiased predictors). Several multivariate linear mixed models were tested using a combination of the F-test (for the fixed component) and the Akaike test (for the random component). The best fit model was: $pib = rb + gi + e$, where *pib* represented the phenotype of the b^{th} replicate of the i^{th} genotype; *rb* the fixed effect of the b^{th} replicate; *gi* the random effect of the i^{th} genotype, and e the residual.

2.3. Statistical analysis and QTL detection

Statistical analyses were performed using R software (R development core Team, 2009). Analysis of variance was applied to estimate genotypic/environmental effects. The broad-sense heritability (h^2_{BS}) values were calculated as $\sigma^2_G/(\sigma^2_G + \sigma^2_E/n)$, where σ^2_G represent the variance in *g* and σ^2_E the residual variance and n the number of blocks; normality, kurtosis and skewness were assessed for each trait with the Shapiro-Wilks test (α=0.05). Correlations between traits were estimated using the Spearman coefficient. QTL detection was based on the Barchi et al. (2012) map, constituted of 415 markers (339 SNPs, 2 HRMs, 3 CAPSs, 11 RFLPs, 33 SSRs and 27 COSII) and spanning 1,390 cM. Both interval mapping and Multiple QTL Mapping (MQM), as implemented in MapQTL v5 software (Van Ooijen, 2004), were used. QTL were considered as major QTL when they explained more than 10% of the total variability for the considered trait. LOD thresholds for declaring a QTL to be significant (α=0.05) were established by applying 1,000 permutations. QTL effects were estimated on the basis of Markov Chain Monte Carlo (MCMC) method. MapChart v2.1 software (Voorrips, 2002) was used to produce visualization of chromosomes and QTL. The syntenic regions of the available genome tomato sequence (build 2.50, http://solgenomics.net/organism/Solanum lycopersicum/genome; tomato genome consortium, 2012) were investigated for identifying candidate genes and transcription factors co-localizing with the eggplant identified QTL.

3. Results and Discussion

3.1. Phenotypic variation and inter-trait correlations

Plants of the parental line '305E40' resulted completely resistant to *Fusarium oxysporum* at 30 DAI and grew vigorously for many weeks without showing symptoms. When inoculated with *Verticillium* in greenhouse, they displayed a partial resistance both at 20 and 40 DAI (Table 1). Unexpectedly, *Fusarium*-inoculated plants of the line '67/3' (lacking of the *Rfo*-sa1 locus) were still alive at 30 DAI; however, they displayed symptomatic leaves (yellowing and necrosis) and grew less than the not inoculated plants. On the other hand they displayed a high sensitivity towards *Verticillium* at both 20 (*Ver20*) and 40 (*Ver40*) DAI. The F_1 plants, like the parent '305E40', were fully resistant to *Fusarium oxysporum,* while showed an intermediate resistance to *Verticillium*. In the F_2 progeny transgressive segregation was observed for *Ver40* compared to '67/3' (8 plants) and for *Fus,* with 10 lines completely susceptible to the pathogen (no alive plants). The broad sense heritability values were high for all the three traits in study, ranging from 0.94 (*Ver40*) to 0.96 (*Fus*) (Table 1). Significant positive inter-trait correlations ($p<0.05$) were detected for *Ver20/Ver40* (0.287) and *Ver20*/*Fus* (0.25). For open field *Verticillium* test, results were not uniform within lines (data not shown) due to a too high environmental effect, and thus were not used for QTL analyses.

Trait	**Trait code**	**Parent means ±SD**		**F1**	**F2 population mean ±SD**	**H^2**
		305E40	**67/3**			
Fusarium 30 DAI	*Fus*	1±0	0.6±0	1±0	0. 68±0.30	0.99
Verticillium 20 DAI	*Ver20*	1.93±0.99	3.14±0.81	2.73±1.10	2.77±0.62	0.95
Verticillium 40 DAI	*Ver40*	2.3±0.96	3.69±0.97	2.75±1.23	2.98±0.81	0.94

Table 1
List of the traits analysed and their code, means, standard deviations (SD), and broad sense heritability (H^2)

3.2. QTL identification

Two QTL underpinning the resistance/tolerance trait to *Fusarium* were mapped in the F_2 population (Table 2 and Figure 1). A large effect QTL was spotted on E2 (*FusE02.01*), which explained about 70% of the PVE and was positively controlled by '305E40'.

In addition, a second major QTL was identified on E11, explaining about 13% of the PVE and positively controlled by '67/3'. As regard the resistance to *Verticillium*, at 20 DAI a QTL was identified on E08, while at 40 DAI a major QTL located on E05 and a minor on E09 were spotted. For all the QTL, the resistance alleles derived from '305E40'.

QTL	CH	GW	QTL	Position	Locus	LOD	PVE
Fus	2	3.9	*FusE02.01*	7.958	21207_PstI L411	55.41	70.1
	11		*FusE11.01*	47.583	C2_At3g51010	16.04	12.7
Ver20d	8	3.1	*Ver20E08.1*	0.000	18202_PstI_L304	3.70	10.8
Ver40d	5	3.8	*Ver40E05.1*	99.515	10016_PstI_L402	7.93	20.7
Ver40d	9	3.1	*Ver20E09.1*	111.870	32063_PstI_L393	3.14	7.3

Table 2:
QTL detected in the mapping population. For each trait, the genome-wide thresholds (GW) at p=0.05 (as determined from 1,000 permutations) is indicated. The position and the closest mapping marker to each QTL are indicated, along with the value of the QTL, the LOD, the percentage of variation explained (PVE).

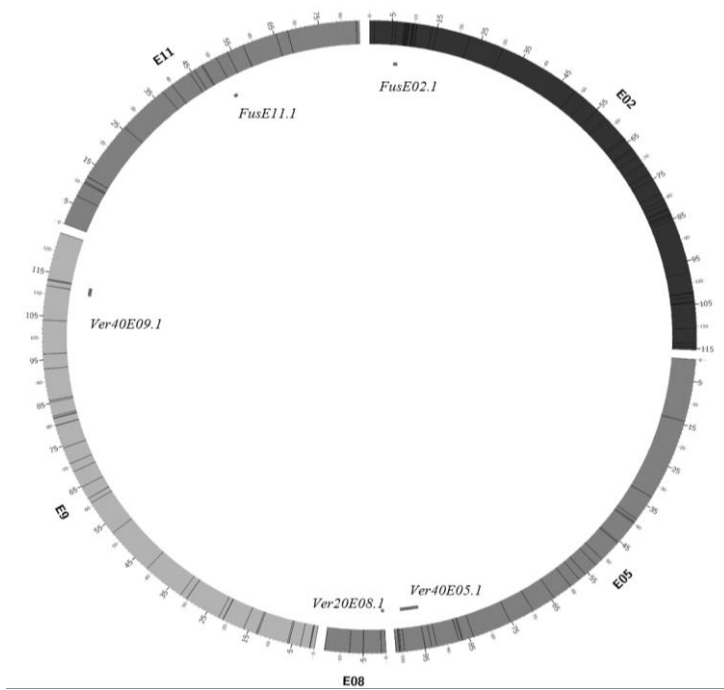

Figure 1
Eggplant chromosomes containing the QTL detected. Each band inside the chromosome represents a molecuar markers, with map distances (in cM) shown outside each chromosome. The length of the band represents the confidence interval of the QTL (LODmax^{-1} interval).

3.3. Candidate gene identification based on evaluation of syntenic regions with tomato

A blastX search was performed on the available tomato genomic sequence (build 2.50: http://solgenomics.net/organism/Solanum_lycopersicum/genome) in order to investigate the sequences underlying regions syntenic with the QTL identified in the present study for candidate genes putatively involved in the control of the traits.

The markers delimiting *FusE02.1*(21207_PstI_L411 and 36006_PstI_L370) are located in the eggplant map 0.3cM apart from each other; on tomato, the former maps at 51,857,044, while the latter at 40,966,245, on chromosome T2, suggesting inversion and other chromosome rearrangements between eggplant and tomato, as already reported by Miyatake et al. (2015). By considering this interval (about 11Mb), we identified 31 genes associated with the keywords "resistance" or "LRR (leucine-rich-repeat)" or both. To narrow the identification of candidate genes, we restricted the search around 1Mb of the tomato genome sequence. For the marker 21207_PstI_L411, three genes were associated to "resistance" or "LRR", and may be selected as candidates; on the contrary, for the marker 36006_PstI_L370, no resistance related proteins were identified. The marker C2_At3g51010, linked to FusE011.1 QTL, maps to T4 at 2,426,283 Mb; the search around 1Mb of this genomic position highlighted the presence of 15 tomato genes associated to resistance" or "LRR.

The marker associated to *Ver20E08.1* is 18202_PstI_L304, which maps at 1,902,945 on tomato T8. In 1Mb around these coordinates, 5 genes were associated to resistance" or "LRR.

The markers delimiting *Ver40E05.1* (10016_PstI_L402 and 12391_PstI_L355) map at 66,653,563 and 66,128,270 of tomato T12 respectively. In the 1Mb region around these coordinates, 10 genes were associated to "resistance" or "LRR". Finally, the marker associated to *Ver40E09.1* is 32063_PstI_L393, which maps on T9 at 7,0144,642 in the tomato genome. In the 1Mb region around this coordinate, three genes were associated to "resistance" or "LRR".

4. Conclusions

Resistance breeding requires continuous efforts of enriching the reservoir of useful genes/alleles to effectively tackle plant diseases. Our intraspecific segregating population derived from two highly contrasting parents for key agronomic traits coupled with a SNP-base linkage map allowed to locate, for the first time in eggplant, QTL affecting the resistance to *Fusarium* and *Verticillium*. Their high value of LOD scores, elevated percentage of variability explained and chromosomal localization make them instrumental in assisting breeders to develop resistant eggplant varieties. Furthermore, the survey of the tomato genome allowed to identify putative orthologous candidate genes, which represent potential additional genomic resources for marker assisted selection programs and for further synteny studies with both tomato and other Solanaceae species.

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Genotyping by sequencing for population structure and genome-wide association analysis for fruit shape and size in pepper (*C. annuum* L.) germplasm

Francesca Taranto, Nunzio D'Agostino, Barbara Greco,
Teodoro Cardi, Pasquale Tripodi

Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria (CREA)– Centro di ricerca per l'orticoltura,Pontecagnano, Italy

Abstract

The exploitation of genetic variation in crops is essential to establish innovative breeding programs in the frame of global population increase and the sustainable intensification of agriculture. DNA fingerprinting provides effective tools for assessing the level and distribution of genetic diversity in germplasm collections. The advent of next generation sequencing technologies and the availability of genome sequences of many crops allowed the implementation of several methods for SNP discovery and genotyping. Genotyping by sequencing (GBS) represents a recently emerged method for exploring plant genetic diversity on a genome-wide scale. It has been successfully applied in several crop species and combined with detailed phenomics, it allows to detect representative and potentially superior genotypes in terms of yield and quality, with enhanced health benefits for consumers. In the present study, a collection of two hundreds *C. annuum* genotypes with different geographical origin, has been subjected to GBS and phenotyped using automated devices for fruit traits, which are the most relevant attributes for varietal selection. Genetic diversity has been investigated using a bayesian hierarchical clustering approach allowing the identification of clusters that reflect the geographical distribution of the accessions. Main traits responsible for the whole variation among genotypes were detected analyzing 38 morphometric traits for a total of 300 K phenomic data points. A preliminary wide association analysis using a General Linear Model (GLM) allowed to identify specific SNP responsible of the phenotypic variation. Mixed Linear Model (MLM) analysis is underway. The identified SNPs associated to pepper fruit shape and size represent potential markers useful for future breeding programs.

1. Introduction

With the growing global population, the exploitation of biodiversity play a crucial role in food security and nutrition. The generation of novel crops and the establishment of innovative breeding programs have become major challenges. Pepper (*Capsicum* spp.) belongs to the *Solanaceae* family, which is a member of flowering plants consisting of about 102 genera and about 2,500 species including tomato, potato, eggplant. Plants belonging to the genus *Capsicum* had their origins in the South America and at present are widely cultivated in tropical and temperate regions. Pepper represents one of the most economically important vegetable crop with a cultivated area extended over a World surface of 3.6 million hectares and a total production of about 35 million tons. Europe represents about 25 % of the World harvest, while China and India are the biggest producers of fresh pepper and chillies with more than 50 % of the World production (FAOSTAT 2013). European consumption is mostly represented by sweet types, however several hot varieties and landraces are cultivated particularly in the Mediterranean area. Given its very large variability and geographical distribution, pepper has multiple uses as food and industrial product and includes a large number of accessions with a considerable variation for several traits including growth habit, seeds, flower and fruit color,

shape and size. Estimates report the existence of about 40 species (www.theplantlist.org), most of them are diploids with 24 and 26 chromosomes. Five species (*C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens* and *C. pubescens*) were domesticated through distinct events at different primary diversification centers [Moscone et al. 2007] and are widely consumed as sweet and hot pepper. Based on morphological characteristics, chromosome banding and hybridization studies, three main gene pools (or complexes) are recognized, namely Annuum complex, Baccatum complex and Pubescens complex (Figure 1) [Mongkolporn and Taylor 2011; Stommel and Albrecht 2012].

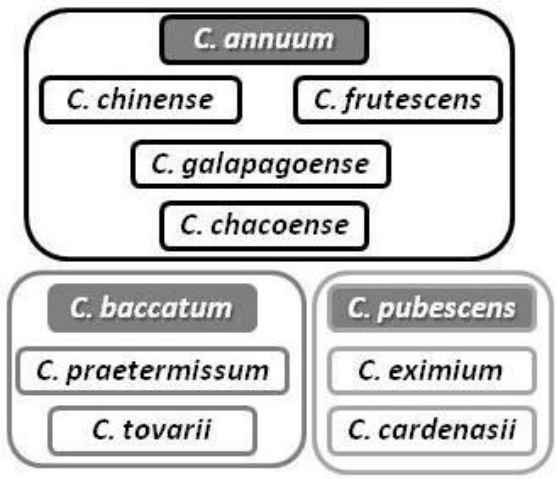

Figure 1:
Main Capsicum complexes (the name of the complex is highlighted)

Inter-specific crosses between species of the same complex can produce partially fertile hybrids, whereas crosses within secondary gene pool generally require aids such as embryo rescue. Among the domesticated *Capsicum* spp., *C. annuum* (2n=2x=24) is the most widely grown species. Consumed as food or processed product, it is the most used in pepper breeding programs. In most vegetables as well as in pepper, breeding programs have led to the selection of a small number of productive cultivars carrying genes for resistance to diseases. As a consequence, genetic variability loss occurred. In this context, plant collections constituted over time as well as wild relatives and underutilized varieties are challenging due to their unexplored genetic potentiality. The evaluation of population diversity and genetic structure in germplasm collections provides vital information for genome-wide association mapping and allele mining studies to be exploited by plant breeders for the development of novel varieties and seed conservation programs [Rodriguez et al. 2015]. Genetic diversity of *Capsicum* spp. have been estimated by different approaches, which include the use of biochemical, morphological and molecular markers. Molecular markers are extremely useful in plants to characterize germplasm collections and improve the conventional plant breeding scheme through marked-assisted selection (MAS). Most of the markers applied in breeding were PCR markers with DNA specificity to loci in genomes such as simple sequence repeat (SSR), cleaved amplified polymorphic sequences (CAPS), sequence characterized amplified region (SCAR) and allele specific PCR (AS-PCR) markers. However, although these markers were widely used for their polymorphic nature per locus, their information content sometimes limited the application for genetic studies. Moreover, gel-based genotyping is very laborious and automated fragment

analysis systems are generally low-throughput even if they provide a moderate level of multiplexing. In the recent years, cutting-edge technologies in biological science, have provided useful tools for genetic analysis, particularly in form of molecular markers, which in combination with conventional phenotype-based selection, define modern plant breeding practices. Next Generation Sequencing (NGS) technologies and the availability of whole genome sequences of many crops allowed the implementation of several methods for SNPs discovery. SNPs, abundant in plants, can be considered the primary choice for many genetic studies, having a number of advantages such as flexibility, speed- and cost-effectiveness [Kim et al 2016]. Recently, the availability of a variety of high-throughput SNP genotyping platforms dramatically reduced costs and time associated with the development of plant breeding schemes. Genotyping by sequencing (GBS) has emerged as an innovative genomic approach for exploring plant genetic diversity on a genome-wide scale [Elshire et al. 2011, Deschamps et al. 2012]. GBS is based on genome reduction with restriction enzymes; it does not require a reference genome for SNP discovery and provides a rapid, high-throughput and cost-effective tool for the investigation of genetic variability in model and non-model species. This marker technology have been used in several crop species and different population type [Nimmakayala et al. 2015, Li et al. 2015, Arbelaez et al. 2015]. The availability of the complete genome sequence of *Capsicum* [Qin et al. 2014, Kim et al. 2014] provides a straightforward tool to estimate chromosome wide molecular diversity and precisely infer pepper population structure enhancing the information derived from GBS data. However, while the development of advanced molecular markers techniques has rapidly progressed within the last years, the understanding of the link between genotype and phenotype did not keep up with this development. Advances in phenotyping as well as the process of quantitative characterization of the phenotype have become the major limiting factors due also to the lack of engineered tools for automated analyses. The gap between the knowledge about genes and phenotypes is particularly large in analyses of plant-environment interactions that are urgently needed for sustainable and resource-efficient crop production in the context of climate change and varying agricultural production conditions. Fruit-morphology related traits are the most relevant attributes to be taken into account for breeding in pepper. Indeed classification of cultivars is mainly based on fruit shape. Genetic mapping studies aiming to discover QTLs in biparental mapping population have been conducted [Ben Chaim et al. 2003], however the limited variation due to genetic variability of the population under investigation as well as the simple method of assessment reduced the possibility to discovery traits related to fruit morphology. Over the past few years, a phenomic freeware, Tomato Analyzer (TA), addressed to the analysis of morphometric fruit traits has been developed [Brewer et al. 2006; Gonzalo and van der Knaap, 2008]. TA analyses a large number of fruit shape traits from scanned images of fruit sections. This software initially developed for the morphological traits of tomato fruits, [Gonzalo and van der Knaap, 2008; Gonzalo et al. 2009; Mazzucato et al. 2010] has been successfully used in other crops such as papaya [Blas et al. 2012] and eggplant [Hurtado et al. 2013]. Aiming to identify traits involved in fruit morpho-metrics in wide genetic background, we determined population structure and estimated phenotypic diversity in a collection of cultivated pepper (*C. annum*) using GBS and TA. The integration of genomic and phenotypic data was useful to describe, at best, the genetic diversity of the pepper collection under investigation, showing significantly relationships among the genotypes. The identified SNPs, associated to pepper fruit shape and size, represent potential markers useful for future breeding programs.

2. Experimental procedures

2.1. Plant material

GBS was performed on a collection of 370 *Capsicum* spp. genotypes from the three complexes (*Figure 1*). For genomics and phenomics only a collection of two hundred cultivated pepper (*C. annumm*) accessions was considered. Genotypes represent over twenty different countries of Europe, Asia, Africa, America. Plant material were initially retrieved from local farmers, associations, research institutes and germplasm banks (CGN, Wageningen, NL and IPK, Gatersleben, DE) and subsequently subjected to cycles of controlled self-fertilization under glasshouse conditions at CREA-ORT. The collected genotypes are characterized by a large phenotypic variability in terms of fruit related traits (morphology, shape and colour), pungency, resistances and uses.

2.2. Genotyping by sequencing

The GBS protocol was composed of major steps including a) sample preparation, b) library construction, c) sequencing, d) SNP discovery e) genetic analysis. Total Genomic DNA was extracted using the DNeasy® Plant Mini Kit (QIAGEN, Germany). DNA quality parameters as well as its concentration were measured by absorbance values at 260 and 280 nm respectively, using a UV-Vis spectrophotometer (ND-1000; NanoDrop, Thermo Scientific, Wilmington, DE, USA). A trial DNA digestion was carried out using the 6-base-cutter *Hind*III. Once main parameters were assessed, digestion with appropriate RE (*Ape*KI) was carried out following the protocol of Elshire et al. 2011. After digestion, fragments are directly ligated to a pair of enzyme-specific adapters, which contain specific priming sites for the Illumina sequencing. Following adapter ligation, the library was assembled pooling up to four amplicons with similar concentrations. A PCR amplification was carried out to generate the GBS library, which was submitted to a single Illumina HiSeq 2500 run (Illumina Inc., USA). After sequencing, raw data were collected. The sequencing produced million reads, split across multiple FASTQ files. All unique sequence tags from each sequence file were captured and then collapsed to generate a master tag file Each sample had two FASTQ files which represented the forward and reverse sequenced reads. Chromosomal assignment and position of GBS markers on the physical map were deduced from the reference genome sequence of *C. annuum* cv. CM334 available at http://peppergenome.snu.ac.kr [Kim et al. 2014]. The alignment of the first 64-bps of the reads to reference genome was carried out using the free software Bowtie2 [Langmead et al. 2012] or BWA, which converted FASTQ format into a "TagsOnPhysicalMap" (TOPM). SNP calling was carried out using the TASSEL-GBS [Glaubitz et al. 2014] software.

2.3. Genetic diversity

Population structure was determined using the parametric Bayesian model-based clustering method implemented in STRUCTURE v.2.3 [Pritchard et al. 2005]. This approach assumes a model in which the individuals are grouped into clusters (K) called ancestral population based on a statistical method known as the allele-frequency admixture model [Pritchard et al. 2000] and used the StrAuto (v0.3.1) program [www.crypticlineage.net/pages/software.html] to assign individuals to K (i.e. the number of cluster in a sample of individuals) according to a membership coefficient (qi). For each K (from 2 to 15) ten runs were performed using the following parameters: 100,000 Markov Chain Monte Carlo (MCMC) repetitions, 100,000 burn-in period and RANDOMIZE=1. The optimal K value was determined by using an ad-hoc statistic ΔK [Evanno et al. 2005] and was estimated with the software Structure Harvester [Earl

DA and vonHoldt 2012]. A genotype was considered to belong to a group if its membership coefficient (qi) was ≥ 0.50 [Jakobsson et al. 2012].

2.4. Phenomics analysis

A total of 20 fruits, at the commercially ripe, per accession was taken. Each fruit was longitudinally cut and scanned with a Canon Scanner Lide 220 scanjet at a resolution of 300 dpi and subjected to morphometric analysis with the TA software. The parameters that were measured are reported in *Table 1*.

2.5. Data analysis

Polymorphic Information Content (PIC), Heterozygosity (H^2) and Gene Diversity were calculated using the Power Marker software [Liu et al. 2005]. Phenomics data were statistically elaborated by using the Statistical Software JMP (SAS Institute). Means were compared by using Tukey's HSD (honest significant difference) test ($P \leq 0.05$) Principal Component Analysis was performed using XLSTAT. Genome-wide association analysis was carried out using Tassel 5.0.

3. Results and discussion

GBS generates 867 million reads, for a total of 7,568,894 master tags, 53% of which aligned to the reference genome (CM334). Uniquely aligned tags were used for calculating distribution of tag density at each position in the pepper genome and for SNP calling. A total of 100K SNP were identified and 30 thousand out of them were selected (minor frequency allele 0.01, coverage > 90%) in order to assess the genetic diversity of the population. Using STRUCTURE, ten independent runs for each K (from K2 to K15) were performed allowing to distinguish several accession groups according to the geographical origin and fruit related traits (*Figure 2*). Based on Evanno's test (K=7), seven clusters were detected. A main group distinguished European accession from those of other countries. Other groups differentiated sweet types from chillies. Blocky types as well as chilli pepper from Calabria region made their own cluster. Morpho-metric analysis allowed to collecting over 300 thousand phenotypic data points.

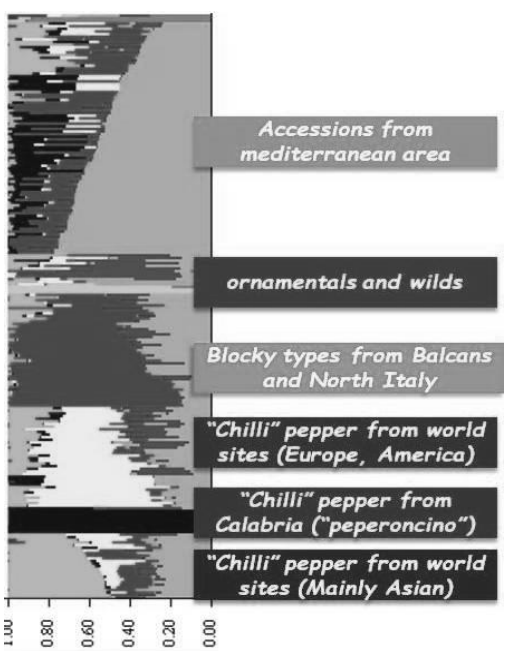

Figure 2
STRUCTURE output and relative clusters (K=7)

Highly significant differences ($P < 0.001$) were found among the pepper accessions for 36 out of the 38 traits (*Table 1*). For proximal and distal eccentricity no significant differences were

detected. Main phenotypic variation was due to fruit size traits (perimeter, area, width mid-height/height mid-width, maximum width and height, curved height) which showed Rsquare greater than 0.85 and high F ratio value. Among fruit shape traits, circular was one of the main discriminant of variation.

Trait	Rsquare	F Ratio	Prob > F
FRUIT SIZE			
Perimeter	0.926	368.300	<0.0001
Area	0.867	190.518	<0.0001
Width Mid-height	0.930	387.213	<0.0001
Maximum Width	0.873	201.999	<0.0001
Height Mid-width	0.809	124.495	<0.0001
Maximum Height	0.920	337.140	<0.0001
Curved Height	0.926	366.658	<0.0001
FRUIT SHAPE			
Fruit Shape Index External I	0.744	85.244	<0.0001
Fruit Shape Index External II	0.719	74.940	<0.0001
Curved Fruit Shape Index	0.875	205.757	<0.0001
Proximal Fruit Blockiness	0.533	33.460	<0.0001
Distal Fruit Blockiness	0.190	6.889	<0.0001
Fruit Shape Triangle	0.136	4.613	<0.0001
Ellipsoid	0.641	52.343	<0.0001
Circular	0.922	345.186	<0.0001
Rectangular	0.546	35.236	<0.0001
Shoulder Height	0.175	6.232	<0.0001
Proximal Angle Micro	0.074	2.341	<0.0001
Proximal Angle Macro	0.174	6.184	<0.0001
Proximal Indentation Area	0.122	4.091	<0.0001
Distal Angle Micro	0.123	4.122	<0.0001
Distal Angle Macro	0.215	8.023	<0.0001
Distal Indentation Area	0.149	5.144	<0.0001
Distal End Protrusion	0.159	5.550	<0.0001
Obovoid	0.122	4.066	<0.0001
Ovoid	0.422	21.414	<0.0001
V. Asymmetry	0.499	29.163	<0.0001
H. Asymmetry.ob	0.092	2.981	<0.0001
H. Asymmetry.ov	0.634	50.716	<0.0001
Width Widest Pos	0.322	13.927	<0.0001
Eccentricity	0.253	9.948	<0.0001
Proximal Eccentricity	0.033	0.997	0.496
Distal Eccentricity	0.029	0.880	0.865
Fruit Shape Index Internal	0.718	74.652	<0.0001
Eccentricity Area Index	0.448	23.822	<0.0001
Lobedness Degree	0.802	118.684	<0.0001
Pericarp Area	0.451	24.094	<0.0001
Pericarp Thickness	0.495	28.775	<0.0001

Table 1
Tomato analyzer data output and principal statistical parameters

The first and second components of the PCA accounted, respectively, for 44.9% and 21.7% of the total variation among accession means.

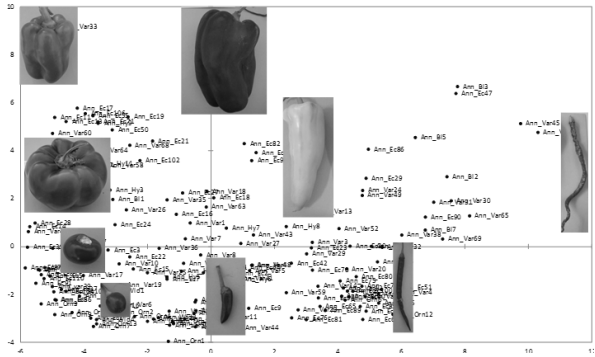

Figure 3
PCA analysis based on 36 morphological traits

The first component was positively correlated with fruit shape traits (i.e. curved fruit shape, ellipsoid etc.), while the second component with fruit size traits (perimeter, area). As shown in *Figure 3*, blocky and rectangular types were quite distinct from horn and roundish types. Although, several TA traits were redundant, the analysis allowed to detect leading traits for pepper breeding. Associations between SNP alleles and morphology were primarily investigated on the basis of General Linear Model. Several SNP highly correlated to the phenotypic variation were identified. For the main traits responsible for fruit size variation as well as for shape traits of high interest in breeding, highly correlated SNP were detected on chromosomes 2, 3, 6 and 9 (*Figure 4*). Mixed Linear Model analysis is underway.

4. Conclusion

GBS has been chosen to identify large number of SNPs useful to precisely define the genetic structure of a *C. annuum* population. Moreover, large-scale phenomics has been carried out for fruit-related traits. A preliminary GWA approach demonstrate the presence of SNP markers responsible of morphological variations.

Information concerning SNP markers and population structure developed in this study are the first step for marker-assisted selection programs in cultivated pepper

5. Acknowledgement

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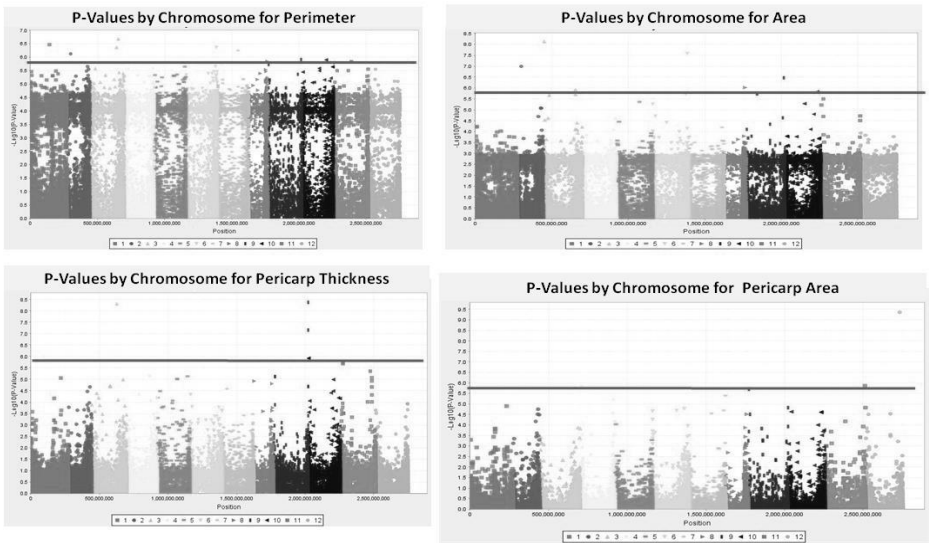

Figure 4
Manhattan plots obtained with GLM analysis for 4 fruit morphological traits

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Cutin deficiency of bell pepper (*Capsicum annuum* L.) results in gluey berries

Jana Leide[1], Anton Hansjakob[1], Franziska Forster[2], Agnes Fekete[2], Gábor Csilléry[3], Zsolt Erős-Honti[4] and Markus Riederer[1]

[1] *University of Würzburg, Julius-von-Sachs-Institute for Biosciences, Chair of Botany II, Würzburg, Germany*

[2] *University of Würzburg, Julius-von-Sachs-Institute for Biosciences, Chair of Pharmaceutical Biology, Würzburg, Germany*

[3] *Budakert Kft., Budapest, Hungary*

[4] *Szent István University, Faculty of Horticultural Science, Department of Botany and Soroksár Botanical Garden, Budapest, Hungary*

Abstract

Representing a dynamic and selective barrier, the cuticle plays a pivotal role in limiting transpirational water loss across primary plant surfaces and shields from various abiotic and biotic stresses like pathogenic invasion. Fruits and leaves of bell pepper (*Capsicum annuum* L.) were used for elucidating cuticle formation. In the course of fruit expansion and maturation, the cuticular waxes, which cover the outermost fruit surface, and the cutin matrix increased, whereas the water permeance declined simultaneously. During the constitutive wax biosynthesis, 7.4 µg cm^{-2} cuticular waxes were accumulated containing mainly triterpenoids, alkanes, and alkanoic acids. Already at early fruit stages, the cutin matrix reached its full amount of 1367.0 µg cm^{-2} dominated by hydroxylated hexadecanoic acid monomers. In contrast to fruits, bell pepper leaves had a distinct reduced cuticular coverage comprising an accumulation of 2.5 µg cm^{-2} cuticular waxes and 4.2 µg cm^{-2} cutin, respectively.

Apart from an organ specificity of the biosynthetic processes, mutations influence the cuticle composition of bell pepper fruits and leaves. The *gluey berry* (*glb*) mutation caused a severe loss of cutin monomers and an altered cuticular wax coverage resulting in reduced transpiration barrier properties and a high susceptibility to pathogens such as *Botrytis cinerea*. A comparative, non-targeted metabolome analysis between red ripe wild type and mutant fruits revealed modifications in sugar deposition on the fruit exterior causing the *gluey berry* phenotype of this cutin-deficient bell pepper mutant.

Anatomical investigations of the *gluey berry* mutant showed a remarkably reduced thickness of the cuticular layer compared to the wild type fruits. However, collenchyma layers of the outer mesocarp were thicker due to an increased deposition of cell wall material and a higher number of cell layers in the mutant fruits. By histological comparisons of $glb^{+} \times glb$ F1 progenies with the parents, epidermal characteristics were found to be similar to that of the wild type, yet the hypodermal collenchyma resembled that of the mutant.

1. Introduction

All aerial organs of the plant that are not lignified are covered by an extracellular membrane, the cuticle. By forming a continuous interphase between the plant interior and its surrounding the cuticle protects against biotic and abiotic stresses e.g. water loss from the plant inside and pathogenic infection or UV radiation from the outside[1-3]. Functionally, the cuticle can be divided into the cuticular layer, which is rich in cutin and polysaccharides, and the cuticle proper

consisting of cutin and cuticular waxes. These waxes provide the main barrier properties by impregnating the cutin matrix with intracuticular waxes as well as covering the cutin matrix with an epicuticular wax film and/or wax crystals[1].

According to the chemistry, the cuticle can be classified into two distinct structures: cuticular waxes that are solvent-soluble and a non-extractable but hydrolysable cutin polymer. Cuticular waxes are complex mixtures of up to 150 different components. They are mainly composed of very-long-chain alkanoic acids and derivatives like alkanals, primary and secondary alkanols, alkanes, alkanones and alkyl esters. In addition to these compounds with chain lengths of 20 to 50 carbon units, cuticular waxes contain pentacyclic triterpenoids predominantly of the lupane, oleanane and ursane group. In minor proportions steroids, flavonoids and tocopherols occur in the cuticular wax mixture[3]. In contrast to the cuticular waxes, the cutin matrix consists of alkanoic acids with chain lengths of 16 or 18 carbon units. These saturated or unsaturated, long-chain alkanoic acids can be modified into mono-, di- or trihydroxy, dicarboxylic, oxo as well as epoxy derivatives. The aliphatic cutin acids are esterified into a complex polymeric network by primary and secondary ester bonds. However, also phenolic acids can be linked within the complex structure. These hydroxy cinnamic acids are for example *para*-coumaric acid, caffeic acid, and ferulic acid. According to the most abundant carbon chain length in the cutin polyester, it can be classified as C_{16} type, C_{18} type or C_{16}/C_{18} mixed type[4].

Thus, the cuticular architecture and composition is highly diverse and varies between plant species, organs and even between tissues. Its formation follows in a well-defined and highly reproducible manner. The biosynthetic origin of the cuticular deposits are the epidermal cells. The precursors originate in the plastids and are modified into the cutin monomers in the endoplasmic reticulum or are extended via the fatty acid elongation complex and are further converted into different wax compounds. Finally, the cuticular components are translocated into the extracellular space[4].

This study is focused on: (i) the visual, functional and chemical characterization of the bell pepper cuticle, (ii) the organ specificity of cuticle formation by comparing vegetative and reproductive bell pepper organs, and (iii) the comparative analysis of a bell pepper wild type and a mutant possessing insufficient cuticle barrier properties.

2. Materials and Methods

Plant material Plants of the bell pepper (*Capsicum annuum* L.) breeding line Kapia were cultivated in a growth chamber with 75% relative humidity, a 14-h photoperiod at 450 µmol m^{-2} s^{-1}, and a day/night temperature regime of 22°C/18°C. Plants were watered daily and fertilized with 2‰ Osmocote (Scotts) and 1‰ Hakaphos Blau nutrient solution (Compo).

Determination of surface area, epidermal cell density, fresh weight, and water content The surface areas of Kapia fruits and leaves were determined via pixel values of a planar surface of the leaves or produced by slicing the fruit and using a reference area (Adobe Photoshop 7.0.1). The epidermal cell density was estimated by counting the number of epidermal cells on nail polish imprints from leaf (adaxial surface) and fruit surfaces. Fresh weights of Kapia fruits and leaves were measured using a balance with a precision of 0.1 g (Satorius AC210S). The dry weight of Kapia fruits and leaves was recorded after lyophilization at -55°C under vacuum for 48 h (Christ Freeze Dryer, Alpha 1-2 LD) and adaptation at room temperature. The percentage of weight loss, representing the total water content, was calculated.

Isolation of cuticular membranes Cuticular membranes were enzymatically isolated from Kapia fruits by incubation at room temperature with pectinase (Trenolin Super DF, Erbslöh), and cellulase (Celluclast, Novo Nordisk AIS) in 20 mM citric acid, pH 3.0, containing 1 mM

sodium azide to prevent microbial growth. Isolated cuticular membranes were extensively washed in deionized water and air-dried.

Light microscopy Semi-thin cross sections were made from the outer pericarp of *glb*$^{+}$, *glb* and *glb*$^{+}$ × *glb* (F1) fruits with a cryostat (Leitz) and investigated (Zeiss, Axio Imager.A2). Morphometric evaluation was accomplished using the AxioVision 4.8 software (Zeiss). Statistical analyses of morphometric data were carried out with the PAST software[5].

Scanning electron microscopy (SEM) Isolated cuticular membranes of Kapia fruits were mounted on aluminum holders using a conductive double-sided adhesive tape (Plannet Plano), 5 min sputter coated with gold/palladium (60/40) at 25 mA using a Bal-Tec SCD coater (Balzers) and examined in a field emission scanning electron microscope (JEOL JSM-7500F) at 15 kV. The sputter conditions, depositing an alloy coat thickness of approximately 20 nm, were optimized for the acceleration voltage in the scanning electron microscope.

Determination of cuticular resistance Cuticular permeance for water (*P*) was investigated for Kapia fruits and the minimum conductance (g_{min}) was obtained from the weight loss of desiccating Kapia leaves at maximally closed stomata in the dark[1]. The attachment site of the pedicel and the petiole were sealed with paraffin (Merck). The amount of water transpired versus time was gravimetrically measured (Sartorius AC210S). Fluxes (*J*) were calculated by dividing the cuticular water flow rates (*F*), which were determined from the slope of a linear regression line, by the total surface area. Between measurements, Kapia fruits and leaves were stored at 25°C over dry silica gel (Applichem). The vapor phase-based driving force (Δc) for transpiration was 23.07 g m^{-3}. For determining water permeance (*P*) and minimum conductance (g_{min}) based on water vapor concentration, *J* was divided by Δc. Cuticular resistance was estimated as the reciprocal of the cuticular water permeability.

Determination of fungal isolate and pathogen assay Fungal material was isolated from fruits of Kapia *gluey berry* opportunistically infected in the field. A single isolate was obtained by consecutive subcultivation of single conidia on potato dextrose agar. The fungal isolate was determined microscopically and based on DNA sequence data[6]. The *Botrytis cinerea* isolate was incubated on potato dextrose plates with 10% homogenized bean leaves at 20°C in a 16 h/8 h day/night regime for ten days until conidia emerged. The conidia were isolated and purified. The conidial suspension was adjusted to 2.5 × 10^{5} ml^{-1} in Gamborg B5 (Duchefa Biochemie) supplemented with 2% glucose and inoculated on the Kapia fruits in droplets of 10 µl. After inoculation, the fruits were incubated at 20°C in 16 h/8 h day/night regime, and the infected surface area was determined.

Analysis of extracellular deposit (RPLC/MS, HILIC/MS) Kapia fruit surfaces were extracted in methanol (Roth) at room temperature for 2 min. Components of the methanolic surface washings were analyzed using reversed-phase ultra-performance liquid chromatography and hydrophilic interaction liquid chromatography coupled to high resolution mass spectrometry. The acquired data were processes as described in Mueller *et al.*[7] with one modification. The reversed-phase separation was performed using a 7 min gradient elution from 0% to 25% acetonitrile in water acidified with 0.1% formic acid. Methanolic extracts of pulverized total fruit aliquots were analyzed as a control.

Analysis of cuticular waxes (GC/FID, GC/MS) For cuticular wax extraction, isolated cuticular membranes of Kapia fruits were immersed for 2 min or intact Kapia leaves were dipped for 1 min in chloroform (Roth) at room temperature. As an internal standard, *n*-tetracosane (Sigma-Aldrich) was added to all extracts and the solvent was evaporated under a continuous flow of nitrogen. Before gas chromatographic analysis, hydroxyl-containing wax compounds were transformed into the corresponding trimethylsilyl derivatives using *N,O*-bis-trimethylsilyl-

trifluoroacetamide (Macherey-Nagel) in pyridine (Merck). The qualitative and quantitative composition of the cuticular wax mixture was determined by temperature-controlled capillary gas chromatography and on-column injection according to Vogg *et al*.[8].

Analysis of cutin monomers (GC/FID, GC/MS) For cutin depolymerization, dewaxed isolated cuticular membranes of Kapia fruits or delipided Kapia leaf disks were transesterified with boron trifluoride in methanol (Fluka) at 70°C overnight to release methyl esters of cutin acid monomers. Sodium chloride-saturated aqueous solution (Applichem), chloroform, and, as an internal standard, *n*-dotriacontane (Sigma-Aldrich) were added to all reaction mixtures. From this two-phase system, the transmethylated cutin components were extracted three times with chloroform. The combined organic phases were dried over sodium sulfate (anhydrous; Applichem). All extracts were filtered, and the organic solvent was evaporated under a continuous flow of nitrogen. Derivatization and subsequent gas chromatographic analysis were performed as described in Leide *et al*.[9].

3. Results and Discussion

Based on the *gluey berry* phenotype mutant, plants of *Capsicum annuum* breeding line Kapia were selected. Irrespective of the abnormal appearance of the mutant fruits macroscopic variations were not detectable in color, size and morphology of stems, leaves and flowers between both Kapia genotypes. In average the surface area of red ripe Kapia fruits was about 89.7 cm^2 constituted by about 4.5×10^6 cm^{-2} epidermal cells (Tab. 1). Simultaneously with the surface area, the fresh weight of Kapia fruits rose during the fruit ripening. However, the fresh weight of Kapia wild type and Kapia *gluey berry* mutant was different at the later stage of fruit development. Red ripe fruits of Kapia wild type had an approximately threefold higher fresh weight compared to Kapia *gluey berry* accompanied by a 2% higher water content. Fully expanded Kapia leaves showed no genotype-specific changes similar to red ripe Kapia fruits. The leaf surface area averaged at 143.8 cm^2, the leaf fresh weight at 1.5 g and the water content at 81%. Thus, the surface area was about 1.5-fold higher, whereas the fresh weight was more than 21-fold and the water content was approximately 5% reduced in fully expanded leaves compared to red ripe Kapia fruits for both, wild type and *gluey berry* mutant. The seed quantity averaged 173 ± 31 seeds per fruit for Kapia wild type and 151 ± 26 seeds per fruit for Kapia *gluey berry* (mean values ± SD, n = 8).

parameter	**red ripe fruit**		**fully expanded leaf**	
	Kapia wild type	**Kapia *gluey berry***	**Kapia wild type**	**Kapia *gluey berry***
surface area (cm^2)	93.3 ± 18.9	86.1 ± 17.7	152.7 ± 19.2	134.8 ± 14.4
epidermal cells (cm^{-2})	$4.5 \pm 0.4 \times 10^6$	$4.5 \pm 0.6 \times 10^6$	$4.2 \pm 0.6 \times 10^6$	$4.2 \pm 0.3 \times 10^6$
fresh weight (g)	79.0 ± 10.2	29.5 ± 21.5	1.6 ± 0.3	1.4 ± 0.2
water content (%)	87.2 ± 0.8	85.3 ± 1.0	81.4 ± 0.6	80.8 ± 0.7

Table 1:
Organ-specific characterization of red ripe fruits and fully expanded leaves of Kapia wild type and Kapia gluey berry. Epidermal cell density was studied on the adaxial leaf side. Data are shown as mean values ± SD (n = 4 - 16).

Histological characterization of the pericarp The epidermis and hypodermis of mature green and red ripe Kapia fruits were investigated[10]. The most striking difference between Kapia wild type and Kapia *gluey berry* was the considerably thinner epidermis composed of cells with significantly thinner cell walls in case of the *gluey berry* mutant (Fig. 1). However, in Kapia *gluey berry* more cell rows comprised the hypodermal collenchyma layer with significantly thicker cell walls. When comparing F1 hybrids, the epidermal characteristics were similar to the wild type parent, whereas those of the hypodermal layer were similar to the *gluey berry* mutant. Differences were found at both the mature green and red ripe stage of fruit development.

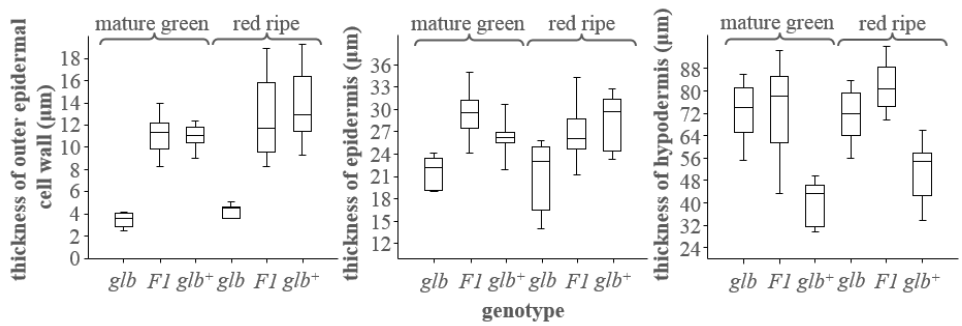

Figure 1:
Histological analysis of mature green and red ripe fruits of Kapia gluey berry (glb), wild type (glb$^+$) and hybrid (F1) by light microscopy (n = 11 - 15).

Visual characterization of the cuticle Red ripe fruits of the *gluey berry* mutant were characterized by a proceeding softness, absence of glossiness and an increasing glutinousness of the fruit exterior compared to Kapia wild type (Fig. 2). During post-harvest storage fruits of *gluey berry* exhibited an elevated water loss. The isolated cuticular membrane of Kapia wild type fruits was extended over four cell layers in the red ripe developmental stage. In contrast, the *gluey berry* cuticle architecture was fragmentary, and the accumulation of the cuticular membrane was substantially reduced. Kapia *gluey berry* developed an extremely thin cuticle, which included not more than three cell layers in red ripe fruits.

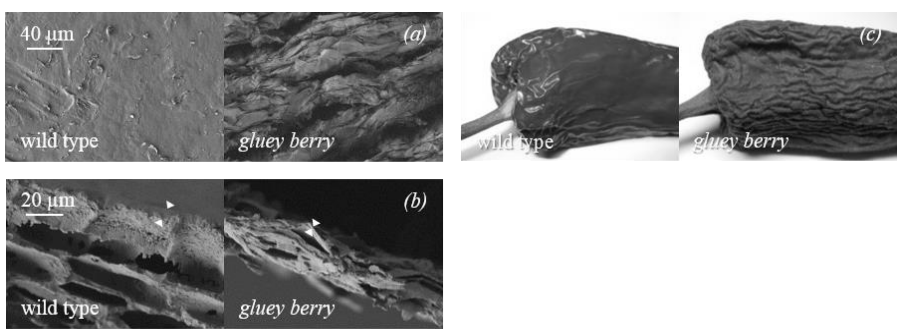

Figure 2:
SEM images of cuticular membranes of red ripe Kapia fruits (a, plan view; b, cross section). The arrow heads indicate the outermost cuticular layer. Kapia fruits after three weeks of post-harvest storage (c).

Functional characterization of the cuticle The cuticular resistance of Kapia wild type and Kapia *gluey berry* was analyzed (Fig. 3). Wild type fruits in the red ripe stage and fully expanded

wild type leaves exhibited a relatively high efficiency to minimize the cuticular water loss of about 18500 s m^{-1}. In comparison, the cuticular resistance of the *gluey berry* mutant was reduced by two-thirds to 6023 s m^{-1} for fruits and by half to 9421 s m^{-1} for leaves.

Similar to a cutin-deficient mutant in tomato (*Solanum lycopersicum* L.)[2], Kapia fruits with the *gluey berry* phenotype had a higher rate of an opportunistic fungal infection in the field. In the growth season of the year 2014 only 8% of the wild type but 42% of the *gluey berry* mutant showed severe symptoms of fungal fruit infection (n = 45). The fungus was isolated from infected fruits, and a single fungal isolate was obtained by subcultivation of conidia. This isolate was confirmed as the necrotrophic plant pathogenic fungus *Botrytis cinerea*. Subsequently, a fungal infection assay was performed under controlled conditions with the *Botrytis cinerea* isolate on red ripe fruits of Kapia wild type and *gluey berry*. The overall infected area per fruit was approximately twice as large on the mutant (57 cm^2) in comparison to wild type fruits (36 cm^2) twenty days after inoculation. The infection of the *gluey berry* mutant was also much faster than on the wild type fruits. The first lesions of the *Botrytis cinerea* infection were already detectable after three days post inoculation whereas the fungal development was delayed on the wild type fruits. Nine days post inoculation the lesions were about five times larger on the *gluey berry* mutant (29 cm^2) compared to the wild type (6 cm^2).

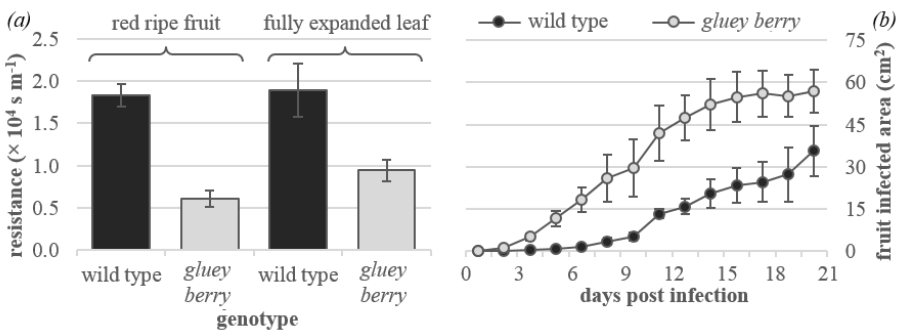

Figure 3:
Gravimetric analysis of the cuticular resistance of red ripe fruits and fully expanded leaves of Kapia wild type and Kapia gluey berry (a). Infection assay of red ripe Kapia fruits inoculated with conidia of Botrytis cinerea (b). Data are shown as mean values ± SD (n = 9 and 15).

Surface washings of red ripe Kapia fruits allowed the non-targeted investigation of changes in the metabolite abundances (Fig. 4). The *gluey berry* mutant showed a striking deposition of monosaccharide derivatives mainly flavonoids like quercitrin and hydroxy cinnamic acids. Furthermore, the disaccharide sucrose was detected as a result of an extensive solute permeability from the fruit interior. Compositional differences between the whole fruits of Kapia wild type and *gluey berry* were not found in the comparative metabolome analysis.

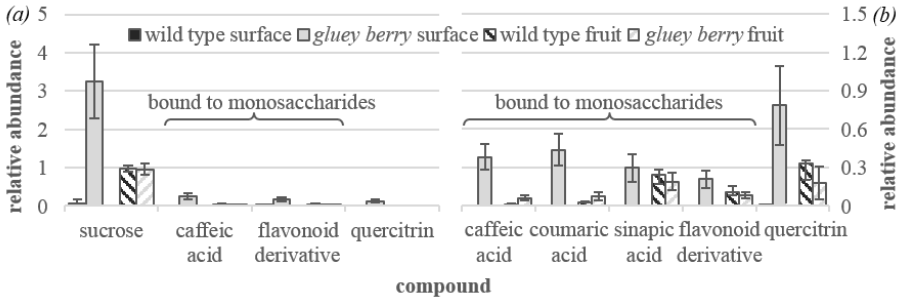

Figure 4:
Liquid chromatographic analysis of surface extracts of red ripe fruits of Kapia wild type and Kapia gluey berry (a, RPLC; b, HILIC). Data are shown as mean values ± SD (n = 5).

Chemical characterization of the cuticle A chemical analysis of isolated cuticular membranes revealed that red ripe Kapia wild type and Kapia *gluey berry* mutant fruits had a cuticular wax load of 7.4 µg cm^{-2} and 5.6 µg cm^{-2}, respectively (Fig. 5). The cuticular waxes consisted mainly of three compound classes: alkanoic acids (26%), *n*-alkanes (19%) and triterpenoids (36%). The dominant components were *α*-amyrin, *β*-amyrin and *n*-hentriacontane (C_{31}). The total cuticular wax amount of red ripe fruits was reduced by a quarter in the *gluey berry* mutant. The organ-specificity of the cuticle biosynthesis likewise reported for tomato[8] caused a distinct lower cuticular wax load of fully developed Kapia leaves compared to the red ripe fruits. However, neither quantitative nor compositional differences were found between leaves of both genotypes. Leaves of Kapia wild type and *gluey berry* possessed a comparable cuticular wax deposition of 2.5 µg cm^{-2} and 2.3 µg cm^{-2}, respectively, consisting predominantly of alkanols (52%) mostly octacosanol (C_{28}) and triacontanol (C_{30}) as well as *n*-alkanes (28%) primarily with carbon chain lengths of C_{31} and C_{33}.

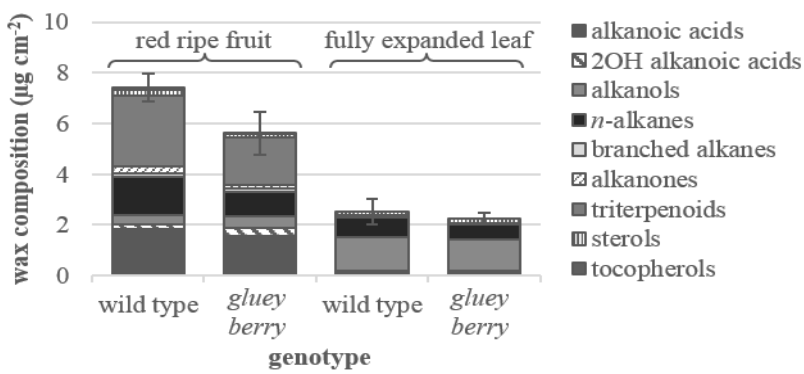

Figure 5:
GC analysis of the cuticular wax coverage of Kapia wild type and Kapia gluey berry red ripe fruits and fully expanded leaves. Data are shown as mean values ± SD (n = 4).

A cutin matrix analysis based on a depolymerization reaction of cuticular membranes was performed. Methyl esters of phenolic and C_{16}, C_{18}, C_{20} aliphatic cutin acids were detected after transesterification reaction (Fig. 6). Quantitative but no qualitative differences were found by comparing Kapia wild type and *gluey berry* mutant fruits. In red ripe mutant fruits the

monomeric cutin level was tenfold reduced. The major compound in the fruit cutin matrix of both Kapia genotypes was 9/10,*ω*-dihydroxy hexadecanoic acid, which is consistent with previous reports[11], with a quantity of 811 µg cm^{-2} in the wild type and a deficient amount of 65 µg cm^{-2} in the *gluey berry* mutant, respectively (C_{16}-type cutin matrix). The amount of cutin monomers in Kapia leaves was much lower compared to red ripe fruits and showed striking accumulations of alkanoic and 2-hydroxy alkanoic acids in addition to 9/10,*ω*-dihydroxy alkanoic acids. Quantitative alterations in the cutin polymer matrix of Kapia *gluey berry* leaves resulted in a reduction from 4.2 µg cm^{-2} to 2.9 µg cm^{-2} compared to wild type leaves. The major leaf cutin monomer was 9/10,*ω*-dihydroxy hexadecanoic acid for both genotypes.

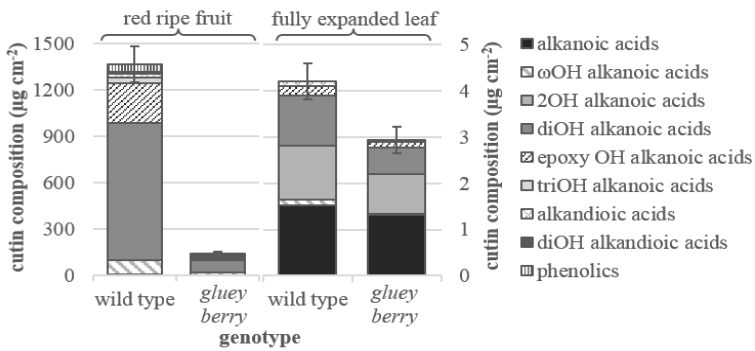

Figure 6:
GC analysis of cutin monomer composition of Kapia wild type and Kapia gluey berry red ripe fruits and fully expanded leaves. Data are shown as mean values ± SD (n = 4).

Decades of research provided insights into the diversity of cuticular structures. However, there is a limited understanding of the biosynthesis, transport, assembly and the functional contribution of cuticular compounds. Our findings concerning the cuticle biosynthesis of bell pepper indicate a suitable model system for comprehensive analyses of cutinization processes of epidermal cell walls. The cuticle of bell pepper fruits is astomatous, trichome-free and rather thick offering a uniform and robust system. By analyzing the cutin-deficient *gluey berry* mutant, the importance of an entire cuticular membrane was substantiated using histological, physiological and chemical approaches. Consequences of the massive cutin reduction on cuticle architecture, and susceptibility of *gluey berry* to water loss, solute leakage, and microbial infection were described. The molecular origin of the *gluey berry* mutation, which has not been analyzed so far, abolishing a crucial function in cutin accumulation exhibited different specificities in reproductive and vegetative organs.

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Comparison of pepper genotypes originated from Turkey and the other countries for anther culture response

Davut Keleş[1], Hasan Pinar[2], Hatıra Taşkin[3], Saadet Büyükalaca[3]

[1] *Alata Horticultural Research Institute, Erdemli, Mersin, Turkey*

[2] *Department of Horticulture, Faculty of Agriculture, Erciyes University, Kayseri, Turkey*

[3] *Department of Horticulture, Faculty of Agriculture, Çukurova University, Adana, Turkey*

Abstract

Anther culture method has been successfully used in pepper breeding programs. However, this success has been changed from genotype to genotype. Therefore, in this study, pepper genotypes originated from Turkey and the other countries were compared in terms of anther culture response to determining this variation. As nutrient medium, Murashige and Skoog (MS) nutrient medium contained 4 mg L^{-1}naphthaleneacetic acid (NAA), 0.5 mg L^{-1}6-benzylaminopurine (BAP), 0.25% activated charcoal, 30 g L^{-1} sucrose and 15 mg L^{-1} silver nitrate ($AgNO_3$) were used. At the end of the study, 2.44% and 10.15% haploid plants were obtained from local and foreign pepper genotypes, respectively. Understanding of the genotypic capacity on haploid plant regeneration is important information for breeding programs. This findings can use to develop more successful breeding program and in shortening the duration of breeding programs.

Keywords: Pepper, anther culture, genotypes, Turkey

1. Introduction

Peppers are one of the sizably consumed vegetable of the world. Fresh, pepper paste, sauce, pickle and spices are the way of consumption in both world and Turkey. Turkey, with 1.8 million tons of production, is the third largest producer after China and Mexico (http://faostat.fao.org/site/567/default.aspx#ancor). There are lots of researches to increase yield and quality of pepper production. Researchers are mainly focused on reducing the negative effect of climatic, pests and diseases. Developing resistant cultivars are the most remarkable way to overcome the problems and increase yield.

Even if there are some conventional methods for obtaining homozygous pure lines those take around 10-12 years in open pollinated and 6-7 years in self-pollinated plants. However, using tissue culture techniques, this period can be decreased to 1-2 years. Haploid plant production methods are significant for plant breeding studies and important for gaining new cultivars. The three main techniques tissue cultures for obtaining haploid plant are gynogenesis (ovule and ovarium culture), parthenogenesis (pollination with irradiated pollen) and androgenesis (anther and microspore culture). The success of these methods depends on kind of species.

In vitro anther culture technique is applied in many plant species as an effective tool for obtaining of haploid-and double-haploid (DH) plant lines. This technique is extensively employed for obtaining of haploid plants. In this manner, by doubling chromosomes in haploid

plants, it is possible to obtain genetically stable homozygous lines in a short time. Such homozygous DH plants have been used in breeding programs for production of pure lines.

The basic principle of an anther culture is to prevent formation of the pollen cells as male gamete form. By this method immature pollen cells is directly inducted as embryos form similar to somatic cells.

Although, anther culture is an important method for obtaining homozygous lines, the yield of haploid plants is low in many species (Kristiansen and Andersen, 1993). Nutrient medium is one of the main factors affecting androgenesis. In addition, the percentage of androgenetic embryo can change depending on the genotype (Comlekcioglu et al. 2001).The first haploid pepper plant was obtained by Wang et al. (1973). In this study, some researchers was followed such as George and Narayanswamy (1973); Saccardo and Devreux (1974); Novak (1974); Harn et al. (1975); Abak (1983); Comlekcioglu et al. (2001); Buyukalaca et al. (2004); Ata (2011); Taşkın et al. (2011); Niklas-Novak et al. (2012); Olszewska et al. (2013), Al Remi et al. (2014), Keleş et al. (2015).

In the some important breeding programs such as obtaining high yield and disease resistance, anther culture technique has been used. The advantage of this method arises from existence of thousands of microspores in each anther. By this way numerous haploid plants can be acquired from a single anther. In pepper, anther culture gives also some successful results. It was known that different pepper genotypes have given different response to anther culture. In this study, we compared the pepper genotypes originated from Turkey and different countries to determine anther culture response.

2. Material and methods

In the current study, fifty two non-Turkish and twenty different Turkish green pepper varieties were used as plant material (Table 1). Pepper seeds were planted into plugs containing two volumes peat and one volume perlite. Throughout the growing period fertilization, irrigation and plant protection practices were applied properly. According to Buyukalaca et al. (2004) the length of corolla should be equal to that of calyx or slightly longer, and almost half of the anthers have anthocyanin at this phase. Therefore, the flower buds at this step were collected in April and May when (for Mediterranean region of Turkey by Buyukalaca et al. 2004; Comlekcioglu et al. 2001; Taşkin et al. 2011 and Ata 2011) and checked by staining with acetocarmine. Flower buds were sterilized by 15% sodium hypochlorite solution including 1 to 2 drops Tween 20 for 15 min, and then rinsed 3 to 4 times with sterile distilled water. In order to test anthers for each variety flower buds were separated. After removal of filaments the anthers were placed 6 cm diameter glass petri dishes with in nutrient medium using sterile forceps and scalpels. Cultured anthers were incubated at 35°C. After 2 days incubation, anther was transferred into growth chamber at 25°C for 8 hours in dark and 16 hours in light photoperiod conditions. Murashige and Skoog (1962) nutrient medium containing 4mg l^{-1} NAA,0.5mg l^{-1} BAP, 0.25%activated charcoal, 30g l^{-1} sucroseand15mg $l^{-1}AgNO_3$ (Buyukalaca et al. 2004; Taskin et al. 2011; Ata 2011) was used as nutrient medium. Embryos obtained were transferred to 15 cm glass tubes containing hormone free MS nutrient medium.

3. Results and Discussion

Totally seventy two different cultivars which fifty two of them were non Turkish were used for green pepper type in this study. For each variety, 300 anthers were examined. It was found that the percentage of haploid plants of foreign genotypes was found to be higher (10.15%) than Turkish local types (2.44%). Among all genotypes tested, while the genotype given the highest

haploid plant number was found to be “CRO6” with 101 plants per 300 anthers, the lowest haploid plant number was obtained from “LM4” to be 1 plant per 300 anthers. In Turkish pepper varieties, the highest haploid plant number was recorded in the genotype “DEM6”. The genotypes “KY3”, “KY5” and “KY6” evaluated in the Capia pepper type gave the lowest haploid plants. In foreign genotypes, while the maximum haploid plant number was obtained from the genotypes “CAO”, “CNO” and “CRO”, “Blocky Red” and “Blocky Yellow” gave the lowest haploid plant number. In terms of different pepper types, Demre and Charleston pepper types had more positive results than Bell and Capia types (Table 1).

As use of haploid plants in plant breeding shorten the breeding process, it has a great importance (Ellialtıoğlu et al. 2002.). Anther culture method which is one of the techniques of haploidization is widely used in pepper. Several studies have been carried out on the developing of a successful protocol related to pepper anther culture. Numerous endogenous and exogenous factors such as genotype, physiological state and growth conditions of donor plants, pollen development stage, pretreatment to flower buds or anthers and in vitro culture medium and conditions affect the embryogenic response of anthers in culture (Atanassov et al. 1995; Datta 2005; Smykal 2000;Wang et al. 2000).

Genotype is the most important one among them and often limiting factor in the pepper androgenic reaction (Comlekcioglu et al. 2001; Rodeva 2001;Wang and Zhang 2001;Rodeva et al. 2004; Koleva-Gudeva et al. 2007). Some pepper genotypes are recalcitrant in terms of induction of androgenesis and formation of haploid regenerations. The frequency of direct embryogenesis varies according to genotype. However, in previous studies were found to be between 0.5 and 75 embryos per 100 cultivated anthers in lines and F1 hybrids (Qin and Rotino 1993; Ltifi and Wenzel 1994; Mityko et al. 1995; Koleva-Gudeva et al. 2007).

It has been reported that different cultivars within a species exhibit different responses in anther culture. For example, 21 cultivars of *Triticumaestivum* were tested and androgenetic response was recorded in only 10 varieties. In rice, *japonica* subspecies have been found to be more productive than *indica*subspecies (Bajaj 1990).In an anther culture study using a large number of Citrus genotypes, positive results were obtained from only one variety of clementine and one variety of lemon (Germana` 2007).

Niklas-Nowak et al. (2012) reported that 31 plants out of 63 plants which were obtained through anther culture were diploid. In an anther culture study conducted by Olszewska et al. (2013), the number of spontaneous doubled haploid plant was given: as three of five plants in ATZ1 breeding line, one of two plants in PO breeding line, four of six plants in F1 (ATZ x PO) line, two of four plants in F1 (ATZ1 x TG), three of five plants in AP40 DH line, six of nine plants in AC7 DH line, one of three plants in F1 (*C. frutescens* x *C. chinense*) interspecific hybrid line and one of three plants in F1 (*C. frutescens*x *C. baccatum*) interspecific hybrid line.

In this study, the percentage of haploid plant efficiency of non-Turkish pepper genotypes was higher than Turkish pepper types. The highest haploid plants were obtained from the genotypes “CRO6” and “DEM6” originated foreign and local genotypes’ respectively. These results showed that although non Turkish genotypes had higher haploid plants when compared with Turkish pepper genotypes, there is important variation among all the pepper genotypes with regard to efficiency of haploid plants.

As a conclusion, beginning of breeding programs, the haploidy efficiency can be calculated for different pepper genotypes. The present results may be helpful for pepper breeders who want use Turkish and non-Turkish pepper genotypes.

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Table 1.
Efficiency of haploid plants %

	MC	MO	MT	NAI	TPN	EHP %
1	BR1	Non Turkish	Blocky Red	300	10	3,33
2	BR2	Non Turkish	Blocky Red	300	1	0,33
3	BR3	Non Turkish	Blocky Red	300	24	8,00
4	BY1	Non Turkish	Blocky Yellow	300	21	7,00
5	BY2	Non Turkish	Blocky Yellow	300	8	2,67
6	BY3	Non Turkish	Blocky Yellow	300	8	2,67
7	CAO1	Non Turkish	CAO	300	6	2,00
8	CAO10	Non Turkish	CAO	300	11	3,67
9	CAO2	Non Turkish	CAO	300	24	8,00
10	CAO3	Non Turkish	CAO	300	11	3,67
11	CAO4	Non Turkish	CAO	300	40	13,33
12	CAO5	Non Turkish	CAO	300	68	22,67
13	CAO6	Non Turkish	CAO	300	45	15,00
14	CAO7	Non Turkish	CAO	300	32	10,67
15	CAO8	Non Turkish	CAO	300	43	14,33
16	CAO9	Non Turkish	CAO	300	13	4,33
17	CNO1	Non Turkish	CNO	300	43	14,33
18	CNO2	Non Turkish	CNO	300	95	31,67
19	CRO1	Non Turkish	CRO	300	88	29,33
20	CRO1	Non Turkish	CRO	300	59	19,67
21	CRO2	Non Turkish	CRO	300	37	12,33
22	CRO2	Non Turkish	CRO	300	72	24,00
23	CRO3	Non Turkish	CRO	300	14	4,67
24	CRO3	Non Turkish	CRO	300	46	15,33
25	CRO4	Non Turkish	CRO	300	69	23,00
26	CRO5	Non Turkish	CRO	300	42	14,00
27	CRO6	Non Turkish	CRO	300	101	33,67
28	DIT	Non Turkish	DIT	300	51	17,00
29	LM1	Non Turkish	Lamuyo	300	16	5,33
30	LM2	Non Turkish	Lamuyo	300	3	1,00
31	LM3	Non Turkish	Lamuyo	300	28	9,33
32	LM3	Non Turkish	Lamuyo	300	12	4,00
33	LM4	Non Turkish	Lamuyo	300	1	0,33
34	LM4	Non Turkish	Lamuyo	300	5	1,67
35	LM5	Non Turkish	Lamuyo	300	6	2,00
36	LM5	Non Turkish	Lamuyo	300	10	3,33
37	LM6	Non Turkish	Lamuyo	300	5	1,67
38	LM7	Non Turkish	Lamuyo	300	15	5,00
39	LM8	Non Turkish	Lamuyo	300	5	1,67
40	RAO1	Non Turkish	RAO	300	6	2,00
41	RAO2	Non Turkish	RAO	300	28	9,33
42	RRB1	Non Turkish	RRB	300	31	10,33

43	RRB2	Non Turkish	RRB	300	44	14,67
44	RRB3	Non Turkish	RRB	300	14	4,67
45	RRB4	Non Turkish	RRB	300	30	10,00
46	RRB5	Non Turkish	RRB	300	26	8,67
47	RRB6	Non Turkish	RRB	300	30	10,00
48	RRB7	Non Turkish	RRB	300	52	17,33
49	RRB8	Non Turkish	RRB	300	25	8,33
50	RRO1	Non Turkish	RRO	300	29	9,67
51	RRO2	Non Turkish	RRO	300	24	8,00
52	RRO3	Non Turkish	RRO	300	56	18,67
	Max.				101,00	33,67
	Min.				1,00	0,33
	Mean				30,44	10,15
1	CHR1	Turkish	CHR	300	12	4,00
2	CHR2	Turkish	CHR	300	13	4,33
3	DM1	Turkish	DOLMA	300	2	0,67
4	DM2	Turkish	DOLMA	300	2	0,67
5	DM3	Turkish	DOLMA	300	1	0,33
6	DM4	Turkish	DOLMA	300	1	0,33
7	KY1	Turkish	KAPYA	300	2	0,67
8	KY2	Turkish	KAPYA	300	2	0,67
9	KY3	Turkish	KAPYA	300	0	0,00
10	KY4	Turkish	KAPYA	300	4	1,33
11	KY5	Turkish	KAPYA	300	0	0,00
12	KY6	Turkish	KAPYA	300	0	0,00
13	DEM1	Turkish	DEM	300	5	1,67
14	DEM2	Turkish	DEM	300	13	4,33
15	DEM3	Turkish	DEM	300	12	4,00
16	DEM4	Turkish	DEM	300	7	2,33
17	DEM5	Turkish	DEM	300	14	4,67
18	DEM6	Turkish	DEM	300	34	11,33
19	DEM7	Turkish	DEM	300	14	4,67
20	DEM8	Turkish	DEM	300	13	4,33
	Max.				34	11,33
	Min.				0	0,00
	Mean				7,55	2,44

MC: Material code, MO: Material origin, MT: Material type, NAI: Number of anthers introduced, TPN: Total plant number, EHP: Efficiency of haploid plants %

POSTER PRESENTATIONS

SESSION 1

Breeding strategies

P1-01

IstvánTúri – The innovative pepper breeder

Lajos Tompos[1], András Andrásfalvy[2]

[1] *Consultant, Szigetszentmiklós, Hungary*

[2] *Consultant, Budapest, Hungary*

István Turi (1933-1999)

Dr. Túri graduated from the College of Horticulture and Viticulture in 1957. From 1958 he was working at the University of Horticulture, the legal successor of the College, as a professor and a researcher. From 1990 he carried on working with his colleagues in his own enterprise breeding pepper and producing seeds of his of varieties.

Besides his main field of research, which was pepper breeding, he established lasting results in other areas too. His most useful achievements supporting the work of the Hungarian growers can be summed up as follows:

- He developed the possibility of growing vegetables on weak sandy soil without organic fertilization
- Establishment and development of plastic-covered facilities
- application of non-heated facilities (portable and non-portable), one and two phase utilization of heated facilities
- Development of different heating systems (multisector and plant heating)
- Development of double-layered covering systems

- Foundation of the theoretical and practical "water curtain" method
- Organization of regular consultations and practical demonstrations for the growers
- Introduction and distribution of one and two stem pruning system
- Research of plant-habit biology, determination of slow, middle and fast growth and how to change conditions related to plant habit.
- Scientific description of the research topic "Növényalkat" (plant habit) - limitations and possibilities increasing the yield
- His publications were user-friendly with a lot of practical advice. He published more than a hundred articles, numerous books and chapters in books that indicate his diverse activities.

István Túri was rightfully most proud of his pepper varieties. He was one of the successful pepper breeders in Hungary who always led the way with his new hybrids and results. The best proof for his success is the practical value and the spread of his hybrids and varieties which are registered and widely recognized.

He did not like the complicated variety collections. He did not believe in breeding materials that contained huge numbers of combinations. He had a good eye for excluding the less promising materials and did not deal with them. He very confidentially picked the most promising parent lines and hybrids. He hardly ever made a mistake.

His first variety was the cv. *Soroksári hajtató* which is was wide-spread and thoroughly used. Those individual plants that were the results of the spontaneous mutation were also found among the open-pollinated plants. These plants played an important role later in the course of pepper breeding.

Although it was never explicitly stated, Túri and his colleagues produced some of the hybrids in Hungary the very first time for certain varieties. As these varieties are local ones, they are not grown or known in other parts or regions of the world. Without doubt, these types were created in the workshop of Túri. The first such hybrid *HRF F1* was nationally registered in 1985.

Figure 1. HRF F1

Figure 2. Pritavit F1

This hybrid was the favourite hybrid of the greenhouse pepper growers for two and a half decades. For the first time commercial amounts of the seeds of this conical white pepper were produced. For a long time, it was the market leader in Hungary in its category.

The hybrid called *Hó F1* stills excels at soilless pepper production. If nurtured properly this type will grow excellent quality and sized fruits.

The hybrid called *Kaméleon F1* became popular in the countries on the Balkan due to its favourable qualities such as its pale green colour, outstanding taste and easy cultivation.

The hybrid *Albatrosz F1* is also white and it is mainly recommended for open field production.

Although designed for protected production the hybrid *Pritavit F1* became the leading variety of the tomato shaped pepper production on open field. The so called internal mold does not occur with this variety thanks to the closed pistil. This plant has high tolerance against bacterial diseases that are very typical on open field.

The production of the kapia type peppers was not well known in Hungary until the millennium. Only a few people got familiar with the grilled kapia peppers during their travels abroad. The introduction of the first kapia hybrid was successful because a unique production technology was created in cooperation with the breeders and the seed retail companies. Their success was justified by the production results. Nowadays, the kapia production area is hundreds of hectares in Hungary. The highest yield can be 80-90 tons.

Figure 3. Karpia (T112) F1

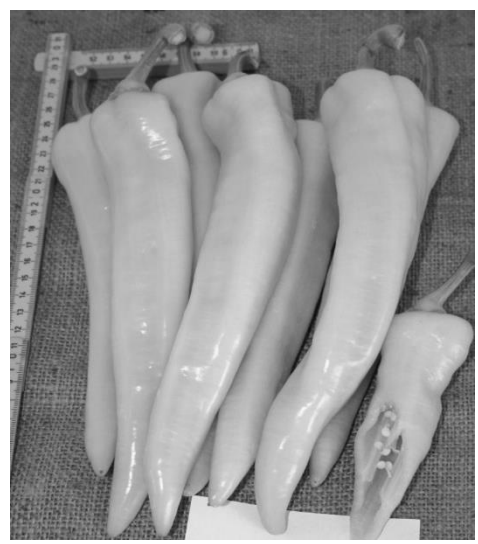


Figure 4. Édes Banán F1

Besides the hybrids listed above, Dr. Túri, along with his colleagues also bred types that only take up smaller volumes of the market. From among the hot pointed ones the hybrid *Titán F1* and the hybrid *Vulkán F1* are worth mentioning. The hybrid *VelenceF1* is a hot conical. Among the growers for small markets the hybrids *Édes banán F1* and *Csípős banán F1* were popular. From among the "apple shape" hybrid *Turul F1* is noteworthy. Túri also participated in the production of four open-pollinated varieties commissioned by a seed company.

Túri often said the following about breeding “It isn’t hard to create new varieties. Everybody can do that. But can we create better ones…?”

He also noted that “It is not sufficient to create a new variety. If there aren’t any seed, it isn’t worth a thing.” He and his colleagues contributed a lot to seed production as well. His patent protected system was recognized by his colleagues both from Hungary and abroad. The seed production of the varieties was organised in their own network. This network which was developed by himself and his team was based on their own technology and it complied with the highest standards.

His work in the fields of research and development was facilitated by colleagues he accepted and trusted to the fullest. He saw the future perspective in them.

Mrs. Gyúrós, Berthold Margit another colleague he used to work with closely also needs to be mentioned here. Without her loyal and personal involvement, the results, listed above wouldn’t have been achieved and the decade long success that followed would not have occurred.

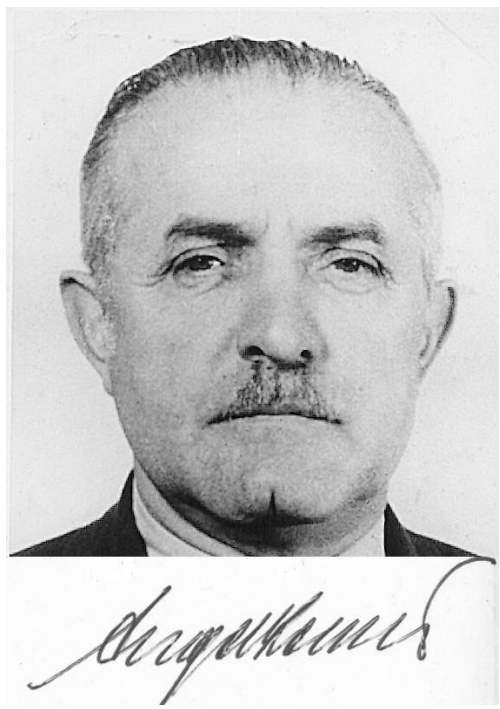
P1-02

Lambert Angeli, pioneering breeder of the first white, sweet variety of bell pepper was born a hundred years ago.

Andrea Moór[1], Lajos Zatykó[2]

[1] *Consultant, Budapest, Hungary*

[2] *Consultant, Budapest, Hungary*



Lambert Angeli (1916-1971)

Dr. Lambert Angeli's birthplace was in Hungary, in the Transdanubian village Isztimér. He was graduated from high school in the nearby city of Székeshehérvár. He received his higher education in Budapest at the Kertészeti Tanintézet (Teaching Institute of Horticulture) starting in 1937, and continued it later at the Kertészeti Akadémia (Academy of Horticulture). After the end of World War II the Academy was renamed Kertészeti és Szőlészeti Főiskola (College of Horticulture and Viticulture), where he was appointed assistant lecturer in the Department of Vegetable Growing. Later he was promoted to lecturer, and finally, to docent.

In addition to his teaching and research work he was doing breeding in the Kertészeti Kutató Intézet (Horticultural Research Institute) without pay or official appointment before receiving an official appointment as senior researcher in 1965. He kept that job until his passing in 1971.

His main field of research was paprika breeding but he worked successfully in various other areas as well, such as mushroom growing, tomato breeding, chickory growing, introducing lima bean and okra for cultivation in Hungary, and developing growing technologies.

Removing the pungency of the "Cecei" paprika, known theretofore as a regional variety, was an epoch-making accomplishment of his efforts. Neither the shape nor the size of the harvested regional variety was uniform and, most importantly, neither was its content of capsaicin. This attribute caused many problems in the flow of commerce both in and outside the country. His objective was to create a unified, homogeneous line. Through a lengthy series of selections made over the years. He chose the mother plants according to their phenotypical attributes and tasted tens of thousands of fruits to arrive at the final result: a sweet, white paprika line, the cv. Cecei édes 3 constant variety that was entered in the national list of species as a state-approved variety in 1958 (Figure 1.). It is thanks to him that Hungarians have become consumers of pepper. While in the 1940's only around 5000 hectares (roughly 12,000 acres) were used for pepper growing, by 1960 the white, sweet variety was cultivated over nearly 20,000 hectares (roughly 49,000 acres). Nowadays just about all seed companies around the world offer white, sweet hybrids, similar to the "Hungarian" paprika. Lambert Angeli's handiwork can no doubt be detected among their genes.

Figure 1. cv. Cecei édes 3

The other important feature of his breeding activity was the use of the bunchy (cluster) gene. He was the first pioneer of doing so for breeding white paprika. Several varieties were created using this method, such as cv. Csokros felálló, cv. Csokros csüngő, cv. Gépi konzerv (Figure 2.) which were very popular for growing in the low air space of the cultivating equipment used, and for the large scale production requirements of the 60's and 70's, and the suitability for machine harvesting of large batches at a time.

It was he who pioneered the breeding of the Hungarian hybrid paprika. The domestic "long and hot" paprika's consumption was radically changed by the first successful hybrid named Tétényi hajtatási zöld F1 (Figure 3.).

The phenotype and genotype of the hybrid's parent lines were completely different. Both the phenotype and genotype of the hot constant mother line selected out of a regional, dark green and thick peeled variety, was heterogeneous. The first thing he did was the creating of a homozygotic mother line. For the father line he used the white, determinate growth, sweet, bunchy gene containing line that he had created earlier. Thus he produced a novel phenotype by crossing two completely different parents.

Figure 2. cv. Gépi konzerv

Figure 3. Tétényi hajtatási zöld F1

Shortly before his early and sudden death he began resistence breeding against the tobacco mosaic virus (TMV). He was the first to make use of the L1 resistence gene for pepper breeding in Hungary. He did not live to see the result of his efforts.

Besides his teaching and research work he also helped pepper growers by writing books for them. His book titled Paprikatermesztés (Paprika growing) was published in 1955. Later on three revised and expanded editions were printed.

His breeding activities were honored with the Rudolf Fleischmann prize in 1971, the year of his passing. On the tenth anniversary of his death, at the initiative of his former workplace, the Kertészeti Egyetem (University of Horticulture) had a bust sculpted in his memory. In 2009, on his former fellow researchers' initiative the municipal authority of Budapest's 22. district named a street after him in the town of Nagytétény. A plaque informs passers-by that he was the pioneer breeder of the sweet, white paprika that all around the world has been known as the Hungarian paprika.

He was a well liked and respected researcher with an uncompromising demand for disciplined work and no tolerance for argumentation. He was an unquestionable authority, a charismatic, conservative, and deeply religious man. He was willing to record and share only facts proven by experimental evidence. We saw him as a balanced, self-assured, contented person, but did not truly know him. He died of a heart attack at the age of 55.

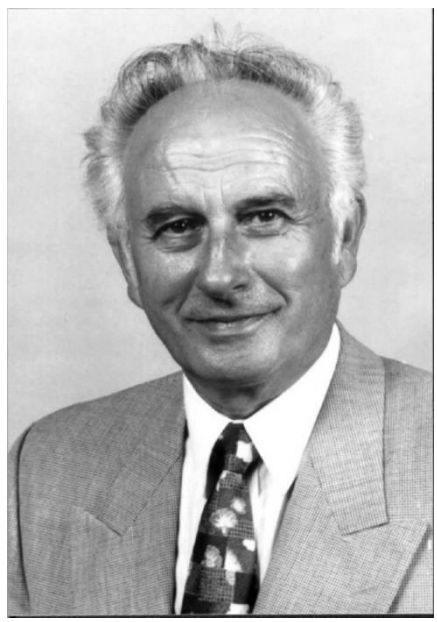
P1-03

The life and work of a paprika breeder Ferenc Márkus

József Kapitány[1], Zoltán Tímár[2]

[1] *Consultant, Budapest, Hungary*

[2] *Univer Plc. Kecskemét, Hungary*



Ferenc Márkus (1935-2003)

An outstanding personality of Hungarian paprika cultivation, Ferenc Márkus sought in all his life to apply the latest methods in the process of selective breeding, to create the most modern varieties according to the need of producers, consumers and the processing industry. As a leading breeder, and in conjunction with his colleagues, he created more new species than had ever existed before during the history of the institution. These species played and still play a decisive role in Hungarian paprika production, enhancing the reputation of Hungarian paprika. The fact that paprika is the only plant in Hungary whose cultivation is based solely on Hungarian varieties demonstrates the value of his work.

Ferenc Márkus was born in 1935 in Homokmégy.He started school at his birthplace, then attended the Piarists secondary school in Kecskemét between 1946-1950 and the Vocational School of Agriculture in Baja from 1950 to 1954. He continued his studies at the University of Agricultural Sciences in Gödöllő between 1954-1959, where he graduated as an agricultural engineer.

He received a postgraduate diploma as plant breeding engineer in 1960, gained his PhD in

1964 and earned a CSc degree in Agricultural Sciences in 1982, and in 2004 he received the posthumous title of Doctor of the Hungarian Academy of Sciences (DSc).

From 1960 he worked in the Duna-Tisza Agricultural Research Institute, in the department of Kalocsa Paprika Research, and in its successor the ZKI Paprika Research Station of Fűszerpaprika Kutató-Fejlesztő Közhasznú Társaság. He worked through all stages of the research career from junior research fellow, to research fellow, to senior research fellow, later becoming deputy director in 1980, and director in 1994. He continued in this position until 2003.

He became a board member of both the National Paprika Product Council in 1994 and the Hungarian Seed Association and Product Council in 1998. In 1986 he was accepted as a member of the Horticulture Working Committee of the Szeged Academic Committee and a member of the Institute of Plant Breeding Committee in 2000. In 2001, he was elected executive president of the National Paprika Product Council, and he became a member of the Knights of St. Stephen in Kalocsa. In 2002 he received an honorary college professor appointment at the College of Kecskemét.

He was awarded the decoration “Excellent Worker of Agriculture” (1968, 1976), the Mathiász János Prize (1980), the Urban Development Prize (1982), the Bács-Kiskun County Science Prize (1998), and the Fleischmann Rudolf Prize (1998).

Ferenc Márkus was an excellent paprika breeder, who loved his profession passionately and did his agricultural research work with diligence and dedication. He was one of those fortunate people who through their consistent and methodical work manage to accomplish the goals set for themselves. He had an exceptional talent for recognizing the potential of different breeding methods. He produced excellent paprika varieties by always searching for newer and better forms. These varieties gained the respect of domestic producers and growers, as well as bringing him international fame and recognition.

He considered Gyula Mészöly, an academician, tomato-breeder and Director of Research Institute, his role model. Gyula Mészöly helped him with infinite goodwill and benevolence, guiding him along the bumpy road of research and providing guidance to his work. As a young researcher Márkus was proud to have received advice that made him receptive to learning about the deeper mysteries of the profession. He often talked about the teachings delivered to him wrapped in humour, teachings that are still relevant, such as: "the breeder should aim to choose from the many gems in his hand the one that shines the brightest, and for the longest time" and "we can take out of the hat only what was previously put into it” to understand the genes and the new combinations.

By using the generative method of cross-breeding and pedigree breeding, he was able to create full varieties of continuously growing, semi-determined and determined breed types (*fasciculate* - *fa* gene) which met the demands of producers, consumers and the industry alike. As a result of his work he was able to find a "gem" that was embodied in the cv. *Kalocsai merevszárú 622* variety, which shone for nearly forty years, providing outstanding color content and a reliable yield in Hungarian paprika production.

The team of researchers, working together for three decades, launched 26 state-certified species in Kalocsa and Szeged, making them available for cultivation together with processing technology recommendations. The value and importance of this kind of work is further enhanced by the fact that paprika is the only plant in Hungary which is grown exclusively from home-bred varieties.

The varieties produced represent national and international standards at a high level in the most important quality values and are favored by customers.

The research work of the resistance breeding program, launched under Ferenc Márkus's leadership in 1992, was performed in a team structure with the participation of the Institute staff and external specialists. He used the most well-known modern breeding approaches. In the course of this activity he used transgenic resistance resources built into the varieties and breeding phylum. In the climatic conditions of the Carpathian Basin the most important paprika diseases are *Xanthomonas campestris pv. vesicatoria* and TMV. Resistance against these diseases is provided by genes *Bs-2, L3* and *gds*.

In the framework of the breeding program he started the production program of the 'resistant hybrid' variety of paprika. The work has resulted in the creation of bacteria resistant constant and hybrid varieties. These can be produced by environmentally friendly technology, providing higher yields and greater efficiency. Ferenc Márkus's life-work contains 26 kinds of paprika which represent the full range of varieties of the past and present.

His scientific works are recorded in 113 Hungarian and English publications, made available at national and international scientific events, meetings and congresses, in academic journals and periodicals. He co-authored four reference books and was editor of another which was published by the Agricultural and the Academy Publishing Houses.

He handed over well founded results, proven by tests, to be used in practice, from the species to the method of cultivation and the processing technology. Through these results the producers of paprika were able to produce and process the paprika efficiently. To use a Biblical example, he provided the fishing net instead of the fish, thus providing a decent living and properity to nearly forty thousand families.

The nearly one hundred year old Kalocsai Paprika Research Centre was at all times a key institution of Hungarian paprika research and achieved sensational results since its establishment. The outstanding achievement of the first 50 years came in 1928, when they discovered and developed here the sweet (non-hot) paprika variety for the first time ever anywhere in the world.

There were two outstanding periods of the past 40 years, in which Márkus made his mark:

The modern types and varieties, along with the related technologies, created between 1970-1995 represent the "Golden Era" of Hungarian paprika research and development.

The size of the cultivation area and yield rose to a level never seen before. This was reflected in people's living conditions and even on the level of the national economy.

These results achieved recognition at international level as well.

The resistant varieties and hybrids marked a new era between 1996-2010 and strengthened the international reputation of Hungarian paprika research.

This is the achievement and merit of all those paprika researchers who were devoted to their work and through their high-quality research contributed to the world reputation of Hungarian paprika. Ferenc Márkus's oeuvre played an important and valuable role in these results and achievements.

His work has been exemplary to all of us who followed his steps and carried on with the scientific legacy he has left us. Hard-working and always forward-looking and proactive, he remains an empowering role model for us and for future generations. Research work is both service and mission at the same time. Ferenc Márkus fulfilled both sides equally with a pure heart and conscience.

Figure 1.
Kalocsai merevszárú 622

Figure 2.
Direct sowed field of Kalocsai merevszárú 622

P1-04

Investigation of obtaining fertile S. *melongena X* S. *torvum* hybrid populations

Atilla Ata[1], Saadet Büyüklaca[2], Nedim Mutlu[3], Davut Keleş[1]

[1] *Alata Horticultural Research Institue Mersin, Turkey*

[2] *Çukuroava University Faculty of Agriculture, Adana, Turkey*

[3] *Akdeniz University Faculty of Agriculture, Antalya, Turkey*

Abstract

Cultured eggplant is too sensitive to many soil-borne diseases and pests. Wild eggplant species called *S. torvum* genotypes contain resistance to soil-borne diseases and pests. Generally, *S. torvum X S. melongena hybrids are sterile.* Until now, it is not reported any character transfer from *S. torvum* to *S. melongena*. *In this study, 7 S. melongena and 5 S.torvum genotypes used for* hybridization in *35 combinations, and plant obtained in 34 combinations. Partially fertile male individuals were obtained in only 1 of these combinations. Pollen viability and germination value of this combination was found 8.75% and 5%, respectively.* These plants were used to backcross, however it weren't obtained BCF1 plants.

In the study, it is also used S. americanum, *S. linnaeanum,* and S. aethiopicum genotypes used for the bridge crossing. In the combinations of S. melongena x S. americanum, S. americanum x S. *melongena S. torvum x S. americanum, S. americanum x S. torvum, S. torvum* x S. *aethiopicum*, and *S. linnaeanum* x *S. torvum* any hybrid plant did not obtained, also *S. torvum x S. linnaeanum* hybrid was sterile. In the S. *aethiopicum x S. torvum* hybrid fruit set has occured but it degeneration in the embryo has become. In the combination of *(S. melongena x S. aethiopicum) x S. torvum* plant are obtained, and determined that these plants are still sterile.

Keywords: Eggplant intercross, *S. torvum*

P1-05

Could quantitative resistance increase the durability of major genes conferring nematode resistance in pepper?

Fulgencio Sánchez-Solana[1], Caridad Ros[1], María del Mar Guerrero[1], Victoriano Martínez[1], Carmen María Lacasa[1], Ana Hernández[1], Jerónimo Torres[1], Alain Palloix[2], Alfredo Lacasa[1]

[1] *Departamento de Biotecnología y Protección de Cultivos, IMIDA, La Alberca, Murcia, Spain*

[2] *Génétique et Amélioration des Fruits et Légumes, INRA PACA, UR1052, Montfavet, France*

Abstract

In pepper (*Capsicum annuum*), the major genes (R-genes) *Me1*, *Me3* y *N*, confer resistance against most serious species of root-knot nematodes (*Meloidogyne incognita, M. arenaria and M. javanica*). In field conditions, development of virulent populations of nematodes has been observed when pepper cultivars carrying *Me3* or *N* genes are growing in same soil for successive years, thus endangering the efficiency of R-genes. Combination of R-genes and quantitative resistance factors in the same genotype is considered as good breeding strategies for increasing the durability of R-genes. The recent discovery of high level of quantitative resistance confered by the pepper genetic background, with broad spectrum of action, protecting pepper against *Me3*-virulent as well as avirulent *M. incognita* isolates, is expected to provide promising applications for preserving the efficiency of nematode resistance. In order to know the ability of this quantitative resistance increasing the durability of R-genes of pepper against root-knot nematodes, five pepper inbred lines, differing in their quantitative resistance level, were combined with *Me1* or *Me3* genes in F1 hybrids. In a greenhouse with soil naturally infected by *M. incognita*, resistance of inbred lines and F1 hybrids (used as rootstocks) were evaluated in two successive growing years. In both years, lines carrying same R-gene were less infected by the nematode when combined with quantitative resistance. An Increase of infection by nematode in second growing year was observed, slightly in lines carrying *Me1* and notably in lines carrying *Me3*, independently of quantitative resistance. Infection level in inbred lines without R-genes was similar in both years. So, we discuss if quantitative resistance increases the resistance level but do not prevent the increase in frequency of virulence towards R-genes once emerged.

Keywords: Capsicum annuum, Meloidogyne incognita, Me genes

P1-06

Determination of reaction of *Solanum aethiopicum* and *Solanum incanum* genotypes against *Fusarium oxysporum f. sp. melongenae*

İsmail Erper[1], Şeyma Sarıbaş[2], Ahmet Balkaya[2], Dilek Kandemir[3]

[1] *University of Ondokuz Mayıs, Faculty of Agriculture, Department of Plant Protection, Samsun, Turkey*

[2] *University of Ondokuz Mayıs, Faculty of Agriculture, Department of Horticulture, Samsun, Turkey*

[3] *University of Ondokuz Mayıs, Samsun Vocational School, Samsun, Turkey*

Fusarium wilt, caused by *Fusarium oxysporum* (Schlechtend: Fr.) f. sp. *melongenae* Matuo and Ishigami (Fomg) is one of the most destructive and widely distributed diseases of eggplant. In this study, a totally twenty two wild eggplant relatives belong to *Solanum aethiopicum* and *Solanum incanum* were tested to evaluate the presence of resistance to eggplant Fusarium wilt. The resistance reactions of 19 *S. aethiopicum* and 3 *S. incanum* genotypes against to highly virulent *F. oxysporum* f. sp. *melongenae* isolate (Fomg-10) were carried out in the controlled plant growth cabin of the Department of Plant Protection during 2015. Inoculation was performed on one-month-old seedlings of genotypes by the method of dipping of the roots for 10 min in conidial suspension ($1x10^6$ conidia mL^{-1}) of the isolate. Thirty days after inoculation, the disease severity index was performed using a scale ranging from 0 (no lesions) to 4 (dead plant) and disease severity percentage was calculated based on the scale values. Then ***level*** of ***resistance [***I= % 0-10: highly resistant (HR), II= % 11-40: moderately resistant (MR), III= % 41-70: slightly resistant (SR), IV= % 71-100: susceptible (S)***] were determined according to*** disease severity percentage. The test results showed that the differences among the reactions of wild eggplant relatives against Fomg-10 isolate were statistically significant ($P<0.05$). From the observations, 14 genotypes belonging to *S. aethiopicum* were found highly resistance (HR), whereas other 5 genotypes were found moderately resistant (MR) against to Fomg-10 isolate. In addition, two genotypes of *S. incanum* were determined slightly resistant (SR), whereas one genotype was susceptible (S) against the same isolate. At the end of this research, selected resistant genotypes will be used in the development of new eggplant rootstock cultivars. The other remaining genetic materials also had some valuable traits. The results provide information on the diversity and breeding values of wild eggplant germplasm.

Keywords: *Solanum aethiopicum*, *Solanum incanum*, Fusarium wilt, resistance, rootstock breeding

P1-07

20 years of non-hypersensitive, non-specific, recessive resistance in pepper - review

Gábor Palotás

Univer Product Plc., R&D department, Kecskemét, Hungary

gabor.palotas@univer.hu

Abstract

Last years a kind of boom was observed in practical application of recessive resistance genes against pepper's bacterial spot disease. These genes encode non-hypersensitive reaction resulting tissue retention and leaf blade thickening instead of necrosis. Clear indication of this process is that the biggest breeding companies have got the access to *bs5* gene and it's marker one after the another in 2014-2015 in order to use it in their pepper breeding programs [1].

The utility of dominant resistance genes do not result long term durability in the field, because „their deployment in monoculture selects for pathogen variants" (Dangl et al. 2013) [2]. But resistance controlled by recessive gene(s) is „not associated with hypersensitive reaction, and for this reason, no specific effector or avirulence factor appears to be involved in interactions with a host gene product to produce a resistance phenotype" (Vallejos et al., 2010) [3]. On the other hand tissue necrosis decreases the plant's assimilation surface which doesn't happen in case of non-hypersensitive reaction.

It requires no further explanation that importance of durable and non-specific resistance is increasing during new challenges for agriculture caused by global climate change.

Discovery and practical application of recessive resistance genes is rightly considered to be paradigm shift in plant breeding. Contribution of conventional pepper breeders and plant pathologists for this change was remarkable. Furthermore kind of milestone achievements were publicated first time on Eucarpia Capsicum and Eggplant Working Group Meetings (1992, 1995).

The aim of this publication is to review the most important scientific publications as well as the public informations on practical and business applications in order to facilitate better understanding the subject's past and present and draw up a few opened questions.

Keywords: *Capsicum annuum*, *Xanthomonas campestris pv.vesicatoria*, non-hypersensitive reaction, recessive resistance, *gds* gene, *bs5* gene, *bs6* gene, *xcv-1* gene

Cook and Stall studied the inheritance of resistance on pepper to bacterial spot since 1958. Already they noticed a „distinct possibility" of multigenic resistance near the monogenic one in case of a resistant accession, PI 163192 (1963) [4].

Sowell and Dempsey (1977) noticed the better durability of resistance of accessions containing PI 163192 and 2 other sources and they explained this finding by a forward-looking argumentation: „new pathotypes will develops slowly following the release of new cultivars having these introductions…" (namely PI 163192, 271322 and 322719) [5].

Hibberd at al. examined the accession PI 271322 as source of a single, dominant resistance gene (*Bs3*) and observed that „the non-hypersensitive reaction of these plants was qualitatively

different from the susceptible reaction in Early Calwonder" (1983) [6].

Kim and Hartmann (1985) already clearly differentiated hypersensitive reaction (HR), resistant non-HR and susceptible non-HR symptoms during study of single dominant gene *Bs3* causing resistance to bacterial spot in PI 271322 accession. In addition, they mentioned the possibility of resistance to be „recessive to susceptibility, with perhaps two genes involved" [7].

Kim (1988) noticed that PI 163192 „showed some tolerance to *P. capsici*" which is an interesting finding in the light of subsequent theories because it suggests bacteria non-specificity of the reaction found in this accession [8].

Poulos et al. (1992) studied the quantitative components of resistance to *Xanthomonas campestris pv. vesicatoria* in pepper line CNPH 703 which is derived from PI 183441. The authors mention CNPH 703 line as a source of „race non-specific, non-hypersensitive resistance", and stated that „at least two genes were influencing the inheritance of quantitative resistance to *X. campestris pv. vesicatoria* in CNPH 703". With this statement Poulos et al. were not far from the theory of next authors [9].

Szarka & Csilléry (1995) studied the defense systems against *Xanthomonas campestris pv. vesicatoria* in pepper and reported observations in connection with a general capacity in plants for tissue retention. They globally named this system of plant features „General Defense Reaction" (GDR). The existence of GDR was proved in host-pathogen and nonhost-pathogen interactions as well [10, 16]. Concerning the resistance of PI 163192 line they were the first to publish a comprehensive description. This resistance is monogenic, recessive, non-hypersensitive and non-specific to bacterial spot. They denominated this gene *gds* („*general defense system*"). This denomination emphasizes that the observed and described resistance is bacteria non-specific [10]. Szarka & Csilléry defined *gds* gene as part of the GDR. Furthermore the authors highlight this resistance is accompanied by leaf blade thickening instead of tissue necrosis and assimilation surface loss caused by hypersensitive reaction.

This recognition opened the way for targeted application of recessive, non-hypersensitive resistance in conventional pepper breeding. Szarka and Csilléry were co-breeders of the first commercial pepper variety in the world which is resistant to bacterial spot disease encoded by one recessive resistance gene (*gds)*. This variety is a cherry-type hot pepper, it's name is Globál, and it was submitted to national registration of varieties already in 2003 [11].

As it became clear after 20 years, this discovery and it's first practical application was a starting point for a paradigm shift in resistance breeding of pepper. After 1995 the authors detailed and completed their theory in several publications [12-18]. Nonetheless this work remained without any citation in the literature, meanwhile the subject reached newer practical applications through further development.

Jones et al. (2002) studied the non-hypersensitive resistance in pepper to the bacterial spot on genotype ECW-12346 and concluded that two recessive genes determine the resistance [18]. They denominated these genes *bs5* and *bs6*. Based on their interpretation *bs5* was derived from PI 271322 accession and *bs6* from PI 264281 and PI 163192. Newer practical applications mentioned before are linked to these genes, finally among them to *bs5*. It should be noted that no exact classification of symptoms is described in this article therefore determination of this resistance by two recessive genes remained an opened question.

Vallejos et al. (2010) targeted to characterize and find molecular markers for *bs5* and *bs6*. They found that „*bs5* confers a greater level of resistance than *bs6* at 25°C, but in combination they confer full resistance to P6 indicating at least additive gene action". So Vallejos et al. confirmed that resistance of ECW12346 reported by Jones et al. is associated with two genes.

Furthermore „a scan of the pepper genome with restriction fragment length polymorphism and AFLP markers led to the identification of a set of AFLP markers for *bs5*" by the authors. In addition they stated that they were „not being able to detect an informative marker for *bs6*" [19].

But already after the work of Jones et al. practical application of *bs5* and *bs6* was started. The first result was the sweet pepper hybrid "9954288" breeded by W. McCarthy (Seminis Vegetable Seeds Inc.), submitted for patent in 2008, publicated in 2009 [20]. This hybrid contains Bs2 gene near *bs5* and *bs6*. Further patented pepper hybrids from W. McCarthy are the followings: PS 09979325 (publicated in 2012), SV3255PB and SV4844PB (both in 2015), which contain Bs2 gene and only *bs5* (without *bs6*!) in order to provide resistance against all races of *Xanthomonas campestris pv. vesicatoria*.

The application of *bs5* will be surely spread in the near future as other leader breeding companies (Sakata Seed, Pepper Research, Bejo Zaden, United Genetics, Rijk Zwaan) have got the license (sequence) of *bs5* from 2Blades Foundation [1]. Using the word „sequence" in these press releases suggests that the sequence of *bs5* gene is already known for the licence owners but obviously not publicated. On the other hand the sequence of *gds* gene became known and public in 2014 thanks to a patent submission entitled "Identification of a *Xanthomonas euvesicatoria* resistance gene from pepper *(Capsicum annuum)* and method for generating plants with resistance" by Gy.B. Kiss et al, owned by 2Blades Foundation as well [21]. Namely the *xcv-1* gene mentioned in this patent is identical to *gds* [22]. So finally the relation between *gds* and *bs5* genes became clear: they are identical, as *xcv-1* and *bs5* are declared as identical by the owner itself [23].

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P1-08

Screening for drought tolerance in eggplant relatives and interspecific hybrids

Mariola Plazas, Azizah Fauziyati Rahma, Adrián Rodriguez-Burruezo, Jaime Prohens and Ana Fita

Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Valencia, Spain

Abstract

Eggplant (*Solanum melongena*) is related to a large number of wild species growing under drougth stress conditions. The latter are of potential interest for breeding programs aimed at increasing eggplant drought tolerance. In this work we evaluated accessions of cultivated *S. melongena*, the wild related species *S. anguivi*, *S. dasyphyllum*, *S. insanum*, and *S. linneanum* and the interspecific hybrids of *S. melongena* with *S. anguivi*, *S. dasyphyllum* and *S. insanum*. Young plantlets (at the stage of 3-4 leaves) were subjected to two treatments: control, in which plants were watered avoiding any drought stress and 50% reduction of irrigation compared to the control. Plant vegetative parameters as well as the photosynthetic rate were assessed. The wild species showed different responses to drought, with *S. anguivi* and *S. insanum* being the most tolerant. Some interspecific hybrids performed better than their parents showing hybrid vigor for tolerance to drought. These interspecific hybrids may be used directly as rootstocks or for backcross breeding programs aimed at improving *S. melongena* drought tolerance.

1. Introduction

Eggplant (*Solanum melongena* L.) is one of the most important vegetable crops worldwide. In 2015, the production of eggplant exhibited an increase reaching a production of 49 milion of tonnes [1]. Most of the production of eggplant takes place in tropical and subtropical areas, where climate change effects, including increased drought stress, have a significant impact. Although eggplant tolerates better drought condition than other crops, likely because of its leaf morphology, a good stomatal control and maintenance of photosyntesis under stress [2], drought is one of the abiotic stresses which may induce severe losses in production and affect the quality of the berries [3,4].

The cultivated eggplant is related to a large number of wild relatives, which grow in a wide range of environments, including desertic areas [5]. These wild materials are a potential source of tolerance to drought, since many of them can be hybridized with the cultivated *S. melongena* [6].

The objective of this work was to assess drought tolerance of several eggplant relatives and their interspecific hybrids in respect to eggplant, with the goal to identify germplams exploitable for eggplant breeding programs .

2. Material and methods

Accessions of *S. melongena* (Mel) as well as of the wild species *S. anguivi* (Ang), *S. dasyphyllum* (Das), *S. insanum* (Ins), *S. linneanum* (Lin) and their interspecific hybrids with *S. melongena* (Mel x Ang, Mel x Das, Mel x Ins) were used.

Seeds were germinated in Petri dishes and then transplanted to 0.5 L pots filled with peat. When the plantlets were grown at the stage of three-to-four leaves, two alternative treatments were applied: i) control irrigation (usually was performed around 100 ml in each watering, which depending on the needs of the plant) and ii) 50% reduction of irrigation. Plants were grown in a climatic chamber with 16 h light / 8 h darkness photoperiod with a temperature of 25 ºC. After 8 weeks of treatment, all plants were measured with an Infrared Gas Analyzer (Li-Cor 6400, Nebraska, USA) for photosynthetic rate (A), transpiration rate (E), stomatal conductance to H_2O (g_s) and intercellular CO_2 concentration (Ci). All measurements were performed in the morning. Intrinsic Water-used efficiency (intrinsic WUE) was calculated from ratio between photosynthetic rate (A) and stomatal conductance (g).

Foliar length and width (from three leaves) were measured with a ruler to estimate Leaf Area as the length per width divided by 2. Also the plant height, aerial and root parts fresh and dry weights were measured. Water use efficiency (regarding biomass) was measured as the ratio of dry weight and total amount of water used to irrigate the plants.

3. Results and discussion

The results of multifactorial ANOVA analysis showed significant effects of the two irrigation treatment on the vegetative parameters (leaf area, plant height, aereal part dry weight and root dry weight), except than for the root dry weight (Table 1). The drought treatment induced an average reduction of 17% in leaf area, 30% in plant height, and 35% in aerial dry weight. These results confirm what previuosly reported in literature [7]. The not significant reduction in the root dry weight is presumably due to a progressive plant adaptation to drought or the maintenance of the water absorptive area [8]. Although ANOVA did not highlighted any significant interaction between genotypes and irrigation treatments (Table 1), the comparison of individual accessions or hybrids revealed that some genotypes (i.e.*Solanum melongena*, *S. dasyphylum* and *S. Linneanum*) were less tolerant to drought (Fig 1). Interestingly, the hybrid Mel x Das performed better that both its parents. *Solanum anguivi*, *S. insanum* and their hybrids with *S. melongena* were not very affected by drought.

		Mean squares			
	df[1]	Leaf Area (cm^2)	Plant height (cm)	Aerial part dry weight (g)	Root dry weight (g)
Main effects					
Accession (A)	7	3695^{***}	34.1^{***}	1.11^{**}	0.37^{ns}
Treatment (T)	1	4733^{**}	43.2^{***}	2.43^{**}	0.11^{ns}
Interactions					
TxA	7	355^{ns}	1.31^{ns}	0.15^{ns}	0.04^{ns}
Error	51	470	3.9	0.31	0.09

Table 1:
Multifactorial ANOVA analyzing biometric values [1] Degrees of freedom; ^{ns}, *, **, ***, mean non-significant, P-value < 0.05, 0.01, and 0.001 respectively

The multifactorial ANOVA over the WUE and instrinsic WUE (A/g_s) also showed significant differences among accessions and treatments (Table 2). An increase of WUE was detected following the water stress treatment. This indicates, as in many other plants, that eggplants tend to adjust their development depending on the availability of water and are more efficient under stress.

		Mean squares	
	df[1]	WUE (g ml H_2O^{-1})	Intrinsic WUE (µmol CO_2 mol^{-1} H_2O)
Main effects			
Accession (A)	7	1.98^{*}	6763^{***}
Treatment (T)	1	6.62^{**}	2264^{*}
Interactions			
TxA	7	0.44^{ns}	904^{ns}
Error	51	0.84	466

Table 2
Multifactorial ANOVA analyzing WUE measures. [1] Degrees of freedom; ^{ns}, *,**,***, mean non-significant, P-value < 0.05, 0.01, and 0.001 respectively

The only genotypes which significantly increased their WUE under water stress were *S. insanum* and its hybrid with *S. melongena* (Fig 2), indicating that these genotypes are the most tolerant to drought Intrinsic WUE was significantly higher under drought for Mel x Ang, *S. insanum* and *S. linneanum* (Fig. 2). This parameter was significantly lower under water stress for Das. In leaves , intrinsic WUE indicates the ratio of the instantaneous rates of CO_2 assimilation and stomatal transpiration. Differences between genotypes in A/gs have been reported to have a genetic basis and breeding for high WUE has become a main goal in other crops [9].

The results showed that there is a wide diversity among wild species in the response to drought. Some interspecific hybrids performed better than their parents displaying hybrid vigor for tolerance to drought. These interspecific hybrids may be used directly as rootstocks or to start a backcross breeding program for improving the tolerance to drought of *S. melongena*.

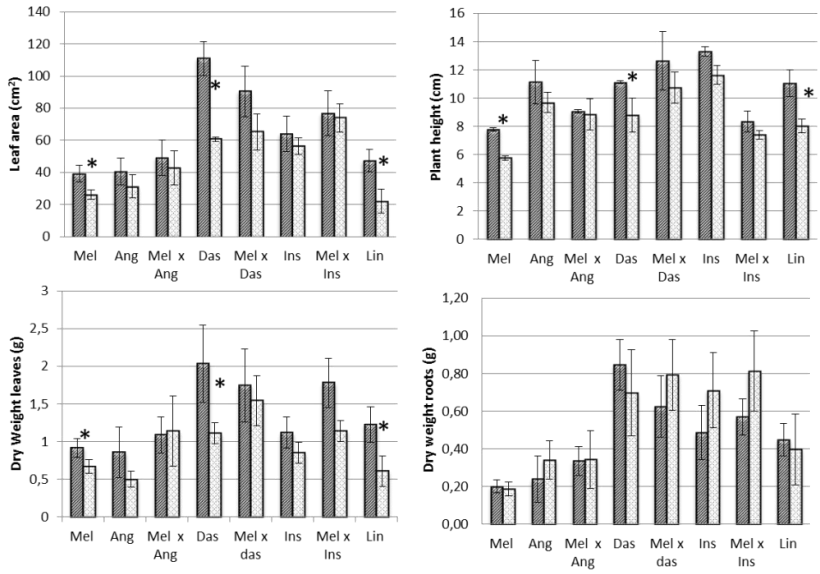

Figure 1
*Average values ± SE per accession and treatment of the vegetatvie parameters evaluated. Each bar is the average of at least 4 plants. * indicates significant differences among avegares of control and drought treatments.*

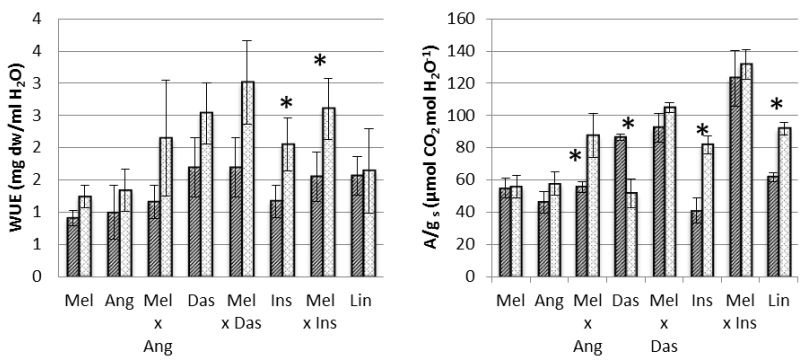

Figure 2
*Average values ± SE per accession and treatment of the WUE, WUE intrinsic (A/g_s) and WUE instantaneous evaluated (A/E).Each bar is the average of at least 4 plants. * indicates significant differences among avegares of control and drought treatments.*

4. Acknowledgements

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P1-09

Breeding Calabrian pepper lines (*Capsicum annuum* L.) for Brazilian agriculture from *sui generis* introduction of germplasm

Cláudia S.C. Ribeiro[1], Renato S. Soares[2], Lucas M. Gomes[3], Lucas G. F. Coelho[3], Francisco J. B. Reifschneider[1]

[1] Embrapa Hortaliças, Brasilia, DF, Brasil
claudia.ribeiro@embrapa.br, francisco.reifschneider@embrapa.br
[2] UNESP-FCAV, Jaboticabal, SP, Brasil
renato_2366@hotmail.com
[3] UnB, Brasilia, DF, Brasil
lucasmatyasgomes@gmail.com

Abstract

Few Calabrian pepper cultivars (*C. annuum* and *C. baccatum*) are available in the Brazilian market, and among these, only BRS Mari (*C. baccatum*) was developed in Brazil, by Embrapa. This work aimed to report on the introduction of germplasm in a *sui generis* way and the initial results of Calabrian pepper breeding at Embrapa Vegetables. Original population was obtained through separation of seeds found in dehydrated Calabrian pepper flakes imported from India. The breeding method was individual selection of plants with progeny test and three generations of selection and self-pollination were advanced. Significant differences ($p<0.05$) were noticed among the 14 S_3 lines obtained, for precocity, length and width of the fruit, weight of the fruit, weight and number of fruits per plant. Five S_3 lines were selected based on the following criteria: average fruit weight above 12 g, early flowering (less than 80 days after sowing), fruit length above 13 cm and fruit width around 1.5 cm, dark green color of leaf and sparse or medium pilosity. In the following S_4 generation, the selected lines will be evaluated under field conditions in order to determine yield, disease resistance as well as capsaicinoid concentration in fruits. New genotypes selected may be released as cultivars that meet the growing demand for dehydrated Calabrian pepper flakes.

1. Introduction

The genus *Capsicum*, represented by hot and sweet peppers, originates from the tropical America, is nowadays widely dispersed and grown all over the world. Brazil is an important diversity center of *Capsicum*, holding domesticated, semi domesticated and wild species (Carvalho *et al.,* 2003; Monteiro *et al.,* 2010). The genus consists of about 30 species, only five being considered as domesticated *C. annuum, C. baccatum, C. frutescens, C. chinense* and *C. pubescens*, the latter being not found in Brazil (Moreira *et al.,* 2006).

Currently, India is a great grower and exporter of dehydrated red pepper fruit (*C. annuum*), whole or in form of flakes. In 2013, India produced about 1.4 million metric tons of dehydrated peppers and exported, in 2011, 260 thousand metric tons of dried peppers. Numerous landraces and local population of different kinds of *Capsicum* peppers are grown in India, and most of them belong to *C. annuum* species. Due to natural great variability, India is known as a secondary center of diversity for *Capsicum*.

C. annuum fruits are among the ten most consumed vegetables in Brazil (Rocha *et al.*, 2006; Moreira *et al*., 2006). This species is represented by sweet peppers, peppers for paprika and hot peppers like jalapeño, cayenne, serrano, cherry, among others, besides ornamental varieties (Reifschneider, 2000; Büttow, 2010). *C. annuum* fruits are consumed green and ripe, *in natura*, in sauces or dehydrated. Calabrian pepper, also known as *peperoncino*, generally belongs to this species and is widely used in Italian cuisine.

In Brazil, Calabrian pepper is not a kind of pepper that belongs to a determined species, but rather it is a product from red pepper dehydration in form of flakes, being frequently used as a spice for the food processing industry. Brazilian Calabrian pepper is obtained from red ripe fruits, mainly from *C. baccatum* and *C. annuum*. Fruits are harvested when ripe and dried in the sun on tarps or in dryers with forced hot air circulation. Once dried, the whole fruits are flaked, including the seeds (Ribeiro *et al.*, 2008).

Fungal diseases and low prices are pointed out as the main reasons for the drastic reduction in cultivation area of Calabrian pepper in Turuçu (SCHNEID, LF, personal communication), previously the most important growing region in Brazil.

There are few cultivars of chile pepper (*C. annuum* and *C. baccatum)* available in the Brazilian market that could be used to obtain Calabrian pepper. Out of these cultivars, only 'BRS Mari' (*C. baccatum*) originated from a Brazilian breeding program, implemented by Embrapa Vegetables (Carvalho *et al*., 2009).

The development of new cultivars heavily depends on the genetic resources available, collected and characterized, in collections or germplasm banks – AGB (Nass, 2007). The enrichment of an AGB is normally carried out through germplasm exchange among research institutions, collecting expeditions and acquisition of commercial seeds available in the market. However, in the specific case of Calabrian pepper accession CNPH 50.000, belonging to the *Capsicum* collection of Embrapa Vegetables, the germplasm was introduced in a *sui generis* way: seeds were obtained from a small batch of the commercial product Calabrian pepper (dehydrated red pepper flakes with seeds) from India, evaluated by an agroindustry as excellent quality. The absence of national cultivars of Calabrian pepper (*C. annuum*) adapted to different Brazilian biotic and abiotic conditions motivated the beginning of this research in Embrapa Vegetables. This work aimed to report results of breeding research of Calabrian pepper (*C. annuum*), from the population CNPH 50.000, as well as the identification and selection of plants and lines with promising agronomic characteristics for developing new cultivars of interest to Brazilian agriculture.

2. Material and methods

This work was carried out at Embrapa Vegetables, Brasília, DF, Brazil (15°55'57.31"S, 48°8'11.36"W).

Genotype used: The seeds of *C. annuum* were obtained from a small sample of dehydrated pepper flakes (Calabrian type) imported from India, and registered in the *Capsicum* Germplasm Bank as CNPH 50.000. This batch of pepper in flakes showed capsaicin content of 32,100 Scoville Heat Units (SHU).

Breeding methodology: The breeding method used was individual selection of plants with progeny test. Original seeds were sown and 10 plants obtained (original plants, OP) which were maintained in a screenhouse and self-pollinated. From each plant, 3-5 self-pollinated fruits were harvested and extracted.

For the following cycle, ten S_1 seeds from each of the 10 original self-pollinated plants were used; 87 S_1 plants were obtained, and Open pollinated as well as selfed fruit were obtained from 73 lines. The OP seeds harvested in the screenhouse were also considered S_2 .

In the next generation, five seeds from each of the 73 S_1 lines obtained in the previous generation were sown and a total of 327 S_2 plants (4-5 plants/line) were transplanted to a screenhouse, directly in the soil. Fourteen plants were selected among and within S_2 lines. Self-pollinated fruits from selected lines were harvested separately and S_3 seeds of these fruits were extracted. In 2015, 14 S_3 selected lines were taken to the field, 5 plants per plot, in two replications, for a preliminary evaluation of agronomic and processing characteristics of interest (earliness, number of side shoots, color of leaves and unripe fruit, plant height, fruit length and diameter, wall thickness, weight of individual fruit, total number and weight of fruits per plant), and identification of lines adapted to Central Brazil. Concomitantly, three plants from each line were maintained in a screenhouse for controlled self-pollination..

Agronomic and processing characterization: A preliminary characterization of fruits of the original population (CNPH 50.000) as well as of the 73 S2 lines was caried out. Due to great variation in number of side shoots among lines, the number of shoots of each plant was also counted for all S2 lines, which were grouped into five classes.

In addition, a subjective evaluation of agronomic value of S_2 plants was carried out, by at least two evaluators. Based on quantitative and qualitative data obtained three S_2 lines were selected. The three selected lines were also evaluated for number of fruits per plant, total weight of fruits (g), total soluble solids (ºBrix), capsaicin content and color of ripe fruit (5 fruits of each plant).

Parameters fruit length, width and wall thickness were measured using a digital caliper MITUTOYO, model 500-144B. To determine total soluble solids (ºBrix), a digital refractometer ATAGO model PR-1 was used, following standard methodology to clean the equipment and to standardize the samples.

Determination of capsaicin: Determination of capsaicin content of three selected S_2 lines was carried out using AOAC Official Method 995.03 (AOAC, 2006) that can be used to determine capsaicinoids content between 750 and 650.000 Scoville Heat Units (SHU).

Determination of fruit color: For analysis of unripe fruit, one fruit from each plant was harvested, from all lines, and a reading per fruit was carried out. Analysis of ripe fruit color was carried out only for the three selected lines (five fruits per plant and a reading per fruit). Color analysis was carried out by using colorimeter (Minolta Chromometer Model CR-400), standard CIE-L*a*b*. Color measurements were carried out in equatorial region of each fruit.

3. Results and discussion

3.1. Evaluation of original population

Evaluation of characteristics of fruits and plants obtained from the original population (CNPH 50.000) that came from Indian Calabrian pepper showed significant variability among individuals. Significant differences were observed through Scott-Knott test ($p<0.05$) for characteristics fruit diameter and wall thickness, which ranged from 0.8 to 1.2 cm and 0.8 to 1.4 mm, respectively. ‘CNPH 50.010’ showed significant difference for number of locules per fruit, with average value of 2.3 locules, whereas other genotypes showed two locules per fruit.

Presence of variability in original population is essential for selecting individuals with superior characteristics. Success in development of new cultivars is directly associated to genetic variability of the population to be improved (Cardoso, 2001; Nass, 2007; Ribeiro *et al.*, 2008). Genus *Capsicum* has significant genetic variability (Inoue & Reifschneider, 1989), and among the domesticated species, *C. annuum* has greater diversity, whereas *C. frutescens* has less variability (Casali & Couto, 1984).

3.2. Evaluation of S2 lines

Significant differences among the 73 S_2 lines were observed (Scott-Knott test, $p<0.05$) for all fruit characteristics evaluated. Values of fruit weight ranged from 2.3 g to 10.9 g; fruit length ranged from 7.5 cm to 13.4 cm; fruit diameter ranged from 0.7 cm to 1.4 cm and wall thickness ranged from 0.87 mm to 1.98 mm. Some of these values are close to the most cultivated variety of *C. annuum* pepper grown in India, known as "Pusa Jwala," which presents fruit length ranging from 7 to 13 cm, fruit width from 1 to 1.5 cm and pungency between 30,000-50,000 SHU.

Color of unripe fruits in the 73 S_2 lines ranged from L*= 26.55; a*= -21.62; b*=6.44 to L*= 60.82; a*= -5.86 and b*= 42.19; differences in the intensity of the green color among fruits could be visually verified (light green and dark green). Color of ripe fruits, however, did not show variation noticeable to the naked eye, and due to this fact, they were not evaluated quantitatively. In relation to side shoots (Figure 1), only one line in class 1 (1%) was observed; six lines in class 2 (8%); 26 lines in class 3 (36%); 32 lines in class 4 (44%); and eight lines in class 5 (11%). A fewer number of side shoots requires less labor for thinning and for fruit harvest. Besides, it also allows a higher level of ventilation and decreases humidity in the microclimate formed around the plants, favoring a better phytosanitary condition.

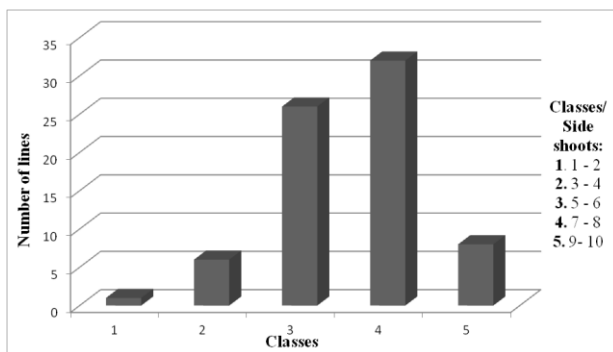

Figure 1. Number of side shoots of 73 S_2 Calabrian pepper lines: average values distributed in shoot number classes. Brasília-DF, Embrapa Vegetables, 2014.

Increase of pruning levels on *C. chinense* pepper plants with consequent decrease of side shoots, resulted in an increase of fruit weight (Jaimez *et al*., 2002). The authors also suggest that with lower amount of shoots, change in distribution of nutrients assimilated by different parts of the plant may have happened. Alsadon *et al.* (2013) reported a significant increase of size and quality of fruits due to pepper plant pruning, keeping only one branch. Density and number of pruning operations per plant are important factors for *Capsicum* production, both under protected cultivation as well as under field conditions. Dasgan & Abak (2003) concluded that peppers grown in a greenhouse with high plant density and reduced number of side shoots per

plant increased significantly the yield per m^2. McCraw & Greig (1986) observed higher yield per plant and fruit weight through pruning peppers grown in field conditions.

3.3. Evaluation of selected S2 lines

In quantitative evaluation, the selected lines CNPH 50.112, CNPH 50.116 and CNPH 50.185 showed significant differences for the characteristics: side shoots, total number and weight of fruits per plant, average fruit weight, length and diameter of the fruit, capsaicin content and color of unripe and ripe fruit (Table 1). The three lines showed fruit length between 9.4 cm and 10.6 cm, fruit diameter between 0.9 cm and 1.2 cm. In Brazil, *C. annuum* cultivars 'Calabrian' peppers (Isla, 2015) and 'Cayenne Dedo-de-Moça' (Feltrin, 2015) present fruit size of 8-12 cm x 1-2 cm and 12 cm x 1 cm, respectively, values close to selected lines. Besides, CNPH 50.112 showed better plant architecture and good fruit yield; CNPH 50.116 showed absence of side shoots and smaller size plants and CNPH 50.185 stood out among others for its excellent fruit yield, color and shape of fruits.

Among the three lines, CNPH 50.185 showed highest average values for total number and weight of fruits per plant, 255 fruits and 1,003 g per plant, respectively. CNPH 50.112 and CNPH 50.116 were less productive, with average of 105 and 86 fruit/plant, respectively.

Table 1. Average values of side shoots, total number of fruits per plant (NTF), average fruit weight (PM), fruit length (CF), diameter (DF), wall thickness (EP), total weight of fruits per plant (PT), unripe fruit color, ripe fruit color, total soluble solids content (ºBrix) and capsaicin (SHU) of three selected S_2 lines. Brasília-DF, Embrapa Vegetables, 2014.

CNPH	Side shoots	Fruits													
		NTF	PM (g)	CF (cm)	DF (cm)	EP (mm)	PT (g)	Unripe color			Ripe Color			ºBrix	SHU
								L*	a*	b*	L*	a*	b*		
50.112	3 b[1]	105 b	6.18 a	10.6 a	1.2 a	1.3 a	647 b	31.83 b	-9.96 b	11.1 b	37.82 a	36.66 b	19.45 b	11.5 a	5246 b
50.116	1 c	86 b	6.42 a	10.5 a	1.1 a	1.4 a	552 b	31.36 b	-8.53 c	9.18 c	37.53 a	36.58 b	19.37 b	10.6 a	5420 b
50.185	6 a	255 a	3.94 b	9.4 b	0.9 b	1.2 a	1003 a	36.49 a	-14.79 a	18.7 a	36.98 a	38.71 a	20.56 a	10.8 a	15727 a
CV(%)	17.1	35.8	13.3	6.4	6.4	10.5	27.4	6.4	13.2	16.8	1.0	2.1	2.9	8.4	

[1]*Means followed by the same letter in the column do not differ significantly by the Scott-Knott test ($p < 0.05$)*

The large number of fruits showed by CNPH 50.185 can be attributed to its high number of side shoots, since a positive correlation between number of fruits and side shoots can be noticed. Dasgan & Abak (2003) obtained variation of approximately 230% in number of fruits per plant when cultivation with one shoot was carried out (spacing of 80 cm between lines x 15 cm between plants) and four side shoots per plant (spacing of 80 cm x 45 cm), in a greenhouse.

CNPH 50.112 and CNPH 50.116 did not differ statistically for length, diameter and wall thickness of fruits. The three Calabrian pepper selected lines showed fruit length greater than the paprika pepper cultivars and 'BRS Mari' (5.4 cm to 6.3 cm), evaluated by Paulus *et al.* (2015).

Regarding the color, CNPH 50.185 showed the highest values in L*a*b* color space, differing statistically from other selected lines, showing unripe fruits with intense/dark green color and ripe fruits with intense bright red color. According to CIELAB chart for colors and pigments, which considers a* b* values, CNPH 50.185 showed color similar to the pigment *Chromium oxide green* for unripe fruits; and color similar to the pigment *Venetian red* for ripe

fruits. Even differing statistically from the lines CNPH 50.112 and CNPH 50.116, for both color of unripe and ripe fruit, the range of values of three lines represented on CIELAB chart was not wide and the color observed in ripe fruits meets the market-required standards. According to Luts & Freitas (2008), colors of pepper fruits come from carotenoid pigments, which for its nutritional value, are among the most important plant pigments. Ripe, red *Capsicum* pepper fruits have 60 times more carotenoids than green fruits, besides having higher concentration of flavonoids and other secondary compounds (Gómez-García & Ochoa-Alejo, 2013).

No significant difference among the three selected lines was observed for soluble solid content, which varied from 10.63 to 11.57 ºBrix. Paulus *et al.* (2015) observed similar results for soluble solids in paprika cultivars (content of 10.30 ºBrix) and BRS Mari (10.2 ºBrix).

CNPH 50.185 presented capsaicin concentration of 15,700 SHU, differing statistically from lines CNPH 50.112 and CNPH 50.116, with contents of 5,200 and 5,400 SHU, respectively. These values are in the range of pungency of Calabrian pepper flakes. Similar values were found by Ziino *et al.* (2009) in Calabrian pepper cultivars 'Amando' (14.700 SHU) and 'Sigaretta' (7.400 SHU).

3.4. Evaluation of S3 lines

Significant differences were detected (P<5%) among the 14 S_3 lines for all parameters evaluated, except for side shoots, plant height and wall thickness. Earliness, determined by number of days until flowering, counted from sowing, ranged from 17 days between the earliest line and the latest line, average fruit length ranged from 9.7 cm to 15.6 cm, average fruit width from 1.2 to 1.5 cm, average fruit weight from 8.1 g to 15.9 g, average fruit weight per plant from 120 g to 730 g and average number of fruits per plant from 17 to 106 fruits. CNPH 50.199 showed low yield due to a severe attack by *Alternaria* sp. observed since transplanting into the field. For color of leaf, average values of parameter b* on CIELAB chart ranged from 18.86 to 20.64 for light green and from 12.55 to 14.79 for dark green and for fruits ranged from 7.47 to 21.25.

Five of 14 S_3 lines (Table 2) were selected based on the following characteristics: average fruit weight above 12 g, less than two side shoots per plant, early flowering less than 80 days after sowing, average fruit length above 13 cm and average fruit diameter around 1.5 cm and dark green leaf; the selected lines did not differ from each other for any of the parameters; and sparse or medium pilosity (CNPH 50.189, 50.192, 50.193, 50.194 and 50.195). It is interesting to highlight that significant increase of fruit size (length and diameter) and average fruit weight of the five selected S_3 lines in relation to the values observed in S_2 lines (Table 1) and OP, from which they derived, was observed. Values of average length and diameter of fruits of selected S_3 lines are close to the highest values observed in Indian local variety "Pusa Jwala" (*C. annuum*), 13 cm and 1.5 cm, respectively. In the next generation (S_4), tests under field conditions will be carried out in order to determine line yield, resistance to diseases as well as analysis of capsaicinoid concentration in fruits.

Limited information, as well as few Calabrian pepper cultivars, are available in the Brazilian market, despite the growing demand for this kind of hot pepper. The results of this work showed that the introduction of germplasm in a *sui generis* way allowed efficient selection of genotypes of interest. The authors found high variability in original population, allowing the selection of new genotypes (*C. annuum*) with superior agronomic and processing characteristics. These materials may be released as cultivars adapted to Brazilian conditions that meet the Brazilian market demand for dehydrated pepper flakes.

Table 2. Average values for fruit weight (PF), length (CF), diameter (DF), days from sowing until flowering (earliness), number of side shoots per plant and stem pilosity observed in five selected S_3 lines and 14 S_3 lines evaluated. Brasília-DF, Embrapa Vegetables, 2015.

Range of average values	PF (g)	CF (cm)	LF (cm)	Earliness	Side shoots	Pilosity
Five selected S_3 lines	12.7 – 15.9	13.3 – 15.6	1.4 – 1.5	70 - 75	1.20 – 1.50	Sparse to medium
14 S_3 lines	8.2 – 15.9	9.7 – 15.6	1.2 – 1.5	70 - 87	1.20 – 1.77	Sparse to medium

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P1-10

Synthesis of a base population of Habanero chile pepper and initial assessment of derived F_3 lines (*Capsicum chinense*)

Luciano L. Nass[1], Karina R. R. de Souza[2], Clebson S. Carneiro[3], Renato S. Soares[4], Cláudia S. C. Ribeiro[2], Francisco J. B. Reifschneider[1]

[1] *Embrapa, Secretariat for International Relations, Brasília-DF, Brazil*
luciano.nass@embrapa.br, francisco.reifschneider@embrapa.br

[2] *Embrapa Hortaliças, Brasilia- DF, Brazil*
karinarrsouza@gmail.com, claudia.ribeiro@embrapa.br
[3] *UFV, 36570-900, Viçosa-MG, Brazil*
clebson.carneiro@ufv.br
[4] *UNESP-FCAV, Jaboticabal, SP, Brazil*
renato_2366@hotmail.com

Abstract

This work reports on the establishment of a base population with a wide genetic base and its use for the derivation of F_3 lines based on the perspectives of future demands for Habanero peppers by Brazilian producers and processors. Thirty one accessions of Habanero pepper from different origins present in the *Capsicum* Active Germplasm Bank (AGB) of Embrapa Vegetables were used. Hand pollinations were carried out using a mixture of pollen from all the accessions under controlled conditions. Flowers that received the pollen mixture were emasculated to avoid contamination by self-pollination. Eighty one F_1 hybrids were obtained, which were harvested in the second semester of 2010. In 2011, the advance of generation F_1 to F_2 was carried out. At this stage, the main objective was the production of a greater number of F_2 seeds per plant. The base population of Habanero was formed by an equal mixture of F_2 seeds from all crosses obtained, using a fixed weight (1 g) per cross, which is close to 150 seeds per genotype. This balanced population was introduced in the *Capsicum* AGB as CNPH 15,469. A little over 1,000 plants of the base population were planted in 2014 at Embrapa Vegetables and ca. 50% were eliminated due to plant characteristics and/or susceptibility to diseases. The 492 surviving plants were individually assessed for several characteristics, which were used to select a total of 17 lines. These lines (25-30 plants/line) were planted in a greenhouse and further selected based mostly on fruit characteristics, plant architecture, leaf and fruit color as well as capsaicin content. Presently, ten selected F_3 lines derived from the base population are being selfed under greenhouse conditions and field evaluated as well. The selected lines present high variability for several traits, such as fruit size and color, with capsaicin levels varying from 0 to over 500,000 SHU. There are several additional possibilities to explore the variability of this base population including its use as a source of additional inbred lines; the use of the base population for selection in specific environments; establishment of new populations from the base population and its use in recurrent selection programs.

1. Introduction

The success of breeding programs depends on the genetic variability available to be used by breeders. Since breeders need to obtain promising results in relatively short term, conditions to promptly exploit the potential variability maintained in germplasm banks are limited (Nass *et*

al., 2012). Thus, the development of base populations with wide genetic variability may stimulate the interaction among germplasm banks and breeding programs, increasing the chances of using the accessions, also because of the possibility of exploiting new combinations originating from the intercross of accessions used when forming these base populations. Despite the fact that examples of the use of multiple parents to establish base populations are found in the literature (Alliprandini & Vello, 2004; Guimarães & Châtel, 2005; Mackay *et al.*, 2014), this procedure is not common, especially due to the difficulty in performing wide crosses, which takes time and can be very complex due to reproduction type.

Taking advantage of the advancement and reduction in cost of genetic techniques, specifically structured populations have been established using several accessions of multiple origins (Cavanagh *et al.*, 2008; Buckler *et al.*, 2009; Yan *et al.*, 2011; Mackay *et al.*, 2014; Huang *et al.*, 2015). These populations allow to understand the genetic basis of complex interest and also are of great interest for breeding programs.

Embrapa Vegetables has a three-decade long *Capsicum* breeding program that relies on the collection maintained in the *Capsicum* Active Germplasm Bank (AGB), currently with more than 4,000 accessions of several species, composed of the domesticated species *C. annuum*, *C. chinense*, *C. frutescens*, *C. baccatum* and *C. pubescens*, besides semi-domesticated and wild species.

The program has included an additional strategy to strengthen its work on populations, with the development of base populations of two pepper types of interest to Brazilian agriculture: the traditional "Malagueta" and a sort of a newcomer to the Brazilian market, the "Habanero".

"Habanero" is among the most pungent peppers in the world. The demand for this type of pepper has been increasing in Brazil. In order to better meet the Brazilian market demand, Embrapa Vegetables' *Capsicum* breeding program has concentrated efforts on developing more productive and uniform genotypes with better nutritional quality and resistance to diseases both in "Malagueta" (*C. frutescens*) and in "Habanero" peppers (*C. chinense*).

With the perspectives of future demands for "Habanero" peppers, this paper reports on the establishment of a base population with wide genetic base and the possibilities it opens for *Capsicum* breeding programs, highlights the advantages of such an approach for vegetable breeding in Brazil and elsewhere, and presents an initial assessment of derived F3 lines.

2. Material and Methods

Embrapa Vegetables *Capsicum* AGB maintains 542 entries of *C. chinense*, from which 31 accessions of "Habanero" pepper from different origins were used to develop the base population (Nass *et al*., 2015).

Hand pollinations of emasculated flowers were carried out using a mixture of pollen from all the accessions. Eighty-one F_1 hybrids were obtained, which were harvested in the second semester of 2010. In 2011, the advance of generation F_1 to F_2 was carried out. At this stage, the main purpose was the production of a great number of seeds per plant. From five to ten fruits per genotype were harvested, followed by extraction of seeds.

The base population of "Habanero" was formed by an equal mixture of F_2 seeds from all hybrids obtained, using a fixed weight (1 g), which is close to 150 seeds per genotype. This balanced base population was introduced in the *Capsicum* AGB of Embrapa Vegetables and received the identification number CNPH 15,469.

In 2014, a little over 1,000 plants of the base population were planted at Embrapa Vegetables, Brasilia-DF, Brazil (15°55′55.31″S , 48°8′11.36″W) and ca. 50% were eliminated due to plant characteristics and/or susceptibility to diseases. The accessions used to form the base population showed high variability (Table 1). The 492 surviving plants were individually assessed for several traits, which were used to select a total of 17 lines. These lines (25-30 plants/line) were planted in a greenhouse and further selected based mostly on fruit characteristics, plant architecture, leaf and fruit color as well as capsaicin content. Presently, 10 selected F_3 lines derived from the base population are being selfed under greenhouse conditions and field evaluated as well.

Table 1
Range of variation of important traits for Capsicum breeding observed in the accessions used to form the base population of Habanero pepper. Brasília-DF, Embrapa Vegetables, 2014.

Trait	**Maximum**	**Minimum**
Plant height(cm)	90	46
Plant width (cm)	99	55
Fruit length(cm)	6.0	2.9
Fruit width (cm)	4.6	2.9
Fruit wall tickness (mm)	3.9	2
Fruit shape*	5	3
Capsaicin (K)	1,000	90
Vitamin C (mg/100 g)	130	54
Yield (t/ha)	48	<10
Disease resistance**	TSWV, GRSV, PepYMV, PVY, PMMoV, CMV, Powdery mildew, bacterial spot, bacterial wilt, root knot nematode	

**1= Elongate (alongado), 2= Round (redondo), 3= Triangular (triangular), 4= Campanulate (campanulado), 5= Others (outros) **TSWV: Tomato Spotted Wilt Virus, GRSV: Groundnut Ringspot Virus, Pep YMV: Pepper Yellow Mosaic Virus, PepYMV: Pepper Mild Mottle Mosaic Virus, CMV: Cucumber Mosaic Virus, Powdery mildew: Oidiopsis haplophylli, Bacterial spot Xanthomonas euvesicatoria and X. gardneri, Bacterial wilt: Ralstonia solanacearum, Root knot nematode: Meloidogyne spp.*

3. Results and Discussion

The accessions used to form the base population showed high variability (Table 1). It should be pointed out that as part of the *Capsicum* breeding program, Embrapa has previously made limited efforts in the exploitation of *C. chinense* variability in Brazil: Ulhoa *et al.* (2010) reported on morphologic characterization of 23 accessions of "Habanero" pepper under field conditions in two environments, Brasília-DF and Catalão-GO. The authors observed great variability among the accessions, mainly associated to color, shape, productivity, size and cycle. Accessions CNPH 15,031, CNPH 15,037, and CNPH 15,045 were considered promising for characteristics of plant, fruit, and yield. Teodoro *et al.* (2013) characterized 22 accessions of

"Habanero" pepper maintained in a greenhouse in relation to vitamin C content. Great variability was observed among accessions, considering that vitamin C contents ranged from 54 to 130 mg/100 g, with an average of 98±24 mg/100g. As a reference, the recommended daily levels of vitamin C are from 75 to 90 mg (Chen *et al.*, 2003).

Presence of variability in the original population, as observed in this work, is essential for selecting individuals with superior characteristics. Success in development of new cultivars is directly associated to genetic variability of the population to be improved (Nass, 2007; Ribeiro *et al.*, 2008).

Ten F_3 lines have been selected from the base population, and high variability for relevant traits such as fruit shape, fruit color and capsaicin have been observed (Figure 1). Capsaicin content among F_3 lines varied from 0 to 503,759 SHU, in genotypes CNPH 15,684 and CNPH 15,693, respectively (Table 2). Usually, Habanero peppers have ca. 300,000 SHU. Therefore, it is possible to select materials with high pungency from the base population, which has been a growing demand from the Brazilian market.

Table 2.
Variability for capsaicin (SHU), fruit length (FL, cm), fruit width (FW, cm), and total soluble solids content (°Brix) of ten selected F_3 lines. Brasília-DF, Embrapa Vegetables, 2015.

Genotype CNPH	SHU[£]	FL[*]	FW[*]	Brix*
15,684	0	3.8	4.4	8.3
15,685	248,163	7.2	3.5	7.4
15,686	219,334	4.9	2.4	8.2
15,687	192,080	3.8	3.6	8.8
15,688	2,066	3.8	3.8	9.0
15,689	104,923	5.8	4.2	7.4
15,690	82,886	6.1	3.6	8.2
15,691	84,370	9.2	3.8	7.6
15,692	190,934	4.1	3.4	9.8
15,693	503,759	7.4	3.0	8.4

*[£] Sample of 30 fruits; * Mean of five fruits*

Aiming to exploit the wide genetic variability present in the population CNPH 15,469, the *Capsicum* breeding program has different possibilities, such as:

a) Selection of specific characteristics of interest to the markets through immediate extraction of promising inbred lines, generating more adapted and productive hot pepper cultivars of Habanero-type; This is the case of the ten F_3 lines under evaluation, which represents a selection pressure of 1% considering the established base population;

b) Use this population in recurrent selection programs;

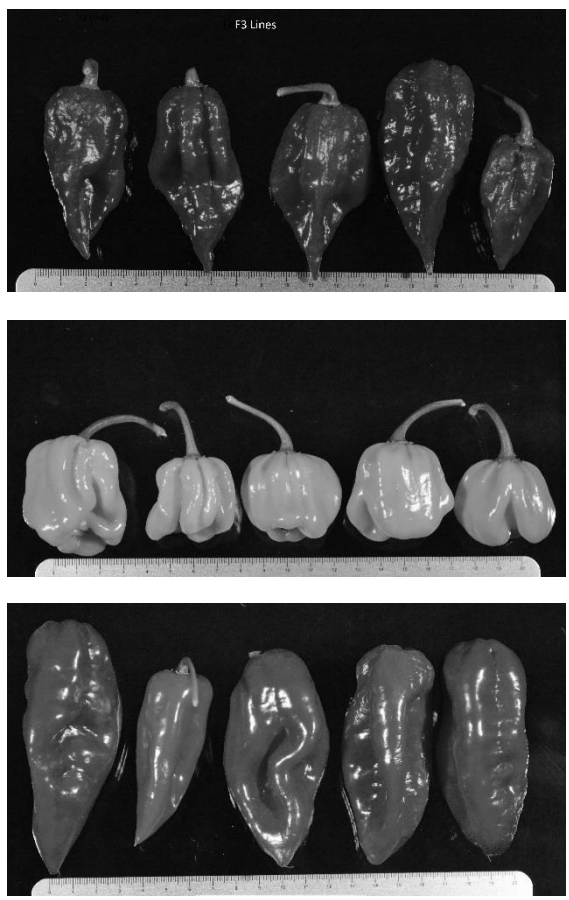
c) Use of these materials as a subject for genetic studies;

d) Develop a case study on the methodology for establishing the base population as a mechanism to exploit wide genetic variability available in germplasm banks.

According to Nass *et al.* (2015), the establishment of base populations, as exemplified by this effort with "Habanero" pepper, provides additional possibilities for the development of new cultivars of interest to the pepper agribusiness; additionally, it provides support to and strengthens Embrapa Vegetables' *Capsicum* breeding program, as well other national and international programs via germplasm exchange.

High variability for capsaicin was detected among F_3 lines varying from 0 to 503,759 SHU, in the genotypes CNPH 15,684 and CNPH 15,693, respectively (Table 2). Usually, Habanero peppers have ca. 300,000 SHU. It is possible to select materials with high pungency from the base population, which has been a growing demand from the Brazilian market.

Figure 1
Variability in fruit shape, size and color observed in selected F3 lines. Brasília-DF, Embrapa Vegetables, 2015.



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P1-11

Conservation, landscape and home garden varieties in South part of Hungary

Attila Rózsás, Zoltán Szögi

Szentesi-Mag Kft., Szentes, Hungary

Abstract

The Szentesi-mag Kft greatest strength is in addition to expertise are the marketed 35 vegetable and 2 winter oil rape varieties bred by Dr. Péter Szalva, László Nagy and a staff off the Company, of which 12 pepper varieties are in Hungarian National list and 3 varieties in Slovakian National List originated from selection to landrace varieties. This varieties represent all types of popular Hungarian pepper.

The number one target audience to supply with traditional Hungarian varieties and technology the several million users of small hobby gardeners. Our goal are that more people and family produce vegetables in their garden around their house to get delicious god tasty fresh and healthy vegetable food.

In 2011 entered into force the Regulation 65/2011 (VII.11) VM harmonizing the Commission Directive 2009/145/EC providing for certain derogations, for acceptance of vegetable landraces and varieties which have been traditionally grown in particular localities and regions and are threatened by genetic erosion and of vegetable varieties with no intrinsic value for commercial crop production but developed for growing under particular conditions and for marketing of seed of those landraces and varieties. The Regulation made possible to collect, preserve and register in the National list and EU Common Catalogue the determining vegetable varieties which were important in the life Landscape in recent decades.

As part of this work plays a major role the tracing of landraces and home garden pepper varieties and rediscovery the deleted old peppers.

In 2015 were registered 4 home garden vegetable varieties the one in four was the Evita white apple type variety for fresh consumption was bred in Szentes (in Southern Great Plains).

Further 3 home garden pepper varieties were are under application procedure from Southern Transdanubia regionin last year and registered in 2016:

- Bogyiszlói vastaghúsú, white hot pepper variety grown for fresh consumption and canning
- Kocsolai, light green sweet pepper variety grown for fresh consumption and long storage
- Belecskai, light green slightly spicy grown for fresh consumption

The traditional Hungarian varieties above originated from selection to landrace varieties deleted from the National List but in the original region had preserved by families.

- Bogyiszlói Kárász landraces spicy pepper variety grown for powder important variety for decades in the in South part of Hungary registered too. Our activities we would like to continue in the future.

Keywords: traditional, landscape, home garden pepper varieties, Commission Directive 2009/145/EC

Introduction

The Szentesi-mag Kft greatest strength is in addition to expertise are the marketed 35 vegetable and 2 winter oil rape varieties bred by highly respected Dr. Péter Szalva his direct fellow breeder László Nagy and a staff off the Company. The company located in Szentes city in southern Great Plans in the riverside "Kurca". The Hungarian National consist 12 pepper varieties and 3 varieties in Slovakian National List (Almapaprika, Szentesi piacos, Szentesi kosszarvú) of the Company originated from selection to Hungarian landrace varieties. This varieties represent all types of popular Hungarian pepper. The number one target audience to supply with traditional Hungarian varieties and technology the several million users of small hobby gardeners.

The variety selection is very rich.

Hungarian conical, white type: Bella, Csilla, Szentesi piacos (hot) and Totál (sweet)

Figure 1.
Totál variety, Hungarian conical type, pale whites yellow,

Figure 2.
Almapaprika, variety apple shape, white to red hot sweet

Narrow pointed type: Record (hot)

Apple shape, hot: Almapaprika, Dalma

Tomato shape: PAZ Szentesi, Szepazar,Szentesi sárga

Horn shape: Szentesi kosszarvú, Zöld kos, Fehér kos

Cherry pepper: Szentesi, Cserkó

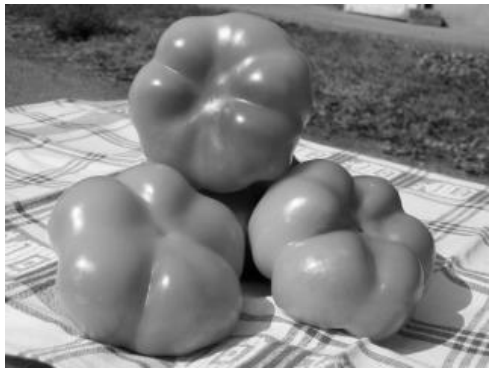


Figure 4.
Szentesi sárga variety, yellow tomato shape

Figure 5.
Zöld kos variety, green horn shape

We started our work by Hungarian Regulation 65/2011 (VII.11) VM to collect landraces varieties and to rediscovery the traditional varieties deleted from the Hungarian National Vegetable list. The Hungarian Regulation harmonizing the Commission Directive 2009/145/EC entered into force in 2009 the providing for certain derogations, for acceptance of vegetable landraces and varieties which have been traditionally grown in particular localities and regions and are threatened by genetic erosion and of vegetable varieties with no intrinsic value for commercial crop production but developed for growing under particular conditions and for marketing of seed of those landraces and varieties. The Regulation made possible to collect, preserve and register in the National list and EU Common Catalogue the determining vegetable varieties which were important in the life Landscape in recent decades.

As part of this work plays a major role the tracing of landraces and home garden pepper varieties and rediscovery the deleted old peppers.

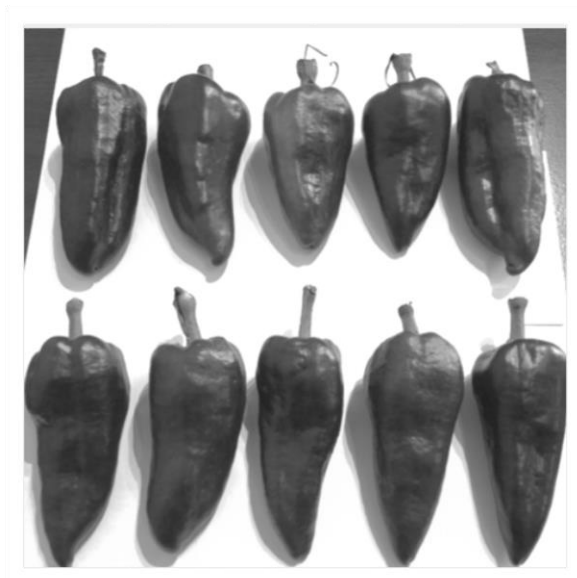
We together with families and Rural municipalities collected and described the varieties. The landrace denominations the names of the villages where it was traditional pepper cultivation.

Bogyiszlói vastaghúsú, white, thick flesh, hot pepper variety grown for fresh consumption and cooking Hungarian Ratatouille and canning.

Figure 6. Bogyiszlói vastaghúsú variety, white hot

Kocsolai, light green sweet pepper variety grown for fresh consumption and long storage till Christmas in pantry.

Belecskai, light green slightly spicy pepper variety grown for fresh consumption turn to red



Bogyiszlói Kárász landraces fish shape, spicy pepper variety grown for powder the important variety for decades for traditional fish sup in South part of Hungary registered too in 2016.

In order to continue the landraces of domestic biodiversity we are working on rescue and preservation of our National value.

P1-12

Higher quality traits - breeding strategies in pepper

Zsolt Sági[1], Anikó Gémesné Juhász[2]

[1] *Rijk Zwaan Budapest Kft. 1016 Budapest, Aladár u. 17-19.*

[2] *Medimat Kft., Budapest, XIV utca 37., H-1224, Hungary*

Abstract

In the Hungarian Wax varieties from the breeding program of Rijk Zwaan Zaadteelt & Zaadhandel B.V., the quality traits of the fuits will be more and more important in the future. Next to the basic resistance requirements (TMV, TSWV), the importance of the quality traits increasing very quickly from the side of the markets.

The extra white fruit color, the smooth skin, the needed size (all the time) and the insensibility for the anthocyanin on the fruits (by the different kind of stresses) are more and more important during the production. The new Hungarian Wax varieties – Bravia RZ, Brigy RZ and Brody RZ - can act as answers for these demands of the markets.

Integrated haploid technology is one of the most effective biotechnological tool to generate valuable material for hybrids breeding.

Figure1
Extra white color, smooth skin, insensibility for the anthocyanin on the Hungarian Wax fruits

1. Introduction

The own Hungarian Wax hybrid pepper breeding program of Rijk Zwaan Budapest Ltd. started in 2005 at Felgyő. This is the biggest and most important type in Hungary in pepper growing. Growers and the market require improved varieties continuously.

The breeders have to give quick reactions by creating new varieties with higher and higher quality traits also. For hybrids production to obtain pure lines is a priority. Haploid technology offers a shortcut to perfectly homozygous lines and provided an attractive solution for production huge amount of pure-lines and new paprika candidates for registration (Sági and Gemes Juhasz, 2012)

An efficient doubled haploid protocol involves evaluating factors which influence induction of embryogenesis, the regeneration of those embryos to plants and fertile doubled haploid (DH) induction from haploid plants (Segui-Simarro *et al*., 2011; Ferrie and Möllers, 2011).

Last years we have been taking an integrated research approach the effectiveness of fertile pepper doubled haploid (DH) production by optimizing in vitro and in vivo culture parameters (Gemes Juhasz *et al*., 1998; 2004; 2010, Lantos *et al*., 2009; 2012).

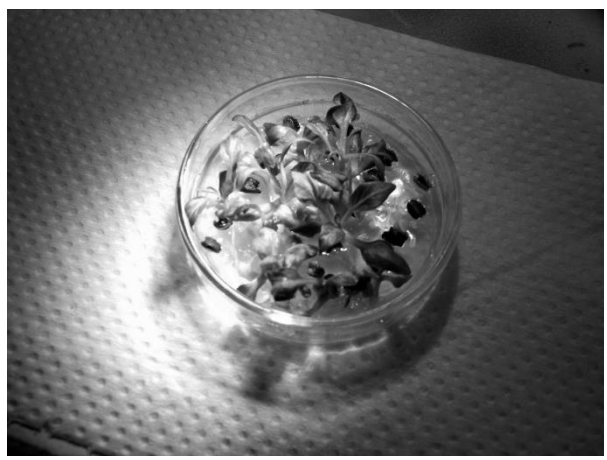


Figure 2
Plants development from anther culture of pepper

2. Results

Bravia RZ is already very popular and successful on the market because of the quality traits of the fruits together with the high resistance level and production. **Bravia RZ** has a pendant fruit type with a big fruitsize (120-130 gr.) and perfect white fruit color. The variety gives extra high setting also. Bravia RZ has anthocyanin less characteristic also for the insensibility of the anthocyanin on the fruits.

Figure 3
Bravia RZ in production, after the harvest and the fruits are ready for the market

The variety **Brigy RZ** is a variety with very high productivity with extra white, bright and big size fruits (130-140g) with the same anthocyanin less character, like Bravia RZ. This variety is adapted to the unheated growing systems and the second cycle (autumn production) growing as well.

Figure 4
Brigy RZ in unheated tunnel and the extra white color fruits in pack

The new introduction, **Brody RZ** can give the answer for the request of the export market with the needed export size, very regular fuits during the season with high resistance level (Tm2, Tswv, Nematode) in the variety.

Figure 5
Export size, regular and smooth fruits of Brody RZ and in production

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P1-13

High quality apple peppers for the canning industry

Csaba Sebesi

ZKI, Kecskemét, Hungary

Abstract

ZKI 110618 F1 and ZKI 110619 F1 high quality apple peppers for the canning industry.

The pepper is important not only for the fresh market, but also for the canning industry. The white waxy „apple" pepper is one of the most used type by the food industry in Hungary. It is known and prefered in Slovakia and Austria also. There are two different processing methods which determine the use of the hybrids. For different use different fruit sizes are needed. Besides the size, the presence or the absence of the pungency plays a big roll in the decision of the canning factories.

Our goal was to breed high yielding hybrids which do the job under different conditions. We have tested the experimental hybrids during two years, in several location, on open fields.

We have categorised the yield as the same way a the canning factories do. At the end of this breeding program, we could realese one hot (ZKI 110618 F1) and one sweet (ZKI110619 F1) apple pepper for the local and the neighbourhood market.

1. Material and methods

The examined parent lines of hybrids were breaded with one, worldwidely used method. These hybrids were tested in two seasons. In the first (2011) year we had only one, in the second year two trials. The testing was based on yield measurent (qualitiy and quantity) and on observation. After the evaluations, hybrids were released for commercial sale.

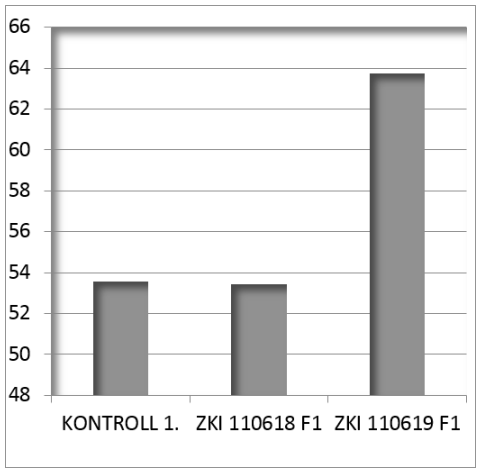
The experimental hybrids: ZKI 110618 F1, ZKI110619 F1 and five controlls.

The trials had to be well prepared and designed. The arrangement of the plots was designed with a randomising softwere. The hybrids were planted in two locations, in one soft and one hard ground. The experiments were observed for two seasons, in representative growing conditions. The measure system was based on the used measuring system of canning factories and it was done three times per trial during the season.

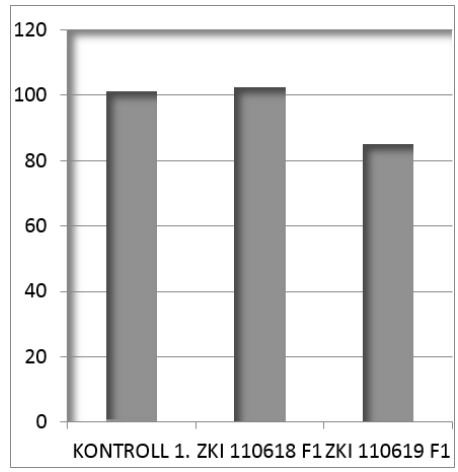
The recorded datas were analysed in variance analysis(ANOVA). We based our final decisions on this analysis.

2. Result

Result of first years' trial:



1.graph
Yields of the first (2011) year's trial (%)

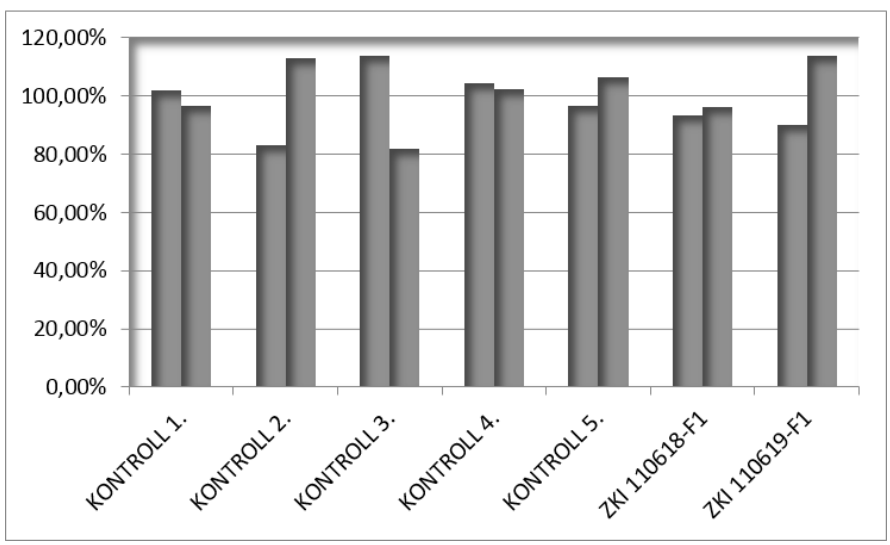


2.graph
Average fruit weights of first (2011) year's trial(g)

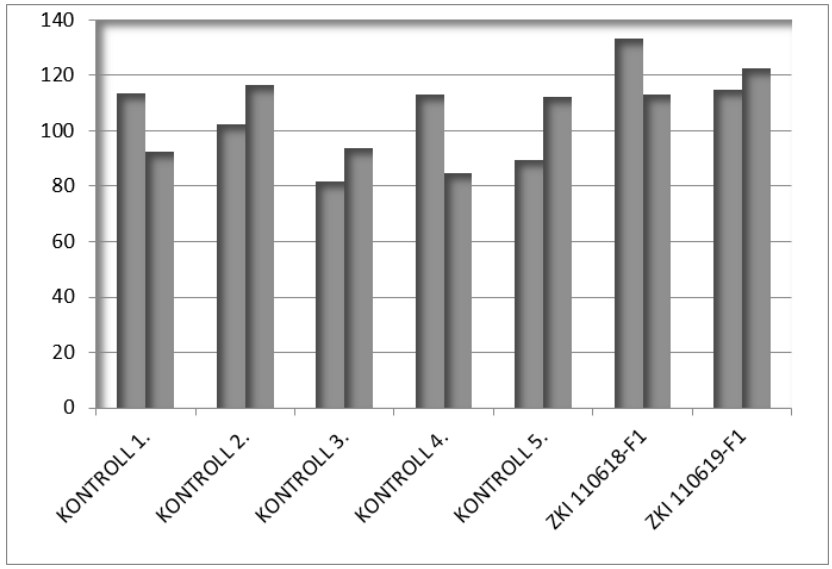
In the first year, based on the testing results, we have selected the best producing hybrids.

Results of second years' trials:

In the year of 2012 we tested them again, comparing with the same control hybrids and with new hybrids also. We had the possibility not only to examine the differences between yield abilities and average fruit size of hybrids (3., 4. graphs), but to test the season's effect also.

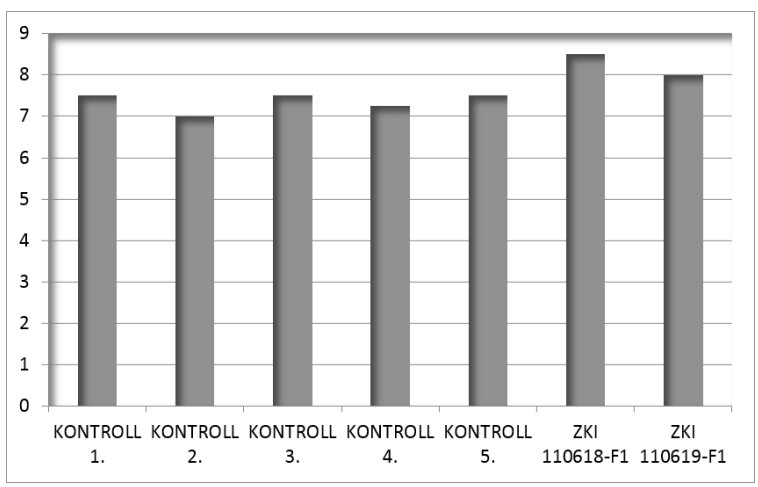


3. graph
Comparison of the yields in 2012



4. graph
Comparsion of the average fruit size(g) in 2012

The results of in graphs show, that both the pungent (ZKI 110618 F1) and sweet(ZKI 110619 F1) hybrids' average fruit weight and yield has not differed significantly from the controlls(3., 4. graphs).



5. graph
Comparsion of the visual review

The datas of the breeder's visual rewiev show some differcies between the hybrids (graph: 5).

3. Conclusion

In conclusion, we can say, that making the final decision of selecting new hyprids, based only on yield measurments, can be misleading. The visual rewiev can be same important just like the measured. Properties are important together in defining „quality" and correlate to each other.

These visual rewiev qualities were:

- fruit shape, colour
- proper size
- strong vigor
- reliable pungency rate or sweet taste
- attractive appearence, meaning to have a good impression for the growers

Sample seeds of new two hybrids which were choosen in the year of 2011-2012, were given to potential growers for marketing purposes. According to their feedbacks, we can state, that our new varieties can fulfill their requirements.

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P1-14

Breeding use of *Capsicum annuum* L. mutant genepool

Olga Timina[1], Oleg Timin[2]

[1] *Taras Shevchenko Transnistria State University, Tiraspol, Transnistria, Moldova*

[2] *National Ecology and Natural Resources, Scientific Research Institute, Bendery, Transnistria, Moldova*

Abstract:

The new mutant of natural origin was isolated, which was characterized by the totality of the changed characters in the vegetative (cotyledon) and generative organs (flower and fruit). Cytological studies showed that the meiosis in new mutant is accompanied by the increased output of bivalent with three chiasma on it meiocyte, i.e., atypical and being rarely encountered. One should use this mutant for increasing the output of rare recombinants. In the breeding practice the mutants with the diverse types of sterility, stability to the diseases, with the high content of β- carotene, and also mutants, with the different duration of vegetal period are used. As a result the peach colored type of vegetable pepper Katyusha was obtained, in which the content of provitamin A increased on 30 % and reached the usual level of the red variety types. The mechanism of the unstable colour of pericarp, which occurs in a number of the crossings was determined also.

The polygenetic nature of early ripeness character was refined and the pleiotropic effects on it of the gene ***fa*** were determined. The use of a gene ***fa*** is effective for creating of early-ripening types or hybrids with the stable productivity.

Radiation mutants with the male sterility successfully are used for conducting the seed-growing of heterotic hybrids. The attraction of sterile maternal line with the production of hybrid pepper considerably increases the profitability of the seed-growing of culture.

1. Introduction

In Moldova and Transnistria pepper is - the traditional and very popular vegetable culture, where it is cultivated according to the data of Moldavian statistics to the commodity purposes over the area of 5000 hectares. And if varieties of universal usage resistant to the verticillium wilt were especially claimed at the first stage, then at present the varieties assortment was transformed and enlarged due to breeding and cultivation of hybrids. The use of a mutant gene pool is one of the methods for higher breeding and genetic studies of the pepper crop. At present there are more than 300 mutants of the spontaneous and artificial origin in pepper [1]. The complete genetic map of the pepper genome and its sequencing, mapping of the peppers quantitative characters became the unconditional scientific breakthrough in genetic studies of pepper [2, 3, 4]. However, the complete development of the correspondence between the revealed markers and the specific economically valuable characters is not solved task. In addition the use of the phenotypically expressed mutants increases the authenticity of the studies and simplifies genetic analysis.

2. Materials and Methods

Plant material. Katyusha variety population (*Capsicum annuum var. annuum*, Grossum variety type) of our own breeding was used in this study [5]. This variety was obtained by the method of individual selection from the population of redly fruitful variety Lastochka during evaluation of 240 thousand plants in the open ground. In turn, the new type of mutant with the hypertrophied calyx was isolated in the population of Katyusha variety. The chosen mutant was designated by the symbol Calcc – Calyx completely closed.

Hybrid analysis was carried out employing the conventional procedure. Calculation of genetic distance between the loci with the alleles of yellow pericarp colour ***y*** and high content of β- carotene ***bc*** was conducted through the formula of Kosambi: $m_K=25 \ln\{(1+2r)/(1-2r)]$.

The cytological analysis of the meiotic cell nucleus division was carried out accordingly procedures of [6]. Daskalov's mutants with the genes ***ms-3*** and ***ms-8*** on basis of which the maternal lines were developed for the heterosis breeding, and also mutant gene ***bc***, coding the high content of provitamin A and the selective lines, developed with its participation also were used in the breeding work. Gene ***fa*** was used widely in breeding earliness traits and uniform fruits ripening.

The general (GAi) and specific (SAi) adaptability, genotype stability (Sgi), and selection value of the genotype (SVGi) were determined according [7]. Plants were grown in a spring–summer or winter–spring growth cycle in open ground, plastic unheated greenhouses, and glass winter greenhouses according to cultivation and selection requirements [8]. Mathematical data processing was conducted with the application of the packet Statistica 6,0.

3. Results and Discussion

New mutant with the completely closed calyx was isolated under the conditions of plastic unheated greenhouse and it was described with the totality of the changed vegetative and generative characters. Hybrid and cytological analyses showed that the new mutant is - the result of two chromosomal mutations [6]. The first mutation was lethal, – the result of the epistatic two dominant genes interactions, and it was phenotypically manifested on the postzygotic phase of the sporophyte at the cotyledon stages, which turned yellow, they did not grow and were not developed, they became white, and the whole plants gradually died. The second mutation was also dominant and caused significant phenotypically expressed deviation in the structure of perianth and pericarp, and it was connected also with homozygote depressed viability (Fig.1). Both mutations are not coupled and segregate independently.

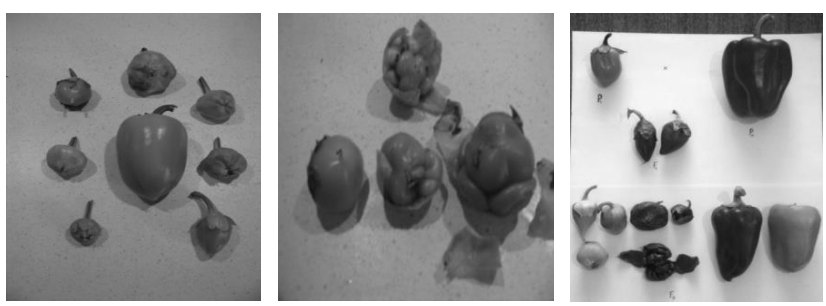

Fig.1. Phenotype of new mutant Calcc and its manifestation in F_1 and F_2.

Cytological analysis showed that the increased quantity of bivalents with three chiasma on it meiocyte, i.e., atypical and being rarely encountered is observed in some families of mutant. Therefore new mutant can be useful in the crossings for a possible increase in the posterity the appearance of nontraditional recombinants. The use of a new mutant is promising also for investigating the genetic network of flower and productivity.

Manifestation and inheritance of the pericarp orange-red colour characters and high content of β- carotene in the dependence on the genetic background in vegetable pepper were studied. It is shown that the orange-red colour is determined by mutant gene ***bc*** and the dominant gene CrtZ-***2***. The linkage group of the mutant gene ***bc***, which controls the high level of β- carotene is determined. It is possible to add the new identified mutant gene ***bc*** to the known genes of pericarps colour and content of β- carotene. Gene ***bc*** is localized in one linkage group with ***y*** gene, and the relative distance between these genes according to our calculations is in the limits m_k=64,67cM. [9]. It is revealed, that the contents of different biologically active pigments were the independent character [9]. Therefore it was possible in the yellow colored fruits to raise the level of β- carotene. The new promising lines were obtained with the gene ***bc.*** On their basis hybrids and cultivars - candidates, included in competitive and state quality testing, were allowed to the use. Good results showed also the cultivar Katyusha. In the new cultivar with the yellowish orange fruits it was possible not only to reach the level of the β- carotene content as in usual erythrocarpous types, but also to exceed it on 30%.

The gene ***fa*** was widely applied in the breeding to the early ripeness. Donors of earlyripeness, simultaneous ripening, and shade and verticillium wilt tolerance were used for developing Moldavian type of the vegetable pepper cultivars with clustered fruits [10]. Now the work started to specify the ***fa*** and ***Mf*** genes interaction in the cross ♀Black Pearl x ♂Dobrynya Nickitich. In F_1 the number of clusters seemed to be the polygenic character, which is in agreement with [11]. But the types of bush fit with determinant type, so both genes determine the clustered fruits but differ by the type of the bush. These genes also have pleiotropic effect on the earliness.

The need for information about the adaptability of donors has vital importance for a breeding process. The identified donors are claimed stable under the specific conditions of environment with the specific unfavorable factors and for creating of cultivars and hybrids with the high productivity. The adaptability of the "phenophase duration" and "early yield" parameters was assessed more precisely for the varieties Topolin, Venti, Lastochka, and Winnie Pooh (Tab.1), which have been shown to be reliable donors of the "early ripening" parameter [12].

Genotype	*X*i, kg/m^2	GAi	SAi	Sg_i, %	b_i	SVGi
Topolin	1,36	0,09	0,94	71,5	1,03	0,72
Lastochka	0,99	-0,28	0,9	95,7	1,05	0,36
Venti	1,28	0,01	1,07	80,6	1,14	0,6
Vinni_Pukh	1,45	0,18	0,65	55,5	0,77	0,92

Table 1
Adaptability parameters of the "early ripening" of bell pepper with regard to fruit yield.

Donor adaptability was studied under open ground, glass winter greenhouses, and unheated plastic greenhouses. Analysis of variation of the crop yield parameters, and the duration of the first and third phenophases under three different sets of cultivation conditions revealed a difference between the genotypes and the interactions of the genotype with the environment.

Adaptability analysis was performed for the "crop yield" parameter and the duration of the first and third phenophases which had the level of significance at 5 and 1%, respectively. The data concerning the phenophase duration demonstrated the advantage of the *Vinni_Pukh* variety with gene ***fa***. Given that the selection with regard to early ripening aims at decreasing the duration of the second phenophase, the low values of GAi, SAi, and SVGi confirm that the character is more pronounced in the *Vinni_Pukh* variety at different backgrounds. The expression of the "first phenophase duration" parameter is unstable, while that of the third phenophase duration is stable (Sgi < 10%), the responsiveness is weak (bi < 1), and the selection value is the highest. The parameters of the other donors are quite similar, the responsiveness being weak (with the exception of Lastochka), and therefore their selection values are almost similar. The results obtained demonstrate donor differentiation with regard to the "early crop yield" feature adaptability. The GAi value was the highest for the *Vinni_Pukh* cultivar. Combination of the "high productivity," "low variance of SAi and Sgi," and "weak responsiveness to cultivation condition changes" (bi < 1) parameters allowed for the classification of this donor as highly adaptive. The combination of productivity and stability is the most favorable, this being manifested by the highest SVGi value. This donor can be used for the developing of early ripening semi-intensive varieties or hybrids characterized by a potentially high ecological productivity and capable of maintaining a stably high, if not maximum, crop yield.

The sterility genes ***ms*** are widely utilized also in the breeding practice. For this maternal lines are transferred into the sterile basis by recurrent selection, alternating individual selections with repeated backcross. As the result the hybrids on the sterile basis Yubileyniy Semko and Vitamin are successfully cultivated in the Russian Federation. The hybrid Vitamin is also improved in the content of provitamin A, it has the original orange-red pericarp colour and it is needed in population. The approaches are studied on the use of different molecular markers for early genotype necessary genes testing, since reliable systems for the genes of male sterility are not developed until now.

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P1-15

Breeding of a high yielding white waxy hibrid ZKI 113485 for the Mediterranean region

Péter Varró[1], Csaba Sebesi[1]

ZKI Zrt., Kecskemét, Hungary

Abstract

The white waxy „cecei" type of pepper (*C. annuum*) is widely used by the Hungarian growers. It is also known in Serbia, Czech Republic, Slovakia and Ukraine In the early '90-s due to the high energy costs, the production under the heating tunnels decreased in Hungary. In the winter and early spring time the Mediterranean area became the most important prodeucers for the Central European market.

The target of our breeding program was to develop a hybrid, which fits the Mediterranean production, and has high and stable yield with excellent quality also in the wintertime.

We conducted the trials in several locations and for more years. The testing tunnels were located in Hungary and Crete. In Hungary our trials were carried out on light sandy soil, on heavy soil and on cocopeat.

We measured the yield and categorised it, according to market classification used by the vegetable wholesalers.

In addition to yield we focused also on the resistance, mainly to the Tomato spotted wilt virus resistance and the Tomato mosaic virus (Tm3) resistance.

After several years of experiment the ZKI Zrt issued in 2015 an excellent high yielding hibrid ZKI 113485 –CTK2 for the Mediterranean market. The hybrid is resistant to TSW and Tobamo viruses.

1. Introduction

The white waxy „cecei" type is the most important pepper type in Hungary. It is also known in Serbia, Czech Republic, Slovakia and Ukraine. In the early '90-s due to the high energy costs, the production under the heating tunnels decreased in Hungary. In the wintertime and early spring the main producer area is the Mediterranean area, mainly North Africa and the Mediterranean basin. The production conditions of these areas are completely different from the European conditions. The transplanting time is at the beginning of September, and the plants stay during the winter without heating. As a result the abiotic stress tolerance of these hybrids has to be different, than the hybrids used only in Europe. One of the most important features is the fruit setting during the winter conditions and the quantity of the marketable yield.

Due to the warm winter the thrips infestation in these areas is high during the whole production period. This causes high pressure of the Tomato spotted wilt virus. To be successful on the market the Tsw resistace is essential for the growers.

Our main goal was to breed such hybrids which satisfy the demand of the European summer production and the Mediterranean winter production also. (1), (2), (3)

2. Material and method

The experiment was conducted between 2013 and 2015 in Hungary and in Crete. In this testing 32 hybrids were involved. As control we used the market leader variety. In Hungary we transplanted the trial in a light heated plastic tunnel in the beginning of March. The first harvest was on the 9th of May 2013. As a substrate we used peat moss filled in 14 l buckets. Each bucket contained 3 plants. We pruned the plants for one stem. One plots contained 12 plants. The fertilization was managed with chemical analysis of the waste water. The row distance was 80 + 40 cm and 25 cm between the plants.

In Crete we sowed the trial at the beginning of August and transplanted on the 8th of September 2014 in soil. The row distance was 200 + 100 cm and 25 cm between the plants. We pruned the plants for two stems.

On both locations we categorised the fruits according to market classification used by the vegetable wholesalers.

- >6 cm fruit shoulder (Extra)
- 5-6 cm fruit shoulder (1st class)
- 4-5 cm fruit shoulder (2nd class)
- out of classes
- damaged fruits

3. Result and discussion

In Hungary we harvested the trial in the early season, when price of peppers on the market was the highest. (table 1.) We made 4 harvests. In this period the total yield of the hybrid 113485—CTK2 reached the control (2.69 kg/ m^2) and the average fruit weight was higher. If we look at the categorised results (table 2), we see that the extra yield (>6 cm) is 1.82 kg/m^2 is much higher than the control. This is due to the high number of fruits in the extra category and the high average fruit weight (137,65 g)

We collected data from the winter production in 2014 and 2015. In 2014 the result of the first few harvests (table 3) shows that the average yield was close to the control (90.35 g) If we look at the yield data (Figure 1), we can see that the yield of the 113485—CTK2 was higher than the yield of the other varieties in the beginning of December. Beginning of the December is a turning point of the summer production, which finishes and of the import which starts.

For the market involved, the most important yielding period is the winter and the early springtime. The hybrid 113485—CTK2 has in this period higher yield (1,05 kg/$m^{2)}$ than the control (0.87 kg/m^2) (Figure 2).

Summarizing, we found that hybrid 113485—CTK2 satisfies demands of the light heated production in Central- Europe as well as the demands of the Mediterranean winter production.

However during both testing periods the TSWV pressure was really high, but we could not observ any kind of virus infection due to the resistace of the hybrid 113485—CTK2 to TSW.

Table 1
Performance of „cecei" hybrids for the yield in the trial managed in 2013 in Hungary

	máj.09. - jún.22.					
	Fruit number	fruit number (%)	Yield (kg)	Yield (%)	average fruit weight (g)	average fruit weight (%)
KONTROL Kontrol	27.78	100	2.69	100	101.5	100
113474-CTK2	19.32	69.6	2.14	79.6	111.04	109.4
113475-CTK2	26.57	95.7	2.8	103.8	105.26	103.7
113478-CTK2	20.53	73.9	2.1	77.8	102.47	100.9
113479-CTK2	18.06	65	1.94	71.9	107.43	105.8
113480-CTK2	17.51	63	1.89	70.2	108.13	106.5
113482-CTK2	17.21	62	1.72	63.9	100.07	98.6
113485-CTK2	22.95	82.6	2.69	99.9	**117.72**	**116**
113486-CTK2	19.93	71.7	2.3	85.4	**113.94**	**112.2**
113487-CTK2	15.1	54.3	1.61	59.9	106.73	105.2
113488-CTK2	22.04	79.3	2.42	89.9	109.48	107.9
113493-CTK2	21.74	78.3	2.41	89.5	111.26	109.6
113535-CTK2	16	57.6	1.73	64.3	107.64	106
113536-CTK2	17.51	63	2.11	78.5	**120.69**	**118.9**
113539-CTK2	18.12	65.2	1.71	63.5	94.86	93.5
113540-CTK2	18.12	65.2	1.98	73.5	109.06	107.4
113541-CTK2	16.91	60.9	1.8	66.8	106.46	104.9
SzD 5% :	8.84	31.8	0.97	35.8	12.16	12
CV% :	17.7		17		5.3	

Table 2
Yield of the hybrids in the extra category in the trial 2013 Hungary

	> 6 cm (Extra)					
	Fruit number	fruit number (%)	Yield (kg)	Yield (%)	average fruit weight (g)	average fruit weight (%)
KONTROL Kontrol	10.57	100	1.38	100	131.37	100
113474-CTK2	11.78	111.4	1.4	101.3	118.99	90.6
113475-CTK2	8.45	80	1.05	76	123.54	94
113478-CTK2	10.27	97.1	1.15	83	111.77	85.1
113479-CTK2	6.83	64.7	0.84	60.5	121.23	92.3
113480-CTK2	8.15	77.1	0.98	70.7	123.72	94.2
113482-CTK2	5.74	54.3	0.64	46.3	109.17	83.1
113485-CTK2	13.29	125.7	1.82	131.4	137.65	104.8
113486-CTK2	9.66	91.4	1.21	87.8	125.59	95.6
113487-CTK2	8.15	77.1	0.95	69	117.09	89.1
113488-CTK2	12.08	114.3	1.46	105.7	121.94	92.8
113493-CTK2	10.57	100	1.26	91.3	119.71	91.1
113535-CTK2	7.25	68.6	0.89	64.6	122.36	93.1
113536-CTK2	9.06	85.7	1.26	91.3	137.24	104.5
113539-CTK2	2.11	20	0.25	18.3	120	91.3
113540-CTK2	7.85	74.3	1	72.5	126.41	96.2
113541-CTK2	5.13	48.6	0.62	45	120.98	92.1
SzD 5% :	7.04	66.7	0.94	67.6	15.66	11.9
CV% :	28.6		29.6		6.1	

Table 3
Yield of the hybrids in the trial 2014 in Crete

	okt.22. - dec.30.					
	Fruit number	fruit number (%)	Yield (kg)	Yield (%)	average fruit weight (g)	average fruit weight (%)
KONTROL Kontrol	148.6	100	13.52	100	91	100
112170-CRK1	156.28	105.2	14.93	110.4	96	105.5
112176-CRK1	183.57	123.5	15.57	115.1	85.1	93.5
112275-CRK1	6.05	4.1	0.64	4.7	112.49	123.6
113485-CTK2	195.66	131.7	17.69	130.8	90.35	99.3
113490-CTK2	171.47	115.4	16.93	125.2	98.92	108.7
114779-CPE2	159.63	107.4	14.62	108.1	91.32	100.4
SzD 5% :	68.13	45.8	5.77	42.7		
CV% :	19.1		17.6		7.1	

Figure 1
Yield of the hybrids in the trial 2014 in Crete

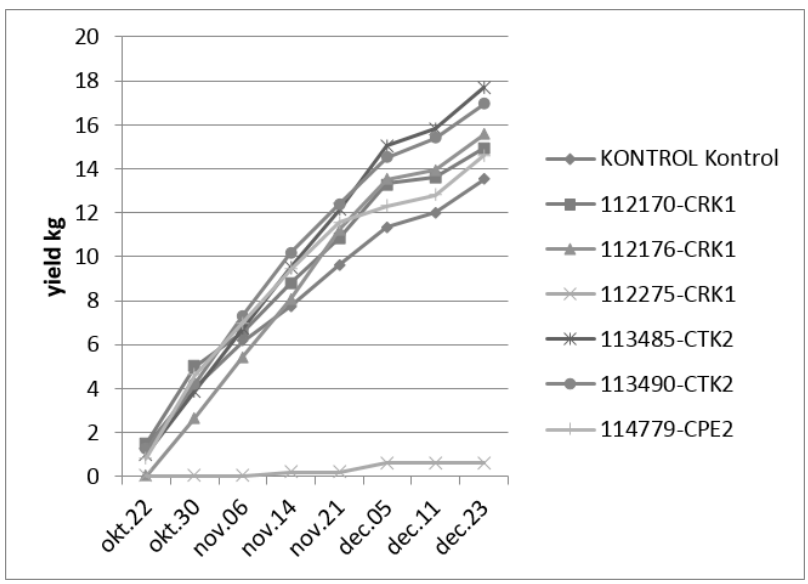

Figure 2
Yield of the hybrids in the trial 2015 in Crete

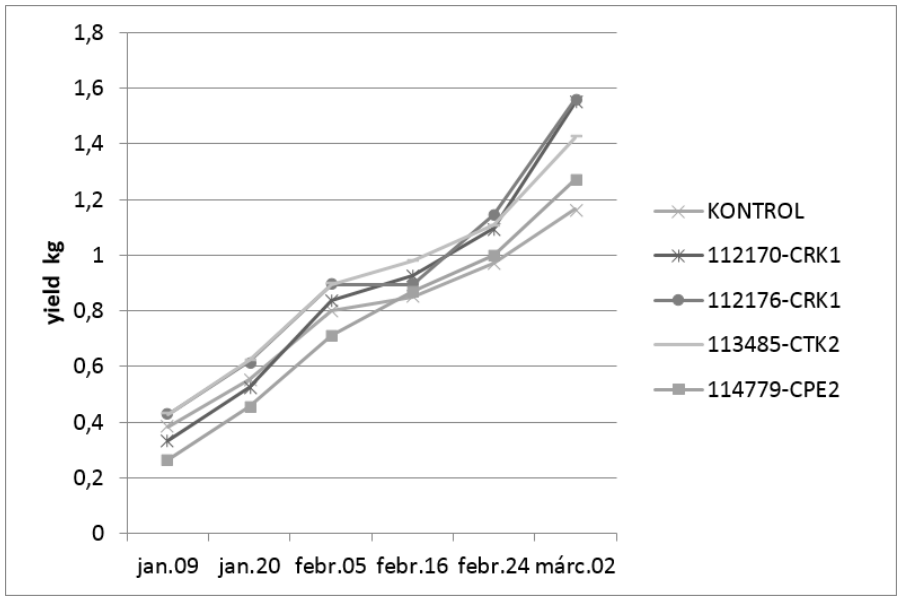

Figure 3. Trial hybrid 113485 –CTK2 in winter production

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P1-16

The role of general and specific combining abilities in pepper hybrid breeding

Lajos Zatykó, Attila Rajki

Orosco Ltd., Orosháza, Hungary

Abstract

An important aim of hybrid breeding is to find high level of specific combining ability (heterosis effect) controlled by the presence of dominance (dominance, overdominance) gene effects appearing in parental combinations . Risk may be involved when selection of the new lines is made on the basis of their general combining ability (primarily yield potential of test combinations) controlled by additive gene effects (Kalloo 1988, Doshi and Shukla 2000).

In case of those crops (maize), where due to depression of inbreed lines, the individual value of the lines becomes visible only in their hybrids, the number of lines can be reduced by the early testing of general combining ability exclusively. Yet, pepper as a self-pollinated species avoids depression of inbreed lines, therefore the number of lines can be decreased by evaluation of individual values, primarily by visual evaluation. In developing lines, factors of yield potential and quality traits allow breeders to achieve good selection gains, their values exhibit close correlation with the values of their hybrids (Allard 1960, Depestre – Gomez – Espinosa 1989).

Due to the possibilities presented above, the most economic way for finding the parental pairs with best specific combination ability, involves partial diallel testing of lines preliminarily screened for individual plant performance. The diallel test is partial because it includes only those combinations that are likely to satisfy the criteria of the targeted product.

1. Introduction

A strict selection for general combining ability (GCA) alone can cause irreversible deficit regarding the important genes of other factors including specific combining ability (SCA).

Even though, finding the best GCA lines and SCA trial combinations are the cardinal points in pepper breeding as well, exploitable traits of the plant have led most of the breeding companies avoiding to follow strictly the "corn model" in their methods to find the best combination. In case of pepper, the reasons of diverging from the classic model are mainly the demand to avoid depression of inbreed lines on the one hand and the possibility of visual evaluation on the other hand.

In developing lines and evaluating combinations, selection based on visual observations is far more feasible in paprika than in the majority of crops.

Within certain limits, the yield potential (early and total yield) can be judged from the number and size of the fruits, while quality traits, which are often a basic criterion, can often be evaluated more accurately by visual observations. In the early stages of breeding, which is still large in numbers, "rapid visual evaluation is better than precise measurements" (Allard 1960) and this is also true of the first selection on test combinations.

The visual evaluation of paprika is promoted by the row of phenotypic manifestation that are in close correlation with yield potential, while the reliability of this classification is enhanced by the high heritability values of these traits.

During the first visual screening of lines and combinations for earliness (rapid development rate, low sensitivity to lack of light) the plants can be scored for branching, start of flowering, date of first fruit setting or start of biological maturity. However, when evaluating earliness it must not be forgotten that the general negative correlation observed in the plant kingdom between earliness and yield potential is also valid for paprika (Chang – Lin – Tseng 1977, Chung 1981). The first visual screening for early and total yield quantity can be used to rank the lines and combinations on the basis of fruit number per plant, fruit size, and perhaps pericarp thickness and vegetative mass. Numerous studies have confirmed that yield potential can be judged from the fruit number, fruit size, vegetative mass and even from the length of the fruit shank, since all these traits are in positive correlation with the total yield (Chang – Han – Ko 1968, Chang – Lin – Tseng 1977, Chung 1981). Though the fruit number per plant is negatively correlated with the mean fruit mass and flesh thickness (Depestre – Gomez – Espinosa 1986). Several authors, including Doshi et al. (2001), have demonstrated that seedling traits are suitable for the estimation of combining ability for total yield.

The heritability values for earliness, fruit number per plant, fruit weight, total yield and plant height are all high (Chang – Han – Ko 1968, Chan – Lin – Tseng 1977, Chang – Chung 1979, Choi – Kim 1986, Depestre 1988, Depestre – Gomez – Espinosa 1989), so visual evaluation is not greatly affected by environmental effects.

According to Kuckuck et al. (1985) the separated testing of GCA can be neglected in case of hybrid breeding of self-pollinated species, where the number of lines can be reduced, and to carry out diallel crosses for genotypes is sufficient. Regarding the value of combination ability, it is also worth noting the findings of Kalloo (1988), who stated that the value of SCA of F1 gives a better indication of the value of the parents than an examination of their general combining ability, while diallel crosses provide information on both general and specific combining ability.

General combining ability is indicative of additive gene effects, while specific combining ability reveals the presence of (non-additive) dominance gene effects (Kallo 1988). Paprika breeders are fundamentally interested in finding parental lines with good specific combining ability, i.e. with non-additive gene effects for as many major traits as possible.

As general combining ability depends more on additive gene effects (Doshi and Shukla, 2000), which in themselves will not make a combination better than the better parent and which are generally only valid for yield potential, strict selection based purely on general combining ability can be risky.

The fact that a paprika line has only moderate general combining ability does not necessarily mean that it does not possess non-additive factors and other traits making it a good specific combiner.

According to Sprague (1946; in Allard, 1960) early testing is worthwhile if there are too many parental lines and if the emphasis is on yield potential, but not if visual evaluation is possible. Allard (1960), Singleton and Nelson (1945), Richey (1945; 1947) and Payne and Hayes (1949) all reported that visual selection was effective in early testing, but warned that early testing might lead to the rejection of potentially valuable lines. Kalloo (1988) raised the possibility of line selection prior to the evaluation of combining ability for vegetable crops.

The visual evaluation of yield potential of inbreed pepper lines such as the early and total yield as well as quality traits offers an opportunity for shortening of the breeding process. Consequently, for finding materials with extraordinary general and specific combining abilities, we apply methods of combined partial diallel testing that are less risky and more suitable for the terms of commercial breeding.

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SESSION 2

Growing and seed production

P2-01

The effect of grafting on the quantitative and qualitative parameters of fresh pepper

Zita Birkás, István Terbe, Márton Mészáros, Gábor Balázs

Szent István University, Department of Vegetable and Mushroom Growing, Budapest, Hungary

Abstract

Fresh pepper (*Capsicum annuum* L.) production is relevant today in Hungary. In 2014 fresh pepper was forced on 1600 ha, which reached approximately a 10 kg/m^2 yield. While in the recent years the forced fresh pepper production area decreased, due to the technological development the yearly yield don't show the same tendency. While in production of tomato the use of grafted plants is relevant today, the practice of grafted plants in cultivation hungaricum fresh pepper, which belongs also to the *Solanaceae* family is not so known and used method. Therefore, the aim of the study was to determine how grafting effects the quantitative and qualitative parameters of forced fresh pepper and to determine appropriate rootstock scion combination.

In the experiment *SV 9702* white type fresh pepper variety was examined as own rooted and grafted on two different rootstocks (*Snooker, Capsifort*). For soil culture the soil of the plastic house and for the soilless culture coconut fiber slabs were used. Grafted and non-grafted fresh pepper was placed at row width and plant spacing of 110 + 40 x 33 cm (4 plants per m^2). After the picking the fruit number and size was measured. From all combination the refraction was measured at the analytical laboratory of the department. The fruits were blended and measured with manual digital refractometer (PAL-1, ATAGO). The results granted in ^{o}Brix.

Due to grafting slight decrease occurred in Brix content, both in soil and soilless culture. Between the grafting on different rootstocks *Snooker* and *Capsifort* resulted the same which can be explained that both rootstocks have approximately the same effect on the scions.

After our studies in soilless culture non-grafted *SV 9702* is recommended, as long as in soil culture both *Snooker* and *Capsifort* rootstock are recommended combination of *SV 9702*.

Keywords: fresh pepper, rootstock- scion, yield, refraction

1. Introduction

Fresh pepper (*Capsicum annuum* L.) production is relevant today in Hungary. In 2014 fresh pepper was forced on 1600 ha, which reached approximately a 10 kg/m^2 yield. While in the recent years the forced fresh pepper production area decreased, due to the technological development the yearly yield don't show the same tendency (Fruitweb, 2014). Nowadays the demand for grafted seedlings are increasing in Europian countries, while in some Asian countries grafting enjoys great popularity (Lee, 1994; Fernández-Garcia et al., 2004).

Grafting has many advantages. The tolerance of the grafted transplants to low (Bulder et al., 1990) and high temperature (Rivero et al., 2003) can increase. Colla et al. (2008) in their experiment examined higher yield of grafted plants, furthermore the refraction level of fruits correlates in case of the grafted and non-grafted transplants. According to Panella (2014) the

abiotic stress tolerance of grafted fresh pepper improved and shows better reaction to low soil temperature and high soil salt content (Edelstein, 2004). Garner (1979) mention three disadvantages of grafting, which are the incompatibility, costliness and possible quality loss. Morra and Biolloto (2006) found out in their experiment, that the choose of appropriate rootstock scion combination is the most important at the beginning of the technology.

While in production of tomato the use of grafted plants is relevant today, the practice of grafted plants in cultivation hungaricum fresh pepper, which belongs also to the *Solanaceae* family is not so known and used method. Therefore, the aim of the study was to determine how grafting effects the quantitative and qualitative parameters of forced fresh pepper and to determine appropriate rootstock scion combination.

2. Material and method

In the experiment *SV 9702* white type fresh pepper variety was examined as own rooted and grafted on two different rootstocks (*Snooker, Capsifort*). The experiment was set up at the research garden of Szent István University in Soroksár in unheated plastic house. The transplants were planted in 18th May 2015 to soil and soilless culture using intensive technology: soil mulch, dripping tube/dripping irrigation system, training system. For soil culture the soil of the plastic house and for the soilless culture coconut fiber slabs were used.

Grafted and non-grafted fresh pepper was placed at row width and plant spacing of 110 + 40 x 33 cm (4 plants per m^2). During the growing season two main stem was evolved twisted constantly to the training system and slightly pruned. The fruits were harvested in economic ripeness every 7-10 days seven times (14th July, 23rd July, 30th July, 6th August, 13th August, 25th August, 8th September) during the season. After the picking the fruit number and size was measured. From all combination the refraction was measured at the analytical laboratory of the department. The fruits were blended and measured with manual digital refractometer (PAL-1, ATAGO). The results granted in °Brix.

3. Results

The yield parameters of pickings are shown on Figure 1. Both soil and soilless culture grafted and non-grafted combinations.

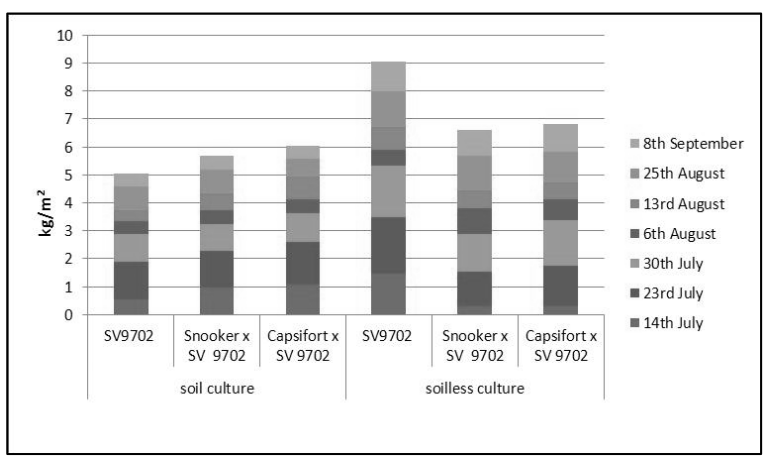

Figure 1
Yield parameters of non-grafted and grafted plants in soil and soilless culture (2015)

According to Figure 1 it can be seen that in soil culture the grafted plants results higher yield than the non-grafted. Between the rootstock-scion combinations *Capsifort x SV9702* reached higher yield. In soilless culture the non-grafted *SV9702* results higher yield with 9 kg/m2. To compare the grafting combination also *Capsifort x SV 9702* gained better yield.

Non-grafted *SV9702* shows almost double yield in soilless culture than in soil culture. In case of the graft combination this difference is not observed between the two cultures.

At the first picking in soilless culture from the grafted plant more fruit were picked, however in soilless culture this tendency was not noticed.

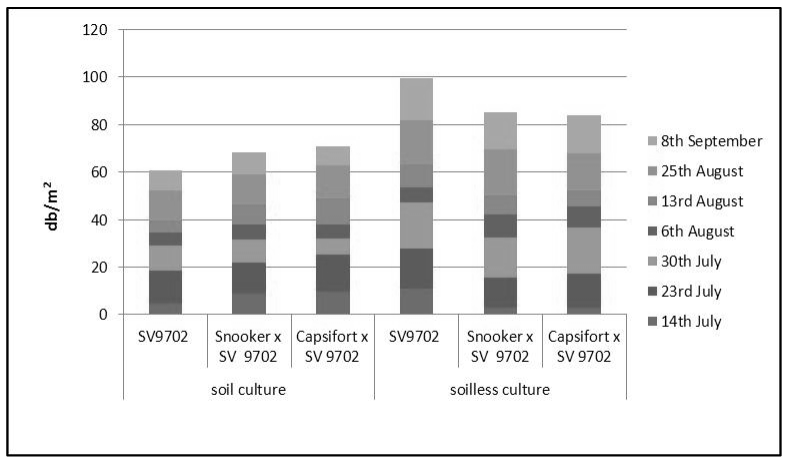

Figure 2
Fruit number of non-grafted and grafted plants in soil and soilless culture (2015)

Figure 2 shows the picked fruits in each picking day (db/m^2). In soil culture at the first picking from the grafted transplants more fruits were picked, while in soilless culture the non-grafted *SV 9702* results better.

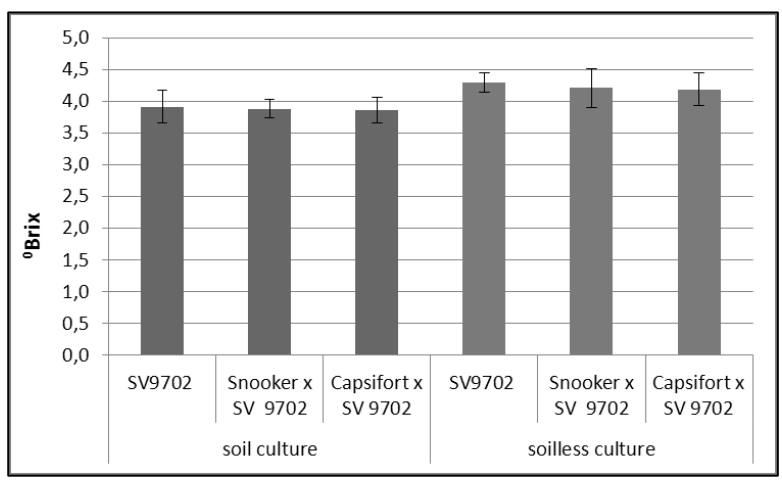

Figure 3
Brix content of non-grafted and grafted plants in soil and soilless culture (2015)

Based on the results, it can be seen, that the Brix content was higher in soilless culture than in soil culture. In soil culture and as well as in soilless culture between the grafted and non-grafted plants there is no significant differences in Brix content (Figure 3).

4. Conclusions

In the course of the yield parameter studies was observed in soil culture, that the grafted plants at first picking doubled both the yield and fruit number than non-grafted plants. This points to the fact that in soil culture the pickings of grafted plants can be started earlier than non-grafted, which can be advantage in forcing. In the second picking decline was not observed, but significant difference wasn't between the two propagation techniques.

Due to the high temperature in July and August the fruit set was inhibited, thus decreased the fruit number and yield. In soilless culture more Ca deficiency fruit was observed, which was caused by the high temperature as well.

Due to grafting slight decrease occurred in Brix content, both in soil and soilless culture. Between the grafting on different rootstocks *Snooker* and *Capsifort* resulted the same which can be explained that both rootstocks have approximately the same effect on the scions.

After our studies in soilless culture non-grafted *SV 9702* is recommended, as long as in soil culture both *Snooker* and *Capsifort* rootstock are recommended combination of *SV 9702*.

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P2-02

Variety and seed use of organic sweet pepper production in Hungary

Anna Divéky-Ertsey[1], László Csambalik[1], Krisztina Madaras[1], Izóra Gál[1], Zita Szalai[1], Peter Pusztai[1]

Szent István University, Department of Ecological and Sustainable Production Systems, Budapest, Hungary

Abstract

Sweet pepper (*Capsicum annuum* L.) is one of the main cultivated vegetable species in Hungary both in conventional and in organic farming, mostly produced under plastic tunnel by unheated technology. Due to more extensive cultivation method organic farmers need to take other variety characteristics into account than in an intensive production system. According to literature the most important variety characteristics in organic agriculture are resistance, flexibility, and nutrient uptake efficiency. Organic agriculture needs versatile varieties to withstand different cultivation conditions, to enhance self-regulation capacity of organic farming systems, and to adapt to the requirements of each farmer (e.g. specific markets). Increasing number of farmers are looking for such varieties in order to achieve seed self-sufficiency which can support genetic diversity within a crop population. However, there are organic farmers using modern varieties and hybrids to be more profitable on the market. The Council regulation (EC) No 889/2008 describes organic seed to be used when available. According to the regulation EU Member States maintain an online database in order to facilitate the acquisition of organic seeds.

In this study names of varieties used in Hungary were collected from different sources: i. The Organic Seed Database, and, ii. authorisations lists on non-treated conventional seeds of Hungarian certification bodies. The aim of the research was to collect and characterize the main properties of sweet pepper varieties cultivated in organic farming in Hungary, and to investigate consciousness of variety selection of farmers. Origin of propagation materials were also analysed.

Today the variety use in Hungary is extremely wide in the organic sector, unreasonably excessive taking the size of the sector into account. In the studied period (2004-2012) organic sweet pepper area (open field and covered) didn't achieved yearly the 40 ha and during this period 77 different variety were produced organically in all which varied from 9 to 47 variety per year.

Keywords: sweet pepper, organic vegetable, organic variety

1. Introduction

The approach of organic farming requires the aim for a closed design when operating an agricultural system. The highest possible share of energy for production, processing and retailing should originate from the farm. Depending on the intensity of the management, the design of a totally closed system is obviously very hard to achieve. However, there is one essential condition, which is required not only by the approach, but the regulation of organic farming: the origin of the seed. The Council Regulation (EC) 834/2007, regulating the whole process of organic farming, describes, that in certified organic farming only the seeds and propagation materials originating from organic farming can be used.

The present regulation contains only limitation with regards to the application of plant varieties in organic farming: the variety cannot be created by genetic modification. Certain organic organizations (e.g. Demeter) which exceeds the criterion- and certification system of EU in strictness, also excludes the use of hybrids from production. At the same time, there are farmers who insist on the hybrids in order to keep their market position and competitiveness.

Two main categories of varieties suggested for organic production used to be mentioned. The first one consists of varieties, which are produced by conservative breeding (except GMO) in conventional circumstances. Their characteristics make them applicable in more extensive, or organic production. The second, smaller group contains the so-called organic varieties, which were produced according to the regulation of organic farming and with the use of breeding tools preferred by the approach (Kovács 2004). Nowadays the production of the varieties belonging to the first group are widespread. In Western Europe organic breeding and the use of organic varieties are more frequent (Lammerts van Bueren 2010).

In order to ensure easier access to organic seed available on the market on a limited scale, The regulation 834/2007 EC obliges the member states to create and maintain an organic seed database. In Hungary, this database is operated by National Food Chain Safety Office (NFCSO) Directorate of Plant Production and Horticulture, in cooperation with the certification bodies (https://www.nebih.gov.hu/szakteruletek/szakteruletek/novterm_ig/szakteruletek/vetomagfel/jegyzekek/OKO_adatbazis). The goal of the organic seed database is to link the supply and demand of organic seed within the country. Organic seed producers can announce the available seed species with the indication of variety, classification, and quantity. According to the extent of organic seed production sites inspected by NFCSO Seed Inspection professionals in the past years (2004-2014), it is clear, that most of the seed supplies were not registered in the database. The reason for this could be the targeted production to foreign countries or the sales via personal contacts. Generally speaking, the predominant part of species present in the database are vegetables, the most of which are imported (Divéky-Ertsey 2014).

In case there is no organic seed available on the market the farmer can apply for a derogation for the acquisition and sowing of non-treated, conventional seeds. The certification body checks the database, and when there is no supply for the species the farmer wants to produce, the body provides the derogation for one vegetation period. This derogation is more typical in the case of arable plants. The farmer can also ask for a derogation even when the seed of the species is provided in the database, but not of the variety the farmer wants to produce. In certain member states of the EU derogations regarding to varieties of the most important plant species are not yet accepted (Döring et al. 2012). There is only Germany where sweet pepper (red-green blocky type) belongs to Category 1 lists, that means the organic farmers didn't get any derogation possibility for conventional seed use (http://www.organicxseeds.de/ 2016).

2. Materials and methods

Data analysed in this study were available from the seed derogation database of certification bodies operating in Hungary, Biokontroll Hungária Nonprofit Kft. and Hungária Öko Garancia Kft.. The survey cover an eight year period between 2004 and 2012 and contains the data of varieties of conventional seed derogations granted for organic farmers.

The characteristics of listed varieties in the database were collected from the databases of breeders and seed retailers, mainly using web sources.

3. Results

Most of the derogations concerned the seed of pepper, tomato and eggplant of Solanaceae family. Among the species mentioned, the highest number of derogations and the highest number of varieties referred to pepper (Fig. 1).

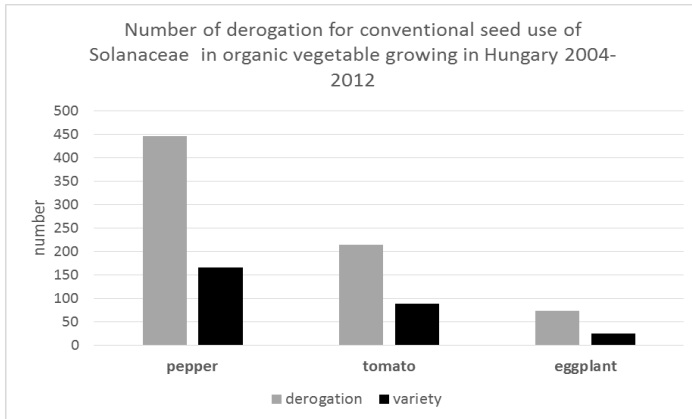

Figure 1
Number of derogations for conventional seed use of Solanaceae in organic vegetable growing in Hungary 2004-2012 (Source: Surveyed data)

Between 2004 and 2012 the two Hungarian certification bodies released a total of 446 derogations within pepper species, consisting of 135 different varieties. This regards only to the cases when the seed of preferred variety was not available on the market. Out of the mentioned 135 varieties 77 were sweet pepper variety. Sweet pepper varieties can be further divided into five groups. The most popular ones among farmers are white conical varieties with white flesh. The second place goes for Kapia types, which are more frequently produced in Hungary. Farmers chose Blocky and Tomatopepper types roughly in equal proportion. The list also included the sweet varieties of Apple pepper types used classically for pickling (Fig. 2).

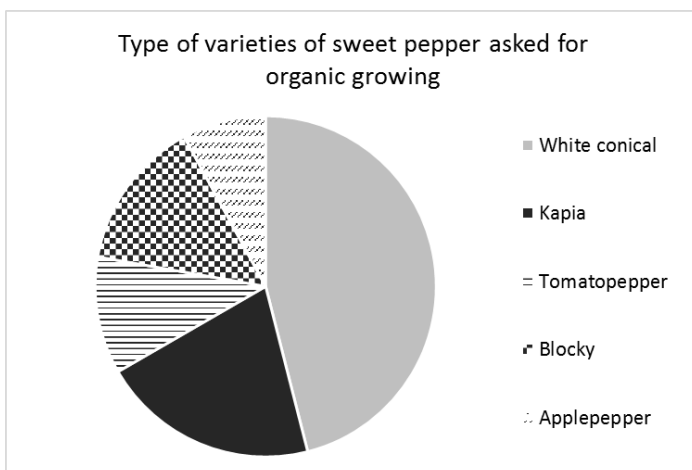

Figure 2
Ratio of different pepper variety types asked for organic vegetable growing in Hungary 2004-2012. (Source: Surveyed data)

The colour distribution represents the Hungarian sweet pepper variety use. Hungarian consumers traditionally prefer white conical type pepper with slightly pointed end as sweet pepper (Fig. 3).

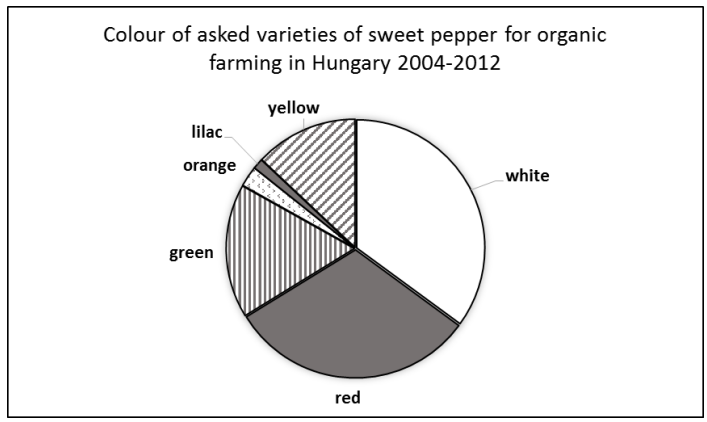

Figure 3
Colour of asked varieties of sweet pepper for organic farming in Hungary 2004-2012.
(Source: Surveyed data)

There were 30 different pieces of F1 hybrids in the derogations. The very high number of varieties consists of the whole time period investigated (2004-2012). The variety 'Cecil' (conical type with white flesh) was the only one, which were applied in the every eight investigated years. The second variety was 'Meteorit' followed by 'Fehérözön'. Both are white conical types.

Besides them, 'Karmen' and 'Karpia' Kapia type varieties were demanded, as well as the 'Greygo' variety of Tomatopepper group. The other varieties were registered only once within the investigated time period, with an occasional manner.

4. Discussion

Hungary is traditionally a good seed producer country. There is a constant marketable demand for good quality Hungarian seeds, both in national and international level. Unfortunately, our professional and environmental potential is not utilized on the organic seed market. In the previous years the domestic organic seed production could not even cover the inland demands.

The selection of an appropriate variety is an essential key point of successful plant production. Today the variety use in Hungary is extremely wide in the organic sector, unreasonably excessive taking the size of the sector into account. In the studied period (2004-2012) organic sweet pepper area (open field and covered) didn't achieved yearly the 40 ha and during this period 77 different variety were produced organically.

Sweet pepper production has a tradition in Hungary, both in forcing and on open field. Hungarian consumers prefer white conical varieties with white flesh and slightly pointed shape –so-called as stuffing pepper- for fresh consumption. The derogations claimed by the organic farmers supported this tendency.

The regulation of organic farming does not exclude the production of hybrids. Their application in production is supported by the relatively high proportion of hybrids among seed derogations.

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P2-03

Colonization of pepper by three entomopathogenic fungi and effect on the probing behavior of green peach aphid

Yiannis Manoussopoulos, Spiridon Mantzoukas, Ioannis Lagoyiannis, Stavroula Goudoudaki

ELGO-Demeter, Plant Protection Institute Of Patras, NEO & Amerikis, Patras, Greece
inminz@gmail.com

Abstract

Entomopathogenic fungi are promising pest control agents, causing insect mycosis by direct cuticle penetration or insect repel and toxicity by volatile organic compound production. A number of entomopathogenic fungal species have a symbiotic phase with plants and this feature could be important for pest control exploitation in agriculture. Of insects, aphids are among the economically most important crop pests, causing direct damage to plants by sucking sap or indirect loss by transmitting destructive plant viruses. Aphid probing behavior at the early moments of plant feeding is important for the transmission of non-persistent viruses. Here we investigate the relationship of three Greek isolates of entomopathogenic fungi, namely, *Metarhizium robertsii, Beauveria bassiana και Isaria fumosorosea*, with pepper and we examine their possible effect on the probing behavior of the aphid *Myzus persicae*, an important pest of pepper and efficient vector of plant viruses. We found that each fungus has an endophytic phase in pepper; Yet, aphids placed on plants inoculated with any of these endophytes, either avoided probing or made significantly less probes than made aphids placed on the control. Our results suggest that the three endophytes may be further exploited for aphid control and for elimination of spread of non-persistent viruses.

1. Introduction

Interactions among entomopathogenic fungi, plants, and insects have led to the evolution of specific mutualistic, antagonistic or other type of biological relationships. Entomopathogenic fungi can affect insects by several modes of action, including direct cuticle penetration [1, 2], and production of entomotoxic or insect repellant volatile organic compounds [3]. Recent reports show that some entomopathogenic fungi such as *Metarhizium robertsii* (Mr), *Beauveria bassiana* (Bb) and *Isaria fumosorosea* (If) have a symbiotic (endophytic) phase with many plant species [4]. Concerning insects, aphids are considered as the most efficient vectors of plant viruses [5] and also as important crop pests. Interaction among aphids and plant viruses has led to the evolutionary selection of specific mechanisms and the adaptation of certain aphid behavioral traits for successful virus transmission. For example, ability of aphids to transmit the so-called non-persistent plant viruses is related to the adoption of a specific mechanism for quick virus particle acquisition and release, and to the exploitation of "short-probing", a prefeeding behavioral trait, part of the aphid host selection process [6].

Although much work has been done on the effect of entomopathogenic fungi on several insect species of agronomic importance, no work has been done for studying the endophytic status of these fungi and its possible effect on aphid behavior. Such knowledge may be useful,

not only for scheming effective methods for aphid control, but also for designing novel and environmentally friendly approaches for controlling plant viruses. In this work we examined the endophytic status of three locally isolated entomopathogenic fungi, in an economically important Greek pepper variety ("Stavros"), and investigated the effect of the endophytic phase of each fungus on the probing behavior of the aphid *Myzus persicae*, an important crop pest and an efficient vector of many plant viruses. Our results reveal a lasting endophytic phase of each of the three fungi in pepper and show a significant decrease in the number of probes in such plants, implying an important effect on pepper infestation by this aphid and on transmission of non-persistent viruses.

2. Material and Methods

Entomopathogenic fungi were isolated by the Galleria bait method [7]. Pepper plants were inoculated at the stage of two true leaves with a suspension of conidia of each fungus [8]. The presence of each fungus in peppers was examined in 20-day intervals by routine isolation in PDA [8], from newly developed leaves.

Aphid colonies were kept at stable temperature (21 ^{o}C) and photoperiod (16h light) in pepper plants. Behavioral experiments were performed during a period of two months. In each experiment a batch of about 80 apterous aphids were starved for one hour [9]. One well developed leaf from each of the three treatments and the control was used in a random order and blind design. Ten aphids were used per treatment. Each aphid was placed on the under examination leaf and its behavior was monitored during the first minute under a microscope. Initiation of probe was marked at the time aphids put their rostrum vertically in the leaf surface and drew back their antennae. End of probing was marked when aphids raised the rostrum and started moving their antennae. Variables measured in the first minute were: "Time of First Probe Initiation", "Number of Probes", and "Duration of First Probe".

For analysis we used one-way ANOVA, considering treatments as fixed effects. When assumptions of equality of variance or normality were not met, we used the non-parametric "Wilcoxon/Kruskal-Wallis" test. Distribution fitting was tested by the Kolmogorov-Smirnov (K-S) Goodness of Fit test. We also used a chi-square test to examine the association between infection status (presence or no presence of each fungus in the plant) and aphid preference in making or not making probes. For this "Number of Probes" was treated as a dichotomous categorical variable. For all tests, p values less than 0.05 were considered significant.

3. Results and Discussion

All pepper plants treated with conidia suspension were colonized by the corresponding fungus and grew without sign of fungal infection. All three fungi were regularly isolated from upper, not sprayed leaves. This suggests a systemic spread of the fungus inside the plant and confirms a lasting endophytic phase.

We examined the possible effect of fungus treated pepper plants on the probing behavior of *M. persicae*. In all plant-fungus combinations and the control, we found that the number of probes, during the first minute, followed a Poisson distribution (K-S test, $p > 0.05$), suggesting a random probing process (Fig. 1). However, the probing rates (λ) were different between the control and the treatments. Thus, for the control, λ (and 95% confidence intervals) was 1.9 (1.54, 2.30), whereas, for Bb, If, and Mr the corresponding values were 0.62 (0.43, 0.86), 0.82 (0.59, 1.1) and 0.86 (0.63, 1.14), respectively, indicating an important change in the probing behavior of aphids placed in treated plants. Focusing on probed and not probed plants, we found that only 10% of the aphids in the control avoided probing, whereas, the rest made at least one probe.

In contrast, in the treated with Bb, If, and Mr plants, about 50%, 42%, 36% of aphids, respectively, avoided probing.

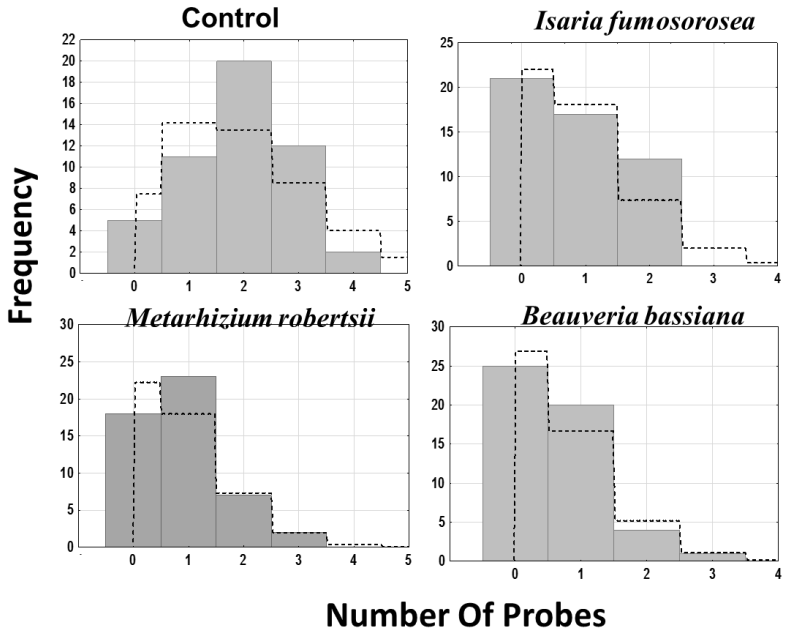

Figure 1
Distributions of the number of probes (0,1,2…) aphids made in the first minute of the prefeeding process in leaves of pepper treated with entomopathogenic fungi and in Control (untreated). Fungus species of each treatment is shown on top. Each histogram shows the observed frequency of the number of probes (solid bars) and the theoretical distribution (dashed line) for the observed mean (λ) of each treatment.

Analysis by ANOVA (Fig. 2) showed that the mean difference between the control and the treatments was highly significant ($p<0.00001$, Tuckey test) but the difference among the treatments was not ($p>0.5$). Assumption for equality of variances for this test was fulfilled (Levene test, $p=0.34$) but normality test was rejected (A-D test, $p<0.05$). The Kruskal-Wallis non-parametric test confirmed the above results and significances. Similarly, analysis by chi-square test for association between plant infection status and aphid preference for probing (not shown), indicated a strong association ($p<0.001$) suggesting a tendency for aphids to avoid probing in plants treated with any of the three fungus. There was no difference in time of first probe initiation, total probing time, probing duration or elapsed time between the first two probes in the used pepper variety.

The above results suggest that pepper plants treated with each of the three endophytes, may be colonized less efficiently by *M. persicae*. Furthermore, they imply an effect on the transmission of non-persistent viruses. Indeed, these viruses are acquired and transmitted by aphids through short superficial probes, made shortly after aphids alight in a leaf and start testing host suitability [9].

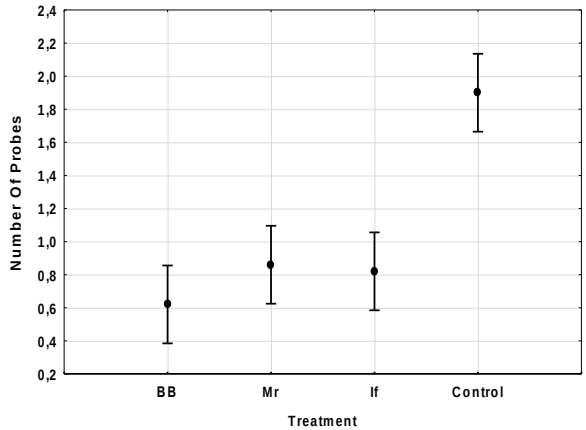

Figure 2
One-way ANOVA of the effect of pepper endophytic entomopathogenic fungi on the number of probes in the first minute of prefeeding process. Dots show the means and whiskers show 95% confidence intervals. BB= Beauveria bassiana , Mr= Metarhizium robertsii, If = Isaria fumosorosea

As a high percentage of aphids, in treated plants in our experiments, avoided probing, it is expected that significantly fewer insects will acquire or release virus particles, when alighting in such hosts. Yet, it is expected that even those aphids that do make probes in plants inoculated with any of the three endophytes, will transmit non-persistent viruses less efficiently, as there is a positive association between number of probes and transmission efficiency [10].

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P2-04

BRS Juruti: The first Habanero pepper cultivar developed in Brazil

Claudia S. C. Ribeiro[1], Karina R. R. Souza[1], Sabrina I. C. Carvalho[1], Francisco J. B. Reifschneider[2]

[1] *Embrapa Vegetables, Brasília/DF, Brazil*

[2] *Embrapa, Secretariat of International Relations, Brasília/DF, Brazil*

Abstract

The Habanero hot pepper group is not well known in Brazil, despite its origin in the Amazon. Nevertheless, domestic and international markets have been increasing the demand for this type of hot pepper. The Brazilian agroindustry is interested in supplying part of this demand with high quality products and competitive prices, and it needs well-adapted cultivars. To fill this gap, Embrapa Vegetables developed the Habanero pepper cultivar BRS Juruti, which meets the demands of both fresh fruit and the mash and sauces markets.

BRS Juruti was derived from segregating population CNPH 4159, which is part of the germplasm collection of *Capsicum* spp. maintained at Embrapa Vegetables. Five generations of plant selection and selfing were performed until the progenies showed no segregation. During each generation, selection for agronomic and processing traits relevant to the industry was undertaken. Selection was based on plant and fruit characteristics such as plant architecture and height, shape and size of fruit, mature fruit color, and pungency, high yield, and disease resistance.

BRS Juruti has red fruits and yielded around 50 t ha^{-1} (36,000 plants ha^{-1}). Fruit pungency is *circa* 260,000 SHU and fruits have high content of vitamin C (122 mg 100 g^{-1}). BRS Juruti has shown field resistance to several viruses, *Oidiopsis sicula*, *Meloidogyne javanica* and presents intermediate resistance to key bacterial diseases; and is highly uniform in comparison with the original population. Cultivar BRS Juruti is recommended for open field (specially adapted to the Central Region of Brazil), as well as greenhouse/screenhouse cultivation. BRS Juruti has been registered (RNC 32010) and protected (DOU 01/09/2015) in the *Brazilian Ministry of Agriculture, Livestock* and Food *Supply* (MAPA). Breeders' seed is being made available to interested parties in the private sector.

1. Introduction

The production of chile peppers in Brazil is of high social importance, and the integration between small farmers and the *Capsicum* processing industry is an important characteristic of this segment (Reifschneider, 2000; Ribeiro et al., 2008).

Habanero is one of the most popular pepper types within *Capsicum chinense* species and was originally grown on the Yucatan Peninsula of Mexico and in Belize (Bosland & Votava, 1999). In addition to the high pungency, fruits of habanero have a particular, fruity, apricotlike aroma and flavor (DeWitt & Bosland, 2009).

Habanero peppers (*C. chinense*) are beginning to be of interest to the Brazilian market; so far, no cultivars specifically adapted to Brazilian agroecosystems have been developed.

Domestic and international markets have been increasing the demand for this type of pepper, particularly in the United States of America and Europe, in the form of pepper paste or mash. Brazilian agroindustry is interested in supplying part of this demand with high quality products and competitive prices, and it needs well-adapted cultivars.

The *Capsicum* breeding program of Embrapa has concentrated efforts on the development of new, uniform, high yielding, high nutrition and disease resistant habanero-type cultivars. The main objective of this work was to develop a habanero-type cultivar adapted to the Central region of Brazil with high pungency, yield, and uniformity, to meet both the demands of both the market for fresh fruit and the processing agroindustry.

Figure 1: Ripe (red) fruits of BRS Juruti

2. Material and Methods

BRS Juruti was derived from segregating population CNPH 4159, which is part of the germplasm collection of *Capsicum* spp. maintained at Embrapa Vegetables.

Five generations of plant selection and selfing were performed until the progenies showed no segregation. During each generation, selection for agronomic and processing traits relevant to the industry was undertaken.

Selection was based on plant and fruit characteristics such as plant architecture and height, shape and size of fruit, mature fruit color, and pungency, high yield, and disease resistance.

3. Results and Discussion

BRS Juruti is a highly uniform cultivar in comparison with the original population. A typical BRS Juruti plant presents intermediate growth habit, and is around 90 cm high by 60 cm wide.

Its pods are lantern-shaped, the standard shape of habaneros, turn from light green to bright red when mature (Figure 1), 5.1 cm long by 4.2 cm wide and 1.9 mm in wall thickness.

The fruits of BRS Juruti are very hot with 260,000 SHU (Scoville Heat Unit) of total capsaicinoids (197,600 SHU of capsaicin, 59,800 SHU of dihydrocapsaicin and 2,600 SHU of norcapsaicin) and vitamin C content reaches 122 mg 100 g^{-1} of fruit (Teodoro et al., 2013).

This cultivar showed field resistance to tospovirus Tomato Spotted Wilt Virus (TSWV) and to potyvirus causing Pepper Yellow Mosaic Virus (PepYMV) and Potato Virus Y (PVY); resistance to powdery mildew (*Oidiopsis sicula*) and to *Meloidogyne javanica*; and intermediate resistance to *Ralstonia solanacearum* biovar 1, *Xanthomonas euvesicatoria* and *X. gardneri*.

BRS Juruti was evaluated in several Brazilian states (SP, MG, GO and DF), demonstrating good adaptation, yielding 26 to 50 t ha^{-1}, depending on the spacing used and the region. In Central Brazil's growing conditions during the dry season, the harvest of ripe fruit begins around 90 days after transplanting, with yields around 50 t ha^{-1} at the density of 36,000 plants ha^{-1}. The open pollinated cultivar BRS Juruti has had yields above the American hybrid 'Caro-Tex-312' (28.9 t ha^{-1}, 32,600 plants ha^{-1}) (Crosby et al., 2013).

The new cultivar has been registered (RNC 32010) and protected (DOU 20/05/2015) in the *Brazilian Ministry of Agriculture, Livestock* and Food *Supply* (MAPA); breeders'seed of this new cultivar is being made available to interested parties in the private sector.

BRS Juruti was developed to meet both the fresh fruit market and the processing industries, particularly for the production of hot pepper paste ("mash") and sauces, in addition to the potential use for the dehydration of whole fruit to obtain spicy paprika. Many gourmet products use habanero pepper for aggregating pungency, flavor and taste, such as in fruit jellies, flavored vinegars, and different kinds of seasoning powder, nuts, potato chips, cookies, cheeses and sausages.

4. Acknowledgements

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P2-05

Protection of a new Brazilian habanero pepper cultivar, Brs Juruti

Sabrina I. C. Carvalho[1], Cláudia S. da C. Ribeiro[1], Luciano B. Bianchetti[2], Francisco J. B. Reifschneider[3]

[1] *Embrapa Vegetables, Brasília/DF, Brazil*

[2] *Embrapa Genetic Resources and Biotechnology, Brasília/DF, Brazil*

[3] *Embrapa, Secretariat of International Relations, Brasília/DF, Brazil*

Abstract

Plant variety protection (PVP) Act was instituted in Brazil in 1997 and regulates intellectual property concerning plant breeding. A new cultivar may only obtain legal protection if it possesses five attributes: innovation, own denomination, distinctiveness (D), uniformity (U), and stability(S) (DUS). The main objective of this work is to present the process and the results of DUS tests required for the new habanero pepper (*Capsicum chinense* Jacq.) cultivar BRS Juruti developed by Embrapa Vegetables. BRS Juruti was derived from segregating population CNPH 4159, one of the over 4,000 accessions of Embrapa Vegetables´ *Capsicum* Germplasm Active Bank. The original population showed variability for fruit and plant characteristics and incidence of viruses under field conditions. BRS Juruti was obtained after five cycles of individual selection and self-pollination until progenies showed no segregation. Plants were evaluated for disease resistance for different pathogens (*Ralstonia solanacearum*, *Xanthomonas euvesicatoria*, *X. gardneri*, *Oidiopsis sicula*, *Phytophthora capsici*, nematodes and viruses - tospovirus and potyvirus) under field conditions. DUS tests were performed in two production cycles in central Brazil. In both tests, three habanero pepper cultivars were assessed: BRS Juruti, CNPH 4159 and BRS Nandaia. A total of 42 plants were grown in each cycle, with six replicates of 7 plants each. Data were collected from three plants of each plot, and a total of 18 plants evaluated for each cultivar. Morphological characterization was based on 49 descriptors established by the Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA) for implementing the DUS tests of *Capsicum*: 16 descriptors for plant, 8 for flowering, and 25 for fruit characteristics. The original population CNPH 4159 showed low uniformity in both assays, particularly for plant stem length, leaf length and width, and fruit length, with late flowering and fruit ripening. The new cultivar BRS Juruti presented high uniformity being distinct from CNPH 4159 and BRS Nandaia, and it was stable during the two evaluation cycles; it is resistant to several viruses, *Oidiopsis sicula*, *Meloidogyne javanica* and presents intermediate resistance to key bacterial diseases. The protection process in Brazil is complex and rigorous, however, fundamental to comply with the Brazilian legislation. BRS Juruti has been protected by MAPA under certificate # 20150097.

1. Introduction

The Plant Variety Protection (PVP) Act in Brazil was instituted in 1997 by Law No. 9,456/97 and it is coordinated by the National Plant Variety Protection Service (SNPC) of the Ministry of Agriculture, Livestock and Supply (MAPA). The SNPC has jurisdiction to consider requests, grants the PVP certificate, and ensures the intellectual property rights of breeders or holder of new cultivars (Santos et al., 2012).

A new cultivar may only obtain legal protection if it is an innovation and distinguishable from other known cultivar through descriptive characteristics, own denomination, homogeneity and ability to remain stable in successive generations, which must be verified by Distinctiveness (D), uniformity (U) and stability (S) (DUS) tests (Carvalho et al, 2009; Santos et al., 2012).

Currently, there are about 1265 varieties protected by SNPC in Brazil; from these, seven are hot peppers (*Capsicum* spp.), and four (BRS Sarakura, BRS Garça, BRS Juruti and BRS Nandaia) were developed by Embrapa Vegetables´ *Capsicum* breeding program (Brasil, 2016).

The new habanero pepper cultivar BRS Juruti (*Capsicum chinense* Jacq.) was developed by Embrapa Vegetable to make available to the Brazilian market a red habanero adapted to Brazil, which was lacking. It is an open-pollinated cultivar developed to meet both the fresh fruit and the processing (mash) markets. In addition to high pungency (about 260,000 Heat Unit Scoville - SHU), a feature of interest to the sauce agroprocessors, BRS Juruti is very aromatic with a high vitamin C content (122 mg $100g^{-1}$). It has a high yield potential, ranging from 26-49 t ha^{-1}, depending on the spacing used and the region.

The main objective of this paper is to present the results of DUS tests for the protection process of the new habanero cultivar BRS Juruti.

2. Material and Methods

BRS Juruti (Figure 1) originated from segregating population CNPH 4159, which is part of the *Capsicum* Active Germplasm Bank (AGB) of Embrapa Vegetables. The original population presented variability for fruit and plant characteristics, as well as for incidence of viruses under field conditions. BRS Juruti was obtained after five generations of individual plant selection and selfing until the progeny showed high uniformity for plant and fruit characteristics and disease resistance. In each cycle, plants were evaluated for disease resistance in controlled conditions for different pathogens: *Ralstonia solanacearum*, *Xanthomonas euvesicatoria*, *X. gardneri*, *Oidiopsis sicula*, *Phytophthora capsici*, root-knot nematodes (*Meloidogyne incognita*, *M. javanica* and *M. enterolobii*) in addition to viruses (Tospovirus and Potyvirus) under field conditions.

The DUS tests were conducted in two cycles (April to October 2013; April to October 2014), at Embrapa Vegetables, in Brasilia, DF, Brazil. In both tests, three pepper habanero type cultivars were assessed: BRS Juruti, CNPH 4159 (original population from which BRS Juruti was derived) and BRS Nandaia. A total of 42 plants were grown in each cycle, with six replicates of 7 plants each. Data were collected from three plants of each plot, and a total of 18 plants evaluated for each cultivar. Morphological characterization was based on 49 descriptors established by the Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA) for implementing the DUS tests of *Capsicum*: 16 descriptors for plant, 8 for flowering, and 25 for fruit characteristics.

Figure 1. BRS Juruti

3. Results and Discussion

The original population CNPH 4159 showed low uniformity in both assays, particularly for plant stem length (ranging from 15 to 37 cm), leaf length (10 to 15 cm) and width (5 to 8 cm), and fruit length (3.5 to 6.3 cm), with late flowering and fruit ripening (Table 1).

The new cultivar BRS Juruti presented high uniformity of plants and fruits, being distinct from CNPH 4159 and BRS Nandaia, and it was stable during the two evaluation cycles.

BRS Juruti has field resistance to several viruses (TSWV, PVY), is resistant to *Oidiopsis sicula* and *Meloidogyne javanica* and presents intermediate resistance to *Ralstonia solanacearum* biovar 1. The protection process in Brazil is complex and rigorous, however, fundamental to comply with the Brazilian legislation. BRS Juruti has been protected by MAPA under certificate # 20150097.

Morphological Descriptors		
Cultivars	**Original Population (CNPH 4159)**	**BRS Juruti**
Plant		
Stem length	15 to 37 cm	~ 24 cm
Leaf length	10 to 15 cm	~ 14 cm
Leaf width	5 to 8 cm	~7.3 cm
Fruit		
Fruit length	3.5 to 6.3 cm	5.1 cm
Time of beginning of flowering (first flower on the second flowering node in 50% of plants)	medium 95 dias	early 85 dias
Time of physiological ripeness (fruit color change on 50% of plants)	medium 116 dias	early 109 dias

Table 1
Plant and fruit characteristics of original population (CNPH4139) and BRS Juruti based on six morphological descriptors

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P2-06

Virological survey in pepper crops in south-east Hungary and first identification of *Tobacco mild green mosaic virus* in Hungary

Katalin Nemes[1], Asztéria Almási[1], István Tóbiás[1], Gábor Csilléry[2] and Katalin Salánki[1]

[1] *Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary*

[2] *Budakert Ltd., Budapest, Hungary*

tobias.istvan@agrar.mta.hu

Abstract

Pepper (*Capsicum annuum* L.) is an important vegetable crop in Hungary. The virus diseases cause enermous losses in terms of quantity and quality of products. In the field production *Cucumber mosaic virus* (CMV), Potato virus Y (PVY) and *Tomato spotted wilt virus* (TSWV) are the main pathogens, while in greenhouses tobamoviruses [*Tobacco mosaic virus* (TMV) and *Pepper mild mottle virus* (PMMoV)] and *Tomato spotted wilt virus* (TSWV) causes severe economical losses. In the spring of 2015 mild mosaic symptoms were observed on the leaf of "Hó F1" and „Nirvin F1"cultivars in the main pepper growing region of South-East Hungary. The symptoms on the fruits were more obvious, characterized by reduction in size, mottling and color changes, brown necrotic streaks and spots. In order to identify the pathogen symptomatic fruit samples were collected and carried to the lab. After description of the symptoms preliminary test plant experiments were conducted. The reactions on *Nicotiana tabacum* cv. Samsun, *N. tabacum* cv. Xanthi-nc, *Nicotiana benthamiana*, *Capsicum annum* cv. Albaregia (*L+*), *Capsicum annuum* cv. Fehérözön (*L1*) and *Capsicum annum* cv. Brendon F1 (*L3*) indicated the presence of tobamovirus isolates belonging into pathotype 0 and/or 2. From the original pepper fruit total RNA was extracted using RNeasy Plant Mini kit (Qiagen, Germany) and served as a template in conventional RT-PCR using universal tobamovirus primers for the coat protein gene. Products of the expected size (700 bp) were obtained and cloned into p-GEM T-Easy Vector (Promega, USA) and sequenced. According to the sequence data *tobamovirus* isolates from Nirvin F1 samples showed highest identity with *Pepper mild mottle virus* (PMMoV) and belonged to the pathotype 2, infecting the pepper varieties containig the *L3* resistance gene.

Tobamovirus isolates from „Hó F1" cultivar were member of pathotype 0 group and blast analysis of sequnces showed the highest identity with a Spanish isolate (P04/17, Accession No. FN594859) of *Tobacco mild green mosaic virus* (TMGMV) (99%). The sequence of one isolate was deposited in GenBank (Accession No. KT374283). To our knowledge, this is the first proven data of TMGMV isolated from pepper in Hungary.

1. Introduction

Pepper (*Capsicum annuum* L.) is an important vegetable crop in Hungary. Virus diseases annually reduce yield and quality of all types of pepper. In field production *Cucumber mosaic virus* (CMV), *Potato virus Y* (PVY) and *Tomato spotted wilt virus* (TSWV) are the main viral

pathogens, while in greenhouses tobamoviruses [*Tobacco mosaic virus* (TMV) and *Pepper mild mottle virus* (PMMoV)] and *Tomato spotted wilt virus* (TSWV) causes severe economical losses in terms of quantity and quality of products (Tóbiás et al 1978, 1981a,b, Csilléry et al 1985, 1995).

One of the possibilities to control virus diseases is a resistance breeding. In Hungary among vegetable crops pepper was the first plant where resistant varieties were introduced in the mid seventies (Fehérözön, D. cecei) containing *L1* resistance gene (Zatykó et al. 1983). Some years later resistance breaking tobamoviruses appeared, causing enermous losses (Tóbiás és Csilléry 1982, 1983). Against these new tobamovirus patotypes *L3* resistance gene from *Capsicum chinense* and *L4* from *C. chacoense* were introduced into all important pepper types. Tobamovirus infection accurs year by year in different pepper growing regions causing economical losses. In the spring of 2015 mild mosaic symptoms were observed on the leaf of "Hó F1" and „Nirvin F1"cultivars in the main pepper growing region of South-East Hungary. Virological studies were conducted to identify the pathogen(s).

2. Materials and methods

Plant. Nirvin F1 and HóF1 pepper varieties were planted in September-October 2014, in March 2015 mild mosaic symptoms on leaves and malformed fruits, discoloration, necrotic spots and depressions were observed (Figure 1). Samples were collected and virological tests were done.

Test plants. *Nicotiana tabacum* cv Xanthi-nc, *N. tabacum* cv Samsun, *N. benthamiana*, *Capsicum annuum* cv Brendon (*L3*), cv. Fehérözön (*L1*) és cv Albaregia (*L+*) were used to identify tobamovirus and to determine the pathotype.

RT-PCR. Total nucleic acid (TNA) was extracted from small piece of fruit flesh by the method of White and Kaper (1989). Tobamovirus specific primers –

for 5'-GATCGCGCGAGTCGTGATTCGTATTTAAATATG-3',

rev 5'-TGGGCCGCCTACCGCGGCGG-3' amplified 700 nt product from the coat protein region. The following conditions were used for the PCR: initial denaturation at 95 ºC for 5 minutes, 30 cycles of 95 ºC for 30 s, 60 ºC for 30 s and 72 ºC for 1 min and a final extension at 72 ºC for 10 min. The PCR product was purified by High Pure PCR Purification Kit (Roche) and sequenced (Baygen, Szeged) or cloned into p-GEM T-Easy Vector (Promega, USA) and sequenced.

The nucleotide homology of the Hungarian and other *Tobamoviruses* retrieved from the GenBank was examined by the BLAST program of NCBI. Phylogenetic trees were composed by the Neighbor-Joining (NJ) method with 1,000 bootstrap replications of Mega program (MEGA 5.2).

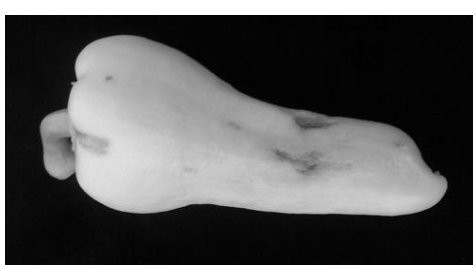
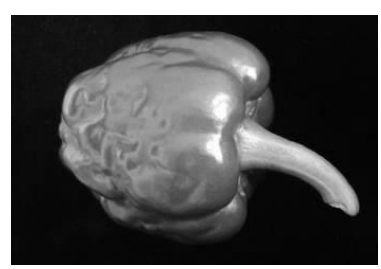
A   B

Figure 1. Symptoms on fruits of Hó F1 (A) and Nirvin F1 (B).

3. Results

Necrotic local lesions on inoculated leaves of *Nicotiana tabacum* cv Xanthi-nc and systemic infection on *N. tabacum* cv Samsun, *N. benthamiana* plants demonstrate the presence of tobamovirus in the collected pepper samples. No sign of presence of other pepper infecting viruses. Hó F1 and Nirvin F1 samples differed in pathotype, samples from Hó F1 infected only the Albaregia pepper cultivar (containing no resistance gene) and belonging to pathothype 0, while samples from Nirvin F1 infected all the tested pepper varieties (containing *L1* or *L3* resistance genes) and belonged to the pathotype 2.

Rt-PCR test produced the expected size of PCR- product (Fig. 2.)

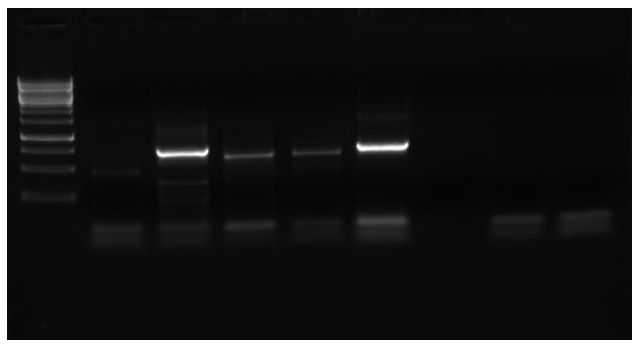


Figure 2. RT-PCR amplified product from Hó F1 and Nirvin F1 pepper samles.

According to the sequence data *tobamovirus* isolates from Nirvin F1 samples showed highest identity with *Pepper mild mottle virus* (PMMoV). Sequence data from Hó F1 isolates were different. Blast analysis showed a 99% identity at nucleotide level with a Spanish isolate (P04/17, accession No. FN594859) of *Tobacco mild green mosaic virus* (TMGMV). The sequence was deposited in GenBank under the accession No. KT374283. To our knowledge, this is the first report of TMGMV on pepper in Hungary. Phylogenetic tree showed high identity with TMGMV and clear differences to other tobamoviruses (Fig.3).

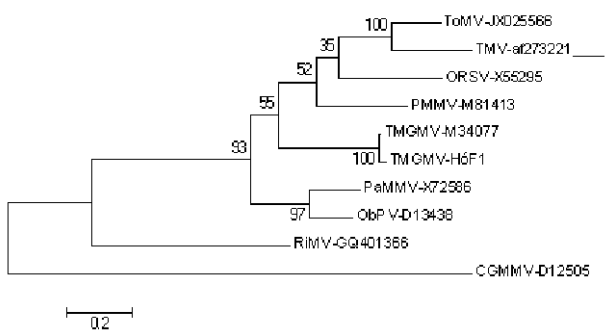


Figure 3. Phylogenetic tree inferred by NJ method based on coat protein gene of various tobamoviruses. Numbers at branches indicate the bootstrap percentages.

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P2-07

Production of sweet and spice peppers in Slovakia

Magdaléna Valšíková, Alžbeta Hegedűsová, Marián Rehuš

Slovak University of Agriculture, Horticulture and Landscape Engineering Faculty, Nitra, Slovak Republic

Abstract

Pepper is one of the most popular types of vegetables which can be used flexibly. Its cultivation has its place and justification in the southern regions of Slovakia. Despite the good conditions, the area and the production of peppers are gradually decreases. In 1985 the area of spice pepper was 2373 ha and production amounted to 3497 t. By 2014, the area decreased to 42 ha and the yield was only 72 t. Bell peppers have a similar downward trend. The largest area of sweet pepper was in Slovakia in 1999 and 2000, when it was grown on area of around 3000 ha with a production of 35000 to 40000 t. Area of this peppers is currently 2000 ha and yield is around 25800 tons. In the Slovak list of registered varieties is enrolled 112 vegetable varieties and 10 varieties of spice pepper. Of the large number of sweet pepper varieties, it is of Slovak origin about 15 % of them.

1. Introduction

In terms of nutritional value, the pepper is the most valuable kind of vegetables (3, 6, 8). Nevertheless, in recent years we have recorded a decline in peppers production (4). This is due to the loss of agricultural land, high costs in the cultivation, a great need of manual labor in the cultivation and harvesting operations (1). These are the reasons why the areas and production of vegetables, including peppers continue to drop (2). Consumption of vegetables and peppers should be increased. This contradiction is currently being dealt by imports of bottleneck species from other countries. (5).

2. Objective and methods

The aim of the work is to show the true evolution of the cultivation and consumption of vegetable pepper and spice pepper. For research and monitoring we used data from the Statistical office of the Slovak Republic (2, 4) and other publications that followed the themes previously (7, 10).

3. Production of Sweet Pepper

To changes in the areas and production of sweet peppers and whole vegetables, refers table 1. Here are the data of arable land, including home gardens. From1993 to 2014 fell pepper harvest area of 2138 ha to 1993 ha. Pepper production has decreased over the same period from 32659 t to 28065 t. The same time decreased the area and production of vegetables together. The areas of peppers formed from 6,28 to 7.59% of the total vegetables area. Production of pepper ranged between 4,93 to 8.92% of the total harvest of vegetables. We have the data separately for arable crops from the statistical office since 2001. Areas of bell pepper and of vegetables were the highest in 2002 and lowest in 2013 and 2014. Bell pepper production in the years 2001-2006 ranged between 7606-9688 tons. In 2014 we recorded the production of Bell

pepper only 5083 t. The average numbers of harvest and consumption of bell pepper and vegetables are in the table 2 (7, 10).

Table 1 Harvested area of sweet pepper and vegetables (arable land + home gardens)

Year	Area of peppers in ha	Area of vegetables in ha	% of peppers	Production of peppers in t	Production of vegetables in t	% of peppers
1993	2 138	32 860	6.51	32 659	535 466	6.10
1994	2 395	34 195	7.04	34 893	483 524	7.22
1995	2 436	37 009	6.58	33 048	498 421	6.63
1996	2 465	38 389	6.42	37 205	559 588	6.65
1997	2 506	39 921	6.28	42 334	594 741	7.12
1998	2 748	42 157	6.52	29 224	593 025	4.93
1999	3 324	46 903	7.09	40 387	685 379	5.89
2000	2 937	43 834	6.70	35 399	468 838	7.55
2001	2 335	32 896	7.10	27 862	406 064	6.86
2002	2 531	33 572	7.54	29 781	363 482	8.19
2003	2 438	34 538	7.06	26 243	368 847	7.11
2004	2 301	32 017	7.19	26 969	380 626	7.09
2005	2 296	30 241	7.59	28 713	353 567	8.12
2006	2 219	29 795	7.45	28 616	351 280	8.15
2007	2 119	28 870	7.34	24 620	307 756	8.00
2008	2 067	28 426	7.27	26 238	307 756	8.53
2009	2 084	28 547	7.29	23 584	312 084	7.56
2010	2 053	30 559	6.72	24 620	284 429	8.66
2011	1 998	30 334	6.58	25 777	314 855	8.19
2012	1 971	29 164	6.76	27 691	310 148	8.92
2013	1 992	28 320	7.03	26 336	325 378	8.09
2014	1 993	28 065	7.10	25 801	326 074	7.91

Table 2 The average yield of peppers in t/ha and consumption of sweet pepper and vegetables

Year	Arable land + gardens t/ha	Arable land t/ha	Consumption of pepper in kg/person/year	Consumption of vegetables in kg/person/year
2001	11.93	12.54	4.2	80.5
2002	11.77	10.97	4.5	77.3
2003	10.77	11.84	3.6	80.9
2004	11.72	13.25	4.6	89.9
2005	11.72	14.51	5.3	86.7
2006	12.90	15.66	6.5	88.0
2007	12.58	16.72	6.1	88.4
2008	11.91	13.98	6.0	100.6
2009	12.59	17.58	6.7	102.5
2010	11.48	14.56	6.1	94.6
2011	12.90	21.11	6.5	100.6
2012	14.05	21.35	7.1	100.9
2013	13.22	18.58	7.3	104.7
2014	12.94	17.40	6.6	104.2

Data source: Statistical Office of the Slovak Republic and VÚEPP Bratislava

4. Production of spice pepper

The consumption of spice pepper per capita is increasing and nowadays it is around 100 – 150 grams (7). Even if the domestic consumption is increasing, its production has been estimated to decrease by dozens of tons per year.

These types of pepper are grown on around 150 ha, which represents around 181 t of overall production. Decreased production is brought about by cheap transported goods from foreign countries, which are nevertheless often less quality (9, 10).

The table 3 shows the course of cultivation of spice pepper since 1985. It also includes the information about harvested area as well as total harvest and yield in individual years. The first more substantial decline in production was recorded in the 90s and the trend has ever been continuing up to now. Nowadays, only about 40 ha of spice peppers are recorded on arable land. The production has been decreasing in accordance with the decrease in areas too. In comparison with 1985, when 3497 t of fresh pepper were harvested, the yield from the year 2014 represented only 72 t (Fig. 1). The yield in $t.ha^{-1}$ has been fluctuating due to weather conditions from 0.56 to 1.89 $t.ha^{-1}$. Despite these fluctuations we can see a mild increase in yield since 1985 up till now (Fig. 2).

In past, the production of sweet pepper was extended in more areas. People used to grow spice pepper in the following regions: Nitra, Trnava, Banská Bystrica and Košice (4). Today, the cultivation of sweet pepper is only limited especially in the Nitra and Trnava regions.

List of registered varieties in Slovakia contains 122 varieties of pepper. Of these are 110 varieties of sweet peppers, 10 varieties of spice peppers and 2 varieties of hobby peppers. Slovak consumers prefer sweet peppers type of "PCR", with light green and yellow-green color, which ripen red. The shape of the fruit has to be elongate and pointed the blossom end. Prevails the demand for sweet taste, but about a third of consumers requires a pungent taste. Of the 110

varieties are 10 Slovak hybrids and 15 hybrids of foreign origin. Of the 110 varieties has Slovak origin 53, Hungary origin 18 and Czech origin 8 varieties. Of the 10 spice pepper varieties, four come from Hungary (Kalocsa) and 6 varieties from Slovakia.-

Table 3 Production of spice pepper in Slovakia from 1985 to 2014

Year	Harvested area in ha	Yield in t	Productivity in t.ha^{-1}
1985	2 373	3 497	1.47
1986	2 147	1 778	0.83
1987	2 153	2 431	1.13
1988	1 954	1 919	0.98
1989	2 204	2 116	0.96
1990	1 835	1 029	0.56
1991	2 546	3 080	1.21
1992	1 480	1 388	0.94
1993	1 031	1 218	1.18
1994	1 070	1 340	1.25
1995	1 343	1 727	1.29
1996	959	1 090	1.14
1997	552	666	1.21
1998	718	1 024	1.43
1999	560	827	1.48
2000	536	540	1.01
2001	333	482	1.45
2002	272	378	1.39
2003	254	228	0.90
2004	460	450	0.98
2005	430	538	1.25
2006	195	225	1.15
2007	152	181	1.19
2008	105	87	0.83
2009	60	73	1.20
2010	65	97	1.50
2011	49	48	0.98
2012	27	51	1.89
2013	36	52	1.43
2014	42	72	1.69

Figure 1 Harvested area and yield of spice pepper in last 30 years in ha

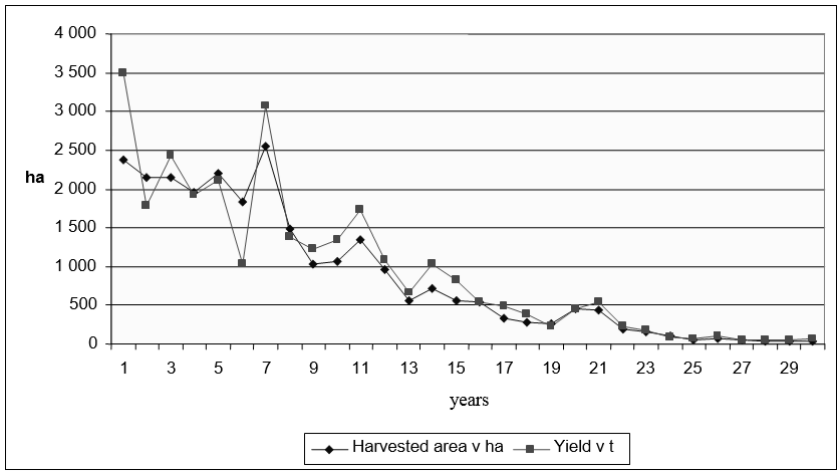
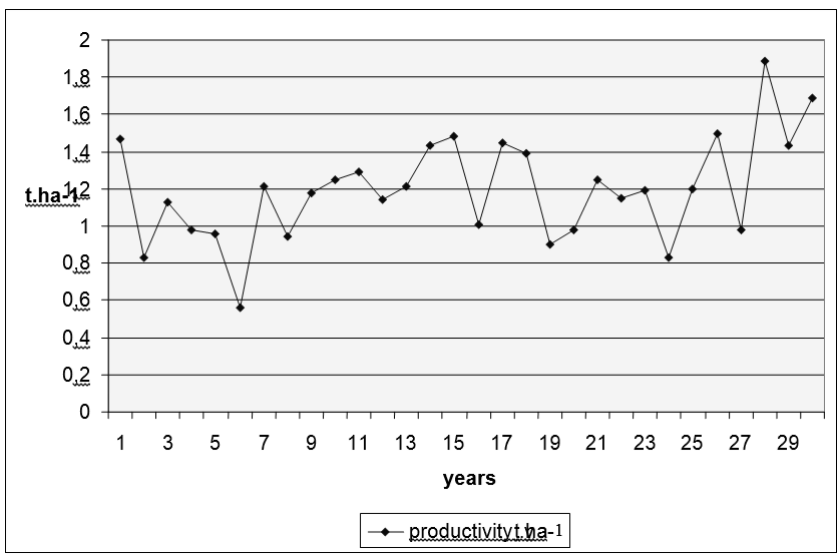


Figure 2 Productivity of spice pepper in last 30 years in t.ha^{-1}



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Contact person

prof. Ing. Magdaléna Valšíková, PhD., Department of Vegetable Production, Horticulture and Landscape Engineering Faculty, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic, e-mail: magdalena.valsikova@uniag.sk

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SESSION 3

Genetic resources

P3-01

The morphological characterization of *Solanum aethiopicum* and *Solanum incanum* accessions

Ahmet Balkaya[1], Şeyma Sarıbaş[1], Ahmet Seçim[2], Zeynep Dağ[2], Dilek Kandemir[3]

[1] *University of Ondokuz Mayıs, Faculty of Agriculture, Horticulture Department, Samsun, Turkey*

[2] *Gento Seeds Ltd., Antalya, Turkey*

[3] *University of Ondokuz Mayıs, Samsun Vocational School, Samsun, Turkey*

Abstract

In this study, twenty accessions of *S. aethiopicum* and three *S. incanum* were characterized using standard morphological descriptors. The results demonstrate that many differences for plant, flower, stem pubescence, leaf, fruit and seed characteristics among detailed *S. aethiopicum* and *S. incanum* accessions. Cluster and Principal Component Analysis (PCA) were performed to determine relationships among accessions and to obtain information on the plant characters for the definition of groups. Cluster analysis based on 12 qualitative and 8 quantitative variables identified 7 different groups. The first six principal component axes accounted for 80.36% of the total multivariate variation among the detailed accessions. Characters with high coefficients in the first PC (stem pubescence, leaf blade sinuating of margin, leaf prickles, corolla colour, fruit colour and 100 seed weight), and the second PC (anthocyanin coloration of stem, leaf blade intensity of green colour, fruit length and fruit stalk length were considered the most important since these axes explain nearly half of the total variation. The dendrogram also was prepared to evaluate morphological similarity between the *S. aethiopicum* and *S. incanum* accessions. Each cluster had some unique characteristics and revealed high variation in this study. These results will be useful for the classification, management of genetic resources and breeding of both *S. aethiopicum* and *S. incanum* accessions in the future.

Keywords: *Solanum aethiopicum*, *Solanum incanum*, characterization, variation, multivariate analysis

1. Introduction

Solanaceae is a plant family comprising about 2300 species, nearly one-half of which belong to the genus *Solanum*. This family has been the source of many morphologically different domesticated species (Sekara et al., 2007). *Solanum aethiopicum* is the most popular native, traditional vegetables in Africa, but the productivity of this crop is still relatively low and data on growing areas and yields are scarce. The diversity centre of *S. aethiopicum* is Western Africa and it is also widely grown in South America (Lester and Daunay, 2003).

Solanum aethiopicum is characterized by many types and forms morphologically different with hundreds of local varieties (Lester, 1986). Lester and Niakan (1986), on the basis of the morphologic traits, classified four varietal groups within the species *S. aethiopicum*: Aculeatum, Glio, Kumba and Shum. Each group has been selected primarily for desirable traits the parts of the plant used for food or ornament. The Gilo group is characterized by large and edible fruits, glabrous and edible leaves characterize the Shum group; both large fruit and glabrous leaf characterize the Kumba group. The Aculeatum group, used as ornamental is characterized by

large and ribbed fruit with prickly leaf (Sunseri, 2010; Prohens et al., 2012). Both *S. macrocarpon* and *S. aethiopicum* have been considered as resources of interest for the genetic improvement of *S. melongena*, as the former present some traits of interest, including to tolerance to *F. oxysporum* f. sp. *melongenae* and resistance to *R. solanacearum* in both species (Gisbert et al., 2011). Resistance to root-knot nematodes has also been reported in S. *aethiopicum* (Hébert, 1985). Another species of interest as a source of variation for developing new eggplant rootstocks is *Solanum incanum*, which is the putative ancestor of eggplant (Lester and Hasan, 1991), and which has been reported as resistant to *F. oxysporium* f. sp. *melongenae* (Yamakawa and Mochizuki, 1979). Sakata and Lester (1994) reported that *S. melongena* and *S. incanum* are very close in phylogeny than the wild species *S. marginatum* L. (Kashyap et al., 2003). Close genetic relationship between *S. melongena* and *S. incanum* was determined by Lester and Hassan (1991).

Characterization and utilization of genetic resources is essential for the sustainability in modern agriculture. Plant breeders can use genetic similarity information to complement phenotypic information in the development of breeding populations (Balkaya et al., 2010; Solieman et al., 2012).Morphological characterization is the first step in the description and classification of genetic resources (Balkaya et al., 2009). Morphological identification using conventional descriptors has proved useful for describing and establishing relationships among cultivar groups and accessions in scarlet eggplants (Lester et al., 1986; Osei et al., 2010; Plazas at al., 2014). Lester et al. (1986) characterized 108 accessions of the scarlet eggplant using morphological traits. In another study, twenty eight accessions of African eggplant from three species of *S.aethiopicum*, *S. anguivi*, and *S.macrocarpon* were characterized by Osei et al. (2010). It was found wide variations between *Solanum* species studied. Otherwise, sixty three accessions of the scarlet eggplant complex (*S. aethiopicum*, *S. anguivi* forms) were characterized, and a large diversity was found between both complexes (Plazas et al., 2014).

Knowledge of the extent of genetic diversity, identification, differentiation, and characterisation of genotypes and populations, are information tools for the detection of duplicates in a collection, their effective extension, and better use in breeding (Hornakova et al., 2003). Modern phenomics tools may be useful for precise characterization and for studying the diversity and relationships in collections of genetic resources (Furbank and Tester, 2011). Diversity present in a group of populations can be displayed by means of cluster analysis and it shows similarity and differences among populations (Balkaya and Ergün, 2008; Balkaya et al., 2010). It has been used by several research groups for identifying morphological variability in *Solanum* species (Demir et al., 2010; Osei et al., 2010; Tumbilen et al., 2011; Prohens et al., 2012; Plazas at al., 2014; Aguoru et al., 2015). In analyzing genetic variation among populations and determining the most important variables contributing to this variation, it seems that principal component analysis (PCA) is most useful.

In this study, we characterized a collection of accessions of *S. aethiopicum*, and *S. incanum* by using morphological descriptors. The first aim of this study was to determine similarities and differences in the morphological variation of *S. aethiopicum*, and *S. incanum* genetic resources. The second aim of the present study was to assess the genotypic variation among these accessions determined.

2. Materials and Methods

This study was carried out cooperatively by the University of Ondokuz Mayis and Gento Seed Company between 2014 and 2015 years. Twenty accessions of the *Solanum aethiopicum* and three accessions of *Solanum incanum* from USDA-ARS National Germplasm bank were used at this work (Table 1). In the previous study, we tested *S. aethiopicum* and *S. incanum* accessions for resistance towards *F. oxysporum* f. sp. *melongenae* and *Verticillium dahliae* (Balkaya et al., 2015). In this study, all materials of both species have been described as resistant to *F. oxysporum* f. sp. *melongenae* and tolerant to *V. dahliae* (Gisbert et al., 2011).

Code	Accession number	Species	Origin	Code	Accession number	Species	Origin
G1	Grif 14165	*S. aethiopicum*	Brazil	G13	44188501	*S. aethiopicum*	Brazil
G2	19416601	*S. aethiopicum*	Serbia	G14	44190001	*S. aethiopicum*	Brazil
G3	24782801	*S. aethiopicum*	India	G15	44190201	*S. aethiopicum*	Brazil
G4	37469501	*S. aethiopicum*	Africa	G16	44190501	*S. aethiopicum*	Brazil
G5	42022601	*S. aethiopicum*	Africa	G17	63610702	*S. aethiopicum*	Brazil
G6	42023001	*S. aethiopicum*	Brazil	G18	66507502	*S. aethiopicum*	Japan
G7	42486001	*S. aethiopicum*	Brazil	G19	66607702	*S. aethiopicum*	Rusia
G8	44183901	*S. aethiopicum*	Brazil	G20	66607802	*S. aethiopicum*	Japan
G9	44185801	*S. aethiopicum*	Brazil	G21	19604301	*S. incanum*	Ethiopi
G10	44186201	*S. aethiopicum*	Brazil	G22	38115501	*S. incanum*	India
G11	44186501	*S. aethiopicum*	Brazil	G23	39021101	*S. incanum*	Japan
G12	44188401	*S. aethiopicum*	Brazil				

Table 1
Code, accession number, and origin of S. aethiopicum and S. incanum genetic resources

The field assays were carried out at the experiment station of Gento Seed Company in Antalya, Turkey. Morphological analyses were carried out on 100 plants harvested from each accessions. The morphological characters measured and their scales are presented in Table 2. All characters were measured in the field and at the normal harvest time. Individual plants were characterized using modified 20 plant descriptors commonly used for cultivated eggplant species and wild relatives characterization (IBPGR, 1990; Plazas et al., 2014).

	Descriptors
(1)	Plant height (cm)
(2)	Anthocyanin coloration of stem (1. absent, 2. present)
(3)	Stem pubescence (1. weak, 2. medium, 3. strong)
(4)	Leaf length (cm)
(5)	Leaf width (cm)
(6)	Leaf blade sinuation of margin (1. weak, 2. medium, 3. strong)
(7)	Leaf prickles (1. absent, 2. present)
(8)	Leaf blade intensity of green colour (1. light, 2. medium, 3. dark)
(9)	Flower corolla colour (1. white, 2. light violet, 3. dark violet)
(10)	Fruit length (cm)
(11)	Fruit diameter (cm)
(12)	Fruit stalk length (cm)
(13)	Fruit shape (1. globular, 2. flattened round, 3. ovoid, 4. obovate)
(14)	Fruit apex (1. indented, 2. flattened, 3. pointed, 4. protruded)
(15)	Fruit brightness (1. dull, 2. shiny, 3. very shiny)
(16)	Fruit intensity of main colour of skin (1. light, 2. medium, 3. dark)
(17)	Fruit colour (1. red, 2. violet, 3. green, 4. yellow, 5. white)
(18)	Seed number per/fruit
(19)	Seed colour (1. light yellow, 2. yellow , 3. dark yellow, 4. light brown, 5. other)
(20)	Weight 100 seeds (g)

Table 2
The descriptors used for the characterization of S. aethiopicum and S. incanum accessions

Statistical analysis was performed using the statistical package SPSS (21.0 for Windows). For a better overview of diversity in the local populations, cluster analysis was also used. Hierarchical cluster analyses were performed using Ward's criteria, minimizing the total sum of squared distances of objects to cluster centres. Ward's criteria were preferred because they tend to produce desirable compact clusters (Zewdie and Zeven, 1997). In the Principal Component Analysis (PCA) and the load coefficient values which relate the values. The PC with Eigen values >1.0 were selected and the characters with load coefficient values >0.6 were considered highly relevant (Jeffers, 1967; Balkaya et al., 2009).

3. Results and Discussion

Principal Component Analysis used for revealing general distances between genotypes as numerical values indicate which traits could be used to differentiate genotypes (Balkaya et al. 2010). The Principal Component axes accounted for 80.36% of the total multivariate variation among the detailed accessions the first PC axis accounted for 31.42% of the variation, whereas the second and third axes accounted for 17.62% and 10.73% (Table 3). The first three PC axes explained 59.79% of the variation, suggesting considerable diversity among the characters (Figure 1).

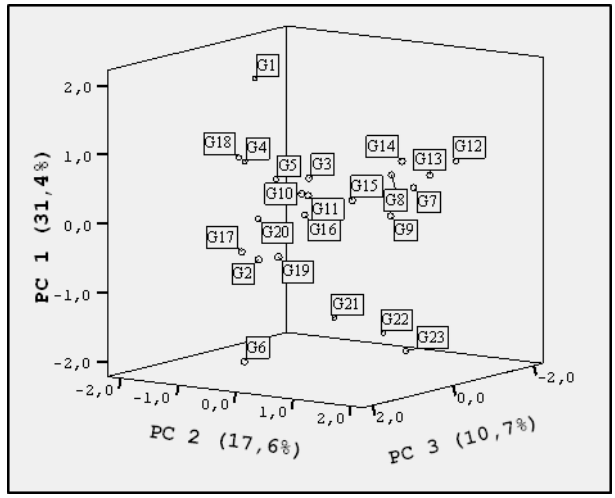

Figure 1
Scatter plot the three principal component axes explain 59.79% of the total variation

Characters with high coefficients in the first, second and the third PCs should be considered more important since these axes explain the biggest share of the total variation. Though clear guidelines do not exist to determine the significance of a character coefficient, one rule of thumb is to treat coefficients >0.6 as having a large enough effect to be considered important (Jeffers, 1967; Balkaya et al., 2009). Characters with high coefficients are: stem pubescence (0.828), leaf blade sinuating of margin (0.622), leaf prickles (-0.743), corolla colour (-0.847), fruit colour (-0.824) and, 100 seed weight (-0.811) for Principal Component 1; anthocyanin coloration of stem (0.645), leaf blade intensity of green colour (-0.71), fruit length (0.738) and, fruit stalk length (0.642) and the second PC, and fruit diameter (0.774) the third PC. This treats are considered as the most important since they define the axes which explain 60% of the total variation (Table 3).

Principal Component Analysis	PC axis					
	1	**2**	**3**	**4**	**5**	**6**
Eigen values	6.28	3.52	2.14	1.63	1.39	1.08
Explained proportion of variation (%)	31.42	17.62	10.73	8.16	6.97	5.43
Cumulative proportion variation (%)	31.42	49.05	59.79	67.95	74.93	80.36
Traits	**Eigen vectors**					
Plant height (cm)	0.166	-0.341	-0.199	0.476	0.580	-0.017
Anthocyanin coloration of stem	0.404	**0.645**	0.379	-0.205	0.112	-0.241
Stem pubescence	**0.828**	0.329	-0.191	-0.093	0.079	-0.113
Leaf length (cm)	0.560	-0,365	-0.018	-0.393	0.062	0.060
Leaf width (cm)	0.524	-0.588	-0.167	-0.491	-0.042	0.067
Leaf blade sinuation of margin	**0.622**	-0.073	-0.091	0.237	0.093	0.242
Leaf prickles	**-0.743**	0.026	-0.021	0.217	-0.335	-0.070
Leaf blade intensity of green colour	-0.009	**-0.710**	-0.195	0.028	-0.170	0.286
Corolla colour	**-0.847**	0.369	-0.084	-0.091	-0.081	0.049
Fruit length (cm)	0.546	**0.738**	0.000	0.100	0.087	0.282
Fruit diameter (cm)	0.517	0.027	**0.774**	0.157	0.011	0.089
Fruit stalk length (cm)	0.580	**0.642**	-0.227	-0.003	-0.122	-0.251
Fruit shape	0.160	0.561	-0.525	0.283	0.125	0.017
Fruit apex	0.376	0.319	0.027	0.054	-0.154	0.776
Fruit glossiness	0.386	-0.408	0.559	0.480	0.077	-0.201
Fruit intensity of main colour of skin	0.356	0.029	0.094	0.545	-0.622	-0.059
Fruit colour	**-0.824**	0.391	0.065	-0.164	-0.149	0.059
Seed number per fruit (unit)	0.365	0.104	0.588	-0.391	-0.184	0.022
Seed colour	-0.592	0.232	0.310	-0.058	0.603	0.155
Weight 100 seeds (g)	**-0.811**	0.028	0.391	0.137	0.060	0.188

Table 3
Principal component (PC) coefficients of each trait in S. aethiopicum and S. incanum accessions. Proportions of variations are associated with first six PC axes, which correspond to eigenvalues greater than 1

In this study, Cluster analysis grouped the populations into seven clusters. The related dendogram is shown in Figure 2. The means and standard deviations of the some traits for each cluster are given in Table 4. Among the seven different groups, Group D was divided into eight subgroups, Group B into two subgroups. Group A, C, E, F, and G did not show differential internal characteristics (Figure. 2). The seven groups and 15 subgroups shown in Figure 2 could be considered to be distinct germplasm pools. This study shows that there is considerable morphological variability between sampled accessions. No association was observed of clusters within the collection zone. This work has also showed the relationship between major *S. aethiopicum* and *S. incanum* groups (Figure 2).

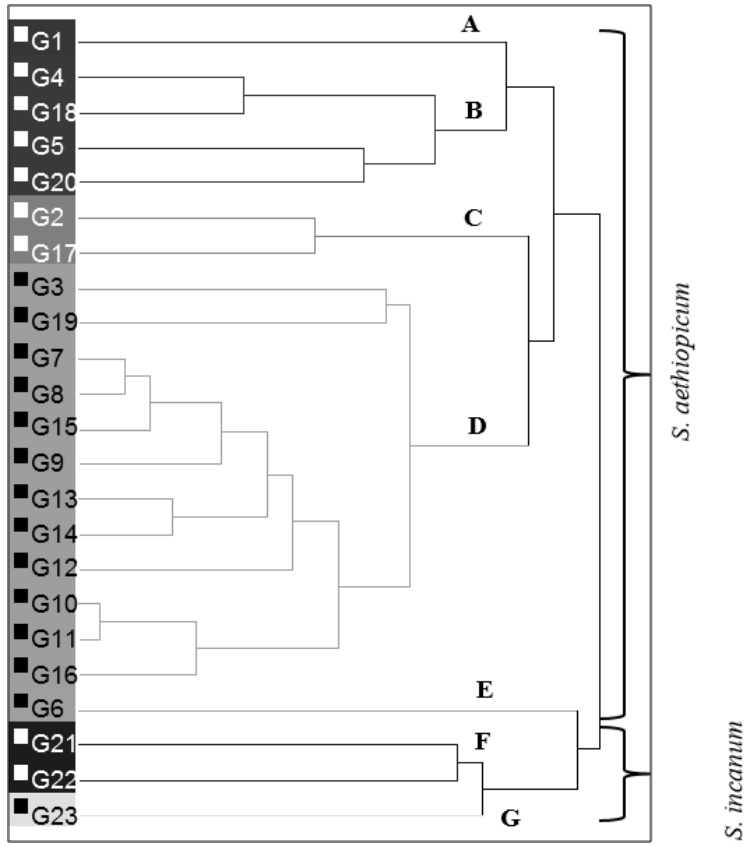

Figure 2
Genetic groupings of S. aethiopicum and S. incanum accessions according to cluster analysis

Traits	A	B	C	D	E	F	G
Plant height (cm)	138.2±4.2	144.2±5.3	158.1±2.8	136.3±9.2	108.4±6.8	137.3±9.5	122.6±5.9
Leaf length (cm)	33.6±7.7	27.1±2.9	25.8±1.4	26.4±2.5	27.9±4.6	19.2±3.9	17.4±1.4
Leaf width (cm)	29.3±2.8	20.2±2.7	18.9±0.8	19.5±1.8	16.2±2.0	13.7±2.7	11.1±0.9
Fruit length (mm)	19.1±1.1	25.2±1.6	47.9±16.7	56.5±9.9	46.2±2.4	31.7±6.3	58.1±5.7
Fruit diameter (mm)	27.1±1.4	38.9±0.4	55.3±2.1	48.6±6.5	54.1±2.5	23.6±0.4	32.6±6.8
Fruit stalk length (cm)	1.2±0.1	1.7±0.2	1.6±0.1	4.1±0.7	2.5±0.4	2.1±0.3	4.2±1.1
Seed number per fruit (unit)	326.0±12.4	257.2±9.9	429.0±22.3	411.3±9.7	963.0±16.7	80.0±8.8	231.0±3.3
Weight 100 seeds (g)	0.22±0.1	0.36±0.1	0.38±0.1	0.24±0.1	0.53±0.1	0.44±0.1	0.43±0.1

Table 4
Mean trait values used in S. aethiopicum and S. incanum accessions group identification

The conclusions of this study are as follows. The multivariate techniques applied to morphological data sets demonstrated the component of plant characters of *S. aethiopicum* and *S. incanum* accessions. A morphometric analysis of plant traits showed that variation level was relatively high among the *S. aethiopicum* and *S. incanum* accessions. In addition, *S. aethiopicum* and *S. incanum* species were classified into seven groups and the number of accessions per group varied considerably. All *S. aethiopicum* and *S. incanum* accessions used for this study are also generated as inbred lines for rootstock breeding programs in another study. These genotypes are an important source of diversity which could be used for rootstock eggplant breeding programs of heterotic hybridization in the near future.

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P3-02

Inheritance of quantitative resistance to *Meloidogyne incognita* in the pepper 'Alcos'

Fulgencio Sánchez-Solana[1], Caridad Ros[1], Elena Sánchez-López[2], Josefa Gomariz[2], Carmen María Lacasa[1], María Carmen Martínez[1], Alfredo Lacasa[1], Rafael Fernández-Muñoz[3]

[1] *Departamento de Biotecnología y Protección de Cultivos, IMIDA, La Alberca, Murcia, Spain*

[2] *Departamento de Hortofruticultura, IMIDA, La Alberca, Murcia, Spain*

[3] *Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora", Universidad de Málaga - Consejo Superior de Investigaciones Científicas, Algarrobo-Costa, Málaga, Spain*

Abstract:

The pepper (*Capsicum annuum*) Spanish cultivar 'Alcos' shows high and stable quantitative resistance against the root-knot nematode *Meloidogyne incognita*. In order to study the inheritance mode of this resistance, a six-generation family composed of 'Alcos', the susceptible cultivar 'DLL' and their F1, F2, $BC1_R$ and $BC1_S$ were obtained and tested for resistance to *M. incognita*. Also, an allelism test was performed using an F2 progeny obtained from the cross between 'Alcos' and the resistant line 'HDA149' (carrying the *Me3* resistance major gene). Mendelian and quantitative genetic analysis of the six-generation family revealed that resistance to *M. incognita* in 'Alcos' is mainly controlled by a single locus with predominantly dominant effects, but with epistatic interactions from 'additive x additive' and 'dominant x dominant' gene effects. The allelism test showed that genetic control of 'Alcos' resistance is different from that conferred by the *Me3* locus and the closely linked loci *Me1* and *N*, suggesting then a new genetic source of nematode resistance in pepper.

Keywords: *Capsicum annuum*, genetic resistance, nematodes

P3-03

Study of the *Yellow spot flower – Ysf* gene in *C. annuum* L. genetic background

András Andrásfalvy[1], Franco Saccardo[2] and Gábor Csilléry[3]

[1] *Consultant, Budapest, Hungary*

[2] *Consultant, Roma, Italy*

[2] *Budakert Ltd., Budapest, Hungary*

csillerygabor48@gmail.com

Abstract

Resistance genes against the main pathogens and pests of *Capsicum annuum* L. are available in wild *Capsicum* species. These resistance genes can be incorporated into *C. annuum.* by conventional crossing methods. *C. annuum* can be crossed easily with white flower species *C. chinense* Jacq., *C. frutescens* L. and *C. chacoense* Hunz., species that contain useful resistance genes against a wide rage of pathogens and pests of pepper. Interspecific crosses of genetically distantly related *C. baccatum* L., *C. baccatum var. pendulum* (Willd.) Eshbaugh and *C. praetermissum* Heiser & P.G.Sm. with *C. annuum* L.. are however more technically challenging. Some degree of success has been enjoyed in certain crosses such as *C. annuum* x *C. baccatum var. pendulum*, where a successful hybridization is marked by the yellow spot corolla in the F1 generation governed by *Yellow spot flower - Ysf* (syn. *Yellow spot – Ys*). The main issue proceeding from the crossing of *C. baccatum var. pendulum* **x** *C. annuum* is that the F2 generation suffers low fertility, leading to the loss of various interesting traits deriving from *C. baccatum var. pendulum*. The reciprocal cross proved to be even more difficult and to our knowledge there is no evidence of successful attempts. In the present paper we discuss the experiments in production of successful interspecific hybrid of *C. annuum* and *C. baccatum var. pendulum* and backcrossing generation.

1. Introduction

According to The Plant List (2010) 40 species belong to *Capsicum* genus, but only 5 of them - *C. annuum* L., *C. chinense* Jacq., *C. frutescens* L., *Capsicum baccatum var. pendulum* (Willd.) Eshbaugh and *Capsicum pubescens* Ruiz & Pav. - are cultivated. Pepper is a facultative self-pollinating species. Depending on cultivar, farming location and other factors cross-pollination varies from 5 % to 10 % of the total successful fertilization events. Insects, which play a pivotal role in cross-pollination, are influenced by colour and odour of the flower. Pepper flowers are white or greenish-white and have very low nectar production and in particular *C. annuum, C. chinense, C. frutescens* and *C. chacoense* Hunz. are not very attractive to pollinators. Conversely, the corolla of some *Capsicum* species is white and coated in different shade of purple and even specific *Capsicum* groups displaying a dark purple corolla were reported. Pollinators tend to prefer bi-coloured corollas (in particular yellow-spotted ones) over totally white flowers. This phenomenon was also observed for yellowish-green, green or purple flowers. Among cultivated peppers only a *C. baccatum* var. *pendulum* has yellow-spotted flowers and produces higher quantity of nectar. This fact can come to the aid of farmers as cultivations that include both plants with white flowers and plants with multi-coloured flowers are more attractive to pollinators.

Some *Capsicum* species are genetically related and easy to cross. *C. annuum* is the most cultivated species worldwide and can be easily crossed with *C. chinense*, *C. frutescens* and *C. chacoense*. In the last few decades several resistance genes from these species were incorporated into *C. annuum* cultivars. Unfortunately, plants resulting from certain crosses suffered from reduced fertility, especially when *C. chacoense* was one of the parents. This problem could however be overcome with several rounds of backcrossing and selection. Crosses between the *Capsicum annuum – chinense - frutescens* group and other *Capsicum* species need to employ a so-called „crossing bridge", like *C. baccatum var. pendulum* and *C. praetermissum* having yellow spot purple coloured flower.

This „crossing bridge" is for example the only way to obtain useful characters from *C. eximium Hunz.*, which has a thin, flimsy and fragile plant habit with small leaves and yellow-spotted or purple flower, *Capsicum cardenasii* Heiser & P.G.Sm. with similar habit, but with purple coloured flower without yellow spots and *Capsicum pubescens* with lying plant habit, hairy leaves and brown seed.

2. Materials and Methods

33 years ago multicomponent interspecific breeding program to incorporate useful properties into *C. annuum* was set up and started (Csilléry 1983), but only a part of the program was realized due to a shortage of funds. Furthermore, some resistance genes were found in *Capsicum* species which are easier to cross with *C. annuum*. Presumably more complicated, labour intensive interspecific breeding program will be required in the future.

Some interspecific hybrids were successfully bred: *C. baccatum var. baccatum* **x** *C. praetermissum, C. baccatum var. pendulum* **x** *C. praetermissum, C. eximium* **x** *C. praetermissum, C. pubescesn* **x** *C. eximium*. Progenies of these hybrids were studied in Hungary (Budatétény, F1-F3 generation in greenhouse), in Rome (ENEA F4-F5 generation in field), in Napoli (Università degli Studi di Napoli Federico II, F4-F6 generation, field) and in Casaleone (North Italy, F5-F8 generation, field). These studies were supported by Prof. Franco Saccardo and Gianni Gatto. Seeds of F6 and F8 generations were stored in our gene bank but, unfortunately, they became progressively less and less viable over the course of time.

The current work is focused on crosses between *C. annuum* and *C. baccatum var. pendulum*, the latter having a yellow-spotted flower. In this kind of cross cytoplasmic male sterility severely impairs fertility of the progeny (Andrásfalvy and Csilléry 1983). Also, the most valuable trait – *Yellow spot flower* (*Ysf*) – of *C. baccatum var. pendulum* could not be stabilised in the progenies.

Seeds obtained from the *C. annuum* **x** *C. baccatum var. pendulum* cross were often not viable and the few F1 plants were deformed and short-lived. In the end *C. annuum* **x** *C. frutescens* F9 breeding line and *C. annuum* cv. Jalapeno (originated from Mexico) items were fertilised using pollen of *C. baccatum var. pendulum*. Both interspecific F1 generations showed the desired trait *Yellow spot flower* character (*Ysf* gene), but were displayed very low fertility. Since the interspecific hybrid made with *C. annuum* cv. Jalapeno mother was more fertile, this hybrid was selected for further backcrosses.

3. Results and Discussion

At present, we are studying homozygote *Yellow spot flower (Ysf)* uniform, nearly isogenic lines (*Ysf/Ysf*) which are in *C. annuum* cytoplasm background and backcrossed (BC8) with different types of spice pepper lines (*Figure 1.*). Colour intensity of the yellow spotting in these lines is variable, but so it is in *C. baccatum var. pendulum* material stored in our gene bank. To evaluate the intensity of yellow spot flower three levels (Spot - S) were defined. In the S1 type lines the yellow spotting is hardly visible, S2 type lines have visible spots and S3 type lines have intensely-coloured yellow

spots. We are currently studying and stabilising 150 nearly isogenic lines: 10 % belong to S1, 40 % to S2 and 50 % to S3 type. Crosses among S1, S2 and S3 constant lines allow us to study the genetic background as we aim at determining the factors influencing the variability. The progeny of F1 population from *Ysf+* **x** *Ysf3* crosses were divided in ratio S1 11 % : S2 57 % : S3 32 % in *Ysf* type.

Hitherto there is no interspecific hybrid of *C. annuum* cytoplasm containing *Ysf* gene. In the future creating interspecific hybrids between *C. annuum* and *C. baccatum var. pendulum* would be important since in *C. baccatum var. pendulum* is a source of several resistance genes against a variety of biotic threats to *C. annuum*. Lastly, there are reports of a new pathotype of *Tomato spotted wilt virus* overcoming the *Tsw* resistance gene from *C. chinense*. A putative resistance gene against this new, aggressive TSWV strain has been identified in *C. baccatum var. pendulum* making it an important element of future breeding efforts. Moreover, according to Bento (2013), is a source of polygenic resistances against *Pepper yellow mosaic virus* (PepYMV), and Kim et al (2008) and also anthracnose (*Colletotrichum acutatum*).

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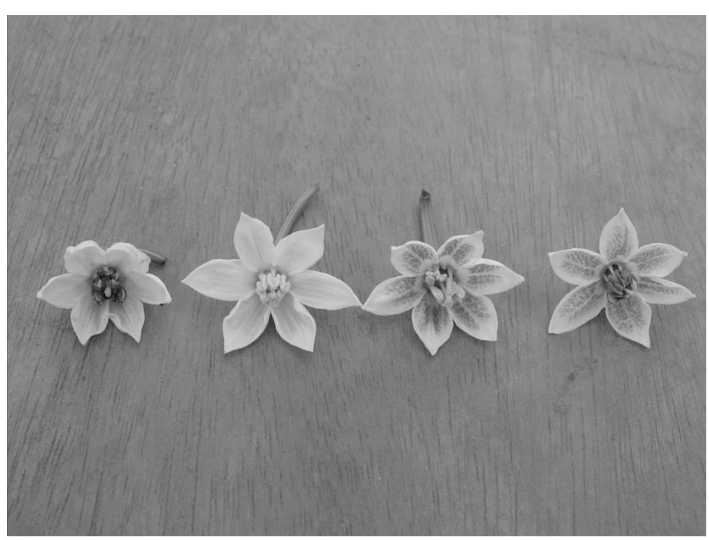

Figure 1:
Normal Ysf+ and Ysf flowers in Capsicum annuum genetic background

P3-04

The *ivory ripe berry – irb* project

Gábor Csilléry[1], Lajos Zatykó[2]

[1] *Budakert Ltd., Budapest, Hungary*

[2] *Consultant, Budapest, Hungary*

csillerygabor48@gmail.com

Abstract

Pepper berry is consumed either economically ripe or biologically ripe. Pepper berry is called economically ripe if it stopped growing, its flesh fully thickened, its shoulder is hard, but colouring has not begun. Then pepper for consumption can be picked and can be stored for 10-14 days at 8-12 ºC without special deterioration in quality. The colour of the economically ripe pepper berry is dark green, light green, or might be lilac, but in Hungary the tradition is other. The best known and favoured Hungarian pepper variety group is called "Cecei" in the word. These types are different from others consumed in other countries, since they are ivory coloured in economic ripe stage. They are often called white but this colour definition is wrong since most of the berries called white are not white, but ivory. This type was called milky in the Hungarian "homeland" of economically ripe ivory pepper, in Cece village**.**

The original Cecei local variety (landrace) had an economically ripe ivory colour berry, but it was hot. The collecting, the selection and the breeding of the Cecei type were made by Lambert Angeli (1954, 1964). The name of the first registered variety was *cv.* Javított Cecei.

In winter shorter days the berry of the early cultivation pepper is not at all white, but ivory, what is more slightly greenish due to environmental factors and the supply technology of nutrient. The cv. Hó F1 variety is the only exception whose economically ripe berry is almost white even in winter shorter days (*snow white berry – swb*). The name of the variety also refers to this because the meaning of snow is "hó" in Hungarian. *(Figure1.)*

The biologically ripe berry colour ranges much more widely. The red, the orange and the lemon yellow are well-known. If chlorophyll does not decompose in green pepper when it ripens, the green and red colour together give brown (Mexican types – cv. Mulato, cv. Ancho). The berry colour of "Permagreen" genotype lines remains totally green, does not colour. The goal of our research is to breed a Cecei pepper whose economically ripe berry is ivory, but does not colour even when biologically ripe, remains almost ivory or slightly yellowish-ivory.

1. Introduction

Some decades ago in western European markets almost 90% of blocky, elongated blocky and Lamuyo peppers got into the fresh market green. By now this proportion has changed significantly and the proportion of green berries is only 30-40 %. The majority of the 60-70% colour pepper is red (70-80%), the rest is lemon yellow or may be orange. In processing industry the proportion of green and coloured is about 50-50% since the colourfulness of the produce is an important factor. Producers of course would like to harvest economically ripe pepper that the development of the plant should not stop, but there is higher price in return if it is picked coloured. In the market of the lately widespread Kapia types the proportion of coloured, only red peppers is 90 %.

The Hungarian market, Hungarian consumption habits are different. In spite of pressure and attempts Hungarian consumers prefer elongated, conical ivory Cecei pepper. The taste of these types is totally different, when economically ripe they are ivory, when fresh they are consumed almost as a fruit. It is usual to slice pepper to add to bread and butter or sandwiches with cheese and salami for breakfast or dinner. The taste of the too early picked soft shouldered, economically not fully ripe pepper of course is not so good. Unfortunately, it is often experienced especially in case of early cultivation pepper. If a Hungarian pepper begins to colour the taste is the best because the decomposition of acids has not begun, the tastes are still in balance. The Cecei Hungarian peppers also differ from blocky varieties in their thinner epidermis. The flesh consistency of the berry is also softer not so hard and crispy (this is what we like) that is why the colouring berry begins to soften earlier, loses some of its value. As a result of this situation the trader buys coloured pepper only in quality under any class.

In the framework of our research we wanted to slow down this initial stage of colour change. We were looking for characteristics inhibiting the development of biologically ripe berry colour which hinder the colouring of varieties (Cecei, HRF, Alma) of Hungarian phenotypes typically ripening from ivory into red. The variety ripening from ivory into lemon yellow seemed to be the simplest. Irish (1888) described the "Ivory Tusk" variety as "ivory white", but we do not know this variety. In our wild *Capsicum* collection there have been and there are items, especially the ones belonging to *Capsicum chinense* Jacq. species whose biologically ripe berry colour was ivory, pink or rather peach colour. (*Figure 2.*)

The initial momentum of the work was temporarily suspended because of the appearance of the new and more aggressive strains of the Tobamo viruses and the research of more important topic.

The publication of Huertado-Hernadez and Smith (1985) again raised our interest since they said they found an item among whose hybrid next generations the biologically ripe colour is determined by three independent gene pairs (*Y, C1 and C2*). Altogether eight phenotypes (red, light red, orange, pale orange, orange yellow, pale orange yellow, lemon yellow) were separated in the red x white cross F2 generation.

The topic raised others' interests too, several publications are known in this topic, but we do not know about biologically ripe ivory coloured pepper variety. Thorup et al (2000) on the basis of the molecular analysis of the almost similar hybrid combination separates only four colours. (In brackets the results of Huertado-Hernadez and Smith). Red (red and light red), peach (orange and pale orange), orange (orange yellow and pale orange yellow), cream (lemon yellow and white).(*Figure 3.).* Quiros (2010), to make it exhaustive also inserts into the line the one remaining green and the one ripening into brown and lists four genes (*Y, C1, C2, cl*), which cause ten types of biologically ripe berry colours: red, light red, orange, pale orange, chocolate, yellow, pale yellow, lemon yellow, white, green. Brand et al. (2013) studied the quantitative genes influencing the pigment contents of the pepper berry.

2. Materials and Methods

In 1985 we began the following research program with the biologically ripe ivory ripening to lemon yellow and orange lines, which we got from Prof. Smith. We chose Hungarian cv. HRF (long conical), cv. Cecei (short conical), cv. Almapaprika (almost round apple form) ripening from ivory into red and long hot (cv. Rapidus F1), from light green to red as mother lines. We used Smith type which in both economically and biologically ripe form is ivory line as father line. We analyzed the next generations of the successful crosses. As expected the F1 plants in economically ripe form were white, in case of long hot type were light green but in biologically ripe form in all

cases the berries were red. In F2 the colour of 665 plants was judged and the following berry colours were seen: Red (58%), light red (4%), orange (10%), peach (11%), lemon yellow 13%), ivory (4%). Due to our lack of practice no more colours were registered.

3. Results and Discussion

After the initial momentum, and several resumptions, then the separation of our research works (1990) we worked on the material divided at that time at different intensity but independent of each other. With different breeding lines we performed backcrosses, but due to limited possibilities later only the biologically ivory, probably berries ripening to lemon yellow were harvested and we had no possibility to analyze the splitting proportions.

We can summarize our results now that we have white HRF, *Cecei* and Alma pepper types of acceptable size which are ivory when biologically ripe. On the basis of several year observations, unfortunately we do not have favourable experiences with biologically ripe ivory berries. The problem with items placed at producers was that if the producers did not pick the economically ripe produce in time the value of the overripe produce deteriorated it was not crispy any more, but gummy, soft. On the other hand in case of Alma peppers ivory in ripe state (where already the producers tried it) another problem is that it was produced only for pickling. The pickled produce will soften during storage even in case of material of beginning biological ripening. In the new backcross programs we try to choose types of thicker and harder flesh.

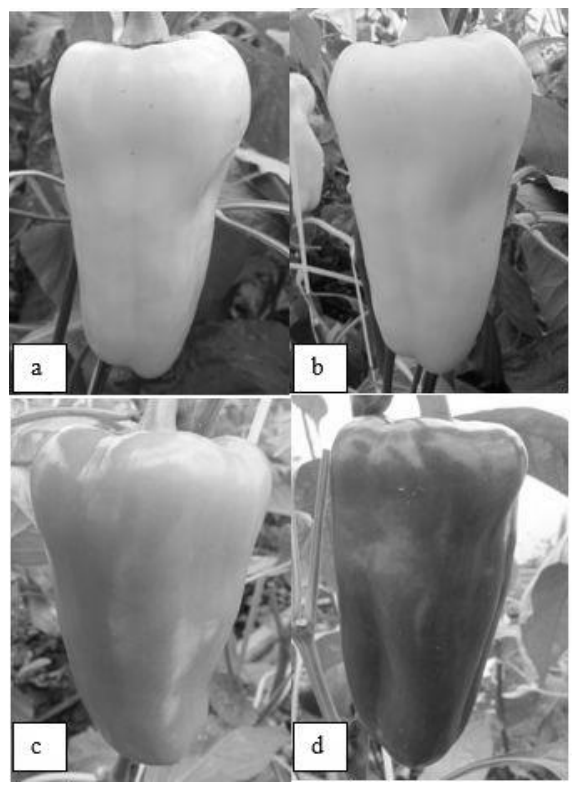

Figure 1 Economical ripe colour berries (a./ snow whit berry, b./ ivory, c./ pale green, d./ green)

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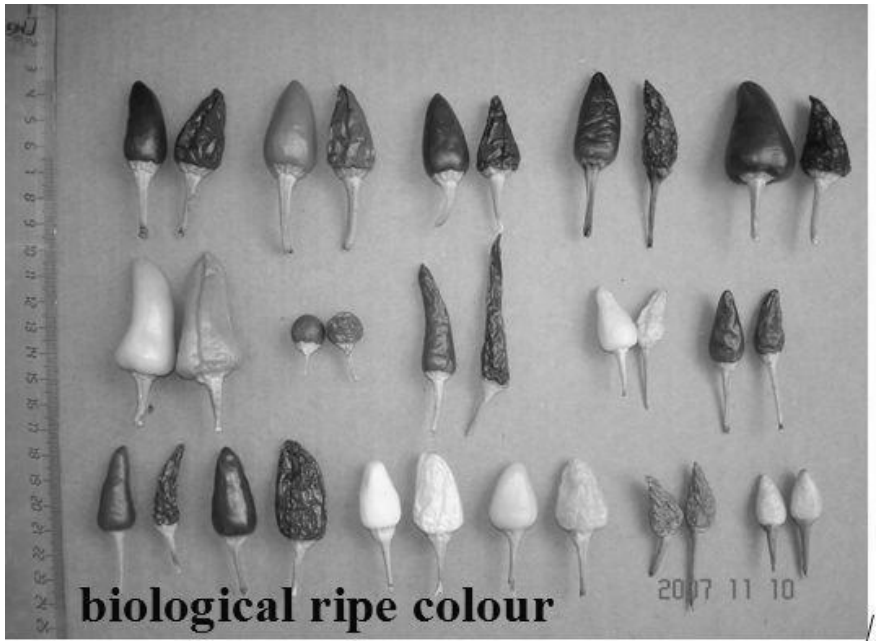

Figure 2
Biological ripe colour berries

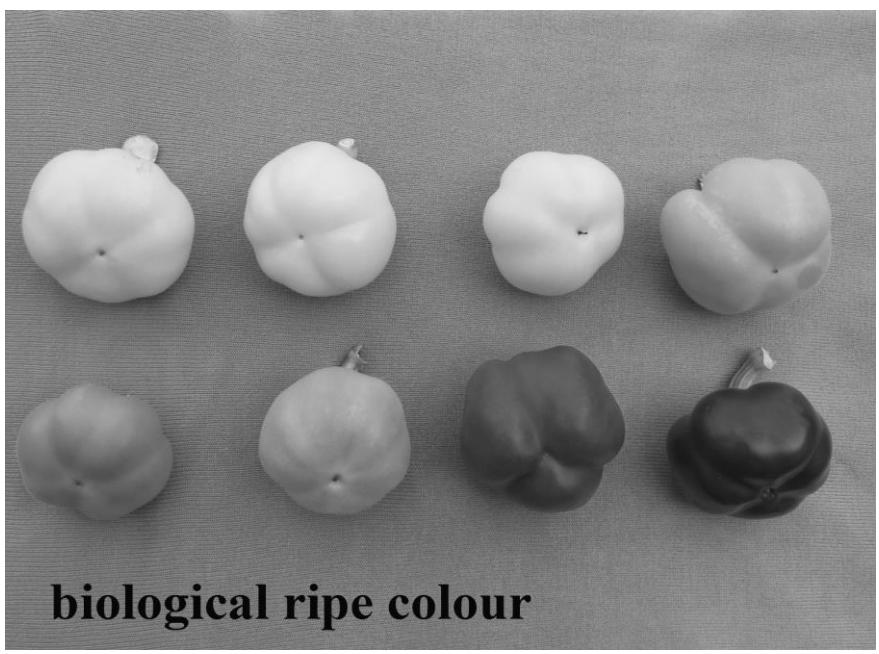

Figure 3
Biological ripe colour berries

P3-05

Genetic study of the *super fasciculate plant - sfx* mutant

Gábor Csilléry[1], Zoltán Timár[2], Lajos Zatykó[3], Ferenc Márkus[4]†

[1] *Budakert Ltd., Budapest, Hungary*

[2] *Univer Product Zrt. Kecskemét, Hungary*

[3] *Consultant, Budapest, Hungary*

[4] *Red Pepper Research-Development Co. Ltd. Kalocsa, Hungary*

csillerygabor48@gmail.com

Abstract

The main axis of the cultivated varieties of *Capsicum annuum* L. ends in one or several flower buds generally located after 8-12 internodes, and then growth continues in 2-3 stems in so-called double cyma bifurcate. After branching the stems do not grow in double cyma bifurcate but in single cyma bifurcate, which means one of the branches is always of stronger and longer, while the second one has shorter internodes. Even in case of bunchy varieties the main axis ends in one-two flower buds but downstream of the double cyma bifurcate the length of the stems will shorten to different extents. Short stems and leaves often gather into one bunch. One recessive gene, the *fasciculate plant – fax (syn. fasciculate - fa)* gene is responsible for this trait. The appearance of the *fax* gene phenotype is however influenced by several unknown genes resulting in several phenotypes of bunchy plants. In Hungary several bunchy varieties were selected within the vegetable pepper and spice varieties. Two varieties, respectively belonging to each of the two groups, have been on the market for over 30 years and their seed turnover has broken all sales records. The cv. Kalocsai merevszárú 622 (Ferenc Márkus) spice variety was registered in 1974, while the cv. Fehérözön (Lajos Zatykó) was registered in 1981.

We have found ones in two continuously growing (no fasciculate types) item of independent stock, whose phenotype at first sight resembled the ones of known bunchy phenotypes. Also in this case the main axis of the bunchy individuals is continuously growing until the 8^{th}-12^{th} nodes but then it may end in up to 4-8 flower buds. A significant difference is that the main axis does not continue in double cyma bifurcate, but stops growing or results in abnormal side stems. The peduncle is definitely upright, but its end curves backwards, it is conspicuously long (10-15 cm) and thin. The new mutant is named *super fasciculate plant – sfx.*

1. Introduction

The origin of the fasciculate (syn. fasciculatum, fastigiate, bunchy, cluster, bouquet, buket, determinate habit, csokros etc.) trait is unknown. However, an increasing number of research groups are investigating this topic. Earlier were cited the publications of the researchers in the 30s and 40s as the first publishers of the fasciculate mutant (Kaiser 1935), Deshpande (1940, 1944). Lately we were able to access earlier researches from Japan Tenjikumamori (1874), France (Vilmorin seed catalogue, 1886) and the USA Surtevant (1888), who was the first to call the cv. Bouquet rouge item, which he received from the Vilmorin firm as fasciculatum. Shore (1908) published the experimental results performed on Red cluster variety of fasciculate cluster habit in the annual report of the New Jersey Agricultural College Experiment Station. He was

the first to examine the inheritance of the normal and fasciculate growth, the pendent and upright berry state. We would like to highlight this publication also for the remarkably interesting pictures of bunchy varieties and F2 generations displayed in it. Later on, Ikeno (1913) worked expanded the work with material received from New Jersey.

2. Materials and Methods

Before summarizing the history Hungarian breeding of fasciculate varieties we have investigate what has caused the popularity of the fasciculate varieties in Hungary and in other Eastern European countries in the past 30-40 years. The traditional cuisine typical of these countries is more meat- than vegetable-based. After the discovery of the American continent, new vegetable species such as potato, tomato and pepper began to spread. In particular, pepper started to be renowned for its spicy taste. Plants with such property usually produced modestly sized fruits that can be dried and stored. By the 18th century these kind of spicy peppers were widely spread. Popular taste changed only in the 19th, 20th century and pepper types suitable for fresh consumption of Western-European and Turkish-Bulgarian origin were incorporated in Eastern European cuisine. After World War II public policies always emphasized the importance of increasing quantity and rather than quality. In addition, due to forced industrialization there was shortage of labour force in agriculture. To overcome these issues direct sowing, mechanical harvesting and mechanical post-harvest drying took the place of the more traditional, manual techniques. New varieties with specific applications such as "Lecsó" - Hungarian ratatouille and "Zakuska" were then developed to meet the needs of the processing industry supplying the huge Soviet market. Varieties containing the fasciculate gene resulting in determinate growth perfectly met this purpose thanks to slightly smaller berry size, making them the most common even nowadays.

We would like to stress the importance of the work of Kormos and Kormos (1956), the first Hungarian researchers who focused on fasciculate mutant and detected six phenotypes while studying bunchy state. Furthermore, an outstanding researcher of Hungarian vegetable pepper is Angeli Lambert, who took part in the breeding of several bunchy varieties (cv. Csokros felálló, cv. Csokros csüngő, cv. Gépi konzerv) (1971). As a student of Angeli Lambert, Ferenc Márkus (Márkus and Kapeller 1971, Kapeller and Márkus 1974, Márkus and Kapeller 1974) began the breeding of fasciculate spice pepper. Several of his pendent (cv. Kalocsai 702, cv. Kalocsai 801) and upright (cv. Kalocsai determinált 601, cv. Kalocsai determiált 621) berry varieties have been appreciated, with cv. Kalocsai merevszárú 622 being its greatest success and still cultivated. This variety has excellent substantive values and is suitable for direct sowing, mechanical harvesting and traditional cultivation. The cv. Kaldóm is the nearly isogenic variety of cv. Kalocsai merevszárú 622, which in addition to *fax* gene also contains *Bs2* gene providing resistance to *Xanthomonas vesicatoria* bacteria (Márkus et al. 2011). Another student of Angeli Lambert is Lajos Zatykó (Zatykó 1980, Zatykó and Moór 1982), who continued to breed vegetable varieties of fasciculate types. In addition to completing the breeding of the cv. Gépi konzerv (the meaning of the variety name is: suitable for mechanical harvesting and canning industry) he produced the cv. Tizenegyes, cv. Suptol and cv. Korona varieties. The greatest success, however, was the cv. Fehérözön which is still well-known, but produced only in smaller area today.

Another Eastern European country, Bulgaria, had a traditionally large production of fasciculate pepper. There, some varieties were bred too and are known as cv. Buketen (Cristov and Popov 1971, Milkova 1974, Cristov et al. 1974).

A special mention should be given to F1 hybrids containing *fasciculate – fax* gene (*Figure 1a: flowering stage of fax, 1b: fruit setting stage of fax* phenotype). The *fax (syn. fa),* as it is known, is a monogenic and recessive trait. The F1 hybrid is of normal growth, but due to unclear genetic factors the varieties containing the *fax* gene develop earlier and of have richer

fructification. Among the Long hot and Cecei ivory hybrids several were obtained thanks to parents that contain the *fax* gene (cv. Tétényi hajtatási zöld F1, cv. Budatétényi F1, cv. HRF F1, cv. Ciklon F1, cv. Emese F1, cv. Danubia F1, cv. Cinema F1, cv. Dimentio F1 etc.).

3. Results and Discussions

We have observed continuously growing Long hot (Hot banana) and Kapia types of pepper displaying a phenotype similar to cultivars that have the *fax* gene. Similarly, the main axis of these new varieties continuously grows up to the 8-12th nodes, but then terminates in up to 4-8 flower buds. A significant difference is that the main axis does not continue in double cyma bifurcate but arrests its growth, or might develop abnormal side stems. The peduncle is clearly *upright (up* gene), but its end curves backwards, is conspicuously long (10-15 cm) and thin. The new mutant was named *super fasciculate plant - sfx* gene (*Figure 1c: flowering stage of sfx, 1d: fruit setting stage of sfx* phenotype).

Crosses were performed among the continuously growing type *(fax^+/fax^+ sfx^+/sfy^+)* with the bunchy *(fax/fax sfx^+/sfx^+)* lines and the continuously growing type with the new bunchy (*super fasciculate – fax^+/fax^+ sfx/sfx*) ones. In both cases, the F1 plants were continuous growing types. Accordingly, in the F2 generation the proportions of fasciculate and super fasciculate phenotypes were almost 25 %.

Cross was also made between the *fasciculate (fax/fax sfx^+/sfx^+)* and the *super fasciculate(fax^+/fax^+ sfx/sfx*) lines. The F1 plants had a continuous growing phenotype and *fax^+/fax sfx^+/sfx* genotype. In the F2 generation of *fax/fax sfx^+/sfx^+ x fax^+/fax^+ sfx/sfx* hybrid the proportion of continuously growing plants was 55 % and that of both bunchy types were 45 %. The *fax/fax* and *sfx/sfx* genotypes could be segregated on the basis of their phenotypes rate was 50%: 50%.

In the F3 generation we have studied the progenies of 10 *fasciculate* plants and 7 *super fasciculate plants* of phenotypes. Out of 10 F3 individuals, 7 showed segregation in fasciculate and super fasciculate phenotypes, while 3 had no segregation and each plant belonged to the fasciculate phenotype. We have not found any segregation among the progenies of super fasciculate plants.

The allele test of the two super fastigiate items (provisional names are *sfx-1* and *sfx-2*) of two different origins but of *sfx* phenotype showed identity. Clarification of the assumed identical gene mutation is currently under research. In conclusion, we believe that in time it will be possible to insert the *sfx* gene in commercially valuable lines, thus improving their value.

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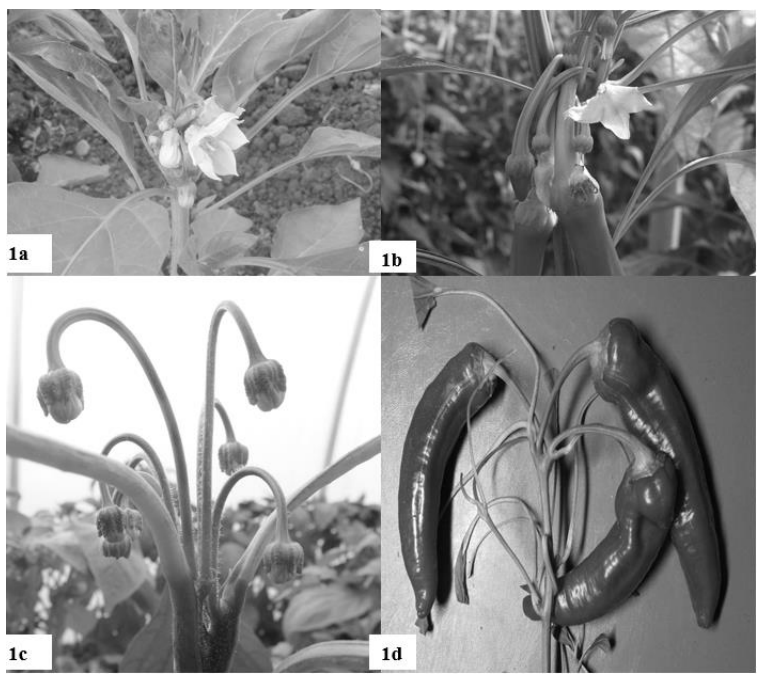

Figure 1a.: flowering stage of fax, 1b.: fruit setting stage of fax,
1c.: flowering stage of sfx, 1d.: fruit setting stage of sfx phenotype

P3-06

Genetic study of the *fragile plant - frx*, the *Micro cracking berry - Mcb* and the *green seed - grs* genes

Gábor Csilléry[1], Zoltán Timár[2], Andrea Moór[3]

[1] *Budakert Kft., Budapest, Hungary*

[2] *Univer Product Zrt., Kecskemét, Hungary*

[3] *Consultant, Budapest, Hungary*

csillerygabor48@gmail.com

Abstract

The Capsicum mutant collection maintained by Budakert Ltd. is certainly unique. In course of pepper breeding, in the early period of the work, the seeds from single plants deemed necessary are collected plant by plant. The great diversity of the material allows to observe an interesting range of spontaneous mutations. This phenomenon is of particular relevance when *Capsicum annuum* L. are crossed with *C. chinense* Jacq., *C. frutescens* L. *and C. chacoense.* It is well-known that as a result of species hybridization the mutation rate is increasing. Over more than 41 year-work we have created several interspecific hybrids mostly to identify resistance genes for breeding programs. In the next generations we have checked great attention to each item from seedling until ripe crop and the disorders have been registered. An extraordinarily high number of spontaneous mutations occurred lately and this may be caused also by environmental changes. High background radiation and strong sunspot activity are often cited as causes but also "modern" cultivation technologies may play a relevant part. For example, plants cultivated on rockwool, coco-peat and perlit receive might display higher mutation rates derived from the artificial substrate. We have published the Hungarian Plant Breeding Scientific Days and the International Eucarpia Capsicum and Eggplant Meeting and the International Pepper Conference about collections gathered from our own research materials and through international relations, which have been maintained since then in spite of smaller or bigger pitfalls.

This research is about three new mutations:

- the recessive *fragile plant – fxp* gene leading to systemic changes in the plant,
- the dominant feature *Micro cracking berry – Mcb* gene leading to small and seemingly insignificant changes on the surface of the berry,
- the *green seed – grs* gene changing the color of the seed as a result of Na OH treatment.
- *fragile plant – frp*

1. Introduction

Stem strength has been studied in other plant systems such as case of corn, wheat, barley, rice, maize, rapeseed, sunflower, alfalfa, hemp, carnation, Arabidopsis, acacia, poplar, etc. Research usually aims at breeding varieties with strong stems and high fiber contents which are not prone to cracking. The relationship with pathogen resistance to cellulose, hemicelluloses, lignin and parasite fungus is clear.

2. Materials, Methods and Results

While screening trials for resistance against pepper nematode (*Meloidogyne* sp.) we found pepper items whose roots remained in the soil and broke despite careful extraction and washing. Our investigations showed that not only the root but all the organs of the plants are fragile like glass. Figure 1.a, 1.b. The extent of fragility was quantified with simple measurement method (kilogram force/cutting or breaking the stem).

Cutting of stem: *frx* phenotype: 1,2 – 1,5 kp, *frx+phenotype* 4,5 - 5,0 kp.

Breaking of stem: *frx* phenotype: 0,4 – 0,6 kp, *frx+phenotype* 2,0 - 2,5 kp.

Seeds of fragile mutants also differ from normal ones because the outer shell cracks like mesh. Figure 1.c. We have made crosses the wild type *frx+/frx+* with *frx/frx* getotype. The F 1 plants exhibited a normal phenotype, but 21 % F2 plants were fragile, whereas 79 % were normal suggesting a monogenic recessive mutation is involved. The mark of the monogenic recessive mutant is: *fragile plant – frx.*

Erős-Honti and Csilléry (2016) have published results on the histological examination of *frx* mutant.

- Micro cracking berry – Mcb:

1. Introduction

Spicy pepper was traditionally rowed after picking, pre-dried in sun, and then dried to 6-8 % moisture content in drying chambers and then ground. New production systems skip pre-drying and post-ripening steps as pepper fruits are artificially dried in energy-intensive processes.

2. Materials, Methods and Results

We have found several individuals that displayed berries with micro-cracked surfaces. This feature was visible already on economically ripe green berries, then the cracks extension increased on the biologically ripe red berries. Economically and biologically ripe items were prone to fast dehydration of wild-type fruits with comparable phenotype and size. We have made crosses the wild type *Mcb+/Mcb+* with *Mcb/Mcb* getotype. Berries of F1 hybrids prepared with spicy pepper items also cracked even if to lesser extent and dried sooner. In the F2 generation almost 70 % of the items cracked and dehydrated easily. The self-fertilized next generations preserved this feature steadily, but the extent of cracking varied Based on our observations we think that feature is regulated by one dominant gene, which was marked as *Micro cracking berry – Mcb* gene.

- green seed –grs:

1. Introduction

Harvested seeds items are usually treated with various chemicals to decrease the chances of viral infections. One of the most efficients and best known treating agents in Hungary is Sodium Hydroxide or Caustic Soda (NaOH). In addition, Trisodium Phosphate or TSP (Na_3PO_4), Sodium Hypochlorite (NaOCl), Hydrocloric Acid (HCl) and Sulphuric Acid (H_2SO_4) are also used for the treatment of seeds.

2. Materials, Methods and Results

The seed of a Cecei type item following sodium hydroxide (NaOH) treatment became greenish-grey. Untreated seeds were not the usual golden-yellow, but matt and slightly grayish-yellow. The resulting color intensity depended also on the quality of the seed. The treatment affected the surface of smaller / weaker seeds more extensively: their color became darker green.

The seeds of the *green seed – grs* mutant have been treated with agents that are not commonly used for pepper seeds. In course of seed treatment we always used the usual concentration (see on Table 1.). For each agent, the seeds were treated for 10, 20 and 30 minutes, and their color alteration photographed. The results are summarized in Table 1.

We have made crosses with items of green seeds *grs+/grs+* with *grs/grs* genotype. The treated seeds of the F1 next generations were yellow, but in F2 almost 25% of treated seed items had green seeds. The monogenic recessive feature was marked with *green seed – grs* gene mark. We have made crosses among the *brown seed – brs* (Csilléry 2013) and *grs* mutants. The treated seed of the F1 plant was yellow, but both *brs* and *grs* phenotypes have been found among the treated seed items of the F2 items in addition to normal phenotypes. Genetic analysis has not been performed so far because of the few numbers of single plants. We can identify and see what the seed color of the double homozygote *(brs/brs grs/grs)* single plants is only after the examination of the self fertilized F2 next generations.

Table 1
Seed treatment trial of normal and green seed – grs mutant

Products name	NaOH 2 %		Na_3PO_4 10 %		NaOCl 1 %		HCl 2 %		H_2SO_4 1 %	
Genotypes / Times	grs^+	*grs*	grs^+	*grs*	grs^+	*grs*	grs^+	*grs*	grs^+	*grs*
10 minutes	yellow	green	yellow	green	yellow	yellow	yellow	yellow	yellow	yellow
20 minutes	yellow	green	yellow	green	yellow	yellow	yellow	yellow	yellow	yellow
30 minutes	yellow	green	yellow	green	yellow	yellow	yellow	yellow	yellow	yellow

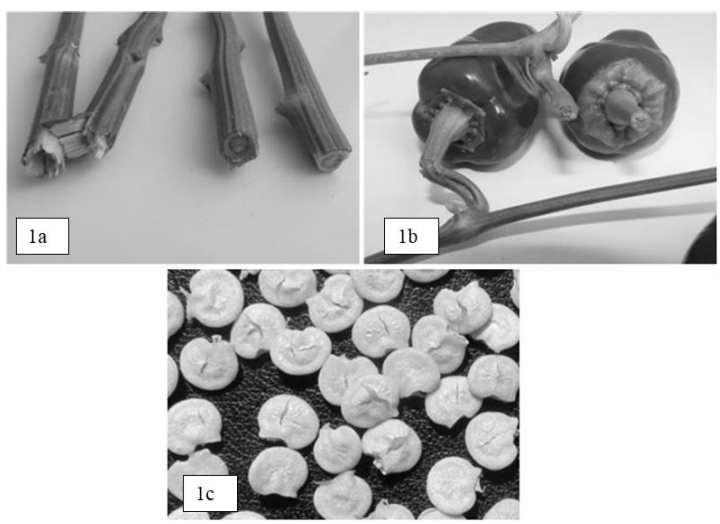

Figure 1a. Stem of the normal and fragile plant – frx, 1b. Peduncle and fruit of normal and fragile palant – frx, 1c. Seeds of the fragile plant - frx mutant

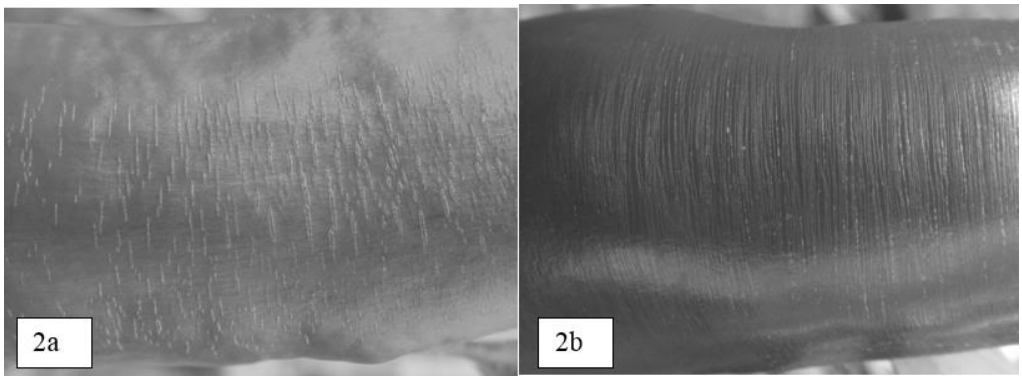

Figure 2a. and 2b. Micro cracking berries - Mcb

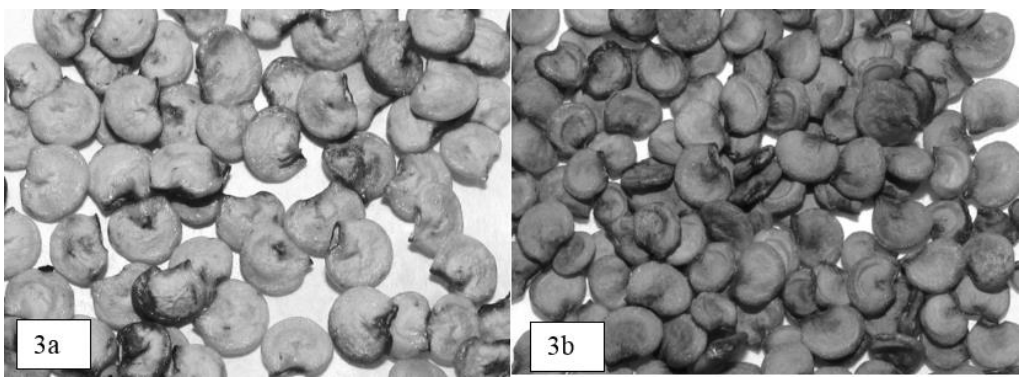

Figure 3a. No treated seeds and 3b. 2 % of NaOH treated (10 minutes) green seed - grs) seeds

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P3-07

Morphological and anatomical characterisation of the *fragile plant – frx* pepper mutant

Zsolt Erős-Honti[1], Gábor Csilléry[2]

[1] *Szent István University– Faculty of Horticultural Science, Department of Botany and Soroksár Botanical Garden, Budapest, Hungary*

[2] *Budakert Ltd., Budapest, Hungary*

Abstract

During the testing of pepper (*Capsicum annuum* L.) parcels for resistance against the parasite *Meliodogyne* sp., some plants were found to possess extremely fragile, glass-like vegetative organs (roots and stems). During the crossing experiments, the mutant trait (signed as *fragile plant – frx*) turned out to be monogenic and recessive. In the present work we are to reveal the anatomical background of the *frx* character by examining comparatively the histology of different organs (roots and stems of different age, leaf petiole, fruit pedicel and pericarp) of wild-type (*frx*$^+$), fragile (*frx*) and hybrid (*F1*) plants. By applying paraffin-embedding method and lignin-specific staining we found that the most obvious difference between wild-type and mutant plants is in the anatomy of the lignified xylem elements. The cell wall thickness of the libriform fibers are significantly thinner in case of *frx* plants compared to that of the wild-type ones. Moreover, the diameter of vessel elements (tracheas and tracheids) was more homogenous in the xylem of wild-type plants. To the contrary, no difference was found concerning the non-lignified elements and the structure of the pericarp.

1. Introduction

Growth and harvest conditions of certain vegetable cultivars are basically determined by the mechanical features of their vegetative and generative organs. Breeding is principally determined to find tough-stemmed, resilient plants of high fiber content that resist to the bending forces. Moreover, growth and harvest conditions of certain cultivars are also basically determined by the toughness of different organs. The mechanical features of the stem of several herbaceous and woody plants was previously studied (e.g. *Arabidopsis* – Zhong et al. 2005, *Populus* – Lee et al. 2009, *Oryza* – Kotake et al. 2011) [*1, 2, 3*], sometimes also revealing the genetic background of the trait. In these cases, the decrease of stem toughness was linked to thinned cell wall of the lignified xylem elements (trecheas and tracheids) together with those of the libriform fibers within the xylem.

During the testing for resistant pepper cultivars against *Meloidogyne* sp., we observed a parcel of plants with remarkably fragile (almost glass-like) roots and stems. We accomplished cross-hybridisations with the mutant plants. The trait was signed as *fragile plant – frx*. All the F1 progenies had wild-type phenotype, yet app. 25% of the F2 generation was found to be fragile. Based on our observations, fragile plant (*frx*) trait is monogenic and recessive.

In the present study we aim at revealing the anatomical background of the *frx*-trait with histological comparisons.

2. Materials and methods

Histological studies were carried out on the mutant (P1 – *frx*) and wild type (P2 – *frx+*) parents, as well as on the offspring of the first (*F1*) progeny generation. Anatomical samples were taken from the young and elder roots, leaf petiole, stem internodia of different stages of development, the fruit pedicel, the pericarp and the seed (esp. seed coat). All the samples were fixed in FAE-solution (formalin : acetic acid : ethanol = 5 : 5 : 90). Organ samples were dehydrated and infiltrated with Ottix Shaper and Ottix Plus solutions (Diapath), according to the manufacturer's instructions prior to paraffin embedding (Thermo Scientific embedding automat and accessories). Thin sections of 10-20 µm thickness were deparaffinised and then stained either with toluidine blue or by the Malachite Green – Bissmarck Brown double staining method so as to differentiate between lignified and non-lignified cell walls. Specimens were studied with light microscopy (Zeiss Axio Imager.A2). Photodocumentation and measurements were carried out using the software AxioVision 4.8.2. Measured data were statistically analyses with ANOVA and Tukey *post hoc* test using PAST 3.05 [4].

3. Results and discussion

When comparing the tissue structure of different organs, we found obvious differences in the anatomy of the xylem, chiefly the secondary xylem. Differences were found concerning the lignified xylem elements (i.e. tracheids, tracheas and libriform fibers). The most striking dissimilarity was observed in the cell wall thickness of the libriform fibers being much thinner in the fragile (*frx*) mutant plants compared to the wild-type (frx^+) ones, however a similar (but not consistent) trend was found in case of the vascular elements (Fig.1., Fig.2., Table 1.)

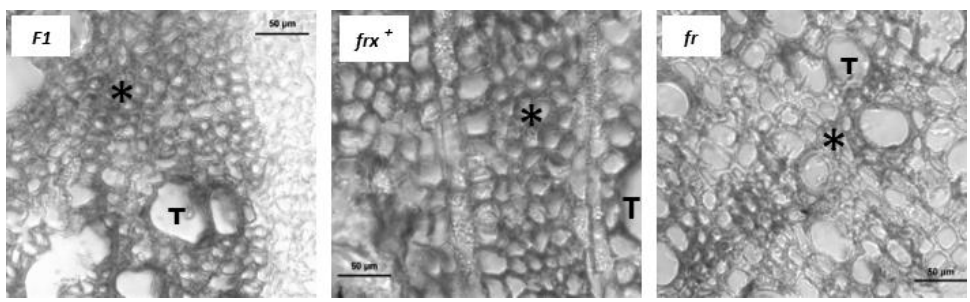

Figure 1
Cross sections from the xylem of the mature root of the first progeny (F1), wild-type (frx^+) and fragile (frx) plants. (: libriform fibers, T: tracheas, bar: 50 µm)*

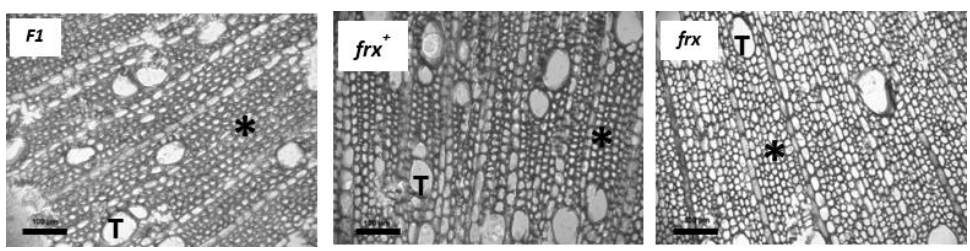

Figure 2
Cross sections from the xylem of the stem of the first progeny (F1), wild-type (frx^+) and fragile (frx) plants. (: libriform fibers, T: tracheas, bar: 100 µm)*

Root

	Libriform fibers			Tracheas		
	frx	F1	*frx*+	*frx*	F1	*frx*+
Cell wall (µm)	1,73±0,24	2,54±0,66	2,70±0,54	2,39±0,54	2,83±0,46	3,63±1,04
Sign. diff.	frx – F1, frx – frx$^+$			frx – frx$^+$		

Stem (mature part)

	Libriform fibers			Tracheas		
	frx	F1	*frx*+	*frx*	F1	*frx*+
Cell wall (µm)	1,20±0,35	2,92±0,73	2,46±0,55	2,20±0,43	3,17±0,69	2,50±0,50
Sign. diff.	frx – F1, frx – frx$^+$			frx – frx$^+$		

Pedicel

	Libriform fibers			Tracheas		
	frx	F1	*frx*+	*frx*	F1	*frx*+
Cell wall (µm)	2,06±0,64	2,68±0,58	2,73±0,53	2,74±0,83	2,66±0,41	3,14±0,52
Sign. diff.	frx – F1, frx – frx$^+$			frx – frx$^+$		

Table 1
Measured values and statistical comparisons of the cell wall thickness of different organs. (Sign. diff.: significant difference revealed by the post hoc test on significance level $p<0.001$)

Besides, another characteristic feature was that the diameter of the tracheas and tracheids was more heterogenous in case of wild-type plants than it was in the fragile mutants. At the same time, in the non-lignified supportive tissue elements (collenchyma) no statistically proven difference was found between the mutant and wild-type plants. Likewise, the pericarp anatomy (and thus the fruit mechanical characteristics) was also similar in case of the wild-type and the fragile plants. The anatomical comparison of the F1 progeny to the two parents was found to correlate with the phenotypical observations during the breeding: F1 plants in most cases showed anatomical similarity to the wild-type parents (Fig.1., Table 1.). The genetic background and inheritance of the *frx* trait is described in details by Csilléry et al. [5].

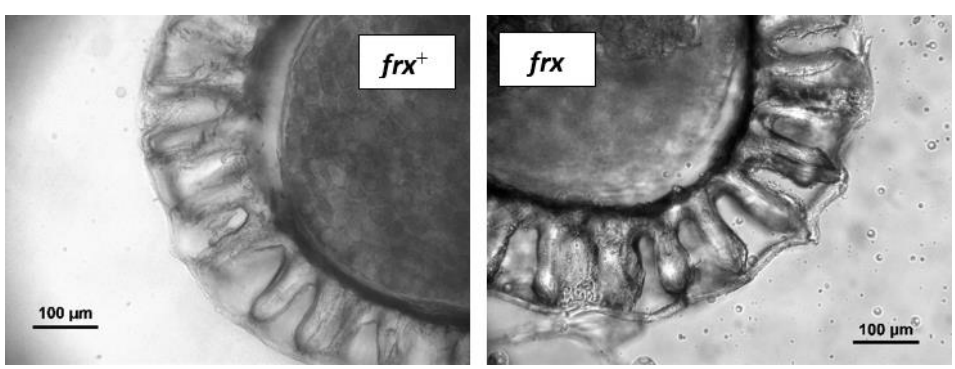

Figure 3
Seed coat cell anatomy a smooth-surfaced wild-type (frx$^+$) and a micro cracked (frx, 12579) seed. (bar: 100 µm)

The morphology of the seeds of mutant and wild-type plant showed a further astonishing dissimilarity. The surface of *frx* seeds were patterned with minor (parcel 12572) or more pronounced (12576, 12579) crevices and cracks, while that of the wild-types (*frx+*) seeds were entirely smooth. As an anatomical background for the trait we supposed that the periclinal and/or the anticlinal cell walls of the seed coat cells would be significantly different between the different types. As a result of our morphometric measurements (Table 2.), however, no such significant differences were found that could be related to the seed coat pattern (Figure 3.). Besides, the significance values of the differences were lower in these comparisons. Consequently, this trait is cannot be related to the cell wall anatomy of the respective cells, rather some compositional alteration of the cell wall may explain the differences, since fragile shoots were found to be related to altered cell wall compound biosynthesis in other plants [3], [6], [7].

Anticlinal walls

	frx (12576)	*frx* (12579)	*frx* (12572)	*frx*$^+$
Thickness (µm)	42,36±1,52	45,18±1,02	45,60±1,42	47,11±1,09
Sign. diff.	frx$^+$ − frx(12576)			

Periclinal walls

	frx (12576)	*frx* (12579)	*frx* (12572)	*frx*$^+$
Thickness (µm)	7,96±0,33	9,22±0,31	8,81±0,23	7,89±0,35
Sign. diff.	frx$^+$ − frx(12572); frx$^+$ − frx(12579); frx(12576) − frx(12579);			

Table 2
Measured values and statistical comparisons of the cell wall thickness of the anticlinal and periclinal cell walls of the seed coat cells. (Sign. diff.: significant difference revealed by the post hoc test on significance level $p<0.05$)

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P3-08

Development of a quick screening bioassay for internal fruit rot in bell pepper (*Capsicum annuum L.*)

Mario Frans[1], Rudi Aerts[1], Sofie Van Laethem[1] Bart Van Calenberge[2], Liesbet Van Herck[2], Kurt Heungens[3], Kris Van Poucke[3], Sanne Van Gool[4], Johan Ceusters[1]

[1] *KU Leuven, Technology Campus Geel, Faculty of Engineering Technology, Dept. Microbial and Molecular Systems, Geel, Belgium*

[2] *Research Station for Vegetable Production (PSKW), Sint-Katelijne-Waver, Belgium*

[3] *Institute for Agricultural and Fisheries Research (ILVO), Plant Sciences Unit, Merelbeke, Belgium*

[4] *Hoogstraten Research Centre (PCH), Meerle, Belgium*

Abstract

In this study, a bioassay was developed to visualize susceptibility differences between different maturity stages (green and coloured) and between different bell pepper cultivars. Based on field trials, an internal fruit rot sensitive and non-sensitive cultivar was selected for each colour (red and yellow). Due to the lack of internal fruit rot observations in spicy peppers, these fruits were also included at different maturity stages. After surface sterilization, fruits were pin-wounded, inoculated with FLASC agar disks and incubated under high humidity for 14 days at 25 °C. After incubation lesion diameter on both outer and inner surfaces was measured at two perpendicular axes. Yellow fruits showed higher lesion areas compared to red fruits and as expected the more sensitive cultivars for both colours showed the biggest lesions. In addition, green fruits from all cultivars showed almost no lesion outgrowth. These observations are all consistent with field trial results and indicate the proposed bioassay to be a reliable and fast screening method for internal fruit rot susceptibility in new cultivars.

1. Introduction

In recent years, sweet pepper or bell pepper have grown in popularity mainly due its wide variety of colours, shapes and sizes [1,2]. Especially greenhouse-grown bell peppers has attracted consumers because of their high quality [3]. However, this high quality standards are still challenged by a common disease known as internal fruit rot caused mainly by members of the *Fusarium lactis* species complex (FLASC) [4,5]. After initial infection via the flower, the disease stays latent until the green mature stage of the fruit [6]. Most pepper types turn from green at the immature stage to variety - dependent colours when they are fully ripe [1]. They can be consumed at the physiologically immature green or full colour ripe stages in either prepared foods or fresh in salads or garnishes [1, 3, 7, 8]. During the colouring stage, the latent fungus can start to proliferate as mycelium on the ovary and/or causes necrosis on the inner fruit wall. Unfortunately, external symptoms such as sunken lesions appears when the fruits are already progressed through the supply chain towards the customer [6]. Nearly all growers are confronted with this disease causing average yield losses of 5% with seasonal peaks up to 20% [9].

From experience, field test and growers observations it is known that internal fruit susceptibility is cultivar-dependent. Internal fruit rot susceptibility of a bell pepper cultivar is desirable before large-scale planting of the cultivars but currently a quick, reliable screening procedure is not available.

2. Material and methods

2.1. Plant Material

The experiments were conducted on four cultivars grown in soilless rockwool bags in a climate-controlled greenhouse at the Research Station for Vegetable Production (PSKW) and Hoogstraten Research centre (PCH). Based on field trails conducted between 2010 - 2015 at PSKW and PCH, fruits from a sensitive and non-sensitive cultivar was selected at different maturity stages (green and coloured) for each colour (red and yellow). Due to the lack of internal fruit rot observations in spicy peppers, these fruits were also included at different maturity stages (Table 1).

Table 1
Overview of tested cultivars and their average internal fruit rot percentages during the growing seasons between 2010 -2015 observed in field trials on both PSKW and PCH. There were no data for 'Shakira' and the Habanero types.

Pepper type	Colour	Cultivar	Internal fruit rot	Breeder
Bell pepper	Red	Redwing	5,50 %	Rijk Zwaan
C. annuum	Red	Viper	9,28 %	Enza Seeds
Bell pepper	Yellow	Sensatio	18,23 %	Syngenta
C. annuum	Yellow	RZ 35-248	5,00 %	Rijk Zwaan
Hot pepper *C. annuum*	Red	Shakira	NA	Enza Seeds
Habanero	Red	Red Savina	NA	/
C. chinense	Yellow	Yellow Goronong	NA	/

2.2. Bioassay

From each cultivar, 10 fruits at different maturity stages were surface sterilized in a 10% bleach solution for 10 minutes. After sterilization, each fruit was pin-wounded, inoculated with a FLASC agar disk of 8,5 mm and incubated under high humidity for 14 days at 25 °C. After incubation, lesion diameter on both outer and inner surfaces was measured at two perpendicular axes using a digital calliper.

2.3. Statistical Analysis

For the lesion diameter between immature and mature fruit in a cultivar, a student T-test was used for statistical analysis. Susceptibility between cultivars at different maturity stages

was analysed by performing one-way ANOVA and Tukey's post hoc test using SPSS software, version 22.0 (IBM Corp., NY, USA).

3. Results

After 14 days of incubation, lesion diameter for immature green fruits were significant lower than for the mature stage in all four bell pepper cultivars (Figure 1). When fully coloured, FLASC could colonize the sensitive yellow cv. 'Sensatio' (4,26 cm²) much faster than the less sensitive 'RZ35-248' (2,26 cm²). A similar result could not be obtained for the red bell peppers types 'Redwing' and 'Viper' (P=0,18)). If the four bell peppers are compared with each other, no significant difference was observed in the lesion diameter at the green immature stage. In the mature stage however, only 'Sensatio' had significant larger lesions then the other three cultivars.

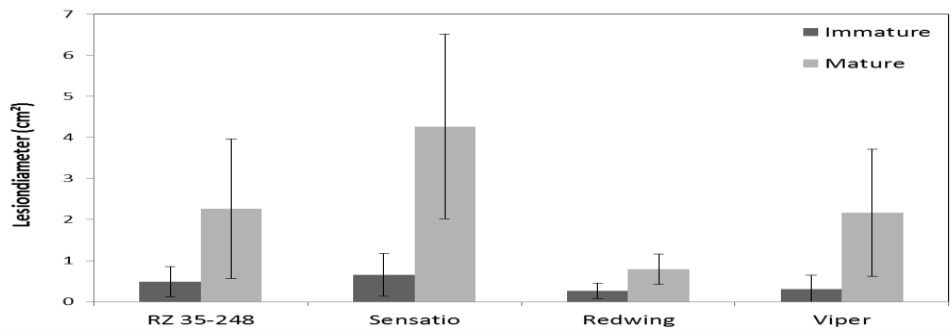

Figure 3
Lesion diameter for the four bell pepper cultivars at different maturity stages. All green fruits showed smaller lesions then mature fruits between a cultivar. The sensitive cv. 'Sensatio' had larger lesions than 'RZ35-248' (P=0,02), 'Redwing' (P=0,00) and 'Viper' (P=0,01). There was no significant difference between the sensitive cv. 'Viper' and 'Redwing' (P=0,16).

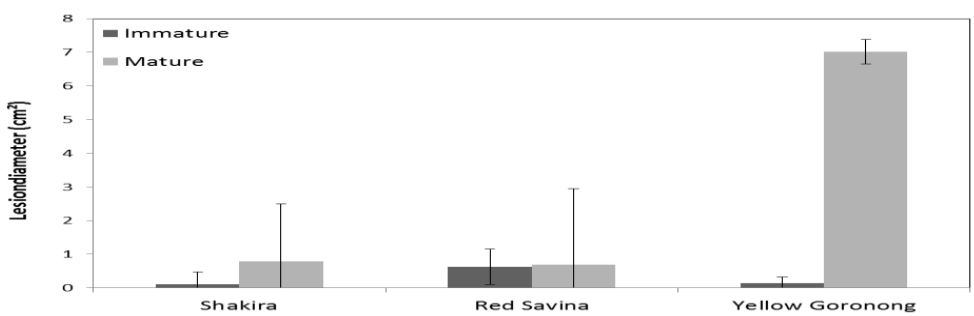

Figure 4
Lesiondiameter for spicey pepper cultivars at different maturity stages. All green fruits showed smaller lesions then mature fruits with the exception for cv. 'Red Savina' (P=0,30).

For the spicy types, similar results were observed. Immature fruits showed significantly smaller lesions than mature fruits with the exception for the red habanero type 'Red Savina' where both maturity stages showed equally sized lesions. At the maturity stage, fruits for the yellow habanero type 'Yellow Goronong' showed to be very susceptible for colonization of FLASC.

At the full-ripe stage, most lesions were significantly greater than the green immature stage. Out of three yellow cultivars, 'Sensatio' and 'Yellow Goronong' showed significant greater lesions than all red cultivars.

4. Discussion and conclusions

Over the years, internal fruit rot has emerged as a major problem worldwide [6,10,11,12]. Yang *et al.* [6] found that infection of bell pepper is initiated via infection of the flower where after the fungus stays latent until it can start proliferate on the inside of the colouring fruit. As observed in field trials (Table 1), the susceptibility towards infection is cultivar dependent. Our study shows that colonization of the fruit is indeed cultivar dependent but moreover, maturity dependent.

In the all pepper cultivars, immature green fruits showed no or small lesions compared to mature coloured fruits with the exception of the 'Red Savina' type (Figure 1). A number of studies observed similar changes in susceptibility between immature and mature fruits towards fungal pathogens such as in *Prunus* species, avocado and tomato [13, 14]. During ripening of the fruits, significant changes of sugars, malate, amino acids and phenols occur [15]. At the colouring stages, yellow cultivars showed larger diameters for 'Sensatio' and 'Yellow Goronong' then red cultivars. Carotenoids are responsible for the colour of the bell pepper fruits. In contrast to red fruits, yellow fruits contains more lutein and violaxanthin as major carotenoids while lutein is completely absent in red fruits [16]. This different composition of carotenoids could attribute to the higher sensitivity to FLASC growth in these cultivars.

Till now, no internal fruit rot observations were obtained in spicy cultivars. Shakira (8000-10000 SHU) and habanero types are recognised for their high concentrations of capsaicin which is believed to work antifungal against Fusarium [17,18]. However, our results showed no difference between sweet and spicy peppers. Even more, lesions on 'Yellow Goronong' had the largest diameter (7 cm^2) of all cultivars.

In conclusion, the results obtained from this bioassay proved to be consistent with the field trials. The proposed bioassay proved to be a reliable fast screening method for the susceptibility for internal fruit rot in bell pepper.

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P3-09

Evaluation of the resistance to *Phytophthora* blight in *Capsicum* genetic resources collected in Laos, Vietnam, and Ghana

Hiroshi Matsunaga and Takeo Saito

NARO Institute of Vegetable and Tea Science (NIVTS), Kusawa, Ano, Tsu, Mie, Japan

Abstract

Phytophthora blight caused by *Phytophthora capsici* is one of the most devastating soil-borne diseases of sweet pepper (*Capsicum annuum*) in Japan. Since attempts at chemical, physical, and cultural control have had little success, developing resistant cultivars offers the only effective alternative for controlling this disease. In Japan, a few cultivars have been developed that possess moderate resistance to Phytophthora blight. However, these are susceptible to new isolates of the pathogen. Several highly resistant rootstock cultivars have been developed in Japan to reduce disease severity, but grafted plants onto these rootstocks have not performed well, with lower yields than non-grafted plants. Therefore, new resistant breeding materials are required. To identify promising *Capsicum* genetic resources, we performed seedling inoculation tests with 134 *Capsicum* accessions to evaluate their resistance: 64 from Laos, 31 from Vietnam, and 39 from Ghana. We found 20 highly resistant accessions. (10 from Laos, 9 from Vietnam, and 1 from Ghana) of which 1 Laotian accession and 2 Vietnamese accessions showed no disease symptoms and will be highly promising breeding materials. We found 18 moderate resistant accessions: 12 from Laos, 5 from Vietnam, and 1 from Ghana. Thus, 34.4% of the Laotian accessions and 46.7% of the Vietnamese accessions were highly or moderate resistant, versus only 5.1% from Ghana. This suggests that it will be relatively easy to find resistant accessions in Laos and Vietnam, but harder in Ghana.

1. Introduction

Phytophthora blight caused by *Phytophthora capsici* is one of the most devastating soil-borne diseases of sweet pepper (*Capsicum annuum*) in Japan. Since attempts at chemical, physical, and cultural control have had little success, developing resistant cultivars offers the only effective alternative for controlling this disease. In Japan, a few cultivars have been developed that possess moderate resistance to Phytophthora blight. However, these are susceptible to new isolates of the pathogen. Several highly resistant rootstock cultivars, such as 'Dai-Power' (Matsunaga et al., 2010a), have been developed in Japan to reduce disease severity, but plants grafted onto these rootstocks have not performed well, with lower yields than non-grafted plants. Therefore, new resistant breeding materials are required.

On the other hand, we have collected many genetic resources in Laos, Vietnam, and Ghana (Monma et al., 1994; Yoshida et al., 1997; Sakata et al., 2008; Saito et al., 2009; Matsunaga et al., 2010b). To identify promising genetic resources, we performed seedling inoculation tests with the accessions collected in Laos, Vietnam, and Ghana to evaluate their resistance to Phytophthora blight.

2. Materials and Methods

Evaluation tests for resistance to *Phytophthora* blight were carried out as three independent trails (experiment 1 to 3). We used 39 accessions from Laos in experiment 1, 16 accessions from Ghana in experiment 2 and 25 from Laos, 31 from Vietnam and 23 Ghana in experiment 3. Control cultivars were used SCM334, AC2258 and 'Berumasari' as highly resistant cultivars and 'Ace' as susceptible cultivar in each experiment.

The *P. capsici* inoculum suspension was prepared according to the method of Matsunaga et al. (2010a). An isolate of *P. capsici* maintained by NIVTS was grown on a V8 juice medium in 90-mm Petri dishes. The dishes were sealed with Parafilm (American National Can, Chicago, IL, USA) and incubated in the dark at 28 °C for 7 days. The Parafilm was then removed and the isolate were further incubated under fluorescent light at 28 °C for 3 days. After the incubation, we poured 10 mL of distilled water into each dish and gently collected the zoosporangia with a paintbrush. The zoosporangia were incubated at 4 °C for 30 min and then at 25 °C for 1 h. The concentrations of zoosporangia were adjusted to 5.5×10^2 mL^{-1} in experiment 1, 2.0×10^3 mL^{-1} in experiment 2, and 3.2×10^3 mL^{-1} in experiment 3.

Table 1. Resistance of the *Capsicum* accessions collected in Laos and Ghana to Phytophthora blight in 2014 (experiment 1 and experiment 2).

experiment 1						experiment 2		
Accessions	Disease Index[z]	Degree of Resistance[y]	Accessions	Disease Index[z]	Degree of Resistance[y]	Accessions	Disease Index[z]	Degree of Resistance[y]
[Accessions from Laos]			[Accessions from Laos]			[Accessions from Ghana]		
07Lao-Veg-002	1.1	S	08Lao-Veg-054	0.7	MR	GJ93/001	0.4	HR
07Lao-Veg-003	2.0	S	08Lao-Veg-055	0.8	MR	GJ93/002	2.0	S
07Lao-Veg-004	1.4	S	08Lao-Veg-056	1.4	S	GJ93/003	1.9	S
07Lao-Veg-008	1.8	S	08Lao-Veg-072	1.1	S	GJ93/008	2.0	S
07Lao-Veg-009	1.3	S	08Lao-Veg-073	1.0	MR	GJ93/009	1.9	S
07Lao-Veg-017	1.1	S	08Lao-Veg-074	0.9	MR	GJ93/010	2.0	S
07Lao-Veg-018	1.3	S	08Lao-Veg-082	1.4	S	GJ93/011	2.0	S
07Lao-Veg-036	1.5	S	08Lao-Veg-088	2.0	S	GJ93/012	2.0	S
07Lao-Veg-049	1.6	S	08Lao-Veg-097	0.4	HR	GJ93/019	2.0	S
07Lao-Veg-056	1.9	S	08Lao-Veg-098	0.4	HR	GJ93/020	2.0	S
07Lao-Veg-074	0.4	HR	08Lao-Veg-099	0.0	HR	GJ93/025	2.0	S
07Lao-Veg-078	1.4	S	08Lao-Veg-100	1.1	S	GJ93/043	2.0	S
07Lao-Veg-079	0.8	MR	08Lao-Veg-106	0.2	HR	GJ93/060	2.0	S
07Lao-Veg-080	1.5	S	08Lao-Veg-107	1.6	S	GJ93/066	2.0	S
07Lao-Veg-089	1.6	S	08Lao-Veg-113	1.5	S	GJ93/076	1.8	S
07Lao-Veg-091	0.1	HR	08Lao-Veg-114	0.8	MR	GJ93/078	2.0	S
07Lao-Veg-106	1.6	S	08Lao-Veg-115	1.7	S			
07Lao-Veg-107	1.8	S	[Control Varieties]			[Control Varieties]		
07Lao-Veg-109	1.7	S	SCM334	0.0	HR	SCM334	0.0	HR
07Lao-Veg-133	1.2	S	AC2258	0.0	HR	AC2258	0.0	HR
08Lao-Veg-006	1.6	S	Berumasari	0.0	HR	Berumasari	0.0	HR
08Lao-Veg-031	1.0	S	Ace	2.0	S	Ace	2.0	S

[z] Diseased Index (DI): Average of symptom index. Symptom index were scored as follows; 0= symptomless, 1= wilting and 2= death.

[y] Degree of resistance; HR (highly resistance) means DI ≤ 0.5, MR (moderate resistant) means 0.5 < DI < 1.0 and S (susceptible) means 1.0 ≤ DI.

In experiment 1, the seeds were sown on 5 September 2014 in sterilized soil in flats and germinated in a greenhouse. On 29 September, seedlings were dug out, and their roots were

washed and then dipped into the inoculum suspensions for at least 1 min. Then 10 seedlings of each accession were transplanted into flats filled with sterilized soil in a controlled-environment system (OMY-4EB, Ozawa-Seisakusho, Kyoto, Japan). The soil temperature was maintained at about 28 °C, and the air temperature was kept above 10°C. For the evaluation of resistance, each plant was scored for Phytophthora blight symptoms on 16 October. The scale of resistance (hereafter, the "symptom index") was 0 = symptomless, 1 = wilting, and 2 = dead. The disease index (DI) for each accession was calculated as the average symptom index. Each accession was classified as highly resistant (HR) = DI ≤ 0.5, moderate resistant (MR) = 0.5 < DI < 1.0, or susceptible (S) = 1.0 ≤ DI.

Similar tests were performed in experiment 2 and 3, with the following exceptions: in experiment 2, seeds were sown on 8 September 2014, seedlings were dug out and inoculated on 3 October, and resistance was evaluated on 20 October; and in experimant.3, seeds were sown on 5 March 2015, seedlings were dug out and inoculated on 2 April, and resistance was evaluated on 23 April.

Table 2. Resistance of the *Capsicum* accessions collected in Laos, Vietnam and Ghana to Phytophthora blight in 2015 (experiment 3).

Accessions	Disease Index[z]	Degree of Resistance[y]	Accessions	Disease Index[z]	Degree of Resistance[y]	Accessions	Disease Index[z]	Degree of Resistance[y]
[Accessions from Laos]			[Accessions from Vietnam]			[Accessions from Ghana]		
08Lao-Veg-143	1.1	S	96VNV046	1.2	S	GJ93/041	2.0	S
08Lao-Veg-144	0.9	MR	96VNV055	1.9	S	GJ93/079	2.0	S
08Lao-Veg-145	1.5	S	96VNV056	0.2	HR	GJ93/081	2.0	S
08Lao-Veg-151	1.4	S	96VNV057	0.2	HR	GJ93/096	2.0	S
08Lao-Veg-160	1.8	S	96VNV063	0.9	MR	GJ93/097	1.6	S
08Lao-Veg-164	1.5	S	96VNV097	2.0	S	GJ93/098	1.3	S
08Lao-Veg-165	1.4	S	96VNV099	1.4	S	GJ93/099	1.6	S
08Lao-Veg-166	1.4	S	96VNV100	1.7	S	GJ93/100	2.0	S
08Lao-Veg-167	0.4	HR	96VNV101	0.2	HR	GJ93/102	2.0	S
08Lao-Veg-175	0.8	MR	96VNV102	0.6	MR	GJ93/103	1.9	S
08Lao-Veg-176	0.4	HR	96VNV116	0.2	HR	GJ93/120	1.2	S
08Lao-Veg-181	1.3	S	96VNV119	1.4	S	GJ93/128	2.0	S
08Lao-Veg-182	1.5	S	96VNV120	1.2	S	GJ93/149	2.0	S
08Lao-Veg-192	0.7	MR	96VNV121	0.3	HR	GJ93/168	2.0	S
08Lao-Veg-193	1.2	S	96VNV122	0.0	HR	GJ93/188	2.0	S
08Lao-Veg-197	1.2	S	96VNV123	0.7	MR	GJ93/222	2.0	S
08Lao-Veg-199	0.3	HR	96VNV124	1.2	S	GJ93/246	1.5	S
08Lao-Veg-200	0.7	MR	96VNV138	1.5	S	GJ93/249	1.5	S
08Lao-Veg-214	0.3	HR	96VNV143	1.9	S	GJ93/260	1.2	S
09Lao-Veg-029	1.1	S	96VNV144	0.6	MR	GJ93/272	1.7	S
09Lao-Veg-030	0.7	MR	96VNV145	0.6	MR	GJ93/287	1.2	S
09Lao-Veg-053	1.1	S	96VNV147	0.0	HR	GJ93/289	2.0	S
09Lao-Veg-060	0.9	MR	96VNV156	1.3	S	GJ93/302	0.8	MR
09Lao-Veg-081	1.5	S	96VNV157	2.0	S	[Control Varieties]		
09Lao-Veg-099	1.7	S	96VNV158	2.0	S	SCM334	0.0	HR
[Accessions from Vietnam]			96VNV159	2.0	S	AC2258	0.0	HR
96VNV011	0.4	HR	96VNV163	1.4	S	Berumasari	0.0	HR
96VNV027	0.5	HR	96VNV165	2.0	S	Ace	2.0	S
96VNV041	1.0	S						

[z] Diseased Index (DI): Average of symptom index. Symptom index were scored as follows; 0= symptomless, 1= wilting and 2= death.

[y] Degree of resistance; HR (highly resistance) means DI ≤ 0.5, MR (moderate resistant) means 0.5 < DI < 1.0 and S (susceptible) means 1.0 ≤ DI.

3. Results and Discussion

In experiment 1, 6 accessions were classified as HR, of which 1 accession, 08Lao-Veg-099, from Laos showed no disease symptoms (Table 1); in addition, 6 accessions were MR, and the remaining 27 were S. In experiment 2, 1 accession was HR and the remaining 15 were S. In experiment 3, 4 accessions from Laos and 9 from Vietnam were HR, of which 2 from Vietnam (96VNV122 and 96VNV147) showed no disease symptoms (Table 2). In addition, 6 accessions from Laos, 5 from Vietnam, and 1 from Ghana were MR. The remaining 54 accessions were S.

We evaluated a total of 134 *Capsicum* accessions (64 from Laos, 31 from Vietnam, and 39 from Ghana), for their resistance to *Phytophthora* blight. We found 20 highly resistant accessions: 10 from Laos, 9 from Vietnam, and 1 from Ghana, of which 1 Laotian accession and 2 Vietnamese accessions showed no disease symptoms and will be highly promising breeding materials. We found 18 moderate resistant accessions: 12 from Laos, 5 from Vietnam, and 1 from Ghana. Thus, 34.4% of the Laotian accessions and 46.7% of the Vietnamese accessions were highly or moderate resistant, versus only 5.1% from Ghana. This suggests that it will be relatively easy to find resistant accessions in Laos and Vietnam, but harder in Ghana.

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P3-10

Examination of bioactive components and aroma compounds in spice paprika

Miklós Pék[1], Helga Molnár[2], Rita Tömösközi-Farkas[2], Nóra Kisbocskói Adányiné[2]

[1] National Agricultural Research and Innovation Center, Vegetable Crop Research Department, Kalocsa, Hungary

[2] National Agricultural Research and Innovation Center, Food Science Research Institute, Budapest, Hungary

Abstract

In the frame of the scientific cooperation, five spice pepper varieties were investigated in order to determine the alterations of the bioactive components and aroma compounds during the postharvest ripening (six weeks period) and storage (1 year). The examined genotypes 'Kaldóm', 'Szegedi 178', 'Szikra', 'Delikát', 'Szegedi 80' are the most commonly grown varieties in the area of Kalocsa and Szeged. In this study the bioactive components and aroma compounds of the Hungarian paprika (spice pepper powder) were determined, as the most specific quality parameters.

Following the common methods of industrial paprika processing some samples were prepared with stalk and compared to samples of paprika powder made without stalk, based on everyday practice of small manufactures. The amount of carotenoids, tocopherols and ascorbic acid were detected by high-performance liquid chromatography (HPLC) and aroma compounds were detected by gas chromatography mass spectrometry (GC-MS). Considering the concentration of carotenoids, tocopherol and ascorbic acid, the values were generally higher in paprika powders made without stalk. The amount of carotenoids and tocopherols significantly increased in paprika ripened after harvest. More aroma components were detected from the hot variety (Szegedi 178) and from the samples made without stalk.

1. Introduction

The spice pepper cultivation was spread in the areas of Kalocsa and Szeged region at the end of the XVIII. century. The continuous development of the growing technology and the thoughtfully selected Hungarian spice pepper (*Capsicum annuum* L.) landraces and varieties which was adapted to the climatic conditions and soil characteristics were the main factors resulting the high quality - worldwide famous – brand of Hungarian paprika (spice pepper powder).

Nowadays both of "Ground paprika from Kalocsa" and "Ground paprika from Szeged" have the registration of protected geographical indications and designations of origin.

Paprika powder, as natural source of carotenoids, is one of the most familiar natural colourant. Spice red pepper is considered as one of the HUNGARICUM, special Hungarian products and as its colour intensity and aroma profile is excellent.

The study was carried out from five relevant spice pepper varieties concerning the effect of the post-ripening phase and the presence of the stalk (CS) in paprika powders from Kalocsa on the quality during storage period.

2. Material and methods

2.1. Spice pepper varieties

Delikát F1 is a sweet, bacterial leaf spot resistant variety, with semi-determinate growth habit and erect fruits. The fruits are suitable to produce high quality paprika powder.

Kaldóm is a sweet, bacterial leaf spot resistant variety with semi-determinate growth habit and erect fruits. This cultivar needs shorter vegetation period than other varieties and suitable for direct seeding. The fruits has special aroma so this variety is recommended for producing high quality paprika powder.

Szegedi 80 is a sweet variety with indeterminate growth habit and pendulous fruits. Nowadays this is the most common growing cultivar for producing paprika powder.

Szegedi 178 is a hot variety with indeterminate growth habit and pendulous fruits. Recently this is the most common growing cultivar for producing hot spicy pepper-cream but it is also suitable to produce hot paprika powder.

Szikra F1 is a hot, bacterial leaf spot resistant hybrid variety, with indeterminate growth habit and pendulous fruits. The fruits are as good for producing hot spicy pepper-cream as for producing hot paprika powder.

2.2. Determination of carotenoid content

Half gram of ground spice paprika sample from each variety was extracted by adding 50 ml of a solvent mixture consisting of 2:1:1 1,2-dichloroethane-acetone-methanol, followed by mechanical shaking for 15 min, filtration through MN 615 type filter paper and evaporation of solvent under vacuum by a rotary evaporator. The residues were re-dissolved in 10 ml of solvent mixture consisting of 55:35:10 isopropanol-acetonitrile-methanol. 20 µl of the extract was injected onto the HPLC column ([2] Biacs et al., 1994).

A Waters Alliance high pressure liquid chromatographic instrument consisting of a Model 2695 Separation Module and a Model 2996 Photodiode Array Detector was used for the analysis of carotenoids. We performed the data processing and the operation by Empower software. The carotenoid extract was separated on Nucleosil C-18, 3 µm, 250 x 4.6 mm column using gradient elution starting with 93% methanol (A) and 7% water (B) for 20 min; changing to 25% methanol and 75% isopropanol-acetonitrile-methanol (55:35:10) (C) for 13 min; changing to 10% methanol and 90% isopropanol-acetonitrile-methanol (55:35:10) for 9 min and returning to 93% methanol and 7% water for 3 min ([3] Daood et al., 2005). The detection was adjusted 470 nm. The flow rate was 0.7 ml/min. Peak identification was based on comparison of retention and spectral characteristics of each sample peak with those of available standards like β-carotene and zeaxanthin and with literature data ([1] Baurnfiend, 1981; [7] Socacia, 2008) when standards were not available.

2.3. Determination of vitamin C component

To extract vitamin C, 1 g sample of ground spice paprika was mixed with 25 ml of 3% meta-phosphoric acid solution and this mixture was shaken mechanically for 15 min at ambient temperature and filtered. The filtrate was further cleaned up by passing through a 0.45 µm filter (Chromafil A-45/25, Cellulose mixed esters) before injection. 20 µl of this extract was injected onto the HPLC column.

An Agilent Technologies 1200 Series high pressure liquid chromatographic instrument consisting of a Quaternary Pump and a Diode Array and Multiple Wavelength Detector SL was used for the analysis of vitamin C. The separation of vitamin C (L-ascorbic acid) was performed on Nautilus Nucleosil C-18, 3 µm, 150 x 4.6 mm column and gradient elution beginning with 1:99 for 3 min, changing to 30:70 for 7 min, to 40:60 for 5 min and returning to 1:99 for 2 min acetonitrile-0.1% potassium-dihydrogen-phosphate solution. Detection was adjusted 265 nm. The flow rate was 0.7 ml/min. L-ascorbic acid was identified and quantified by using a standard material.

2.4. Determination of ASTA Colour Value

ASTA Colour Value of the samples was determined according to the MSZ 9681-5:2002 standard. 0.1 g sample of ground spice paprika was extracted by adding 100 ml of acetone; it was shaken and put to dark place for 2 hours. The extract was filtered and measured the absorbance at 460 nm with a Jasco UV/VIS spectrophotometer (Model 7850) instrument using acetone as a blank. The ASTA Colour Value of the samples was determined from the measured absorbance value with the help of formula.

2.5. Determination of tocopherol content

From 0.5 g of ground paprika tocopherols were extracted by adding 5 ml 30% methanolic KOH, 0.5 g ascorbic acid and 20 ml methanol followed by refluxing the mixture for 35 min at the boiling point of methanol ([8] Speek et al., 1985). After cooling with cold trap water, the tocopherol fraction was extracted twice by shaking with 40 ml n-hexane in a separating funnel. The hexane fractions were pooled and washed 3 times with distilled water. The hexane phase was dried over anhydrous Na2SO4 and the solvent was evaporated with vacuum by a rotary evaporator ([4] Daood et al., 2014). The residue were re-dissolved in 5 ml of HPLC grade n-hexane and injected onto the HPLC column.

A Jasco HPLC (880-PU) instrument consisting of a Shimadzu C-R6A Integrator and an RF-535 Fluorescence HPLC Monitor Detector was used for the analysis of tocopherols. Under normal-phased chromatographic conditions the separation was performed on Nucleosil-100, 5 µm, 250 x 4.6 mm column with an isocratic elution of 99.6:0.4 n-hexane-ethanol. The fluorescent detector was adjusted at 295 nm and 320 nm as extinction and emission respectively. The flow rate was 0.9 ml/min. Identification was based on standard materials.

2.6. SPME analysis of paprika aroma

1 g of ground paprika was placed in headspace vial (40 ml volume) and capped. It was heated at 50°C for 30 min, then the SPME needle was pierced through the septum, and the plunger was depressed to expose the fibre to the headspace region of the sample vial. The fibre was exposed to the optimum headspace sampling condition of heating at 50°C for 30 min. The volatile was desorbed at 250°C in the GC injection port and flushed into the GC column ([6] Mazida et al., 2004).

An HP 5890/II gas chromatograph (Hewlett Packard) equipped with a 30 m x 0.25 mm x 0.25 µm RH-5ms+ capillary GC column and a 5971 mass selective detector were used to analyse the volatile compounds of spice paprika powders. The initial oven temperature was 60°C and then increased to 280°C at a rate of 3°C/min. The injector was operated in splitless mode at 250°C. The detector was run at 280°C. Helium of 4.8 grade was used as a carrier gas. The

detection was performed in the 35-350 mass range ([5] Csóka et al., 2012). The SPME used in this experiment was the manual type, consisting of a SPME fibre holder (manual) and a 100 µm PDMS coated fused silica fibre (Supelco). Compound recognition was based on mass spectra identification spectrum-library. The identification of the mass spectra recorded in total ion chromatograms of the samples were performed by manual mode search in the Wiley275.L spectrum library after individual background correction.

3. Results and discussion

In each sample the concentration of free capsanthin was greatly decreased during post-ripening and storage, however the total amount did not changed so much. Among fresh samples the Szegedi 80 (Sz80) paprika powder had the highest, 286 µg/g, and the Delikát F1 (D) paprika powder had the lowest, 180 µg/g, free capsanthin content. After post-ripening these values changed to 248 and 144 µg/g. During storage the free capsanthin concentration further decreased, so in fresh stored Szegedi 80 (Sz80) and Delikát F1 (D) it changed to 201 and 151 µg/g. In terms of post-ripened stored Szegedi 80 (Sz80) and Delikát F1 (D) these values were 191 and 118 µg/g. The level of free capsanthin concentration decreasing was the lowest in all Szikra F1 (Sz) paprika powder. (Figure 1.)

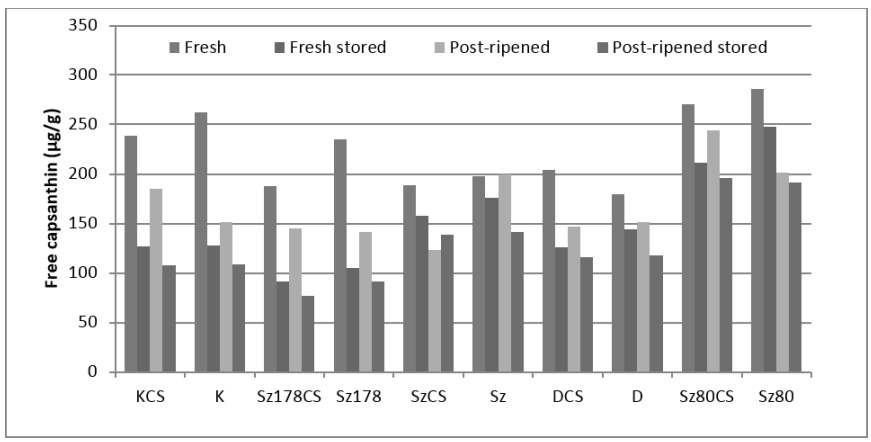

Figure 1
Free capsanthin content of paprika powders

The content of total carotenoids was significantly increased during post-ripening, but during storage it was decreased in all sample. Due to the presence of stalk the concentration was lower with about 10%. During post-ripening the amount of total corotenoids were increased with about 10% in Kaldóm (K) and Szegedi 178 (Sz178) samples. It was the lowest level of increasing. At harvest the Szikra F1 stalk (SzCS) paprika powder had 2477 µg/g total carotenoids content, that was the lowest. After post-ripening the value of total carotenoids was the highest in the Szegedi 80 (Sz80) paprika powder, 5272 µg/g. As long as the concentration of total carotenoids in post-ripened stored samples were decreased with 12%, in fresh stored samples with 30%, so post-ripening is important. (Figure 2.)

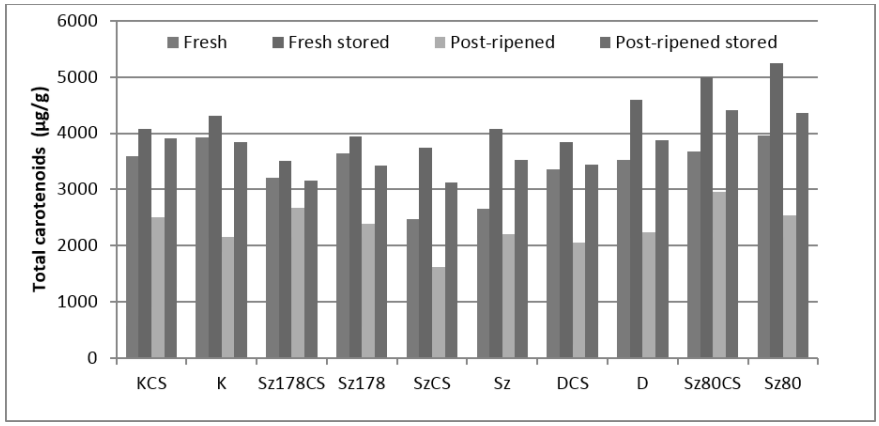

Figure 2
Total carotenoids content of paprika powders

During post-ripening the vitamin C content of red pepper fruit was greatly decreased, and during storage it was also slightly decreased in paprika powder. The quantity of vitamin C was 8548 µg/g in the fresh Kaldóm (K) paprika powder and 7438 µg/g in the fresh Delikát F1 (D) paprika powder. These were the highest vitamin C values. In the fresh-stored Szikra F1 (Sz) paprika powder the value of vitamin C was 126 µg/g, it was the lowest.

Total tocopherols content of paprika powders significantly less changed than previously mentioned parameters. While during post-ripening it was increased slightly (~8%), during storage it was clearly decreased. The total tocopherols content in fresh stored samples showed higher rate of decrease (~27 %) than in the post-ripened stored ones (~8%). In paprika powders the presence of the tocopherols is important because of its antioxidant properties, they prevent colour fading occurred in oxidative processes. (Figure 3.)

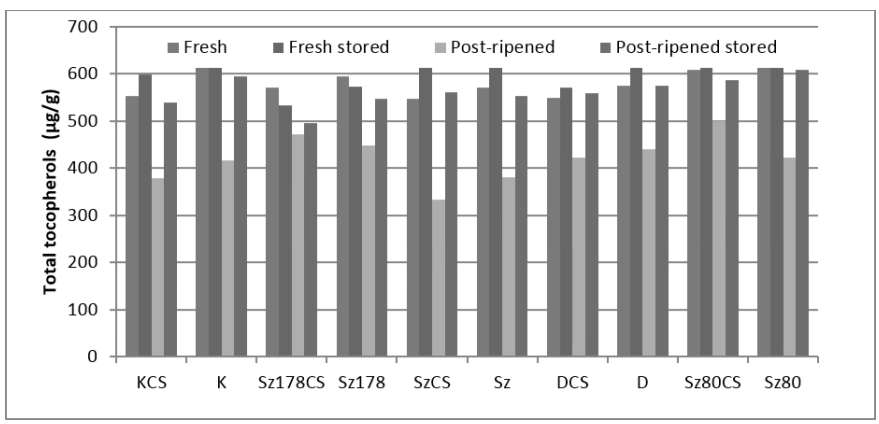

Figure 3
Total tocopherols content of paprika powders

In paprika powders without stalk more aroma components were detected than in paprika powders with stalk. During post-ripening the samples were changed aromatic and it was further raised during storage. In case of Szegedi 178 (Sz178) paprika powder without stalk the

following aroma components formed meanwhile post-ripening: 3-hydroxy-2-butanone, heptadecane, 3,8-dimethyl-decane, trans-caryophyllene, α-longipinene, alloaromadendrene, 2-propanone. β-selinene, aristolen, benzaldehyde, eremophilene, β-himachalene and 2-methyl-pentadecane were detected in the Sz178 post-ripened stored sample. The ß-ionone terpene compound occured just in stored samples. In Sz178 we detected the α-longipinene and ß-himachalene sesquiterpene aroma components as this sample was a pungent paprika. The 2-methyl-tridecane hydrocarbon was detected in Sz178 fresh, fresh stored and post-ripened stored samples with 13.33, 7.19 and 9.01 area %. The β-elemene sesquiterpene and the 3,8-dimethyl-decane hydrocarbon were detected in Sz178 post-ripened sample with 7.11 and 9.42 area %. (Figure 4.)

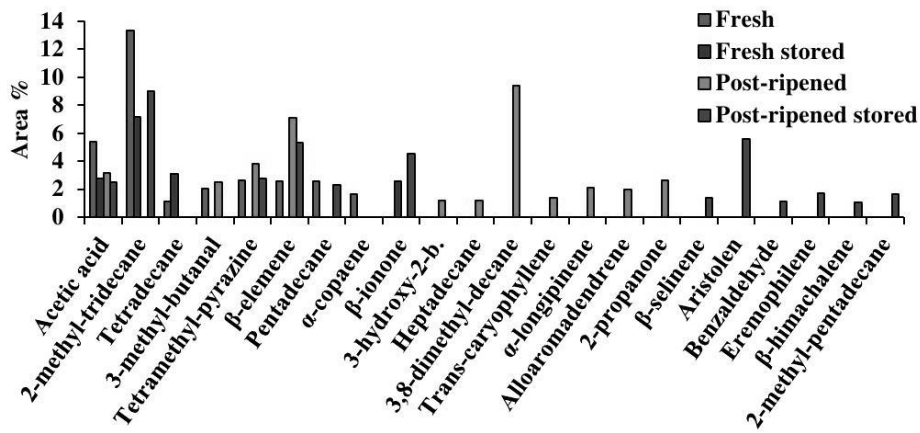

Figure 4
Aroma components in Szegedi 178 fresh, fresh stored, post ripened and post ripened stored paprika powders without stalk

4. Conclusions

According to our results Kaldóm variety had the highest free capsanthin and vitamin C content but its aroma composition was the poorest. By contrast the most varieties of aroma components were detected in Szegedi 178 pungent variety. In case of Szikra F1 pungent variety the concentration of total carotenoid and vitamin C was the lowest. The Szegedi 80 variety was outstanding in terms of total carotenoid and free capsanthin content. Among the 3 not pungent variety (Kaldóm, Delikát F1, Szegedi 80) the Szegedi 80 was mostly outstanding as regards the concentration of bioactive components and among the 2 pungent varieties (Szegedi 178, Szikra F1) the Szegedi 178 was remarkable. The parameters of harvest, post-ripening, processing and storage influence the bioactive components of paprika powders. In terms of aroma composition, total carotenoids and total tocopherols content post-ripening is important.

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P3-11

Root morphology and phosphorus starvation adaptation in pepper

Leandro Pereira-Dias[1], Daniel Gil Vilar[1], Adrián Rodriguez-Burruezo[1], Salvador Lopez-Galarza[2], Alberto SanBautista[2], Vicente Castell[2] and Ana Fita[1]

[1] *Instituto de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV), Universitat Politècnica de València, Valencia, Spain*

[2] *Departamento de Producción Vegetal, Universitat Politècnica de València, Valencia, Spain*

Abstract

Breeding plants for lower inputs in terms of fertilizers is a breeding objective which is gaining attention. In general, one of the bases of the record yields of modern agriculture is the use of synthetic fertilizers. However, climate change and other challenges force us to a more sustainable agriculture. Lowering the inputs needed to grow crops is economically desirable and genetically possible. It has been demonstrated that in general our crops are not very efficient in terms of use of resources, especially if talking about fertilizers. Phosphorus is after N the most limiting mineral in plant nutrition, and the phosphate rocks which are used to produce the phosphate fertilizers are limited. Studies in some vegetables have demonstrated that there is genetic diversity for the use and adquisition of P. In addition, the root system plays a crucial role in the adquisition of the minerals from soils. The objective of this study was to correlate root characters in pepper with a better adaptation to P starvation. To check whether there is a correlation within the root system and a better adaptation to P starvation six accessions of *Capsicum sp* were tested: five of *C. annuum* and one of *C. chinense*. Plants were grown in summer cycle in 10 L pots filled with a 1:1 mixture of soil and sand in a greenhouse. Plants were irrigated with normal nutrient solution for pepper and one without any source of P. At 60 d after transplanting, plants were harvested and the root systems carefully collected. In each root system lateral roots of diameter < 0.5 mm were removed and scored as 'fine roots'. Then each root system was spread over a transparent sheet to be scanned. Every image was analyzed using specific software (WinRhizo Pro) which measured the total root length, average root diameter and length of roots of a certain diameter. Aerial part, lateral roots and fine roots, fresh and dry weights were recorded. P content in the plants was analyzed through ICP to then calculate the phosphorus uptake efficiency (PUpE) and the phosphorus use efficiency (PUE) of each genotype analyzed. The results showed that P deficiency had a great effect on the percentage of fine roots within a root system, increasing it. The correlation analysis of the root parameters and the PUE, PUpE showed that the only root parameter related to PUpE was the percentage of fine roots. The differences in the response of the assayed genotypes to P starvation confirmed that it is possible to breed peppers adapted to low inputs of P.

1. Introduction

Record yields achieved by modern agriculture are based on the use of improved varieties and improved agricultural practices which include: irrigation, use of pesticides and fertilizers. The sustainability of this model of agriculture is starting to be questioned, specially regarding the new challenges imposed by the climate change scenario. Lowering the fertilization inputs is economically desirable for the farmers and environmentally needed to reduce eutrophyzation of waters. This is particularly true in the case of phosphorus fertilization as P rock is a limited

resource, and therefore P fertilizer prices will increase in next years [1].

Improving the ability of the crops to uptake and use P is a breeding objective that can be achieved as long as there exists genetic diversity. This diversity has been demonstrated in some crops [2,3]. In addition, part of the efficient response to low P inputs is due to an adapted root system [4]. The objective of this study was to correlate root characters in pepper with a better adaptation to P starvation.

2. Material and methods

Six accessions of *Capsicum sp* were tested: five of *C. annuum* and one of *C. chinense*.

Table 1
Accessions assayed in the experiment

Species	Accesion	Code	Origin
C. chinense	Chinense 2	Chin	Ecuador
C. annuum	Bola	Bola	Cons. Reg. I.G.P. Pimentón de Murcia
	Piquillo	Piq	Cons. Reg. D.O.P. Piquillo de Lodosa, Navarra
	Jalapeño Espinalteco	Jal	México
	Pasilla	Pas	México
	California	Cal	Experimental line

Nine plants per genotype and treatment were grown in summer cycle in 10 L pots filled with a 1:1 mixture of soil and sand in a greenhouse. Plants were irrigated with normal nutrient solution for pepper (15 mmol L^{-1} NO_3^-, 1.5 mmol L^{-1} NH_4^+, 1.9 mmol L^{-1} K^+, 1.6 mmol L^{-1} Ca^{2+}, 3.25 mmol L^{-1} Mg^{2+}, 1.5 mmol L^{-1} P) which was the control and the same solution without any source of P (NoP). At 60 d after transplanting, plants were harvested and the root systems carefully collected.

In each root system lateral roots of diameter < 0.5 mm were removed and scored as 'fine roots'. Then each root system was spread over a transparent sheet to be scanned. Every image was analyzed using specific software (WinRhizo Pro) measuring the total root length and average diameter of the roots. The total length of i) roots with a diameter within 0.5 mm a 1 mm and ii) roots with a diameter >1 mm (the rest of roots) were also recorded. On the basis of both data, the percentage of roots with a diameter within 0.5 mm a 1 mm on the total root length was calculated. Aerial part, lateral roots and fine roots, fresh and dry weights were recorded. P content in the plants was analyzed through ICP to then calculate the phosphorus uptake efficiency (PUpE) as the increase in total plant P content when passing from low to high P conditions:

$$PUpE\ (mg\ P) = ([P_{control}]*DW_{control}) - ([P_{NoP}]*DW_{NoP}).$$

The phosphorus use efficiency was then obtained as the increase in yield per unit increase in plant P content:

$$PUtE\ (mg\ DW\ mg^{-1}\ P) = (DW_{control} - DW_{NoP}) / [([P_{control}]*DW_{control}) - ([P_{NoP}]*DW_{NoP})]$$

3. Results and discussion

There were significant differences among genotypes for all analyzed traits. The genotypes also responded differently to P starvation. NoP treatment did not affect the root length, which only signficantly increased in California (Fig 1). The effect of the NoP treatment on the average root diameter was reducing it, so it passed from an average of 0.66 mm in control conditions to 0.60 mm in NoP treatment (Fig 1). One of the biggest effects of the treatments was the increase of the proportion of roots with diameter between 0.5-1 mm (Fig 1) under NoP. Under control conditions this parameter was 83%, rising up to 88% under NoP conditions. The effect of the P starvation on the dry weight of roots < 0.5 mm of diameter (evaluated as percentage from the total root dry weight) was very variable, with increases for *C. chinense*, reductions for Jalapeño espinalteco and any significant difference for the others (Fig 1).

In addition to the root morphology parameters the levels of P and the PUpE and PUE were also measured (Fig 2). Piquillo and Jalapeño outstood for their PUpE whereas Bola and Pasilla outstood for their PUE. This indicates that there exists diversity in the response to the P levels in soil.

The Pearson correlation calculated among the PUpE, PUE values and the root parameters showed that PUpE was significantly correlated to the percentage of fine roots of the root system with r^2=0.85 in control conditions and with r^2= 0.88 in noP.

The results indicate that the conditions of P starvation induce changes in the root morphology, mainly increasing the absortive area through the production of fine roots and elonganting roots. This response has been reported earlier in other crops [4]. The high correlation between PUpE and the percentage of fine roots point this parameter as a good candidate to phenotype pepper plants for a good phosphorus adquisition. PUE measures the efficiency in using the P already uptaken by the plant, therefore it depends on the metabolic pathways of mobilization and remobilization of P rather than in the adquisition of it from the soil [5]. As a result it is not surprising the lack of correlation between PUE and the root parameters.

4. Conclusions

Breeding to improve the adaptation to low P supply is complex and depends on many factors. However these preliminary results point to a key role of the root system in the adquisition of P, being specially important the fine roots which increase under low P conditions.

In addition the different PUpE and PUE values for the assayed genotypes indicate that there exists genetical diversity useful to breed new pepper cultivars with improved ability to use and adquire P.

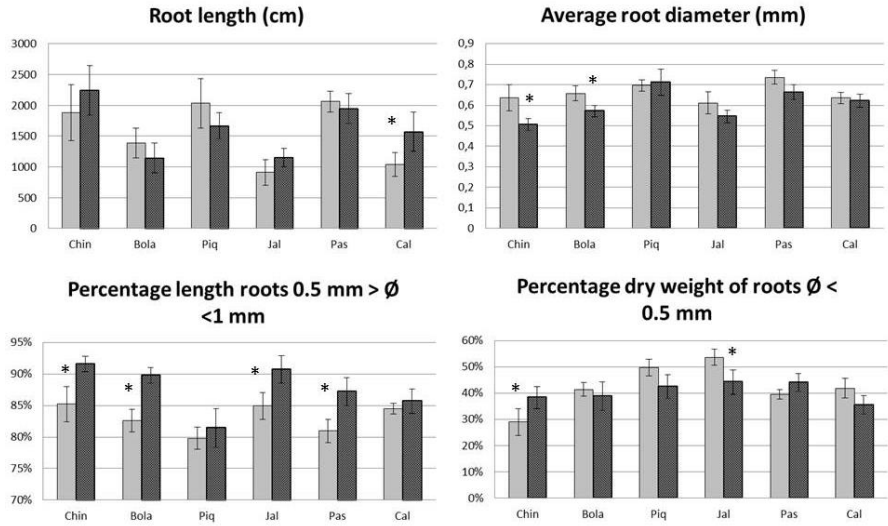

Figure 1
*Average values ± SE per genotype and treatment of the root parameters evaluated. Each bar is the average of at least 9 plants. * indicates significant differences among avegares of control and NoP treatments.*

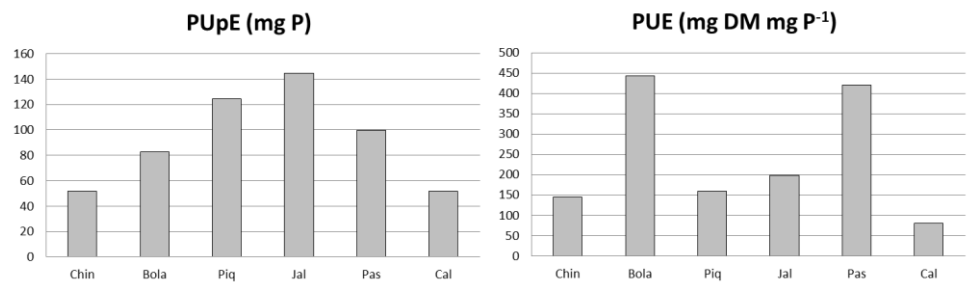

Figure 2
Phosphorus uptake efficiency (PUpE) and Phosphorus use efficiency (PUE) calculated in the different genotypes assayed.

5. Acknowledgements

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P3-12

Morphological diversity and resistance to soil-borne diseases in a core collection of eggplant developed at the NARO Institute of Vegetable and Tea Science

Takeo Saito, Hiroyuki Fukuoka and Hiroshi Matsunaga

NARO Institute of Vegetable and Tea Science (NIVTS), Tsu, Mie, Japan

Abstract

Eggplant (*Solanum melongena* L.), a solanaceous plant native to India, has long been one of the most important vegetable crops in the world. Great variations have been found in eggplant agro-morphological characteristics (plant morphology and fruit characteristics), its resistance to diseases, and environmental adaptation. The National Institute of Agrobiological Sciences (NIAS, Japan) and the NARO Institute of Vegetable and Tea Science (NIVTS, Japan) have gathered and maintained a collection of nearly 1100 eggplant accessions. However, screening of these germplasms for traits of interest is laborious and costly. Establishing subsets of a collection, so-called core collections, which represent the diversity of the entire collection, makes screening more practical. A core collection of 100 eggplant accessions selected from among nearly 1100 accessions from the NIAS Genebank collection is being developed. In the present study, morphological diversity and resistance to soil-borne diseases were assessed in this core collection. Morphological traits, such as characteristics of the whole plant, stems, leaves, flowers, and fruits were examined in the entire core collection, and resistance to bacterial wilt and Fusarium wilt were evaluated in a subset of the core collection. A wide range of variations of 35 descriptors was recorded.

1. Introduction

Eggplant (*Solanum melongena* L.) is one of the few cultivated solanaceous species originating from the Old World (Daunay, 2008). The major eggplant producers are China, India, Iran, Egypt, Turkey, Iraq, Indonesia, Japan, Italy, and the Philippines (FAO, 2013). In Japan, it is grown on 9,700 ha with a total production of 321,200 t (MAFF, 2013). The National Institute of Agrobiological Sciences (NIAS, Japan) and the NARO Institute of Vegetable and Tea Science (NIVTS, Japan) have gathered and maintained a collection of nearly 1100 eggplant accessions. Cultivated types have various morphology, physiology, and quality (Kumar et al., 2008). However, it is difficult to evaluate, manage and utilize a large number of accessions.

Core collections, which are subsets of accessions selected to represent the genetic diversity in the collection and minimize repetitiveness, have been proposed as a good way of maintaining and managing germplasm collections for their further utilization in breeding programs (Frankel, 1984). There is increasing interest in developing core collections in many germplasm collections, including those of eggplant (Weihai et al., 2008; Gangopadhyay et al., 2010). A core collection of 100 eggplant accessions selected from among nearly 1100 accessions from the NIAS Genebank collection is being developed (Fukuoka et al. in preparation). In the present study, morphological diversity and resistance to soil-borne diseases were assessed in this core collection.

2. Materials and Methods

The core collection (100 eggplant accessions) was planted at NIVTS in open field culture in 2014 and 2015. Variation in 35 traits, including characteristics of the whole plant, stems, leaves, flowers, and fruits, was evaluated according to the descriptors of NIAS Genebank (https://www.gene.affrc.go.jp/manuals-plant_characterization_en.php). Resistance to bacterial wilt, Fusarium wilt, and Verticillium wilt was tested according to Saito et al. (2010).

3. Results and Discussion

All examined quantitative and qualitative descriptors varied widely (Figure 1 and Table 1). Several accessions were resistant to one or two of the soil-borne diseases. This core collection will be a useful resource for genomic studies of eggplant and for initiatives aimed at developing eggplant with improved agronomic traits. This core collection will be distributed by NIAS Genebank after sufficient amount of seeds of each accession becomes available.

Figure1
Fruits of some accessions from the eggplant core collection developed at the NARO Institute of Vegetable and Tea Science (NIVTS)

4. Acknowledgements

This work was supported by a grant (PGRAsia project) from the Ministry of Agriculture, Forestry and Fisheries of Japan.

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Table 1. *Characteristics of the 100 accessions of the eggplant core collection developed at the NARO Institute of Vegetable and Tea Science (NIVTS).*

No.	NIVTS ID	Name	Origin	Bw[1)]	Fw[2)]	Vw[3)]	Fruit skin color [4)]	Fruit weight (g) [5)]
15ECC001	ES010	GJ93/108	Ghana	S	S	S	G	115
15ECC002	ES016	GJ93/261	Ghana	S	S	S	G	433
15ECC003	ES090	SM5/2	Italy	S	S	S	W	454
15ECC004	ES091	SM5/21	Italy	S	S	S	LP	468
15ECC005	ES099	CCR3	Italy	S	S	S	P	390
15ECC006	ES123	EPL-1	Japan	S	S	S	DP	253
15ECC007	ES173	WHITE EGG	Pakistan	S	S	S	W	73
15ECC008	ES215	96VNV20	Vietnam	S	S	S	P	44
15ECC009	ES218	96VNV25	Vietnam	S	S	S	G	43
15ECC010	ES221	96VNV35	Vietnam	S	S	S	W	43
15ECC011	ES222	96VNV45	Vietnam	S	S	S	W	25
15ECC012	ES223	96VNV50	Vietnam	S	S	S	W	20
15ECC013	ES225	96VNV53	Vietnam	S	S	S	G	31
15ECC014	ES235	96VNV107	Vietnam	S	S	S	W	7
15ECC015	ES238	96VNV150	Vietnam	S	S	S	G	39
15ECC016	ES288	BATHAGODA	Sri Lanka	R	R	S	WP	164
15ECC017	ES301	KHA YAN THEY (TOT SAYA)	Myanmar	S	S	S	WP	83
15ECC018	ES311	KHA YAN (PHAN PYAUT)	Myanmar	S	S	S	G	184
15ECC019	ES328	KHA YAN	Myanmar	S	S	S	LP	57
15ECC020	ES345	KHA YAN PYAR	Myanmar	S	S	S	LP	648
15ECC021	ES376	MAKKHEUA HAMMA	Laos	S	S	S	DP	214
15ECC022	ES403	MAKKHEUA	Laos	S	S	S	W	32
15ECC023	ES405	Unknown	Laos	S	S	S	G	121
15ECC024	ES438	KHEUA KHEUNE	Laos	S	S	S	G	41
15ECC025	ES440	KHEUA VANE	Laos	S	S	S	G	66
15ECC026	ES446	KHEUA KHEUNE	Laos	S	S	S	G	41
15ECC027	ES454	MAKKHEUA	Laos	S	S	S	G	52
15ECC028	ES471	MAKKHEUNE	Laos	S	S	S	G	241
15ECC029	ES482	MM 00261	France	S	S	S	P	624
15ECC030	ES483	KHEUA KHEUNE	Laos	S	S	S	G	29
15ECC031	ES491	MAKKHEUA POM	Laos	S	S	S	W	29
15ECC032	ES492	MAKKHEUA POM	Laos	S	S	S	G	279
15ECC033	ES500	VIOLETTE DE TOULOUSE	France	S	S	S	LP	586
15ECC034	ES501	NOIRE DE CHATEAURENARD	France	S	S	S	P	360
15ECC035	ES509	ANNAMALAI	India	R	S	S	LP	145
15ECC036	ES511	AUB. TRIVAINDRUM	India	S	S	S	WP	83
15ECC037	ES512	FINGER SOLANUM	Indonesia	S	S	S	G	38
15ECC038	LS0038-03	GIGANTE	Brazil	S	S	S	G	1655
15ECC039	LS0038-04	FLORIDA MARKET	Brazil	S	S	S	P	440
15ECC040	LS0046-02	DANUBIANA 166	Romania	S	S	S	P	531
15ECC041	LS0222-04	Unknown	Unknown	S	R	S	LP	246
15ECC042	LS0222-12	Unknown	Unknown	S	R	S	W	385
15ECC043	LS0222-14	Unknown	Unknown	S	S	S	LP	447
15ECC044	LS0222-16	Unknown	Unknown	-	-	S	LP	439
15ECC045	LS0222-19	Unknown	Unknown	-	-	S	LP	943
15ECC046	LS0225	BRINJAL ROUNO	India	-	-	S	G	1095
15ECC047	LS0228	VIOLETTE LONGUE	France	-	-	S	LP	505
15ECC048	LS0335	C 9 D-0-0-1-0	Indonesia	-	-	S	G	115
15ECC049	LS0356	ISLAMPURI	Bangladesh	-	-	S	WP	1067
15ECC050	LS0616-01	JAPANEE 1	Bangladesh	-	-	S	WP	308
15ECC051	LS0720	OOTOSHI	Japan	-	-	S	LP	472
15ECC052	LS0751	MUKTAKESHI A	Bangladesh	-	-	S	WP	278
15ECC053	LS0755	D.R.CHOUDHURY	Bangladesh	-	-	S	LP	376

Table 1. *(continued)*

No.	NIVTS ID	Name	Origin	Bw[1)]	Fw[2)]	Vw[3)]	Fruit skin color [4)]	Fruit weight (g) [5)]
15ECC054	LS0765	KHATKHOTIA	Bangladesh	-	-	S	WP	259
15ECC055	LS0766	SINGNATH	Bangladesh	-	-	S	WP	309
15ECC056	LS0778	GOWRI WHITE	Bangladesh	-	-	S	G	411
15ECC057	LS0817	BLACK BLAZER	Canada	-	-	S	P	410
15ECC058	LS0818	PURPLE CAPE	Canada	-	-	S	P	661
15ECC059	LS0967	KITSUTA	Japan	-	-	S	BP	308
15ECC060	LS0982	YATSUBUSA	Japan	-	-	S	LP	157
15ECC061	LS0988	Unknown	Japan	-	-	S	G	170
15ECC062	LS1005	OOMARU	Japan	-	-	S	DP	432
15ECC063	LS1027	0047	Egypt	-	-	S	W	295
15ECC064	LS1384	HETA MURASAKI	Japan	-	-	S	DP	157
15ECC065	LS1466	Unknown	Myanmar	-	-	S	WP	237
15ECC066	LS1506	Unknown	Malaysia	-	-	S	WP	404
15ECC067	LS1718	ARKA SHEEL	India	-	-	S	P	195
15ECC068	LS1719	ARKA SHIRISH	India	-	-	S	G	297
15ECC069	LS1721	P K	India	-	-	S	P	273
15ECC070	LS1722	PUSA PURPLE CLUSTER	India	-	-	S	P	43
15ECC071	LS1859	41-2-1	Malaysia	-	-	S	WP	258
15ECC072	LS1860	41-2-2	Malaysia	-	-	S	G	126
15ECC073	LS1896	49-5-2	Malaysia	-	-	S	G	160
15ECC074	LS1920	ARKA KUSUMAKAR	Malaysia	-	-	S	G	20
15ECC075	LS1951	417-3-3	Malaysia	-	-	S	P	512
15ECC076	LS1967	418-4-2	Malaysia	-	-	S	WP	301
15ECC077	LS2112	BAHANTA 1	Nepal	-	-	S	WP	112
15ECC078	LS2118	BAHANTA 4	Nepal	-	-	S	WP	452
15ECC079	LS2206	Unknown	Malaysia	-	-	S	WP	485
15ECC080	LS2219	Unknown	Malaysia	-	-	S	LP	310
15ECC081	LS2228	Unknown	Malaysia	-	-	S	WP	581
15ECC082	LS2234	Unknown	Malaysia	-	-	S	P	559
15ECC083	LS2235	Unknown	Malaysia	-	-	S	G	14
15ECC084	LS2237	Unknown	Malaysia	-	-	S	LP	562
15ECC085	LS2242	Unknown	Malaysia	-	-	S	G	13
15ECC086	LS2249	Unknown	Malaysia	-	-	S	G	32
15ECC087	LS2262	Unknown	Malaysia	-	-	S	G	33
15ECC088	LS2264	Unknown	Malaysia	-	-	S	WP	43
15ECC089	LS2283	Unknown	Malaysia	-	-	S	LP	469
15ECC090	LS2436	56	Malaysia	-	-	S	G	127
15ECC091	LS2439	59	Malaysia	-	-	S	LP	199
15ECC092	LS2440	60	Malaysia	-	-	S	G	189
15ECC093	LS2499	IK 29	Nepal	-	-	S	P	96
15ECC094	LS3632	IK 32	Thailand	-	-	S	W	142
15ECC095	LS3809	NAKATE SHINKURO	Japan	-	-	S	P	203
15ECC096	LS3872	GIANT OF BANARAS	India	-	-	S	W	2530
15ECC097	LS3996	SHITEN	Japan	-	-	S	W	76
15ECC098	LS4103	Unknown	China	-	-	S	G	106
15ECC099	LS4106	OKITSU 1	Japan	-	-	S	DP	221
15ECC100	LS4167	CHANG QIE ZI	China	-	-	S	LP	206

1) Resistance to bacterial wilt: S = sensitive, R = resistant, - = not yet examined.
2) Resistance to Fusarium wilt: S = sensitive, R = resistant, - = not yet examined.
3) Resistance to Verticillium wilt: S = sensitive, R = resistant.
4) Immature fruit: W = white, G = green, WP = whitish purple, LP = light purple, P = purple, DP = dark purple, BP = black purple.
5) Mature fruit.

P3-13

Tracing back the history of pepper (*Capsicum annuum* L.) in the Iberian Peninsula

Cristina Silvar[1], Ana Garcés-Claver[2], Cristina Mallor[2], Federico Pomar[1], Filomena Rocha[3], Ana M. Barata[3]

[1] *Grupo de Investigación en Bioloxía Evolutiva, Departamento de Bioloxía Animal, Bioloxía Vexetal e Ecoloxía, Universidade da Coruña, A Coruña, Spain*

[2] *Centro de Investigación y Tecnología Agroalimentaria de Aragón, Zaragoza, Spain*

[3] *Banco Portugues de Germoplasma Vegetal, Instituto Nacional de Investigação Agrária e Veterinária I.P., Braga, Portugal*

Abstract

Capsicum annuum was the first *Capsicum* introduced into Europe, likely trough Spain and Portugal, at the end of the XVth century. Therefore, the Iberian Peninsula constitutes a significant secondary diversification centre, where part of the original pepper genepool brought back from America might be preserved in the form of landraces. A previous work suggested that the diversification process of *C. annuum* in Spain may occur from an ancient population, still represented by some landraces with ancestral traits. The current work is focused on clarifying the origins and genetic relationships among the pepper landraces from the Iberian Peninsula. For that purpose, a larger number of Spanish peppers, a collection of Portuguese landraces and a panel of *C. annuum* resources from Mexico, including the wild relative *C. annuum* var. *glabriusculum* were genotyped with the DArTseq technology. Sequencing output consisted of 27,159 tags, of which 5,007 SNPs were selected for further analyses. Clustering and *STRUCTURE* approaches clearly differentiated wild and Mexican peppers from those originating in the Iberian Peninsula. Various Spanish and Portuguese accessions clustered within the Mexican group, while the others were primarily organized following a geographical pattern, although their genomic composition was not extremely different.

1. Introduction

Pepper (*Capsicum* spp) is one of the most important vegetables and spices in the worldwide trade. The genus has its origins in the tropical South American region centered in what is now Bolivia. The five most common cultivated species (*C. annuum*, *C. chinense*, *C. frutescens*, *C. baccatum* and *C. pubescens*) were independently domesticated as far back as 6000 B.C. in either Mesoamerica or South America [1]. After the first trip of Columbus and subsequent voyages of exploration to the New World, peppers moved worldwide, suffering from additional diversification at the secondary centers, which resulted in the awesome phenotypic variability that can be observed nowadays. *C. annuum* was domesticated in Mexico from the wild bird pepper (*C. annuum* var. *glabriusculum*) and it was likely the first pepper that entered Europe, being Spain the place of arrival [2]. Afterwards, the extensive trading routes of Spanish and Portuguese helped to disperse peppers around the globe. Therefore, the Iberian Peninsula constitutes a significant secondary diversification centre, where part of the original pepper genepool brought back from America might be preserved in the form of landraces. Landraces

represent native varieties empirically selected by farmers over time and well adapted to specific agro-climatic conditions. In the Iberian Peninsula, hundreds of phenotypically very diverse pepper landraces can still be found all over the territory due the heterogeneity of the land and the versatility of agro-climatic regions. However, the origins and relationships among these landraces have remained under-researched. In a previous work, thirty-nine Spanish landraces from the Vegetable Germplasm Bank of Zaragoza were investigated with a broad set of microsatellite (SSRs) markers. Results suggested that the diversification process of *C. annuum* in Spain may occur from an ancient population, still represented by some landraces with ancestral traits, such as the erect fruits [3]. The main goal of the present work was to investigate the genetic diversity, structure and population dynamics of pepper in the Iberian Peninsula. For that purpose, a larger number of Spanish peppers and a collection of Portuguese landraces were analysed and compared to a set of *C. annuum* resources from Mexico, including the wild relative *C. annuum* var. *glabriusculum*.

2. Materials and Methods

2.1. Plant material

A total of ninety-four pepper landraces from Spain (62), Portugal (19) and Mexico (10) were selected from the Vegetable Germplasm Bank of Zaragoza (BGHZ) (Spain), the Portuguese Plant Germplam Bank (BPGV) (Portugal), the Center for Genetic Resources (CGN) (Netherlands) and the Gene Bank at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) (Germany). All accessions were chosen based on the passport data stored at each gene bank, trying to cover a wide range of geographical areas and the maximum phenotypic variability. Three *C. annuum* var. *glabriusculum* from the germplams collection at INRA were kindly provided by Dr. Alain Palloix.

2.2. Genotyping

Total genomic DNA was isolated from young leaves of each accession using the NucleoSpin Plant XL kit (Macherey–Nagel), following the manufacturer's instructions. Genotyping was performed with a genotyping-by-sequencing approach (DArTseq) provided by the company Diversity Arrays Technology (Canberra, Australia, http://www.diversityarrays.com/). This system combines complexity reduction methods with next-generation sequencing platforms, targeting primarily genic regions.

2.3. Data analysis

DArTseq output was received as a file with tags sequences with SNP polymorphisms aligned through BlastN to the pepper reference genome (release v.1.55, http://peppergenome.snu.ac.kr).

Genetic relationships among accessions were depicted by using the Neighbour-Joining (NJ) algorithm. The software *STRUCTURE v2.3.4* was employed to assign individuals to populations based on their genomic composition. The tests were performed using an admixture model with correlated allele frequencies. Each run consisted of a burning period of 50,000 steps and 50,000 MCMC repetitions. The most likely number of populations (*K*) was inferred with the Evanno correction method [4]. The accessions were sorted into sub-populations based on their maximum membership probabilities (threshold level of 70%). The ones not showing a clear membership were classified as admixture.

3. Results and Discussion

3.1. Genotypic analysis

In total, 27,159 DArTseq tag sequences presented SNP polymorphisms. Of these, 22,531 (82.9%) were successfully aligned to any of the twelve pepper chromosomes (Table 1). The number of assigned tags ranged from 1,337 (chromosome P8) to 2,699 (P3). SNPs with over 10% of missing data or heterozygous alleles were removed, leaving 19,988 tags. Those SNPs with a major allele frequency above 0.95 were recorded as monomorphic. In total, 5,007 curated polymorphisms were used for further analyses.

Table 1
Statistics of the DArTseq output

Chr.	# tags	# P	%P	PIC
P1	2,340	430	23.28	0.294
P2	2,062	360	20.93	0.288
P3	2,699	561	26.70	0.304
P4	1,653	263	20.38	0.327
P5	1,636	283	24.39	0.297
P6	2,147	354	21.44	0.297
P7	1,740	349	28.26	0.302
P8	1,337	255	23.69	0.296
P9	1,721	363	32.55	0.328
P10	1,775	337	27.80	0.317
P11	1,814	348	28.24	0.324
P12	1,607	300	25.32	0.313
NA	4,628	804	25.47	0.317

Chr: pepper chromosome; # P: number of polymorphic tags; % P: percentage of polymorphism; PIC: Polymorphism Information Content; NA: not assigned

The chromosome P8 showed the lowest number of polymorphic loci (255), whereas chromosome P3 displayed the highest (561) (Table 1). The percentage of polymorphism slightly varied among chromosomes, ranging from 20.38% (P4) to 32.55% (P9). The lowest average Polymorphism Information Content (PIC) value was found in chromosome P2, while the highest was observed in chromosomes P4 and P9 (Table 1). The expected heterozygosity (H_e) for the whole set of *C. annuum* accessions was 0.29. Similar parameters of genetic diversity (polymorphism around 25% and H_e values close to 0.29) were reported in a diverse panel of *C. annuum* lines genotyped with the Affymetrix® GeneChip® Pepper Array [5].

3.2. Cluster and structure analyses

The NJ tree clearly separated the wild *C. annuum* and the Mexican accessions in a well-defined branch (group I), differentiated from peppers originating in the Iberian Peninsula (Fig. 1). Interestingly, four Spanish and two Portuguese peppers were also clustered within this group.

As previously reported by Nicolai *et al.* [6], the *C. annuum* var. *glabriusculum* PM669 and PM670 from Panama and Colombia, respectively, appeared closely related and slightly distant from the Mexican one (PM647). The majority of accessions from Portugal were comprised in groups III, VII and VIII, while the others are mostly represented by Spanish landraces (Fig. 1).

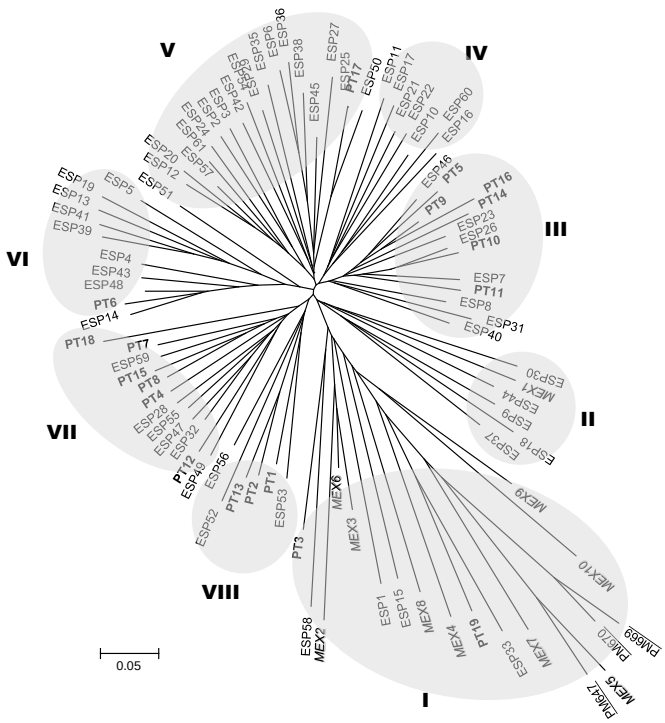

Figure 1
*NJ tree of 94 C. annuum accessions based on 5,007 SNPs. ESP: Spanish accessions, PT: Portuguese accessions (in **bold**), MEX: Mexican accessions (in italics). PM647, PM669 and PM670 (underlined) are wild C. annuum.*

The analysis with the software *STRUCTURE* pointed to the presence of three populations (ΔK=3), after the Evanno correction [4]. The accessions were sorted according their membership coefficients in three groups: A, comprising 7 genotypes, B, including 8 and C, consisting of 42 accessions. The remaining 36 *C. annuum* did not show clear memberships and they were considered to possess a mixed genomic composition, either between clusters A and B or between clusters B and C (Fig. 2). Accessions in population A primarily corresponded to those in the group I defined by the NJ tree, population B comprised accessions from groups II and VI and finally population C included mostly accessions from clusters III, IV and VII (Fig. 2). Clustering patterns derived from both analyses partially responded to geographical factors. Thus, accessions from the border regions in Spain and Portugal tended to group together. Similarly, Northern Spanish accessions primarly separated from those originating in the South. The genetic organization of peppers attending to morphological fruit traits is also expected [3]. However, owing to the phenotypic characterization of several accessions, mainly from Portugal, is on the way, clear morphological groups could not be established yet. Further work will also cover the exploration, based on the DArTseq tag sequences of those genomic regions controlling

traits of interest, such as pungency.

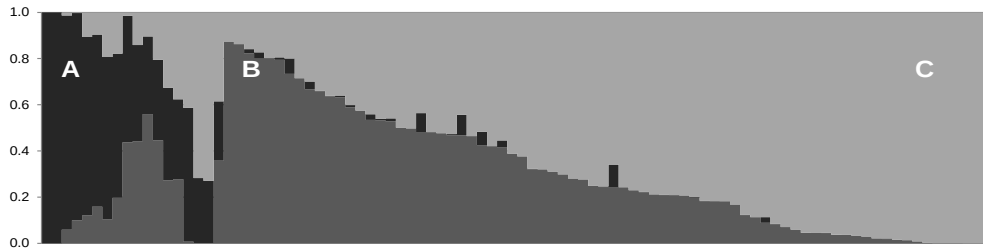

Figure 2
STRUCTURE analysis on 94 C. annuum accessions based on 5,007 SNPs. Accessions are ordered according to their membership coefficients. Cluster A is shown in black, cluster B in dark grey and cluster C in light grey.

4. Acknowledgements

This work was supported by the Spanish Institute for Agricultural and Food Research and Technology (INIA), co-financed by the European Regional Development Fund (FEDER) (Project RTA2011–00118–C02–02) and by Xunta de Galicia (Project EM2014/048). We gratefully acknowledge provision of germplasm by BGHZ, BPGV, CGN and IPK. We are highly grateful to Dr. Alain Palloix for providing the *C. annuum* var. *glabriusculum* accessions.

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P3-14

Variation among Spanish pepper accessions of different traditional varieties

Willem van Dooijeveert[1], José Vicente Valcárcel[2], Enrique Muñoz[2], Eva Solbes[2], José Torres[2], María José Díez[2]

[1] *Center for Genetic Resources The Netherlands, Wageningen University and Research Centre, Wageningen, The Netherlands*

[2] *Instituto de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV), Universitat Politècnica de València, Valencia, Spain*

Abstract

The genebank of the COMAV-UPV holds an important collection of Spanish traditional varieties of pepper. The collaboration between the Center for Genetic Resources, The Netherlands (CGN) and the COMAV of the Polytechnic University of Valencia made it possible to rescue and characterize a group of these Spanish traditional varieties of pepper collected in 1984. One hundred and twenty six accessions were grown during 2014 and 2015 in the spring-summer cultivation period. Several descriptors for plants, flowers and fruits were recorded, using the IPGRI descriptors. Seventy per cent of the accessions studied were sweet peppers and were grouped according to the Pochard classification for sweet peppers. Most of them belonged to B1 (fruits with rectangular longitudinal section), B2 (similar to B1 type but longer fruits) and C2 (fruits with triangular longitudinal section) Pochard's types. A remarkable variability was found among the accessions included in these types. Other shapes, such as Cherry, sub-spheric and heart shaped fruits were identified. Many traditional and appreciated Spanish varieties were found in the group of accessions characterized.

1. Introduction

Spain is the world's fifth largest producer of peppers and holds a magnificent variety of these vegetable. The center of pepper cultivation on an intensive scale is the province of Almería, where peppers account for as much as 40% of all the available surface area in plastic greenhouses. But besides this large scale cultivation there is another type of production based on high quality traditional varieties, little known outside their places of origin and linked to high quality demanding markets. These varieties offer an unsuspected wealth of variable shapes and aromatic flavours frequently ligated to local cultures and traditions in different regions of Spain.

The genebank of the COMAV-UPV holds an important collection of pepper. Collecting expeditions of vegetable crops started in the early eighties by the COMAV institute in Spain, financed by IBPGR, the predecessor of Bioversity International. Safety duplicates of 198 pepper accessions collected, were sent to the CGN and subsequently to the Asian Vegetable Research and Development Center (AVRDC) because pepper had no priority in the CGN collection at that time. With the time about 100 of these accessions were not available because they were never regenerated or regenerations failed. In addition, the viability of a low proportion already available in the COMAV genebank decreased dangerously and needed to be regenerated. With the intervention of CGN, the safety duplicates were recovered from the AVRDC and so two regeneration assays could be conducted in 2014 and 2015 at the COMAV.

The CGN and the COMAV joined work force to rescue this collection of Spanish traditional varieties which resulted in the regeneration and characterization of 126 accessions. Here we present some of the most outstanding results.

2. Material and Methods

One hundred and twenty six accessions were cultivated in two consecutive years, 89 in 2014 and 37 in 2015, some of them were common to both assays. Seeds were provided by the COMAV genebank and from the AVRDC genebank through the intervention of the CGN. The accessions were selected because of their low viability and/or low amount of seeds stored in the genebank. The selected group represents most of the Spanish traditional types of pepper from different Spanish Autonomous Communities. Several accessions of each type were included to study the variation inside each type.

In the first assay, conducted in 2014, plants were cultivated in soil under mesh tunnel in order to avoid crosspollination. Transplants was made in April and the harvest period extended from mid July to the end of September. Fifteen plants per accessions were cultivated in a plot. Accessions of different types were arranged randomly in order not to group accessions of the same type. In the second assay plants were cultivated in pots under mesh tunnel. Fifteen plants per accession were cultivated. Transplants took place in April and the harvest period lasted from July until the end of November.

Descriptors published by IPGRI (PGRI, AVRDC and CATIE. 1995) were used. A total of 17 descriptors, which correspond to different plant, flower and inflorescence, and fruit characteristics were recorded, giving priority to fruit descriptors. Plant and flower descriptors were taken at accession level but variation among plants of the same accession were recorded. Fruit description was emphasized to determine the differences among the different varietal types and the variation inside each of them. At least fifteen fruits per accession were characterized. Additionally, peppers were classified according to the scale developed by Pochard (1966) for sweet peppers. This scale classifies peppers according to their shape in different categories. "A" type includes fruits with quadrangular longitudinal section, "B" type stands for rectangular longitudinal section, "C" type includes fruits with triangular longitudinal section, "F" type includes tomato-like peppers, "N" subspheric peppers and "P" type heart shaped peppers. Types A, B and C have several subdivisions according with size, longitude, rate longitude/width and peduncle shape of the fruits.

3. Results ands Discussion

Eighty nine out of the 126 accessions studied were sweet peppers and 37 belonged to the hot pepper type (Table 1). The most common types for sweet peppers were B1, B2 and C2 (Figure 1). For hot peppers the most predominant fruit type was very long and think. A great variability was found among the accessions included in each of these types.

The B1 type includes fruits with thin pericarp and weight between 200 and 250 grams, sometimes lower (Nuez et al., 1996). The Spanish pepper called "Cuatro cascos" (four locules) belongs to this type. They are used for fresh consumption. B2 type comprises fruits longer than B1. The Spanish variety "Largo de Reus" (Long from Reus) belongs to this type. C1 type includes peppers with very thin pericarp, 2-3 locules and about 90 grams. Usually these peppers are consumed fryed at immature stage. Spanish varieties called "Cornicabra" (Goat horn) and "Cuerno de toro" (Bull horn) belong to the subdivisions of C type. One of the very famous varieties, especially in the Northwest of Spain, is the so called "Pimiento de Padrón". While most of these peppers are naturally mild in flavour, some of them develop a spontaneous

piquancy. They are sometimes hot, sometimes not. These peppers are small, long and with thin pericarp, they can be included in B4-C4 type. The P type includes peppers triangular shaped and with very thick pericarp, usually used for canning (Figure 1, P). They can also be used to fill with meat. An important Spanish type is the so called "Ñora" (Figure 1, N). This is a N type pepper, small in size and sub-spheric in shape. These peppers have very thin pericarp which facilitate their drying. They are used for the production of paprika (pimentón) and oleoresin. This industry is specially important in Murcia province, but also in Extremadura and La Rioja. The N shape is not the exclusive type of pepper for the production of powder, other types of pepper, longer and with very thin pericarp are also used for the production of this type of industrial product. The main requirement is the very thin pericarp to avoid rotting during the drying process.

Sweet pepper				**Hot pepper**			
Type	**Year**		**Total nº accessions**	**Type**	**Year**		**Total nº accessions**
	2014	**2015**			**2014**	**2015**	
A1	3	0	3	B1-like	1	0	1
A3	3	0	3	B4-like	2	0	2
A4	2	0	2	Guindilla long	14	5	19
B1	13	9	22	C2-like	5	4	9
B2	14	7	21	C3-like	1	0	1
B3	1	0	1	Cherry type	2	0	2
C1	2	1	3	OTHER	1	2	3
C2	10	7	17		26	11	37
C3	1	4	5				
C4	0	2	2				
N	2	2	4				
P	2	3	5				
OTHER	1	0	1				
	54	35	89				

Table 1
Number of accessions belonging to each type of pepper according to Pochard's classification (Pochard, 1966)

Hot peppers are called "Guindillas". The most typical ones are very thin (both the fruit and the pericarp) and very long (Figure 1, C1). They are usually preserved in vinegar. Other shapes are more similar to the Pochard classification and have udes the same nomenclature to clasify them. Finally, small and round peppers called "Cerecilla" (Figure 1, Cherry type) are also used for pickling.

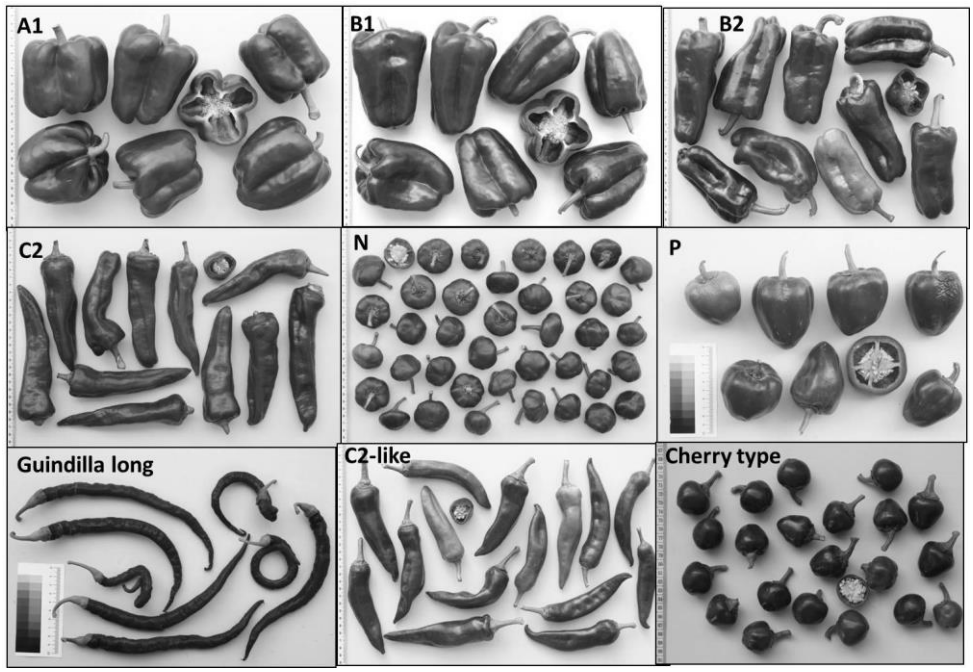

Figure 1

Some of the Spanish traditional pepper varieties. Letters located in the upper left corner of each image represents the Pochard classification, except the Cherry type. From A1 to P (two upper rows) are sweet peppers and from C1 to Cherry type (bottom row) are hot pepper.

4. Conclusions

Rescueing this material shows how fruitfull cooperation between genebanks can be. Sharing responsibilities and funds resulted in a group of old cultivars with interesting features which would have been lost and are available for research and breeding now. Seeds and characterization data of successful regenerated accessions will be made available at CGN and COMAV.

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P3-15

Greenhouse eggplant production in France: Success and failures of grafting for controlling *Verticillium* sp.

François Villeneuve[1], François Latour[1], Théophile Théry[1], Christian Steinberg[2], Véronique Edel-Hermann[2], Marie-Christine Daunay[3]

[1] *Ctifl, Centre de Lanxade, Prigonrieux, France*

[2] *INRA, UMR Agroécologie - Pole IPM Bat. Dommergues, Dijon, France*

[3] *INRA, UR1052, Domaine St Maurice, Montfavet, France*

Abstract

After many years of utilisation of grafting on *Verticillium* wilt resistant rootstocks, mainly tomato rootstocks, growers observe new wilt like symptoms on their grafted eggplants. Accordingly, Ctifl, in close collaboration with INRA, regional extension services, and seed companies, set up a research designed to get a general picture of the various soil borne phytosanitary problems, and to identify their cause(s). The principle of the study was to monitor pathogen distribution changes in eggplant crops in the two main production regions in France. The results show the prevalence of *Verticillium* wilt and, in the majority of cases and all production areas, the simultaneous presence of a number of other bioagressors, including fungi and nematodes. The isolates of *Verticillium* were identified at the species level (*V. dahliae*, *V. isaacii)* and characterized for their race (0 or 1) and their aggressiveness. Surprisingly, the aggressiveness of most isolates was relatively low. To our knowledge, this is the first report of *V. isaacii* in France, but its impact on production across France is unknown at this time.

1. Introduction

France is traditional area of production of eggplant (*Solanum melongena* L.) and *Verticillium* wilt is a long lasting problem for growers. Resistant cultivars are not available (Lockwood and Markarian, 1961; Alconero *and al*., 1988; Yu *and al.*, 2015). Research on grafting started in France in the sixties (Messiaen and *al.*, 1967; Beyries, 1974). Presently, 63% of eggplant surface is grafted (Torres and Brand, 2015) and the rootstocks generally used are interspecific hybrids of tomato species; the use of *S. torvum* is less frequent.

After several years of utilization of tomato resistant cultivars and rootstocks, homozygous or heterozygous for the Ve gene, damaging wilt-like-symptoms are often observed on tomato as well as on eggplant, grafted or not. So, it was necessary to determine the cause of this re-emerging problem. The aim of this study, conducted by the Centre Technique Interprofessionnel des Fruits et Legumes (Ctifl) in association with INRA, regional experimental stations (APREL and Invenio), seed companies and growers, was to identify the major pathogen(s) causing the wilting on Verticillium resistant material and to determine their aggressiveness and virulence.

2. Materials and method

2.1. Sampling plant material and isolates

Plant samples were collected from French production areas on plants displaying wilt symptoms. In each location, three plants (whatever eggplant) were sampled at random and sent to Ctifl (Lanxade) for analysis. On delivery, the samples were unwrapped and rinsed with water in order to permit a detailed analysis of the root system. The number and types of samples are shown respectively in Tables 1 and 2

Provence	South-West France	Center-West
45	13	3

Table 1
Geographic distribution of the eggplants sampled from 2008 to 2015.

13 non grafted	40 grafted onto tomato rootstock	4 grafted onto *S. torvum* rootstock

Table 2
Status of eggplants sampled.

Microbiological isolates were then collected from each plant using potato dextrose agar (PDA) medium amended with 100 mg/L^{-1} of ampicillin in order to isolate the various pathogenic fungi potentially related to the wilting and root decay problems. Plant colonisation by pathogens was analyzed by sampling tissues from roots, collar level, grafting point and shoots. Small sections (0.5 to 1 cm) were cut with a sterilized scalpel, superficially disinfected by dipping in 0.5% sodium hypochlorite for 3 min, rinsed three times in sterile distilled water, and deposited on Petri plates, which were then incubated at 21°C in the dark for 1 week. After few days (5 to 10) actively growing fungal colonies were transferred onto new PDA plates for further purification, sporulation and identification.

2.2. Pathogenicity studies

To identify the *Verticillium dahliae* races and the aggressiveness of the isolates, bioassays were performed using several controls:

- Tomato 'Marmande verte' susceptible to *Verticillium dahliae* race 0 and 1;
- Tomato 'Marmande VR' resistant to race 0 and susceptible to race 1;
- Tomato 'IRAT L3' and 'Mel 26681 70 G' partially resistant to race 0 and susceptible to race 1;
- Eggplant 'Liu Yé Qié' susceptible to *Verticillium dahliae* race 0 and 1.

The aggressiveness of each *Verticillium* isolate was estimated, on the basis of stem vessels browning of each control, by calculating mean values using the following formula, VD = vascular discoloration:

$$\textit{Index of}\ \text{Aggressiveness} = I_A = [((VD\ Marmande\ verte)+(VD\ Marmande\ VR\ x\ 2)+(VD\ IRAT\ L3\ x\ 3)+VD(\ Mel\ 26681\ x\ 3))_{strain\ i} / ((VD\ Marmande\ verte)+(VD\ Marmande\ VR\ x\ 2)+(VD.\ IRAT\ L3\ x\ 3)+(VD\ Mel\ 26681\ x\ 3))_{reference\ strain}] - 100$$

We have imputed different coefficients to the tomato and eggplant accessions, according to their level of resistance. For facilitating the comparison between strains, we imputed the level "0" to the reference *Verticillium* strain ('race 0 Toreille'). Each test consisted of 2 replications of 20 plants per control, grown in individual mini-clods, in order to avoid cross-contamination. For each plant, 3 ml of the concentration of the spore suspension adjusted to 4.10^5 conidia/mL^{-1} was applied.

3. Results and discussion

3.1. Prevalence of soil borne diseases on eggplant

The most frequently observed soil-borne pathogen is *V. dahliae. V. albo-atrum* has not been isolated. In the most frequent cases, *V. dahliae* was isolated together with one or more bioagressors, in particular *Colletotrichum coccodes*, *Pythium* sp., *Rhizoctonia solani* and *Macrophomina phaseolina.* Generally, these fungi were associated to *Meloidogyne* spp.

The situation was slightly different between the two main French production areas. In Provence, we observed more samples with *V. dahliae* alone, and only a few samples with *V. dahliae* associated with other pathogens, generally *C. coccodes*, *Pythium* sp. and *R. solani*. In the South-West area, *V. dahliae* was mostly associated with one or more fungi (Fig. 1). In the case of South-West, the species and race of *Meloidogyne* have been identified as *M. hapla* in some samples, and *M. arenaria* race B in others (Blancard *and al.,* 2007). These nematodes are not controlled by the tomato gene *Mi*.

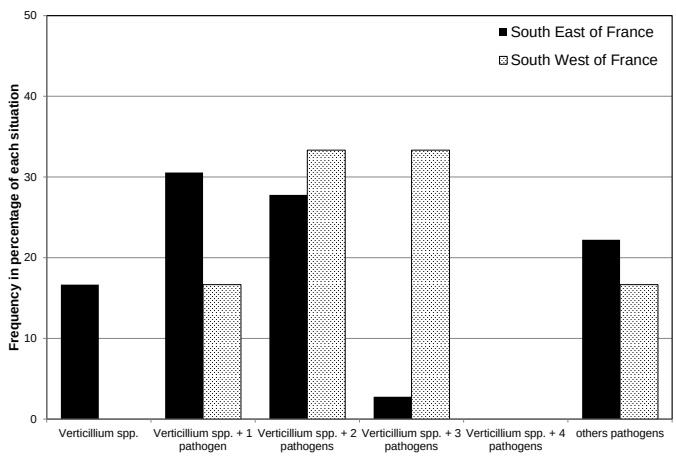

Figure 5
Relative abundance of Verticillium spp. and associated soil borne pathogens isolated from eggplants in two French production areas.

We did not observe any difference neither between grafted and non-grafted eggplants (Fig. 2) nor between the rootstocks used, mainly Beaufort, Maxifort and Imperador (data not shown).

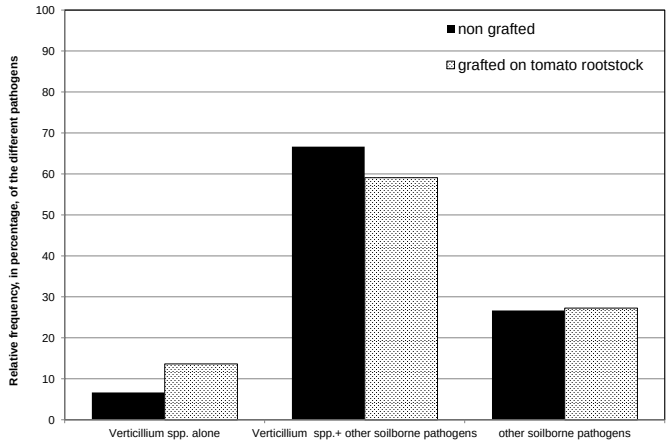

Figure 6
Interaction between grafted or non-grafted eggplants and the structure of the soil-borne pathogenic fungal complex.

3.2. Characterization of isolates of V. dahliae

The characterization of isolates of *V. dahliae* was difficult because symptoms such as yellowing and wilting on tomato and eggplant are unclear and strongly depend on environmental conditions. Vessels browning were the most discriminant symptom between susceptible and resistant controls.

Isolates are generally divided in defoliating pathotypes and non-defoliating pathotypes (Hagiwara, 1990; Usami *and al.*, 2002). The defoliating pathotypes are generally virulent. All the tested strains were non-defoliating but they induced symptoms on tomato and eggplant. Out of the 12 isolates of *V. dahliae* that we could characterized as belonging to a specific race, three belonged to race 0 (common race) and nine to race 1. This result is not surprising given the majority of the isolates originate from tomato rootstocks hosting the (race 0 resistant) gene *Ve.*

Eleven isolates out of the 12 tested presented a lower aggressiveness (Fig. 3) than the reference strain (*V. dahliae* race 1 Toreille). This may be in relation with the presence of a complex of other bioagressors affecting in synergy wilted eggplants. Unraveling the interactions between these different pathogens will be necessary in the future. Different studies have proved the existence of a positive interaction between *Meloidogyne* or *Pratylenchus* and a higher infection level by *V. dahliae*. (Bowers *and al.*, 1996 ; Scholte and s'Jacob, 1989).

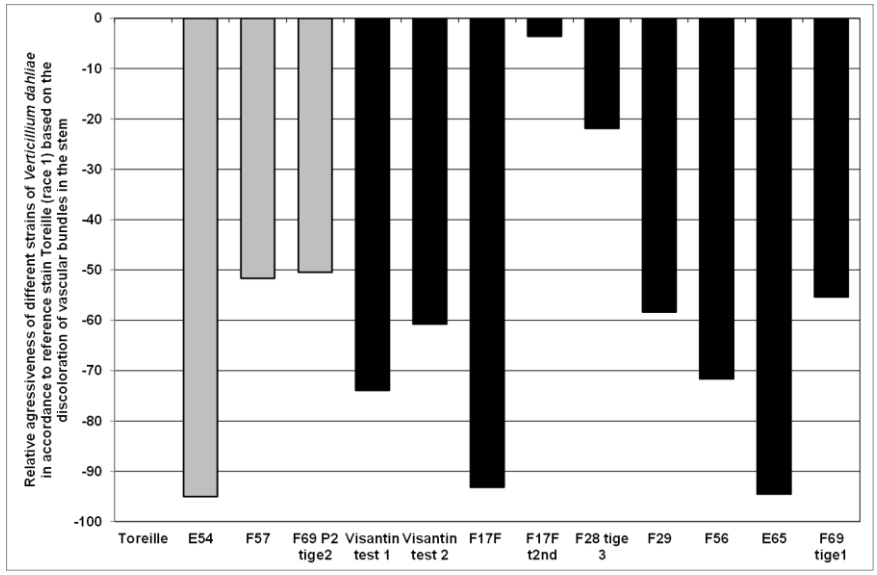

Figure 7
Aggressiveness of the various strains of Verticillium dahliae sampled on eggplants in different French areas. Reference strain ('Toreille') is attributed the level 'zero' for aggressiveness.
: race 1 strains. *: race 2 strains.*

4. Conclusion

After many years of using grafting on resistant rootstocks, complexes of soil-borne fungi develop in intensively cultivated soils and damage formerly resistant plants. The frequency of this phenomenon is increasing since the use of soil chemical fumigation, in particular methyl bromide, is forbidden since 2005. The most frequent situations observed are the following:

- For eggplant grafted onto tomato rootstocks, presence of race 2 of *Verticillium* that bypasses the resistance conferred by the tomato gene *Ve*,
- In addition to the presence of *Verticillium* on wilting plants, two or more additional fungi are isolated, thus revealing the development of pathogenic complexes in the soil.

Hence our future prospect is to combine the use of rootstocks or varieties resistant to a wider range of soil-borne pathogens, together with complementary control methods such as biocontrol, soil organic amendments, and crop rotation.

5. Acknowledgements

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P3-16

Genetic diversity and population structure of pepper (*Capsicum* spp.) in China

Xiaozhen Gu[1], Zhenghai Zhang[1], Yacong Cao[1], Baoxi Zhang[1], Xixiang Li[1], Alain Palloix[2], Lihao Wang[1]

[1] *Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, China*

[2] *INRA-PACA, UR1052, Génétique et Amélioration des Fruits et Légumes, Montfavet, France*

wanglihao@caas.cn

Abstract

To solve the problem of genetic redundancy and further to utilize. Based on 1904 accessions of pepper genetic germplasm conserved by national vegetable germplasm repository, using 29 SSR markers, we analyzed the genetic diversity and primary core germplasm resource. Allele richness, gene diversity, PIC and index of heterozygosity were calculated. Population genetic structure was analyzed. We built primary core collection with M strategy and evaluated its representative. The gene diversity index and polymorphism information index respectively range from 0.016 to 0.883, 0.02 to 0.87, and respectively the average was 0.486 and 0.46.29 SSR markers generated 459 alleles. Analysis of population genetic structure, neighbor-joining tree and principal component showed that there are two different pepper genetic groups in China and admixtures are mostly located in between two populations. Group 1 contains 1411 accessions, characterized by triangular and horn shaped peppers. Its geographical distribution mainly concentrated in the southern region in our country, such as Hunan, Hubei, Sichuan, Yunan and Guizhou. Group 2 contains 493 accessions, characterized by large fruited peppers with blocky or rectangular shape, mainly distributed in the northern regions, such as Heilongjiang, Jilin, Liaoning and Hebei. M (M method) and R (Random method) predicted the optimal sample size of core germplasm. Two sampling methods showed that M score is higher than R, no matter how sample size change, M method sampling alleles is significantly more efficient than R method. M method was used to extract respectively 10, 20, 40, 80 and 160 samples. Five sample sizes of core collection captured 28%, 50%,57% and 69% alleles of raw materials. Considering the practicability of core collection, we selected characteristic accessions in different regions. The final core collection of 344 accessions (supplementary material) captured 81% of the SSR, including all common alleles, 80 (81) less common alleles, 138 (159) rare alleles and 68 (133) vary rare alleles. Out of the 344 accessions, 227 from group 1 and 117 from group 2. Its genetic diversity index and PIC index were 0.527 and 0.5 respectively, higher than those of raw materials (0.486, 0.46). Using 29 SSR markers to analyze the neighbor-joining tree, result show that the final primary core accessions evenly distributed in raw materials and had high representative.

Keywords: Germplasm, *Capsicum*, Genetic diversity, core collection

SESSION 4

Physiology and nutritional value

P4-01

Silicon nutrition ameliorates salt stress of *Capsicum annuum* L. by ion regulation

Ozlem Altuntas[1], H. Yildiz Dasgan[2], Yelderem Akhoundnejad[2]

[1] *Inonu University, Agricultural Faculty, Horticultural Department, Malatya, Turkey*

[2] *Cukurova University, Agricultural Faculty, Horticultural Department, Adana, Turkey*

Abstract

Salt sensitive Demre variety pepper plants were subjected to 75 mM NaCl stress, and 2 mM Si were applied to alleviate the stress. Potassium, Ca, Na and Cl concentrations in leaf and root tissues were investigated. Potassium and Ca concentrations in leaves of the salt-sensitive genotype were increased by 2.6% and 19.0%, respectively and K and Ca were also increased in roots by 10% and 17% in salt by the silicon. However, Na and Cl concentrations in salt stress were decreased in leaves by 13% and 2.8% respectively and in roots by 15% and 0.5% respectively by silicon. The Cl ion in leaves was decreased 2.8% and in roots decreased by 0.5% by Si treatment

Silicon can be an effective nutrient for amelioration of salt stress in pepper genotypes. Under the mild salt stress (75 mM), the salt-sensitive pepper cultivar benefited from2 mM Si from K_2SO_3. Silicon application increased the K and Ca and decreased Na in shoot and root tissues. The increasing Ca/Na and K/Na ratios with Si help plants to cope with the detrimental effects of the salinity.

1. Introduction

Silicon (Si) is an element that presents on earth surface. Yet, its availability to plants is normally low [1]. The main Si resources in soil is monosilicic acid (Si(OH)4) and acid polisilicic which are soluble and weakly adsorbed. [2]. According to [3], silicon is a beneficial element for the plants. Some physiological events can enhance its beneficial effects such as, its absorption and deposition in several organs' cell wallslike stem, root and leaf [4]. Consequently, it has been mostlyrelated to molecular, physiological, nutritional, and morphological circumstances in plants [5]. Pepper (*Capsicum annuum* L.) is one of the most important crops for Mediterranean region, because of its economic success. Late studies have informed varied responses of pepper to salinity stress. For pepper plants, salinity doses ranging from 0 to 2 dS/m are acceptable. However, increasing salinity doses ranging between 8% to 15% might cause linear decrease in yield [6],[7]. All over the world, one of the most important factors that limits the plant growth and yield is salt stress [8]. High salt concentration in the soil causes high osmotic potential of soil which results in physiological drought in plants. Further, higher concentration of Na^+ and Cl^- bring about toxic effects in plants such as ion irregularity. So, amount of reactive oxygen species increases in plants while plant growth and yield decrease [9]. It has been widely reported that Si application may increase salt tolerance in many important agricultural crops such as wheat [10], [11], rice [12], [13], [14] maize [15],[16] barley [17] sorghum [18], [19] tomato [20], [9] and soybean [21]. In this study, we investigated the effect of Si on pepper plants grown under salt stress.

2. Materials and Methods

This study was carried out in the climate chamber of Cukurova University, Faculty of Agriculture, Department of Horticulture in order to determine the effects of silicon nutrition on pepper plants grown under salt stress conditions.

2.1. Materials

In the study, a local pepper variety, Demre (sensitive to salt stress) was used. The plants were treated with 75mM salt dosage. Salt-treated plants were compared with non-treated (control) plants. The silicon with 2 mM concentration was used from K_2SiO_3.

2.2. Methods

Plant growing medium was vermiculite. Hoagland nutrition solution was used for plant nutrition Two liter capacity pots filled with vermiculite and pepper seedlings were planted into each pot on 8th November 2013 and the design of the study was randomized block design with 3 replicates. Each replicate contained 4 plants. The salt treatment (75mM) was done to the required pots. NaCI added with 25 mM increments on every day and in 3 days final 75mM concentration was reached. When the reaching of the final salt concentrations, the day after, 75mM salt dosage was ordinarily applied into the pots. After 30 days under salt stress, on day 31, plants were harvested into the root and leaves for the mineral analysis. Whole root systems and representative leaves from the shoot were used for the ion analysis. Also, silicon was applied into the nutrient solution by 2 mM from K_2SiO_3 for the Si-treated plants.

Potassium, Ca, Na analyzes were done by the method of dry-ashing. The samples were dried and grinded, after then, they were burned at 550 ^{0}C, following, they were soluted in 3.3% (v/v) HCl solution and the elements of K, Ca, Na readings were done by the atomic absorption spectrophotometer [22]. Tissue Cl concentration was determined by the Mohr method [23].

The data were subjected to factorial analysis of variance using Statistical Package for Social Sciences (SPSS version 20) and means were separated by Duncan's Post Hoc Tests at the 5% level $(P < 0.05)$.

3. Results

Sodium concentration in shoots and roots were measured markedly higher for Demre cultivar in the presence of NaCl (75mM) than in the absence of NaCl (0mM) regardless of Si treatment, but added Si significantly decreased sodium concentration in both leaves and roots of salt-treated plants compared with Si-untreated plants (Table 1). Calcium concentrations in leaves obtained from Si treated 75mM salinized was significantly higher than plants untreated with Si but salinized with 75mM NaCl. Potassium and Ca concentrations in leaves of the salt-sensitive genotype were increased by 2.6% and 19.0%, respectively and K and Ca were also increased in roots by 10% and 17% in salt by the silicon. However, Na and Cl concentrations were decreased in leaves by 13% and 2.8% respectively and in roots by 15% and 0.5% respectively in salt by the silicon. The Cl ion in leaves was decreased 2.8% and in roots decreased by 0.5% by Si treatment (Table 1).

The salts interfered with the absorption of K and Ca and decreased their uptake in pepper plants. Addition of Si inhibited the uptake and transport of Na from roots to shoots. Potassium and Ca uptakes, K/Na and Ca/Na ratios, were increased by the addition Si in the shoot and root. In this study, Si-enhanced salt tolerance in salt-sensitive-pepper was ascribed to decreased

Na concentration and increased K and Ca with a resultant improvement in Ca/Na and K/Na ratios, which are the good indicator to assess plant tolerance to salt stress.

In this study, amelioration of salt stress by Si nutrition on pepper plants is believed to be associated with decreased sodium concentration and increased Ca and K concentrations in the leaves and root tissues (Table 1). The enhanced uptake of Ca and K by Silicon treatments under saline stress conditions was thought to alleviate the plants to tolerate salt damage. In rice [24], sugar cane [25] and tomato [26] reported that Si application under the salt stress increased the plants growth and decreased the Na and Cl contents in shoot and root of parts of the plants. In barley plants 1 mM Si under 120mM NaCl stres reduce sodium and increase potassium concentrations in shoots and roots of salt-stressed barley. Sodium uptake and transport into shoots from roots was greatly inhibited by added Si under salt stress conditions. However, Si addition exhibited little effect on calcium concentrations in shoots of salt-stressed barley [27], in our study Ca uptake was higher than K in both leaves and roots. In another barley study under 100 mM salt stres 2 mM Si decreased Na concentration and increased K and Ca with an improvement in Ca/Na and K/Na ratios [28]. Silicon is reported to reduce the effect of salinity on wheat (*Triticum aestivum* L crop by decreasing plant Na uptake and shoot : root Na distribution of a salt-resistant as well as a salt-sensitive wheat genotype. Reduced shoot Na concentration and increased shoot K: Na ratio led to improved plant growth [29].

		In leaves				**In roots**			
Si Apply	**Salt Dosages**	**K**	**Ca**	**Na**	**Cl**	**K**	**Ca**	**Na**	**Cl**
Silicon (+)	0	3.51 a	1.2 a	0.32 c	0.17 b	1.58 a	0.74 a	0.48 c	0.13 b
	75	2.76 b	1.01 b	1.93 b	2.82 a	1.11 b	0.62 ab	1.03 b	2.02 a
Silicon (-)	0	3.51 a	1.18 a	0.3 c	0.21 b	1.37 a	0.70 ab	0.51 c	0.18 b
	75	2.69 b	0.85 c	2.22 a	2.90 a	1.01 b	0.53 b	1.21 a	2.03 a

Table 1
Ion concentrations in leaves and roots of pepper (Demre cv) plant under salt stress with or witout Si

4. Conclusion

Under the mild salt stress (75 mM), the salt-sensitive pepper cultivar benefited from1mM Si from K_2SO_3. Silicon application increased the K and Ca and decreased Na in shoot and root tissues. The increasing Ca/Na and K/Na ratios with Si help plants to cope with the detrimental effects of the salinity.

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P4-02

The effect of shading on chlorophyll fluorescence and pungency of outdoor cultivated chili hybrids

Zsuzsa Nagy[1], Daood Hussein[2], András Neményi[1],
Szonja Szimóna Burján[1], Lajos Helyes[1]

[1] *Institute of Horticulture, Szent István University, Faculty of Agriculture and Environmental Sciences, Gödöllő, Hungary*

[2] *Regional Knowledge Centre, Szent István University, Gödöllő, Hungary*

Abstract

Pungent peppers are valuable sources of many phytochemicals such as capsaicinoids, ascorbic acid and carotenoids. The present study focuses on the different shadings effect on chlorophyll-a fluorescence (Fv/Fm) and pungency. In order to reveal the relation between pungency and photosynthetic activity, two hybrids; a yellow skinned 'Star Flame' and a red skinned 'Fire Flame' were investigated. Three different coloured nets (white, green, red) and a non-shaded control were set above the plants cultivated outdoor in the experimental field of Institute of Horticulture, Szent István University, Gödöllő. The harvests were made during the period of end of August until the end of September, which period covers variable environmental circumstances. Lower fluorescence of 'Star Flame' ranged from 0.682 to 0.755 and in 'Fire Flame' from 0.721 up to 0.779. We found that lower values signify unfavourable condition that could lead to higher pungency in *Capsicum* pods.

Keywords: chlorophyll-a fluorescence, 'Star Flame', 'Fire Flame'

1. Introduction

In 2012 the worldwide yield of chili pepper was estimated to be 31 million tonnes, and 3.3 million tonnes of processed products (FAO 2014). Chili peppers belong to the genus *Capsicum*, which contains different species such as *C. annuum*, *C. frutescens*, *C. baccatum*, *C. chinensis* and *C. pubescens* (Votava et al. 2005), the word chili refers to extremely pungent, but non Hungarian paprika genotypes. The major attribute of chili pepper is the pungency level, which is determined by capsaicinoid compounds (Iwai et al. 1979). Those compounds are synthetized by the glands in the joints of placenta and pod wall. Biological factors such as ripening stage (Barbero et al. 2014) and genotype (Giuffrida et al. 2013) determine the pungency of the pepper pods.

The utilization of net shading reduces air movement in the level of plant culture, reduces the radiation reaching the plants regardless of its colour and often increases relative humidity compared to the above measured value (Stamps 2009). According to Elad et al (2007) netting negatively influenced powdery mildew infection, but positively the yield. The quality attributes, such as vitamin c has been investigated in sweet pepper (Kong et al. 2013 Ilic et al 2011), and a variable effect of shading among harvest times were found.

Chlorophyll-a fluorescence has been investigated and found to be an indicator of physiological stress caused by drought and high temperature (Baker and Rosenqvist, 2004). The

relation of the leaves' chlorophyll-a fluorescence and the pods' pungency have been investigated by Guang-cheng et al. (2011); a decrease in the Fv/Fm values was observed during water deficit in pungent pepper. The relations of shading on pungency and chlorophyll-a fluorescence have not been studied in pungent peppers according to our knowledge. The aim of the recent study was to reveal how different shadings affect the pods' final pungency and also to measure the photosynthetic activity through chlorophyll-a fluorescence (Fv/Fm) in 'Star Flame' and 'Fire Flame' hybrids.

2. Material and Methods

2.1. Plant material

The study was conducted in 2014 at the experimental field of Institute of Horticulture, Szent István University, in Gödöllő, Hungary (lat. 47°61' N, long. 19°32' E). The soil of the experimental field is sandy loam classified as Cambisol with 1.8-2% humus content and pH value around 7. Two hybrids with distinct appearance were chosen; 'Star Flame' which reaches yellow colour at the end of maturation and 'Fire Flame' characterised by a deep red final colour. The seeds were purchased from Seminis, DeRuiterSeeds (Kecskemét, Hungary). We covered the pepper plants with different net shadings: red (Ginegar, ChromatiNet, Israel), white, green (Első Magyar Kenderfonó, Hódmezővásárhely) and a non-shaded control was also set up. For the measurement of chlorophyll-a fluorescence we used a mobile fluorimeter (PAM 2500, Walz-Mess und Regeltechnik, Germany). Four developed top leaves of the appointed 4 plants per treatment were measured following 35 min dark adaption by leaf clips during 4 weeks before both harvests. Fast kinetics was measured and Fv/Fm the maximum photochemical quantum yield of PSII was calculated using PamWin 3.0 software (Regeltechnik, Germany).

2.2. Capsaicinoid extraction

Three grams of blended pepper sample were crushed in a crucible mortar with quartz sand. To the macerate 50 ml of analytical grade methanol was added gradually and then the mixture was then transferred to a 100 ml Erlenmeyer flask with stopper. The mixture was subjected to a 4-min long ultrasonication in a bath ultrasonic device (Raypa, Turkey) and then filtered through paper filter (Munktell, Germany). The filtrate was more purified by passing through a 0.45 mm PTFE syringe filter before injection on the HPLC column (ISIS from Macherey-Nagel, Düren, Germany) The determination of total capsaicinoid amount including nordihydrocapsaicin, capsaicin, homocapsaicin, nordihydrocapsaicins was carried out following the method of Daood et al. (2015). The total capsaicinoid content was calculated from the sum of individual capsaicinoid compounds that appeared on the chromatogram.

2.3. Statistical analyses

Statistical analyses were performed in IBM SPSS 23 software (IBM, New York) and data arrangement was made in Microsoft ® Excel 2007 Analysis Toolpack (Microsoft Corporation, Redmond, Washington). Prior to ANOVA model fitting the normality of each variable was checked with Levene's test. We used harvest time and shading as explanatory variables, and their interaction as a potenial variable. Tukey HSD post hoc test was also made for pair comparison. Throughout the study α was set to 0.05.

3. Results and Discussion

3.1. Pungency

Total capsaicinoid of 'Star Flame' was significantly influenced by the harvest time ($F_{1,24}$=56.107, p<0.001, Table 1); the second harvest resulted in higher values in all shadings, while the effect of shading could not be detected. During the intense period of fruit ripening before the first harvest the average temperature was 18 °C, and before the second harvest this value decreased to 14 °C. In total the combination of the non-shaded control at second harvest resulted in the most pungent peppers 61.14±4.53 mg/100g FW. Yellow skinned *C. annuum* type peppers are not as common as red skinned pods. Another yellow skinned pepper variety 'Caribe' with lower pungency (approx. 93 μg/g FW) and smaller fruit size was investigated by Ornelas-Paz et al. (2010).

In the case of 'Fire Flame' the total capsaicinoid value was significantly influenced by the interaction of shading and harvest time ($F_{3,24}$=8.155, p<0.001, Table 1); at the first harvest the non-shaded plants produced more than the shaded ones, but during the second harvest this advantage disappeared. Although, the effect of shading itself could not be detected in any of the hybrids, we found it interesting that the non-shaded 'Fire Flame' peppers at the first harvest outperformed the shaded ones. In the red hybrid 'Fire Flame' the white shading at second harvest resulted in the highest total capsaicinoid content (33.81±2.57 mg/100g FW).

Table 1. The average ± SD total capsaicinoid values in mg/100g fresh weight (n=4)

	'Star Flame'		'Fire Flame'	
	1. harvest	2. harvest	1.harvest	2.harvest
White	43.64±8.11 Aa	57.67±5.16 Ba	15.29±2.50 Aa	33.81±2.57 Bb
Red	42.79±7.91 Aa	59.15±5.31 Ba	15.07±1.86 Aa	20.23±2.43 Ba
Green	37.57±6.37 Aa	57.85±4.91 Ba	17.26±2.45 Aa	37.08±1.41 Bb
Control	48.94±3.68 Aa	61.14±4.53 Ba	24.78±0.59 Ab	33.75±8.40 Ab

The same capital letters indicate no difference in total capsaicinoid between harvest times, whereas the same small letters denote no difference in total capsaicinoid among shadings according to Tukey HSD post-hoc tests.

3.2. Chlorophyll-a fluorescence

The Fv/Fm value was significantly influenced by the harvest time in 'Star Flame' ($F_{1,24}$=82.406, p<0.001, Table 2). According to Bolhar-Nordenkampf et al. (1989) the Fv/Fm ratio between 0.72-0.85 signifies a stress free optimal state for plants, which range the peppers at second harvest did not reach.

In case of 'Fire Flame' the shading ($F_{3,24}$=5.02, p=0.008, Table 2) and harvest time also ($F_{1,24}$=113.46, p<0.001) determined the Fv/Fm value. The non-shaded plants showed significantly lower values compared to the red shaded plants. According to Cui and Zhang (2003) a lower sun exposure could enhance chlorophyll content of pepper leaf than direct sunlight. And also, high temperature induced stress was observed to inhibit photosynthetic activity in sweet pepper by Hanying et al. (2001).

In the yellow skinned 'Star Flame' a negative and significant relation was detected; r = -0.663, p<0,01 (Figure 1). In case of 'Fire Flame' a similar relation was observed; r = -0.741,

p<0,01 (Figure 2). At the second harvest in 'Star Flame' the Fv/Fm values were lower than 0.72, and those measurements were accompanied by 57.67-61.14 mg/100g FW total capsaicinoid values. It was found by Hanying et al. (2001) that photosynthetic activity is supressed by heat stress, from which peppers in the absence of shading could suffer.

Table 2. Chloropyll-a fluorescence given in Fv/Fm rate

	'Star Flame'		'Fire Flame'	
	1. harvest	2. harvest	1. harvest	2. harvest
White	0.749±0.009 Ba	0.699±0.012 Aa	0.762±0.006 Ba	0.732±0.01 Aa
Red	0.757±0.013 Ba	0.689±0.014 Aa	0.775±0.005 Bb	0.743±0.008 Ab
Green	0.747±0.014 Ba	0.714±0.025 Aa	0.774±0.001 Bab	0.739±0.012 Aab
Control	0.747±0.009 Ba	0.700±0.02 Aa	0.762±0.007 Ba	0.729±0.012 Aa

The same capital letters indicate no difference in Fv/Fm between harvest times, whereas the same small letters denote no difference in Fv/Fm among shadings according to Tukey HSD post-hoc tests.

4. Conclusion

The hybrids responded in different ways to the distinct harvesting times and shadings also, though when analysing the relation of total capsaicinoid and Fv/Fm, both hybrids showed very similar correlation. Presumably this attribution is influenced by environmental factors.

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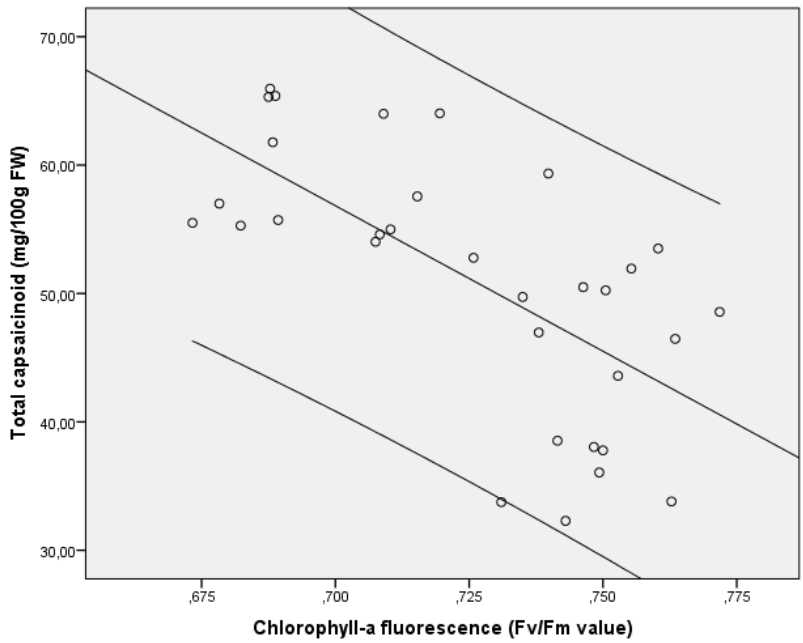

Figure 1
The relation of total capsaicinoid (mg/100g FW) and chlorophyll-a fluorescence (Fv/Fm value) of yellow skinned 'Star Flame' hybrid. The 95% confident interval is marked.

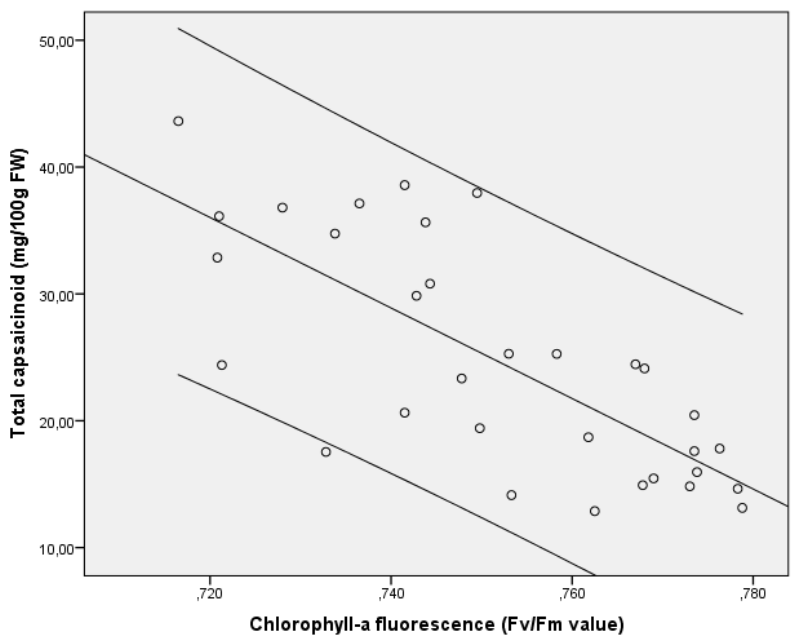

Figure 2
The relation of total capsaicinoid (mg/100g FW) and chlorophyll-a fluorescence (Fv/Fm value) of red skinned 'Fire Flame' hybrid. The 95% confident interval is marked.

P4-03

Digestion of *Chrysanthemum stunt viroid* (CSVd) by leaf extracts of *Capsicum chinense* attributes to strong RNase activity

Boubourakas Iraklis, Eriko Miyashita, Munetaka Hosokawa

Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto, Japan

Abstract

There are several reports on the antiviral and antiviroid agents contained in many plant species, and active agents have been identified. *Capsicum* plants are also known to contain antiviral agents, but the mechanism of their activity has not been determined. The aim of this study was to determine the mechanism of the antiviroid activity of *Capsicum* extract.

Capsicum extract was effective to digest CSVd only when it was sprayed before inoculation. When inoculation was performed at pre-sprayed positions, no infected plants were observed. No plants except *Capsicum* digested the CSVd, and in the *Capsicum* treatment, small digested CSVd products were observed. Strong CSVd-digesting cultivars belonged only to *C. chinense*.

Capsicum extract was revealed to contain strong RNase activity. This activity was concluded to be the main mechanism preventing infection of viroids by spraying before inoculation. The strong activity was cultivar dependent and species specific. Among the reported antiviroid and antivirus activities, this is the first report of digestion activity by RNases and may represent a new mechanism. And our report indicated that the strong RNase activity is a dominant trait and governed by one gene.

1. Introduction

Viroids are the smallest RNA pathogens, with 246–401-nt viroids being known to infect plants. This infectious agent is a single-stranded circular molecule with the capacity to replicate autonomously in host plants (Diener and Lawson, 1973). Viroid genomes contain no protein-coding regions, but replicate and accumulate in the host cell (Flores et al., 1998). Chrysanthemum stunt viroid (CSVd) belongs to the *Pospiviroidae* family that infects chrysanthemums (*Chrysanthemum morifolium* Ramat.), one of the most important ornamental species worldwide. CSVd disease was first reported in the USA in 1945 (Dimock, 1947), and symptoms such as plant stunting, reduced rooting ability, small flower size, and disturbances of the photoperiodic response for flower initiation have been described (Brierley and Smith, 1949; Horst and Langhans, 1977; Hosokawa et al., 2004a).

Difficulties in managing viroid diseases are mainly caused by their high transmissibility and durability. In most cases, viroids are transmitted from plant to plant on scissors or knives. If viroid-infected plants are mixed with mother plants, viroid infection is spread to propagated plants by these tools. For prevention of viroid transmission on tools, washing with sodium hypochlorite is reported to be effective (Roistacher et al., 1969; Matsuura et al., 2010; Li et al., 2015). Some plants contain antivirus and antiviroid agents in various organs. Examples of antivirus disinfectants have been reported for the root extracts of *Mirabilis jalapa* (Kubo et al., 1990; Habuka et al., 1990), *Bougainvillea* (Verma・Dwivedi, 1984; Balasaraswathi et al., 1998;

Bolognesi et al., 1997), *Phytolacca americana* (Picard, 2005), and *Capsicum* (Mckeen, 1956; Matsuura et al., 2010). Identification of the mechanisms of action of extracts against infectious agents is important because a combination of several chemical and natural agents with different mechanisms of action is crucial for the development of a new disinfectant. The antiviral mechanisms of *Mirabilis*, *Phytolacca*, and *Bougainvillea* are attributed to a ribosome-inactivating protein that has beta-glucosidase activity resulting in the inactivation of plant ribosomes and viral nucleotides. However, the mechanism of action of *Capsicum* extract has not been identified.

There are at least two mechanisms for the prevention of infection by chemical treatment: one is the direct modification or digestion of the virus and viroid nucleotides, and another is indirect action, such as the inhibition of infection or induction of resistance in treated plants. In this study, we identified the mechanism of action of *Capsicum* as that of disinfection.

2. Result and Discussion

In treatment with 'Sy-2' extract before CSVd inoculation, one infected plant was observed in each of the 14 and 47 plants in the first and third trials, respectively, and none were observed in the second trial (Table 1). In contrast, 35–80% of the CSVd-inoculated plants sprayed with buffer (control) were infected (Table 1). In 1–4-days post-treatments following CSVd inoculation, no clear effect was observed (Table 2).

Our results suggest that *Capsicum* extract has a direct effect on virus or viroid molecules as a kind of RNase and does not induce resistance in sprayed plants.

Table 1
Spraying of crude extract of freeze dried leaves of Capsicum chinense 'Sy-2' to Nicotiana benthamiana plants before CSVd infection.

Treatment	No. of treated plants	No. of infected plants (%)	Chi-square (*P*-value)
1st experiment			
Mock	12	8(66.7)	
Sy-2	14	1(7.1)	26.4 ($2.8 \cdot E^{-7}$)
2nd experiment			
Mock	15	12(80.0)	
Sy-2	15	0(0)	60 ($9.5 \cdot E^{-15}$)
3rd experiment			
Mock	37	13(35.1)	
Sy-2	47	1(2.1)	31.2 ($2.3 \cdot E^{-8}$)

Table 2
Spraying treatments of crude extract of freeze dried leaves of Capsicum chinense 'Sy-2' to Nicotiana benthamiana post-inoculation of CSVd.

Spraying time after CSVd inoculation	No. of treated plants	No. of infected plants (%)	Chi-square (*P*-value)
1 day			
Mock	12	5(41.7)	
Sy-2	12	2(16.7)	1.29 (0.079)
2 day			
Mock	12	5(41.7)	
Sy-2	12	3(25.0)	0.57 (0.24)
3 day			
Mock	10	8(80.0)	
Sy-2	11	5(45.5)	8 (0.0025)
4 day			
Mock	10	5(50.0)	
Sy-2	13	7(53.9)	0.17 (0.39)

No plant showed digesting ability in its crude extract except *Capsicum* 'Sy-2' and *Pelargonium* (Fig. 1A). For *Capsicum* plants, the concentration of CSVd calculated by real-time RT-PCR was approximately 1/2000 relative to the control (buffer treatment), but for *Pelargonium* the concentration decreased to only 1/6 relative to the control (Fig. 1A). RNA gel blot analysis suggested that CSVd was completely digested by 'Sy-2', but other plants showed no effect on CSVd (Fig. 1B). The substrate-based gel assay showed that spots indicating RNase-like activities were detected at relatively small molecular sizes in *Mirabilis* and *Bougainvillea*, whereas strong RNase-like activity of high molecular weight was detected in 'Sy-2' (Fig. 1C), and this signal was detected as a smear band. RNase-like activity was not detected in *Pelargonium* (Fig. 1C).

Possible explanations for the smeared signal are that the different RNases had multiple molecular weights or that various complex molecules combined with specific RNases were present.

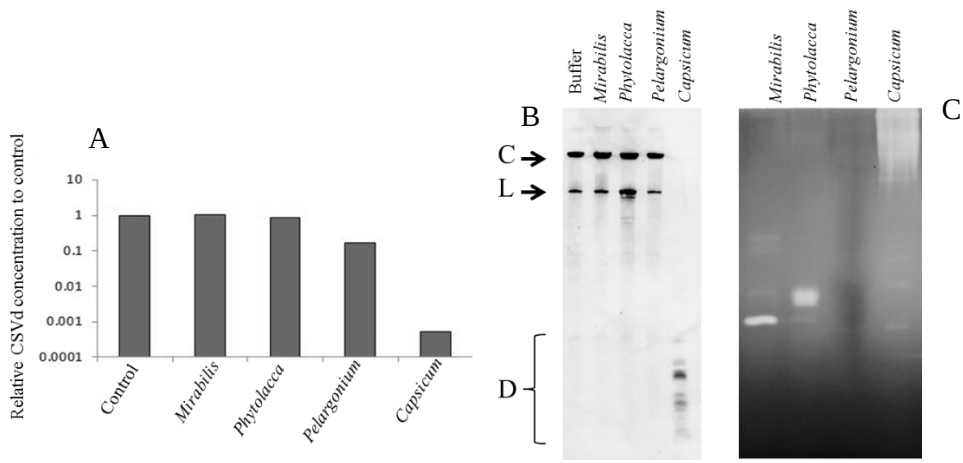

Fig. 1 CSVd digesting activities between several plant cepcies.

A: CSVd digesting activities determined by real time RT-PCR.
B: Result of polyacrylamide electrophoresis after mixing treatment with each plant leaf. Circular and linear CSVd are represented as C and L, and digested small RNAs are represented as D beside the figure.
C: Substrate-based gel assay of crude extract of four plant species. RNases in separated proteins digest the trula RNA contained in gel resulting in blue-white bands.

CSVd-digesting activities were different between cultivars of *C. chinense* and *C. annuum* (Fig. 2AB). Very strong cultivars of RNase activities were found only in *C. chinense*. In *C. chinense*, undigested CSVd was not detected in 19 of the 103 cultivars (Fig. 2A), whereas *in C. annuum* no cultivar digested CSVd completely (Fig. 2B).

The RNase activities were cultivar-dependent and the activities were quantitative. Very strong cultivars and weak cultivars can be used to determine the loci by such means as genetic mapping in cross progenies from these cultivars.

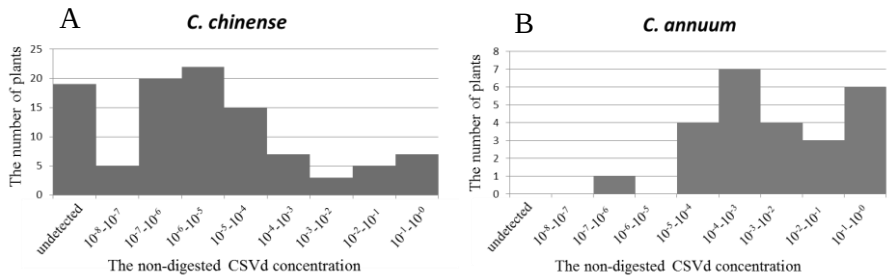

Fig. 2 Histograms of the number of plants with each RNase activities.

'murasaki' is a *C. annuum* cultivar and its RNase activity is very weak. CP is a *C. chinense* cultivar and its RNase activity is very strong. Interspecific hybrid of 'murasaki' and CP has a strong RNase activity. Moreover among 200 individuals of ('murasaki' ×CP)×'murasaki', 106 individuals have strong RNase activities and 94 individuals have weak RNase activities (Fig. 3). This result suggests that the strong RNase activity is a dominant trait and governed by one gene.

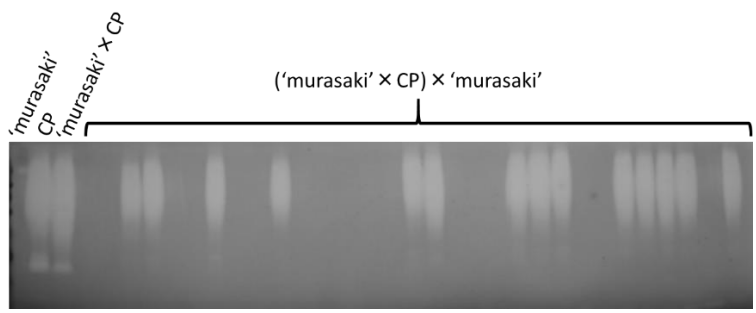

Figure 3
Substrate-based gel assay of 'murasaki', CP, F1 and ('murasaki'×CP)×'murasaki'.

Reference

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P4-04

The effect of mycorrhiza inoculation, water supply and harvest time on eggplants' photosynthetic activity and the fruits' total polyphenol content

Szonja Szimóna Burján[1], Zsuzsa Nagy[1], Daood Hussein[2], András Neményi[1], Lajos Helyes[1], Zoltán Pék[1]

[1] *Institute of Horticulture, Faculty of Agriculture and Environmental Sciences, Szent István University, Gödöllő, Hungary*

[2] *Regional Knowledge Centre, Szent István University, Gödöllő, Hungary*

Abstract

Eggplant (*Solanum melongena* L.) is not only known for its high vitamin and mineral content, but also recognized as a rich source of important phytochemicals, which have antioxidant activity. One of the most important nutrients is polyphenols. Their quantity and quality in the fruits are not homogeneous; therefore the investigation of factors influencing – for instance arbuscular mycorrhizal fungi (AMF) inoculation, water supply and harvest time – on total polyphenol content is an exciting research area. A 1-year open field experiment was carried out at Gödöllő in 2014 to observe the effects of AMF inoculation, different irrigation volumes and different harvest times on the amount of total polyphenol in eggplant fruits, and to examine the eggplant leaves' photosynthetic activity (F_v/F_m of PSII). Mycorrhized treatments were planted out with 25 g AMF per seedling. The irrigation treatment had 2 levels: the optimal irrigation volume was calculated from the daily potential evapotranspiration and was compared to a treatment utilizing 50% of the optimal water volume. For analytical measurements, fruits were harvested at two times: 3rd of September and 2nd of October. The following results were obtained. There were 12 measurements during the vegetation period, with chlorophyll fluorometer. The F_v/F_m value was measured in the range of 0.609 to 0.782. The total polyphenol content ranged from 0.13 to 0.24 mg gallic acid equivalents (GAE) per g fresh weight (FW).

Keywords: eggplant, mycorrhiza inoculation, photosynthetic activity, total phenol

1. Introduction

Solanaceae vegetables are considered to be a staple food in many areas all over Hungary, Europe and the World. This family includes over 2000 species; some of them are the most popular foods consumed today, such as tomato. The most economically important vegetable genera in the family are *Solanum* (potato and eggplant), *Lycopersicon* (tomato) and *Capsicum* (pepper). Botanically, the eggplant is a short-lived perennial in the tropics, but in temperate climatic zones it is cultivated as an annual, and growth is determinate (Somos 1983, Rubatzky and Yamaguchi 1997, Toensmeier 2007). The harvested area of eggplant was estimated at 1.85 million hectare worldwide with 26.13 tonnes/hectare in 2012. More than 80 % of the total area is in China and India, which is not surprising, since the gene pool centre of eggplant is in India – it spread in China during the 5th century BC, then in West Asia, North Africa and Europe (Daunay et al. 2001, Faostat 2015, Mutegi et al. 2015). Eggplants – as members of the warm-season crops (Markov-Haev's temperature range) – do not tolerate frost, and grew best at

22±7ºC. The start time of the fruits' harvest depends on some factors, for example the cultivar, seed germination, environmental parameters. The duration is also variable – it may be as little as 10 days, or as long as 40 (Somos 1983, Rubatzky and Yamaguchi 1997). The fruit is a large, pendant, glossy berry without a cavity. Fruits are good sources of digestion-supportive dietary fibre (Dhingra et al. 2012, Helyes et al. 2015), ascorbic acid (Hanson et al. 2006) and bone-building manganese and magnesium, enzyme-catalysing molybdenum and heart-healthy potassium (Raigón et al. 2008, Dias 2012). Besides being rich in nutrients, eggplant provides relevant quantities of phenolic substances, which compounds have antioxidant capacity. Phenolic compounds are found in both the pulp and skin of the fruits. Nasunin, an anthocyanin pigment isolated from purple eggplants' peel, belongs to the flavonoids, induces the fruit's dark purple colour, and it is known to inhibit peroxidation (Noda et al. 2000). The fruit flesh also presents a major phenolic compound, chlorogenic acid – it plays a role in plant defence, as well as an antioxidant (Prohens et al. 2013).

There are several factors that affect eggplants' plant physiology and fruit quality, for instance biotic factors, such as mycorrhizas, and abiotic factors, such as water supply and cultivation period. The effects of an arbuscular mycorrhizal fungi (AMF) inoculation on *Solanaceae* vegetables have been reported by many authors. Ortas (2012) found that the AMF inoculum increased the eggplants' root colonization and nutrient uptake. The association between the fungus and the root is a partnership. The establishment of this mutualistic association allows the fungus to acquire carbon, and helps the plant to acquire nutrients, especially phosphorus and water (Baslam et al. 2011). The later parameter is probably the most important, because irrigation is essential in eggplant production in all regions, where only few precipitation is available during the growing season (Chen & Li 1996). Karam et al. (2011) found that deficit irrigation significantly decreases the mean of eggplant fruit weight, but the timing deficit irrigation for 2 weeks prior to flowering may relieve the rate of decrease. Therefore under limited water-supplied circumstances, the simultaneous application of timed deficit irrigation and mycorrhiza inoculation may also support the host plants' protection against oxidative stress induced by water deficit. In addition, the cultivation period/harvest time may also have an impact on the fruits' nutritional quality. Helyes et al. (2015) found that harvesting eggplant in autumn, after some relatively cold days, may cause better fruit quality, for a higher quantity of total polyphenol content.

These above mentioned factors (mycorrhiza inoculation, water supply, harvest time) may have measurable effects on the plants' physiological processes also. Barzana et al. (2012) examined mycorrhized and non-mycorrhized tomato plants' leaf water potential under well-watered and drought stress conditions. They found that drought stress decreased leaf water potential in both treated and non-treated plants, but the decrease was more pronounced in non-treated plants. Kirnak et al. (2001) reported that water stress results in significant decrease in dry matter and chlorophyll content in eggplant leaves. Partelli et al. (2009) examined the particularly cold sensitive *Coffea* genus's photo protective mechanisms related to low positive temperatures, and found that a gradual temperature decline decreased the maximum quantum efficiency of the photosystem II [F_v/F_m] and the maximum fluorescence [F_m] in every case, but the recovery period was different between genotypes. According to this result, measuring the leaves photosynthetic activity is not just a reliable method to monitor the plants' physiological mechanisms, but it can contribute to selecting tolerant genotypes and improving crop management.

2. Materials and methods

2.1. Experimental details

A medium vigorous eggplant cultivar, Barcelona F1 (medium long, very uniform, nice coloured fruit, with firm flesh) was cultivated with conventional horticultural practices for the present study at Gödöllő (Gödöllői Agrár Központ Kht., Horticultural experimental field, 47°61' N, 19°32' E) with soil classified as Cambisol (sandy loam soil), in 2014. The experimental fields' water capacity is low, but the hydraulic conductivity is good. The subsoil water is below 4 m. Seeds were sown on 8th of April. Seedlings were planted out in open field on 27th of May, in simple rows, with a plant density of 5 plants/m^2. All of the area was arranged for irrigation treatment – one half was the optimal irrigated (100), the other part was the half amount irrigated (50). Both plots were divided in half for the application of the mycorrhization treatment (M). In AMF treated parcels, before transplantation, the seedlings were inoculated with 25g of Symbivit® mycorrhiza product. This contains the reproductive forms of 6 different *Glomus* fungi species: *G. intraradices* BEG140, *G. mosseae* BEG95, *G. etunicatum* BEG92, *G. clarodideum* BEG96, *G. microaggregatum* BEG56 and *G. geosporum* BEG199. The control parcels did not receive inoculums (C). Thus, we got four treatment-combinations: 50C, 100C, 50M, 100M. Irrigation water quantity was prepared based on the estimated daily potential crop evapotranspiration (PET), and was applied three times a week: Monday, Wednesday, and Friday. The amount of water was the difference between the sum of precipitation amounts fell on previous days and the sum of following days' PETs. The PET was calculated using the estimated daily mean temperature and the crop coefficient.

2.2. Vegetation measurements

To observe the effects of the factors described above on the plants' physiological processes, photosynthetic measurements were carried out during the vegetation period with a PAM-2500 portable chlorophyll fluorometer (Walz Heinz GmbH, Effeltrich, Germany). This device is used for the measurement of the quantum efficiency of photosystem II (PSII) photochemistry, which can be used to estimate the linear electron transport rate. We applied F_v/F_m test using fast kinetics method on a weekly basis. Four repeats, on four dark-adapted fully developed top leaves of a single plant in each replicate were measured. Leaf samples were dark adapted for 35 minutes, using a leaf clip. The ratio of variable fluorescence to maximum fluorescence (F_v/F_m) was determined for each measurement using PamWin 3.0 software (Goethem et al. 2013).

2.3. Analytical measurements

To determine the effects of the factors described above on the plants' fruit quality, analytical measurements were carried out after harvesting with a SPECORD 200 PLUS UV-visible spectrophotometer (Analytik Jena AG, Jena, Germany). This device is used for quantitative measurements of solutions' transmittance or reflectance. For the measurements we used two harvest times' fruits: 3rd of September and 2nd of October. Fruits were harvested at market maturity, when they reached market size and the skin became glossy. 4-4 plant's fruits were chosen per replicate. After delivery to the Regional Knowledge Centre's Laboratory, the samples were immediately cleaned, chopped into small pieces, placed into closable plastic jars, and stored at -18°C until measurement. For total phenol content determination Folin-Ciocalteu method were used, according to procedure described by Nagy et al. (2014). Results were expressed as gallic acid equivalents fresh weight extract (mg GAE/g FW).

2.4. Statistical analysis

Statistical analyses of the data was performed using Microsoft Excel (Microsoft Co., USA) and SPSS modules (IBM Co., USA). For all statistics, α was set to 0.05. Weather conditions are illustrated in Excel chart builders' combine diagram. Linear model was made for "Photosynthesis" as dependent variable with the following explanatory variables: "Irrigation", "Mycorrhization", and "MeasurementDay". For graphical representation, we choose Excel chart builders' combine diagram. Since the distribution of total polyphenol data was skewed, we log-transformed them before analysis. Then, we fitted general linear model for "LogPolyphenol" as dependent variable with the following explanatory variables: "Irrigation", "Mycorrhization", and "Harvest". For graphical overview, we choose SPSS chart builders' boxplot visualization – it helps to understand the distribution of a sample, and its shape, central tendency, and variability. Parts of the boxplot, from top to bottom: 95 percentile, 5 percentile, median and mean, 25 percentile, 5 percentile.

3. Results and discussion

3.1. Weather conditions

The year 2014 ranks as Earth's warmest since modern-day record-keeping began in 1880. Before the experiment, from January to March, there were the biggest positive anomalities, which had a negative effect on pest management. June, July and September also gave big positive anomality without drought and heat waves. Figure 1 shows the weather information under the eggplant cultivation in 2014.

Based on the Hungarian Meteorological Service's data, 2014 was the 9th rainiest year in Hungary since 1901. During the eggplants' vegetation period, only June was considerably drier than usual, with 6 mm precipitation, but during the entire period more than 500 mm precipitation fell. This would have been sufficient for eggplant, but the distribution and intensity were unfavourable. Almost 75% of the rainfall fell only during 14 days. As a result there were warmer and wetter weather conditions prevailing during the experiments' summer and autumn.

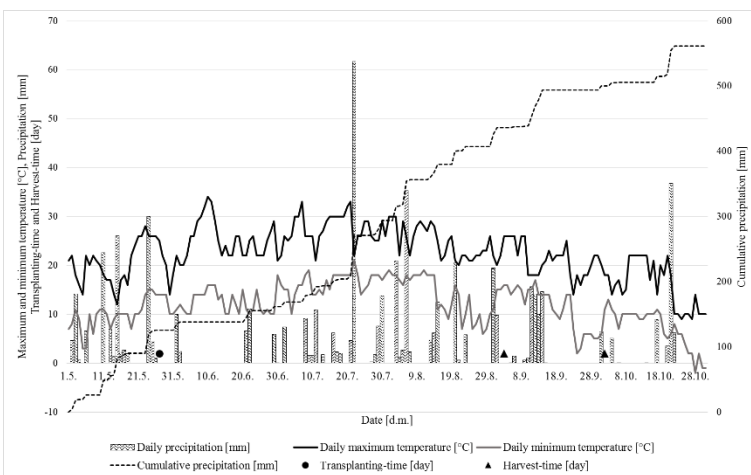

Figure 1
Weather information under the eggplant cultivation in 2014, based on data measured in the Experimental Farm

3.2. Photosynthetic activity

There were 12 measurements during the vegetation period, from 24th of July, to 07th of October. The F_v/F_m value was measured in the range of 0.609 to 0.782. The maximum quantum yield measured on dark-adapted leaves can help to estimate the potential efficiency of PSII, and through this the most types of plant stress. Several studies described that water stress had no effect on F_v/F_m values (Lu & Zhang 1999, Pankovic et al. 1999). This result is partially confirmed by the current study also, since the analysis showed no significant difference between the treatment combinations' curve characteristics, and statistical analysis was invalid (Levene's test, F=2.267, p=0.013), thus we couldn't make conclusive inferences from it. Therefore we used the mean of the measurement times for interpretation (Figure 2).

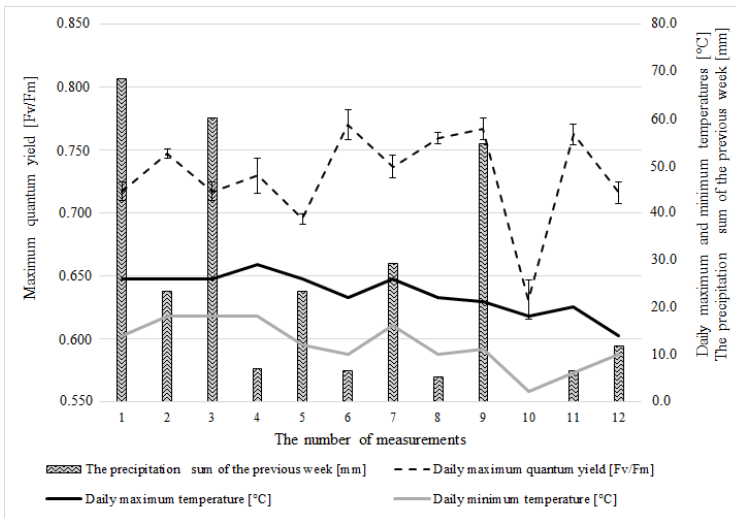

Figure 2
Weather information under the eggplant cultivation in 2014, based on data measured in the Experimental Farm.

The first four measurements showed a downward trend. The 5th measurements' value (19th of August) exceeded the lower limit of the optimum range. The daily maximum temperature was considered to be a good value, but the minimum temperature was lower than the eggplants' optimum. Furthermore the precipitation volumes that fell before this time were extreme high. From the 6th measurement to the 9th measurement, the trend was nearly balanced with an average of 0.758. The daily minimum temperatures were a little bit low, but the maximum temperatures were good, and the precipitation amounts were not excessively high. During the week before to the 10th measurement, the daily minimum temperature decreased to 2 °C. This is much lower than the eggplants' optimum and was reflected in the F_v/F_m value: 0.631 ± 0.031. The 11th and 12th measurements' values demonstrated that the plants were able to regenerate themselves relatively quickly.

3.3. Total polyphenol content

The total polyphenol content results presented in Table 1 show a wide variation between the various treatment combinations. Strongly significant differences were obtained for harvest time

on total polyphenol content ($F_{1,24}$=24.772, p<0.001). Higher levels were measured in fruits harvested on the 3rd September. These results are not in accordance with Helyes et al. (2015) who highlighted, based on spectrophotometric results, that late harvested eggplant fruits have increased total polyphenol content. According to the literature the vegetables' polyphenol production is more influenced by temperature extremes. Nevertheless we assumed that the water stress caused this increase. Nearly 30 mm precipitation fell in the experimental field during the three days prior to the 1st harvest, which may have caused groundwater saturation.

Total polyphenol content [mg GAE/g FW]		
	1st harvest time	**2nd harvest time**
	3rd September	*2nd October*
50C	0.25 ± 0.02	0.14 ± 0.01
100C	0.21 ± 0.07	0.14 ± 0.01
50M	0.23 ± 0.05	0.16 ± 0.03
100M	0.17 ± 0.04	0.15 ± 0.03

Table 1
Mean and standard deviation of the measured total polyphenol contents (n=4) in different treatment combinations' eggplant fruits (100: optimal irrigated, 50: half amount irrigated, M: mycorrhizated, C: control for myorrhization).

We found a significant effect of the water supply on total polyphenol content ($F_{1,24}$=5.066, p=0.034). The fruits harvested in half amount irrigated plots contained more polyphenol. We consider that this result can be attributed to the dilution effect described by Helyes et al. (2012). The highest polyphenol amounts were observed in half amount irrigated plots harvested on 3rd of September, with 0.25 ± 0.02 (50C), and 0.23 ± 0.05 (50M) mg GAE/g fresh weight. Unfortunately these values are lower than the maximum amount reported by Nagy et al. (2014): 0.72 mg GAE/g fresh weight. The interaction between harvest time and water supply was not significant ($F_{1,24}$=2.336, p=0.140).

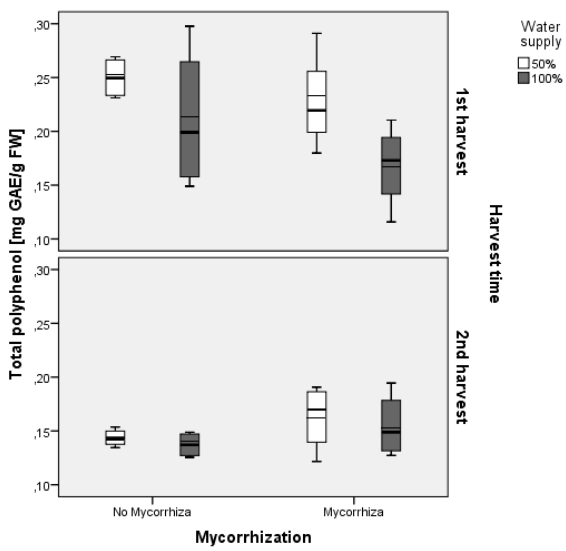

Figure 3
Eggplant fruits' total polyphenol content results as a function of factors (100: optimal irrigated, 50: half amount irrigated, bold line: median, thick line: mean).

In case of mycorrhization, based on the data, the fruits in treated plots contained less polyphenol at 1st harvest, and more at 2nd harvest. The statistical analysis did not confirm this assumption. Mycorrhization had no significant effect either alone ($F_{1,24}$=0.129, p=0.722) or in interaction with the other factors: water supply ($F_{1,24}$=0.156, p=0.696), harvest time ($F_{1,24}$=3.976, p=0.058). This can be more clearly seen in the boxplots of Figure 3. In both harvest time and water supply, mycorrhiza inoculation did not result in noticeably more or less total polyphenol amounts.

4. Acknowledgements

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P4-05

Development of protocols to circumvent fresh seed dormancy in eggplant

Ana Fita, Anastasia Atucha-Zamkova, Mariola Plazas, Adrián Rodriguez-Burruezo and Jaime Prohens

Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universitat Politècnica de València, Camino de Vera 14, 46022 Valencia, Spain

Abstract

Rapid and uniform germination of the seeds is beneficial for commercial production of seedlings and for breeding programmes in eggplant (*Solanum melongena*). In the case of eggplant, fresh seeds frequently display dormancy and germination improves dramatically a few months after seed extraction. In order to test different treatment potentially useful to remove dormancy of fresh seeds, eight different combinations (C1-C8) of seven factors were tested on fresh seeds (less than two months from harvest) of the H15 variety. The seven factors evaluated included: i) soaking of seeds, ii) scarification with sodium hypochlorite (NaClO), iii) application of gibberellic acid (GA_3), iv) use of potassium nitrate (KNO_3) as a moistening agent, v) cold stratification, vi) application of a heat shock, and vii) light irradiation during germination. Two germination parameters, early germination and 7 d germination rate, were recorded. Out of the eight treatments tested, the one consisting in application of soaking, GA_3, KNO_3, and heat shock (C6) resulted in a 100% germination rate in just three days. Other combinations wich dramatically improved germination over the control included C8 (soaking, NaClO, KNO_3 and light), C5 (soaking, GA_3, cold and light), and C2 (KNO_3, cold, heat and light). The ANOVA analysis of the treatments' simple effects in the different combinations, showed that on average, soaking, KNO_3, and light during germination had significant positive effects on germination, whereas NaClO and cold had significant negative effects. GA_3 and the heat shock treatments did not show a significant effect on germination. Based on the results the C6 combination is recommeded for rapid germination of fresh seeds, and on the basis of the simple effects we propose testing an easy and inexpensive combination consisting of soaking, KNO_3 and light.

5. Introduction

Seed dormancy is common in some materials of eggplant (*Solanum melongena* L.) as well as in related species [1]. Rapid and uniform germination of the seeds is beneficial for commercial production of seedlings, as well as for breeding programmes; in this latter case it allows the use of seeds just extracted from the fruit and consequently saving time in the breeding process.

In the case of eggplant, germination improves dramatically a few months after seed extraction [2]. This might be due to the reduction of abscisic acid (ABA) levels, which play a major role in seed dormancy. In eggplant, 2-month old seeds of non-dormant cultivars had a concentration twice as low as 2-month old seeds of dormant cultivars [2]. These authors also found that, in general, ABA concentration in dormant seeds decreases considerably after 12 months [2].

Giberellic acid, (GA_3) usually has a major effect on seed germination, due to its antagonist function with ABA [3]. *Solanum sarrachoides* seeds only germinate when a certain thermal

time has been given (seeds have received a quantity of heat during a number of days), but a high GA_3 concentration reduces the thermal time required [4]. In *S. rostratum*, gibberelic acid also broke physiological dormancy [5], thus reducing germination time.

Among the factors that may improve germination of dormant seeds, potassium nitrate (KNO_3) acts on seed dormancy by being a nitrous oxide donor, a molecule involved in seed germination [6]. In this way, KNO_3 also had an effect in breaking seed physiological dormancy in *S. rostratum* [5]. Also, soaking seeds in water is a highly efficient way of breaking physical or physiological dormancy. This makes sense, because imbibition of seeds by water is indispensable for seed germination. However, not only water is necessary; sometimes, the absence of some key nutrients in the solution may have an effect on seed germination, such as the absence of nitrogen or phosphorus [7].

Physical dormancy can be broken using other physical methods, such as scarification with sandpaper, sulfuric acid (H_2SO_4), or sodium hypochlorite (NaClO) bleach [3]. H_2SO_4 and sandpaper scarification were found to increase germination rates in *Solanum viarum*, a tropical wild shrub [7]. H_2SO_4 is not only effective in breaking dormancy; it also is a powerful disinfectant [8], which may make it useful for *in vitro* culture of seeds. NaClO, commonly in the form of commercial bleach is also used for disinfecting seeds, including those used for *in vitro* culture.

The objective of this study was to develop protocols for the rapid germination of fresh eggplant seeds displaying dormancy.

6. Materials and methods

To obtain a suitable protocol for rapid eggplant seed germination an experiment testing different treatments consisting of combinations of seven germination factors on fresh (less than 2 months from harvest) seeds of eggplant from the H15 variety [9] was performed as described in [10]. Eggplant variety H15 belongs to the traditional Almagro type, which is very popular in the center of Spain for making pickles. This variety presents dormant seeds resulting in a very irregular germination.

The seven factors evaluated included: i) soaking of seeds in water for 1 d, ii) scarification by immersion of seeds for 10 min in a solution of commercial bleach at 30%, resulting in a solution with a final concentration of 1.2% NaClO, iii) application of gibberellic acid (GA_3) by soaking seeds in a 500 ppm solution for 1 d, iv) use of 1000 ppm KNO_3 solution for watering the Petri dishes, v) cold stratification applied by placing seeds on Petri dishes with a moistening agent at 4ºC for 7 d, vi) application of a heat shock, placing seeds on Petri dishes with moistening agent at 37ºC for 1 d and vii) light irradiation during germination. A total of eight different combination as follows; C1 = control with no factors being applied; C2 = KNO_3, cold, heat, and light; C3= NaClO, GA_3, heat, and light; C4= NaClO, GA_3, KNO_3, and cold; C5 = soaking, GA_3, cold, and light; C6= soaking, GA_3, KNO_3, and heat; C7= soaking, NaClO, cold, and heat; and, C8=soaking, NaClO, KNO_3, and light.

For each combination, six replicates, each of which consisted of a Petri dish (8.5 × 2.5 cm) containing 25 seeds were used. Seeds were germinated in the Petri dishes on a layer of 0.5 cm of hydrophilic cotton covered by filter paper. After all the factors had been applied (day 0) the Petri dishes were placed in a climatic chamber with a 16 h light / 8 h darkness photoperiod and a 25 ºC temperature. GRO-LUX F36W/GRO (Sylvania, Danvers, MA, USA) fluorescent tubes were the source of light. Petri dishes were covered with aluminium foil, except for those combinations that involved the treatment with light. The humidity in the dishes was kept constant by covering them with a lid and adding moistening solution if necessary.

Two germination parameters, namely days to first seed germinated and 7 d germination rate, were recorded in fresh eggplant. Data were subjected to analysis of variance (ANOVA) to detect differences among the combinations studied. Orthogonal decomposition was used to evaluate the simple effects of each of the factors evaluated.

7. Results and discussion

The ANOVA analysis of the results (Table 1) showed that there were significant differences among different combinations in terms of days to first seed germination, and number of germinated seeds at 7 d.

	Days to first germination		**Seeds germinated at 7 days**	
	df	**Mean squares**	**df**	**Mean squares**
Treatment	4	28.64^{***}	7	648^{***}
Error	20	0.38	40	10

Table 1
ANOVA analyzing the effects of the treatment combination d.f: Degrees of freedom; ***, mean significant at P-value < 0.001. Combinations for which no seeds germinated were excluded from the ANOVA for days to first germination as their value is infinite

Some combinations resulted in seeds germinating very soon. For example seeds subjected to combination C6 (soaking, GA_3, KNO_3, heat and darkness) began to germinate at day 3, whereas other combinations, such as C1 (control), C3 (NaClO, GA_3, heat and light) and C7 (soaking, NaClO, cold and heat) failed to germinate at all. Combinations C2 (KNO_3, cold, heat and light), C5 (soaking, GA_3, cold, light) and C8 (soaking, bleaching, KNO_3, and light) startet to germinate seeds around the 6^{th} day. Combination C4 (NaClO, GA_3, KNO_3 and cold) germinated the first seed at day 14 (Figure 1).

Combination C6 clearly stands out as the best performer, with a 100% germination rate (Figure 2). Other combinations with good results were in decreasing order C8, C5 and C2.

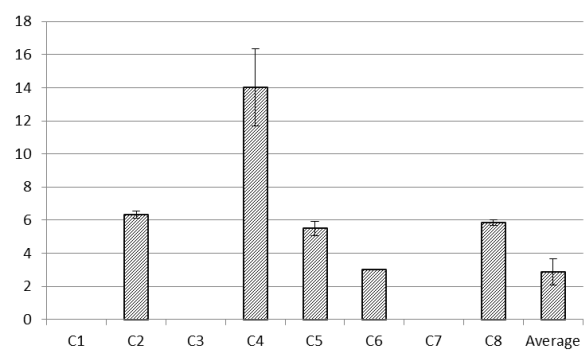

Figure 1
Bar chart showing the number of days it took in each combination to germinate at least one seed. No bar indicates no germination. Error bars correspond to the standard error

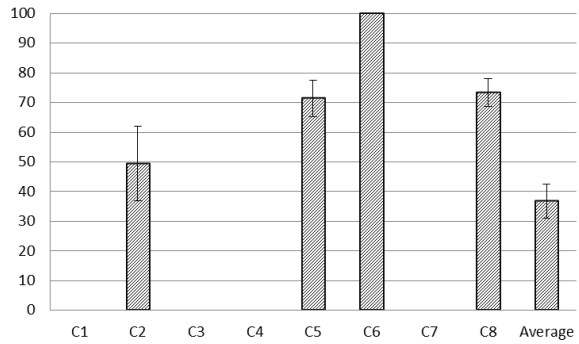

Figure 2
Bar chart showing the germination rate at 7d. No bar indicates no germination. Error bars correspond to the standard error

The three most successful combinations had in common the most critical and significant factor: soaking. The ANOVA analysis decomposition of the simple effects using an orthogonal array design [10] showed that soaking (11.04), KNO_3 (10.63) and light (7.04), had significant positive effects on germination, whereas bleach (-10.38) and cold (-2.13) had significant negative effects. Surprisingly, GA_3 (1.88) and heat (1.46) did not show a significant effect on fresh seeds.

Overall, the results show that combination C6 (soaking, GA_3, KNO_3, heat and darkness) allow a rapid and even germination. However, the results indicate that a combination not tested (soaking, KNO_3 and light) shall probably produce good results in improving germination of eggplant.

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P4-06

Determination of *C. baccatum var. pendulum* aroma components

Martijn Eggink[1], Yury Tikunov[3], Chris Maliepaard[3], Suzanne de Wit-Maljaars[2], Gerald Freymark[2], Han de Rooij[2], Aat Vogelaar[2] , Evert Gutteling[2], Jair Haanstra[1], Arnaud Bovy[3], Richard Visser[3], Eike Rademacher[1]

[1] *Rijk Zwaan Breeding B.V., De Lier, The Netherlands*

[2] *Rijk Zwaan Breeding B.V., Fijnaart, The Netherlands*

[3] *Wageningen UR Plant Breeding, Wageningen University and Research Centre, Wageningen, The Netherlands*

Abstract

The flavour of a fruit is a complex multifactorial trait composed of the experience of its taste, smell and texture. All three flavour components are influenced by environmental factors such as growth conditions, storage or preparation of the fruit as well as intrinsic genetic determinants. We have recently developed a framework to describe flavour in sweet peppers (*C. annuum*) and constructed a population of near-isogenic lines (NILs) to capture flavour components from a hot *C. baccatum* var. *pendulum* accession (PEN45) in a *C. annuum* background. Research on this NIL population revealed several QTLs that influence fruit flavour by altering either taste, smell or texture of the fruit. In particular, presence of a QTL located on linkage group 3 of PEN45 resulted in an aroma described as "tropical fruit aroma". We have found four volatiles that potentially contribute to this aroma and are searching for enzymes that might constitute the respective biosynthetic pathways. Sequencing of PEN45 as well as the *C. annuum* parent used to generate the NILs is expected to reveal several of the underlying PEN45 derived genes. The respective ongoing work will be presented at the conference and might give a starting point to the targeted composition of new paprika flavours.

Keywords: Biochemical Profiling, Volatiles, *C. baccatum var. pendulum*, Flavour

1. Introduction

Ají peppers (*Capsicum baccatum* L. var. *pendulum*) are a common ingredient of the Andean cuisine, frequently used as condiment in local dishes. As such, Ají peppers have undergone centuries of selection and offer a rich variation in tastes and aromas.

Kollmansberger et al. (2011) analysed the composition of volatiles underlying aroma profiles of two *C. baccatum* accessions that were described by a taste panel as having a green, earthy aroma. Of the ca. 100 volatiles they identified by GC/MS in the headspace of ripe *C. baccatum* fruits, only 13 were found to be contributing to the respective aroma by sniffing analysis. Next to 2-Heptanethiol these are several terpenes, phenols and pyrazines, that make up the green, earthy aroma of *C. baccatum* fruits. In addition a few esters were indicated to provide the "fruity, exotic" aroma component found in one of the *C. baccatum* accessions.

Despite their widespread consumption in the Andean countries and their apparent flavour qualities, *C. baccatum* accessions have rarely been used in *C. annuum* breeding programs. This

is due to post-fertilization genetic barriers that greatly hamper interspecific hybridization (Yoon *et al*. 2006).

We have recently developed a *C. annuum* x *C. baccatum* var. *pendulum* mapping population and detected a QTL for "fruity, tropical" aroma in some of these lines (Eggink *et al*., 2014). Here we report our progress in identifying the underlying changes in fruit volatile composition.

2. Materials and Methods

C. baccatum var. *pendulum* accession PEN45 was backcrossed with three Rijk Zwaan cultivated *C. annuum* breeding lines to develop a multi-parent mapping population. For validation and further testing, near-isogenic lines (NIL) were developed from the mapping population by one generation of backcrossing with one of the *C. annuum* parents and two selfing steps. For a detailed description of the QTL mapping and line development, the reader is referred to Eggink *et al.*, 2014.

Metabolic profiling followed Eggink *et al*., 2012.

3. Results and Discussion

Sensory evaluation of fruits for the QTL mapping of fruit traits derived from *C. baccatum* var. *pendulum* PEN45 revealed that several plants produced fruits with a more intense aroma, described as "fruity" or "tropical" by members of the taste panel. Mapping of this trait resulted in the identification of a QTL (LOD 8.0, 38.7% explained variance) on linkage group 3 of the respective genetic map.

To further characterize the compounds underlying this novel taste, we generated 23 NILs, 6 of which contained the LG3 fragment either in homo- or heterozygous state within the genetic background of one of the parental *C. annuum* lines. Sensory evaluation of these NILs revealed that presence of the LG3 fragment resulted in significantly higher scores for the measured attributes "Aroma", "Flowers", "Spices (non-pungent)", "Celery" and "Chives", while "Grassiness" was scored significantly lower.

Profiling of volatiles present in the headspace of NILs containing the LG3 fragment showed an increase in intensity of 6-methyl-4-oxo-heptenal (13.0x) as well as of an undefined compound (8.6x) in comparison to NILs lacking the *C. baccatum* fragment. At the same time a reduction in (Z)-butanoic acid 3-hexyl ester (0.2x) and 2-isobutyl-3-methoxypyrazine (0.3x) was measured in these NILs.

All three known compounds have been described as having a "green" to "green apple" like aroma (The Good Scents Company, 2016) and hence seem not to be directly linked to the "fruity, tropical" aroma found in the NILs. However human perception of aroma is complex and affected by interactions between volatile compounds. Thus it might be possible that the "fruity, tropical" aroma of NILs and plants containing the LG3 fragment from PEN45 is present in the respective plants but masked by the presence of (Z)-butanoic acid 3-hexyl ester and 2-isobutyl-3-methoxypyrazine. In this case, changes in the abundance of all 4 of the above volatiles might lead to a change in "green" aroma perception and a shift towards "fruity" aroma perception.

Alternatively the LG3 fragment of PEN45 might contain genes that encode for enzymes either involved in the biosynthesis or conjugation of volatiles, that we did not pick up during our analysis due to their low odour threshold and hence low concentration in the headspace of the fruits.

Analysis of the PEN45 genomic fragment contained in the NILs with the “fruity, tropical” aroma will give new insights into the processes leading to the respective flavour. Additionally, comparison of flavour profiles from other *Capsicum* species might highlight common volatiles that contribute to specific aroma characteristics and narrow the range of volatiles underlying the particular aroma of the PEN45 derived material.

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P4-07

Effect of the genotype and growing conditions on the main volatile compounds in *Capsicum* peppers

Ana M. Ribes-Moya[1], Estela Moreno Peris[1], Ana Fita[1], María D. Raigón[2] and Adrián Rodriguez-Burruezo[1]

[1] *Instituto de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV), Universitat Politècnica de València, Valencia, Spain*

[2] *Departamento de Química, Universitat Politècnica de València, Valencia, Spain*

Abstract

A plethora of studies have demonstrated that growing conditions (e.g. saline conditions, low irrigation, early cultivation, organic production), as well as the genotype, may affect quality factors in fruits and vegetables. Most studies have been aimed to the levels in compounds related to taste (e.g. sugars, acids, tanins) and/or functional compounds (e.g. vitamins, phenolics, carotenoids, antioxidants). However, the knowledge about the volatile fraction is very scarce or nil. In this regard, volatiles are not only responsible for the aroma of food and beverages but also are key factors for flavour as they are released during mastication and detected at the retronasal area. Moreover, most consumers of organic food consider that these products show a better flavour, although this preconception must be still clarified.

In the present work, we show the preliminary results of a study aimed to assess the effect of organic cultivation on the volatiles of *Capsicum* peppers. The volatile fraction of fully ripe fruits from two *Capsicum annuum* (cv. Valenciano and Jalapeno *M*) and one *C. chinense* (ECU-994) accessions corresponding to different varietal types and cultivated under organic and conventional growing practices were extracted by head space solid phase microextraction (HS/SPME) and analysed by gas chromatography/mass spectrometry (GC/MS).

As a whole, organic conditions provided higher total volatile content and number of compounds compared to conventional conditions. Thus, the number of volatiles in fruits from the organic trial ranged between 34 and 40 in Valenciano and Jalapeño respectively, while this number was lower than 30 in fruits from the conventional trial. Also, total volatiles content was 2.5-fold and 5-fold respectively in *Valenciano* and Jalapeno M from organic trials compared to those levels from conventional trials, while similar values from both trials were found in *ECU-994*. Moreover, considering individual volatiles, organic conditions increased the levels in cultivars Valenciano and Jalapeno M in comparison to conventional conditions, particularly in the case of α-himachalene and methyl salicylate. Genotype-by-environment interaction in many volatiles was also found, which is also discussed.

1. Introduction

Many studies have demonstrated that growing conditions (e.g. saline conditions, low irrigation, early cultivation, organic production), may affect quality factors in fruits and vegetables. Most of them have been aimed to the levels in compounds related to taste (e.g. sugars, acids, tanins) and/or bioactive compounds (e.g. vitamins, carotenoids, phenolics, antioxidants). However, studies involving flavour and aroma are still scarce. The aroma is a key factor in the organoleptic quality of fruits and vegetables, since volatile compounds, together with sugars and acids, are the main factors for flavour, and encompasses both taste and olfactory

stimuli. In addition, aroma and flavour affect dramatically consumer´s preferences. In this regard, most consumers of organic food consider that these products show a better flavour, although this preconception must be still clarified.

Previous reports on the volatile fraction of *Capsicum* fruits have highlighted the presence of a plethora of compounds. In pioneer experiments Keller et al. [1] found one hundred volatiles and Haymon and Aurand [2] isolated more than 120 volatile in the essential oil from the varietal type "Tabasco". Recently, studies like those from Rodríguez-Burruezo et al. [3] and Kollmannsberger et al. [4] identified approximately 350 volatile compounds from different cultivars of the five cultivated species of *Capsicum*.

In the present work, we show the preliminary results of a study aimed to assess the effect of organic cultivation on the volatile fraction of *Capsicum* peppers, which have been found to encompass a range of aromas and volatile profiles among different cultivars.

2. Materials and methods

The study was performed on three different varietal types of *Capsicum*: C. annuum Valenciano (bell/pimento pepper) and Jalapeno M (jalapeno), and *C. chinense ECU-994* (Figure 1), grown in two different trials following organic and conventional practices. Both trials were performed in 2014 and located in Bonrepos and Mirambell, in the periurban area of Valencia (Spain).

Three samples per accession x growing system combination were prepared from fully ripe fruits of the mentioned accessions. Each sample included 2 g from 3-5 different fruits, depending on the size of the fruits. Each sample was prepared in small cuttings of 2x2 mm squares.

Figure 1
Fruits from Valenciano, Jalapeno M and ECU-994 (from left to right).

The isolation of volatiles was conducted by Head-Space Solid Phase Microextraction (HS-SPME) technique using Supelco SPME fibers (SUPELCO, Bellefonte, USA). Each sample was placed in a 20 mL headspace vial.

For the analysis of volatiles a 6890N Network gas chromatograph system (GC) coupled to a 5973 mass spectrophotometer (Agilent, Spain) were used. A silica capillary column (5% phenyl-95% methylpolysiloxane as stationary phase, 30 m × 0.251 mm × 0.25 mm) was used. Helium was used as carrier gas with a flow of 1 μL/min, 250 ^{0}C (division ratio 1:8). The column was programmed (Gerstel Master software) with an initial temperature of 40 ^{0}C and 1 minute hold time. The first temperature ramp was programmed until 200 ^{0}C with a ratio of 5 ^{0}C/min and a hold time of 1 minute. The second ramp up to 250 ^{0}C, ratio of 15 ^{0}C/min and 3 minutes

hold time. The transfer flow was held at 220 ^{0}C. Detection by the mass spectrometer was performed in electron impact mode (EI) (ionization energy 70 eV). The acquisition was carried out in the scanning mode (mass range *m/z* 35-350 amu).

Identification of major volatiles was done by comparing the mass spectrum and corresponding RI values with the NIST library (MS Search 2.0.) and reference compounds.

3. Results and discussion

Our study showed that organic practices increased the total number of volatiles, with an interval from 32 to 40 compounds in *ECU-994* and Jalapeno M, respectively, while the range of volatiles in fruits from conventional practices was considerably lower, from 25 to 29 in Valenciano and Jalapeno M, respectively (data not shown). Quantitatively (GC peak area), total volatiles in Valenciano and Jalapeno M were higher under organic cultivation than conventional cultivation (2.5 and 5-fold respectively), while the contrary was true for ECU-994, for which total volatiles under conventional system were 1.8-fold those observed in the organic system (Table 1).

In terms of the 27 main volatiles identified in the fruits of the three accessions, most compounds showed higher levels under organic practices (O/C ratio > 1), although qualitative and quantitative differences were found depending on the genotype (Table 1).

Table 1

Profile of identified volatiles (GC peak area × 10^{6}) in the fruits of Valenciano, Jalapeno M and ECU-994 accessions varieties in organic (ORG) and conventional (CONV) growing systems, and organic/conventional ratio (O/C) for total and individual volatiles.

Volatile compounds	RI[1]	Valenciano			Jalapeno M			ECU-994		
		ORG	CONV	O/C	ORG	CONV	O/C	ORG	CONV	O/C
Sesquiterpenoids										
α-Cubebene	1344	-	-	-	-	-	-	40.67	98.28	0.4
Ylangene	1221	-	-	-	-	-	-	2.28	3.06	0.7
Copaene	1221	3.19	1.21	2.6	6.33	1.62	3.9	37.58	69.59	0.5
β-elemene	1398	2.00	0.30	6.7	1.61	2.30	0.7	-	-	-
Caryophyllene	1494	-	-	-	0.22	-	-	18.77	36.56	0.5
β-cubebene	1339	0.04	-	-	0.07	-	-	4.68	12.69	0.4
Bergamotene	1430	0.20	-	-	1.08	0.57	1.9	-	-	-
γ-cadinene	1435	-	-	-	-	-	-	7.60	14.64	0.5
β-cadinene	1440	-	-	-	0.87	-	-	34.06	59.07	0.6
α-Himachalene	1494	-	-	-	22.30	0.22	100	14.54	20.18	0.7
Monoterpenoids										
limonene	1018	0.17	0.16	1.1	-	-	-	-	-	-
Ocimene	976	0.88	0.37	2.4	-	-	-	1.62	1.06	1.5
Phenol derivatives										
methyl salicylate	1281	10.41	0.18	59.3	4.10	0.58	7.1	59.77	48.54	1.2
Norcarotenoids										
β-cyclocitral	1204	0.03	-	-	0.04	-	-	0.19	0.16	1.2
Alcohols										
Ethyl hexanol	995	-	0.16	-	0.36	0.05	7.1	0.06	0.03	1.9
6-methyl-1-octanol	1094	0.36	0.27	1.3	0.25	0.12	2.0	-	-	-
2-nonanol	1078	0.27	0.21	1.3	0.14	0.10	1.3	0.04	-	-
Lipoxygenase excision										
hexanal	806	0.05	-	-	-	0.13	-	-	-	-
Nonanal	1104	0.06	0.04	1.5	0.25	-	-	-	-	-
decanal	1204	0.18	0.14	1.3	-	0.15	-	-	-	-
Furanes										
2-pentylfuran	1040	2.53	-	-	0.82	1.47	0.6	-	-	-
Other compounds										
Pyrazine, 2-methoxy-3-(2-methyl propyl)	1204	3.11	-	-	13.38	7.25	1.9	-	-	-
Total Volatiles [2]		46.60	17.01	2.7	171.44	34.00	5.0	593.64	1061.3	0.6
Total number of volatiles [2]		34	25		40	29		32	30	

[1] *Retention index.*

[2] *Including identified (listed above in the table) and still unidentified volatile compounds.*

Thus, in most cases organic farming conditions provided higher levels of individual volatiles. Particularly in Valenciano and Jalapeno M peppers. This trend was specially obvious in the case of α-himachalene in Jalapeno M (O/C ratio 100) and methyl salicylate in Valenciano (O/C ratio about 60) and to a lesser extent in β-elemene in Valenciano and methyl salicylate and ethyl hexanol in Jalapeno M (O/C ratio 6-7) (Table 1). By contrast, *C. chinense ECU-994* showed a different behaviour. Thus, although some volatiles showed higher levels in *ECU-994* fruits from organic cultivation, many others increased under conventional practices, particularly sesquiterpenoids such as α- and β-cubebene, copaene, β- and γ-cadidene, or caryophyllene (Table 1).

The favourable effect of organic practices in the volatile profile of *Capsicum* fruits was also observed at a qualitative level. Thus, considering those volatiles detected exclusively in one growing system (and not in the other), six volatiles identified in Valenciano were only detected in fruits from organic trials (b-cubebene, bergamotene, b-cyclocitral, hexanal, 2-pentylfuran, and the bell pepper pyrazine), while only ethyl hexanol was found exclusively in fruits from conventional trials. A similar behaviour was found in Jalapeno M (five in organic vs. two in conventional) and to a lesser extent in *ECU-994* (one in organic vs. no one in conventional) (Table 1).

4. Acknowledgements

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P4-08

5-5’ dicapsiate: Product of the oxidation of capsiate by cationic peroxidases from pepper (*Capsicum annuum* L.)

Alberto Lema[1], Teresa Martínez-Cortés[1], Ana Garcés[2], Cristina Mallor[2], Oreto Fayos[2], Gerardo F. Barbero[3], Cristina Silvar[1], Federico Pomar[1]

[1] *Grupo de Investigación en Biología Evolutiva, Departamento de Bioloxía Animal, Bioloxía Vexetal e Ecoloxía, Universidade da Coruña, A Coruña, Spain*

[2] *Centro de Investigación y Tecnología Agroalimentaria de Aragón, Zaragoza, Spain*

[3] *Departamento de Química Analítica, Facultad de Ciencias, Universidad de Cádiz, Cádiz, Spain*

Abstract

Capsiate, dihydrocapsiate, and nordihydrocapsiate are part of a group of metabolites from *Capsicum annuum* which are known as capsinoids. They are structurally and functionally similar to capsaicinoids, the substances that causes pungency in hot peppers, but the capsinoids are non-pungent compounds. The only structural difference between the capsaicinoids and capsinoids is the way in which the carbon chain is bound to the aromatic ring: by an amide moiety in capsaicinoids and by an ester moiety in capsinoids. Secretory plant peroxidases may be directly related to capsaicinoid metabolism, and the oxidation of capsaicin and dihydrocapsaicin by a pepper peroxidase was reported earlier. The aim of the present work was to study the ability of pepper peroxidases to oxidize capsiate. In order to obtain an extract enriched in basic peroxidases, a crude extract from local peppers was subjected to a two-step chromatography protocol. This extract oxidized capsiate following a Michaelis-Menten kinetic. The use of specific inhibitors confirmed the main role of secretory peroxidases in this oxidation. The MS spectrum (Orbitrap) of the principal oxidation-products, revealed a molecular ion of m/z=609, according to a 5-5’ dicapsiate structure. This compound is analogous to the main product previously described for the oxidation of capsaicin [1].

1. Introduction

Capsinoids are a group of secondary metabolites from *Capsicum annuum* which include capsiate, dihydrocapsiate, and nordihydrocapsiate. Capsinoids were recently isolated from some sweet peppers as non-pungent compounds similar to capsaicinoids in terms of structure and biological activities. The structural difference between the capsaicinoids and capsinoids is the way in which the carbon chain is bound to the aromatic ring: by an amide moiety in capsaicinoids and by an ester moiety in capsinoids. Secretory plant peroxidases (EC 1.11.1.7; hydrogen donor: H_2O_2 oxidoreductase, Prxs) may be directly related to capsaicinoid metabolism since the vanillyl moiety of capsaicin is easily oxidized by this enzyme [2]. The first report of capsaicin oxidation by a peroxidase enzyme was from Boersch *et al.* [3]. Very soon after, Bernal *et al*. [1] reported the first data of capsaicin and dihydrocapsaicin oxidation by a pepper peroxidase. The dependence of the oxidation rate on capsaicinoids and H_2O_2 concentrations shows a kinetic behavior of the Michaelis-Menten type at low substrate concentrations, with inhibition at high substrate concentrations. The aim of the present work was to study the ability of pepper peroxidases to oxidize capsiate. In order to obtain an extract enriched in basic

peroxidases, a crude extract from local peppers was subjected to a two-step chromatography protocol. The use of specific inhibitors (tropolone/ferulic acid/potassium ferrocyanide and potassium ferrycianide) allow us confirm the main role of secretory peroxidases in the oxidation of capsiate. The MS spectra obtained by the use of an Orbitrap spectrometer provide information about the chemical structure of the main compounds obtained from the oxidation of capsiate by pepper peroxidases.

2. Material and Methods

2.1. Semipurification of pepper peroxidases

To perform peroxidase purification, we followed a three-step protocol including ammonium sulphate precipitation, adsorption chromatography on phenyl sepharose and cationic chromatography on SP sepharose. After the ammonium sulphate precipitation we considered two protein fractions, the first one from 0 to 80% of ammonium sulphate and the second one, from 80 to 95% of ammonium sulphate. Each fraction was pooled into a phenyl sepharose chromatography. The peaks from the adsorption column were then loaded into a cationic exchange chromatography, and the peroxidase bound to SP sepharose matrix was eluted with a linear gradient of 0 to 1 M KCl.

2.2. Spectrophotometric determinations

The oxidation of capsiate by the *C. annuum* basic peroxidases, in the presence and in the absence of H_2O_2, was assayed spectrophotometrically at 25ºC in a reaction medium containing 50 mM Phosphate buffer (pH 5.5), different capsiate concentrations (0.22 mM, 0.44 mM, 0.87 mM, 1.31 mM), and hydrogen peroxide concentration (0.5 mM) using the ε_{230}= 0.27 mM^{-1} cm^{-1}. The specific inhibitors tropolone, ferulic acid, potassium ferrocyanide and potassium ferrycianide are added at a 1 mM concentration one minute before the capsiate addition.

2.3. HPLC/MS Orbitrap analysis

The reversed-phase HPLC analyses were carried out on Thermo Accela high-performance liquid chromatograph, equipped with a Thermo LTQ Orbitrap Discovery detector. The HPLC column was a C18 SunFire (5 mm particle size, 150 mm x 4.6 mm i.d.) from Waters. The oven temperature was set at 30 ºC. Extracts were passed through a 0.45 mm filter (Millex-HV, Millipore) and a volume of 10 mL of solution was injected. The flow rate was 0.8 mL/min and the mobile phase consisted of 2% acetic acid as solvent A and acetonitrile as solvent B. The gradient profile was 85% A at 0 min, 20% A at 15 min, 0% A at 35 min, 0% A at 40 min and 85% A at 45 min. The mass spectrometry system was a LTQ-Orbitrap® Discovery mass spectrometer (Thermo-Fisher Scientific) equipped with an electrospray ionization (ESI) source operating in negative ionization mode. The ESI source conditions were: source voltage -3.51 kV, heated capillary temperature 350 ºC, capillary voltage -35 V and sheath gas and auxiliary gas, 50 and 10 (N_2 arbitrary units). For full scan MS analysis, the spectra were recorded in the range of m/z 80 to 800 with a scan speed of 1 scan/s. The analysis was carried out with source fragmentation at 35V and the mass resolution was set at 30.000.

3. Results

The purification procedure used allowed us to obtain a semipurificated extract of cationic peroxidases from pepper (figure 1). Two major peroxidase isoenzyme groups can be distinguished in *Capsicum* by their individual isoelectric points. The first major group is composed of peroxidase isoenzymes of acidic isoelectric point named APrx, and the second group corresponds to peroxidase isoenzymes of basic isoelectric point (BPrx) [1]. Basic peroxidases are located in the cell walls and the vacuoles, where there is only the strong basic isoenzyme B6. According to the vacuole as the hypothetical place for the accumulation of capsiate, this extract obtained seems adequate to carry out the study of oxidation of the capsiate by pepper peroxidases.

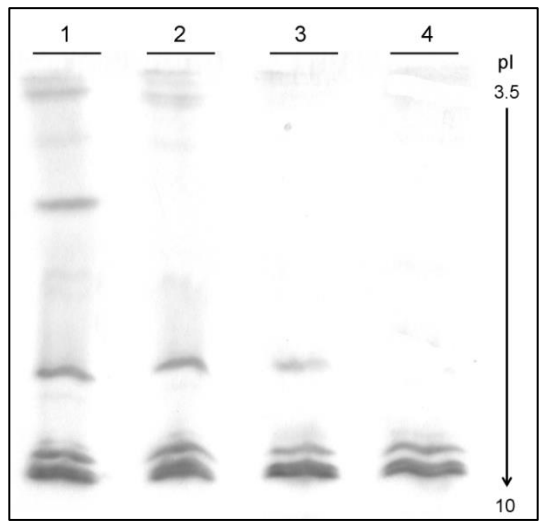

Figure 1
Isoelectrofocusing of the semipurification steps: (1) crude extract, (2) ammonium sulphate precipitate, (3) hydrophobic chromatography, (4) cationic chromatography

The oxidation of capsiate by pepper peroxidases was monitored by the increase in absorbance in the ultraviolet region (figure 2), showing a maximum change at 230 nm. The dependence of the oxidation rate on capsaicinoids and H_2O_2 concentrations shows a kinetic behavior of the Michaelis-Menten type at low substrate concentrations, with inhibition at high substrate concentrations.

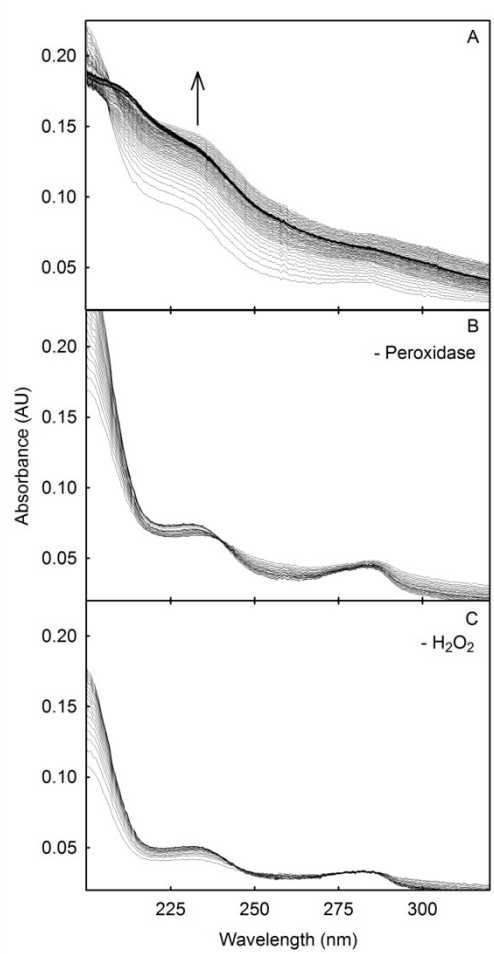

Figure 2
Oxidation of capsiate by C. annuum peroxidases. A) Complete reaction medium, B) reaction medium without peroxidases, C) reaction medium without hydrogen peroxide

The use of different inhibitors allows us to confirm the peroxidase nature for the activity detected (table 1). The activity of peroxidase was suppressed by 1 mM ferulic acid but not by tropolone. These results strongly support a role for the *C. annuum* basic peroxidases as the responsible for the oxidation of capsiate. Further evidence was obtained from the use of a peroxidase substrate, ferrocyanide ($K_4Fe[CN]_6$), as a competitive inhibitor of the enzyme. The advantage of using ferrocyanide is the possibility of having an analog, ferricyanide ($K_3Fe[CN]_6$), which is not a peroxidase substrate, and may be used for evaluating any collateral effects on cyanide/iron complexes [4]. Ferrocyanide inhibits the capsiate oxidation, this observation and the fact that ferricyanide had no noticeable effect, suggest that the capsiate oxidation is due to a Class III peroxidase activity.

Table 1
Effect of inhibitors on the activity of C. annuum peroxidases

	Capsiate oxidation (nmol s^{-1})
Control	1.61 ± 0.39
Ferulic acid (1mM)	0.00 ± 0.00
Tropolone (1mM)	2.69 ± 0.68
($K_4Fe[CN]_6$) (1mM)	0.00 ± 0.00
($K_3Fe[CN]_6$) (1mM)	1.55 ± 0.57

The analysis of the products from de oxidation of capsiate by HPLC-MS, shows several compounds. One of these compounds has a MS spectrum with a maximum m/z fragment of 609.34 (figure 3). This spectrum supports a 5-5'-dicapsiate structure for this compound. According to the molecular weight of 5-5'-dicapsiate (610.35), a negative ionization generates a fragment with a m/z of 609.35. The figure 4 shows a hypothetical reaction of capsiate and peroxidases that produces the 5-5'-dicapsiate.

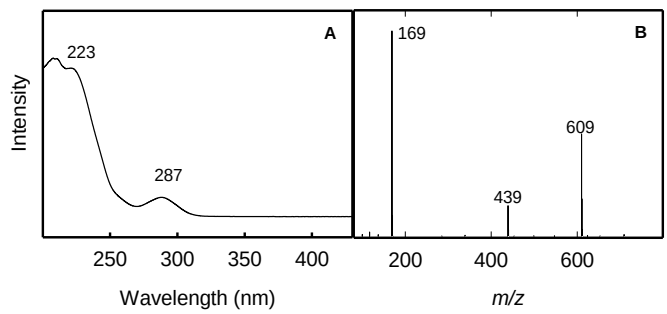

Figure 3
Spectra for the compound obtained by oxidation of capsiate A) PDA spectrum, B) MS spectrum

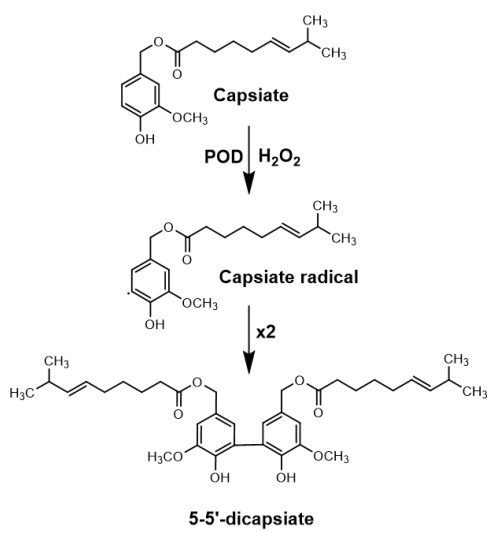

Figure 4
Reaction proposed for the synthesis of 5-5'-dicapsiate throw the oxidation of capsiate by C. annuum peroxidases

4. Conclusions

From the results obtained we can concluded that basic peroxidases from *C. annuum* are able to oxidize capsiate. One of the main products of these oxidation shows a MS spectrum that suggests a 5-5'-dicapsiate structure.

5. Acknowledgments

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P4-09

Phenylpropanoid biosynthesis and regulation in *Solanum melongena* cv. "Lunga Napoletana"

T. Docimo[1], G. Francese[2], A. Ruggiero[1], G. Batelli[1], M. De Palma[1], L. Bassolino[3], L. Toppino[3], G .L. Rotino[3], G. Mennella[2], M. Tucci[1]

[1]*Consiglio Nazionale delle Ricerche, Istituto di Bioscienze e BioRisorse, UOS Portici, Portici, Italy*

[2]*Consiglio per la ricerca in agricoltura e l'analisi dell'economiaagraria, Centro di Ricerca per l'Orticoltura (CREA-ORT), Pontecagnano, Italy*

[3]*Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Unità di Ricerca per l'Orticoltura (CREA-ORL), Montanaso Lombardo, Italy*

Abstract

Delphinidin-3-rutinoside (D3R), and chlorogenic acid (CGA) are the major phenylpropanoids metabolites in eggplant tissues, with CGA constituting over 90% of total phenolics. These metabolites have strong antioxidant properties and confer high nutritional value to eggplant fruits. To gain insight in the CGA and anthocyanin biosynthesis and regulation in eggplant, we performed biochemical analysis to characterize the distribution of phenylpropanoids in different tissues (young and mature leaf, stem, root, flower, fruit skin and flesh) of eggplant cv. "Lunga Napoletana" by LC-MS. Parallely, we evaluated the expression of key structural genes related to phenylpropanoid biosynthesis by qRT-PCR, i.e. *PAL*, *HQT*, *DFR*, *ANS*. We found a positive correlation between transcription of most of the CGA biosynthetic genes and CGA accumulation, as well as between *DFR* and *ANS* genes and D3R accumulation in eggplant anthocyanic tissues. *In silico* analyses of *cis* acting elements in the promoters of *PAL*, *HQT* and *ANS* revealed the presence of several Myb regulatory elements. Therefore, we isolated putative MYB regulatory gene, which is located in a phylogenetic clade strictly close to R2R3-MYB proteins, with a relevant function for regulation of anthocyanin and phenolic acids biosynthesis. Two-hybrid assay confirmed interaction of the isolated eggplant MYBTF with a heterologous bHLH protein, thus supporting its role as an activator of the anthocyanin pathway. Moreover functional analysis by transient expression of the isolated MYB in *N. tabacum* showed a doubled amount of CGA as well as activation of anthocyanin biosynthesis in transformed tobacco leaves. Further, since several R2R3-MYBs belonging to the anthocyanin- and phenolic acids-activating class have been identified in many *Solanaceae* except eggplant, we are currently extending our studies to other MYB TFs in order to understand their regulative role in eggplant.

Keywords: Phenylpropanoids, R2R3-MYBTF, Eggplant

1. Introduction

Among Solanaceae, eggplant is the second most consumed fruit crop after tomato. The high nutritional value of its fruits is due to phytonutrients like phenolic compounds and flavonoids, whose antioxidant activities (Raigón et al., 2008), confer high nutritional value and extraordinary health-promoting properties to this vegetable (Stommel and Whitaker, 2003).

Chlorogenic acid is the main phenylpropanoid metabolite in eggplant, and its accumulation peaks in fruits, ranging from 75 to 90% of total phenolics. Other phenylpropanoid compounds include purple and red anthocyanic pigments (D3R and Nasunin) and flavonols, which are major antioxidant constituents in eggplant fruit skin. As reported for other phenylpropanoids-accumulating species, the biosynthesis of these compounds proceeds thorough the general phenylpropanoid pathway, where the early steps of the pathway provide intermediates to be fuelled into chlorogenic acid and flavonoid biosynthesis. Namely, the high energy intermediate Coumaroyl-CoA ester formed by the concerted activities of phenylalanine ammonia lyase (PAL), cinnamate 4-hydroxylase (C4H) and 4-coumaroyl CoA ligase (4CL), can be either esterified by the hydroxycinnamoyl CoA-quinate transferase (HQT) enzyme to form chlorogenic acid, or serve as the substrate for the chalcone synthase (CHS) enzyme to form naringenin, the entry molecule of the flavonoid pathway (Vogt, 2010). Apple, tomato, onion and potato, skin and flesh tissues are often characterized by a distinct metabolite composition or content (Mintz-Oron et al., 2008;). Similarly, eggplant skin and fruit flesh differ for their phenylpropanoid profile. Namely, a higher accumulation of CGA is found in flesh fruit whereas the purple color of the fruit is due to the high content of delphinidine 3-rutinoside in the skin. This tissue specific production of metabolites suggests that their degree of accumulation is tightly regulated, as also indicated by literature data (Mennella et al., 2010; Mennella et al., 2012;). In this regard, along with the central role of structural genes, the production of phenylalanine-derived compounds in plants is strongly regulated by R2R3- MYB proteins. These TFs are considered the largest class of secondary metabolism modulators (Stracke et al., 2007), and more than a hundred seems to be present in eggplant (The Italian Eggplant Genome Consortium, unpublished).

In this study, we report metabolite distribution in several tissues and organs of the Occidental eggplant cv. 'Lunga Napoletana' by LC-MS analysis and their correlation with the quantitative expression of key structural and regulatory genes. Genes and metabolites tissue specificity guided the isolation of *PAL*, *HQT* and *ANS* and the *in silico* analysis of their isolated promoter sequences highlighted the presence of several Myb *cis* acting elements. Further we isolated a 'Lunga Napoletana' *SmMyb 1* variant, whose regulatory function on metabolite production interestingly suggested that its function is not limited to the activation of anthocyanin biosynthesis but might also be important for the regulation of CGA accumulation.

2. Materials and methods

2.1. Plant Material

S. melongena cultivar "Lunga Napoletana", with purple-black oblong fruits, was cultivated in the greenhouse of the CNR-IBBR UOS Portici, (Italy). Samples were all harvested when fruits reached a commercially ripe stage (Mennella et al., 2012). Samples from 3 different plants were frozen in liquid nitrogen and stored for further molecular and biochemical analysis.

N. benthamiana plants for transient transformation assays were grown in growth chamber of CNR-IBBR UOS Portici, (Italy) at the temperature of 22°C with a photoperiod of 16h light/8h dark, after one month, youngest leaves were used for transient assays. *N. benthamiana* leaf samples were collected in liquid nitrogen and stored at –80°C for further biochemical and molecular analyses.

2.2. Metabolite analyses

Anthocyanins, flavonoids and chlorogenic acid were analyzed by mass spectrometry in different tissues and organs of the *S. melongena* cultivar "Lunga Napoletana" and in *N. benthamiana* leaves. Metabolic analyses of *N. benthamiana* agro-infiltered leaves were carried out on three independent replicates collected for each infiltration. Phenylpropanoids were extracted according to Docimo et al. (2016).

2.3. RNA isolation and qRT-PCR

Total RNA was extracted from 100mg of eggplant tissues using a RNAsy kit (Quiagen, Valencia, CA, USA). Using a SuperScriptII™ kit (Life Technologies, Carlsbad, CA, USA), first-strand cDNA was synthesized by reverse transcription (RT) with oligo-dT primers following the manufacturer's instructions. Gene expression was analyzed by qRT-PCR with an ABI7900 HT (Life Technologies, Carlsbad, CA, USA). Normalization of eggplant genes transcription levels was performed using Adenine phosphoribosyl transferase (*APRT*) as internal reference gene. Expression analysis on *N. benthamiana* was performed on RNA extracted from leaves at 5 days post agro-infiltration and normalization was performed by using *α-Tubuline* as housekeeping gene. Results were analyzed using the $^{\Delta\Delta}$Ct method (Pfaffl, 2001, 2004) and reported as relative expression levels, compared to the respective internal calibrator, whose expression was unitary.

2.4. Nucleotidic sequence isolation and bioinformatic analyses

Isolation of *PAL*, *HQT* and *Myb1* nucleotide sequences from the Occidental traditional cv. 'Lunga Napoletana' was performed by 5'3'RACE. Promoter sequences for the *SmANS* and *SmMyb1* genes were amplified by the Genome walking strategy (Clontech, Mountain View, CA, USA), using gene specific primers designed in order to amplify the 5'UTR region. Analysis of cis-regulatory elements in the isolated promoter sequences was performed thorough the Genomatix platform (https://www.genomatix.de).

2.5. Yeast two hybrid and SmMyb1 transient expression in Nicotiana benthamiana

For yeast two-hybrid experiments, the prey plasmid pGADT7 (Clontech, Mountain View, CA, USA) was used. The full-length coding sequence of *SmMyb1* was PCR amplified and cloned in frame into pGADT7 between *Eco*RI and *Xho*I restriction sites. Plasmids were sequenced to rule out PCR-induced mutations. The bait plasmid StbHLH1pGBKT7 was previously described (D'Amelia et al., 2014). *SmMyb1* cds was cloned in the 35SCaMV expression cassette of pGWB411 (Nakagawa and Kimura, 2009) using the Gateway recombination technology (Invitrogen, Carlsbad, CA, USA) and used to transform *Agrobacterium tumefaciens* LBA4404. *N. benthamiana* plants were agro-infiltered according to the protocol of Voinnet et al. (2003).

3. Results and discussion

We investigated the correlation between the expression profiles of the key biosynthetic genes for Chlorogenic acid and flavonoid formation and the accumulation patterns of the phenolic acid CGA and of the anthocyanin D3R in *S. melongena* tissues. As shown in Figure 1, the D3R content mirrored anthocyanic pigmentation of flower and fruit skin, while Chlorogenic acid was detected in all the tissues, reaching the highest amount of 3000µg/100 mg dwin fruits.

Expression levels of *PAL* and *HQT* were notably high in fruits. The D3R biosynthetic genes, *DFR* and *ANS* showed a similar pattern of transcript accumulation, with higher expression levels detected in anthocyanin-pigmented tissues. Indeed, the expression of the two genes was almost 25 and 35 times higher in fruit skin than in flowers, respectively.

Correlation analysis between molecular and biochemical data highlighted that the coordinated expression of the eggplant *PAL* and *HQT* genes may account for the high accumulation of CGA in eggplant (correlation coefficient $r=0.756$, $p<0.05$), confirming similar findings in tobacco and tomato (Niggeweg et al., 2004; Payyavula et al., 2014). In pigmented tissues, like fruit skin and flowers, the extremely high expression levels detected for the flavonoid structural genes, *DFR* and *ANS*, correlated with the D3R content($r=0.991$, $p<0.05$, and $r=0.992$ $p<0.05$, respectively).

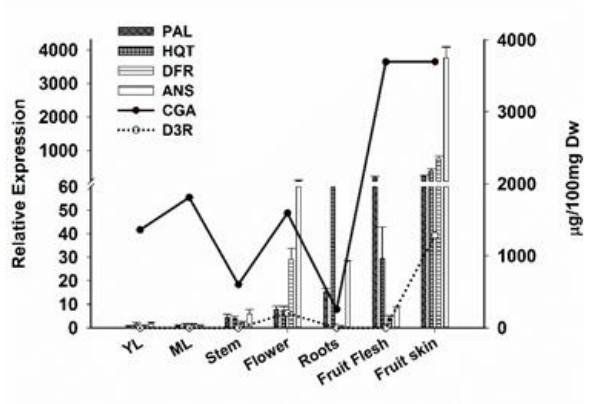

Figure 1

CGA and D3R accumulation and PAL, HQT, DFR, and ANS relative expression in eggplant tissues. LC-MS metabolite analysis is reported in dotted and solid lines (solid for CGA and dotted for D3R) Relative expression of PAL, HQT, DFR, and ANS is reported in bar graph, using young leaves as calibrator tissue.

In order to characterize CGA biosynthesis, we isolated the full length cDNA sequences of *SmPAL* and *SmHQT* from eggplant fruit flesh tissue by conventional 3'5' RACE. Moreover,to investigate whether CGA and anthocyanin accumulation might be differentially regulated in eggplant, *PAL*, *HQT* and *ANS* gene promoters were isolated by genome walking and their upstream sequences were *in silico* scanned for regulatory elements. Matinspector analysis (Cartharius et al., 2005) showed multiple cis-acting elements, including fundamental and specific elements associated with defense signaling and hormone regulation in the *PAL*, *HQT* and *ANS* promoters, along with several Myb regulatory signals (Table 1).

To gain insight in the regulation of phenylpropanoid biosynthesis in eggplant, we isolated a MYB TF homologous CAI, a regulator of CGA and flavonoids biosynthesis in *S. tuberosum* (Rommens et al., 2008).This MYBTF from *S. melongena* cv.'Lunga Napoletana' was found to contain four SNPs in respect to *Sm*MYB1 from the cv. 'Zi Chang' (Zhang et al., 2014), and to be identical at the amino acidic level to a sequence annotated as ANT1 in the eggplant draft genome (Hirakawa et al., 2014).

Along with the structural genes, the promoter region of *S. melongena Myb1* was also isolated and *in silico* scanned and compared with the promoter regions of other MYBTF known as

positive regulator of phenylpropanoid biosynthesis, namely *S. lycopersicum ANT1*, *S. tuberosum AN1* and *CAI*, and *V. vinifera MybA1*, whose sequences were retrieved from the respective genomic resources. *SmMyb1* promoter shared the same light, circadian rhythm and sucrose responsive elements found in the *PAL*, *HQT* and *ANS* promoters, thus suggesting that these genes may be coordinately expressed and supporting the idea that CGA and anthocyanins accumulation in eggplant is controlled by the same environmental factors. Interestingly, the *SmMyb1* promoter was also characterized by the presence of some unique elements, such as the TATCCAT motif, which is required for alpha-amylase expression during sugar starvation. Moreover, the presence of additional and distinctive elements involved in the response to phosphate/sugar starvation, phytocrome/plastid regulation, sporamine formation and cell proliferation and growth, gives an indication that the activation of this TF is also induced by different factors than the other TF and may play various and different physiological roles in eggplant.

Table 1
List of major cis acting regulatory elements in the promoters of structural biosynthetic genes, of SmMYB1 and of other TFs from other species.

Regulatory elements	***SmANS***	***SmHQT***	***SmPAL***	***SlANT1***	***StAN1***	***StCai***	***VvMYBA1***	***SmMYB1***
Myb Plant	3	4	4	3	2	3	2	0
MybAT	1	3	1	2	2	5	4	3
Mybgah	3	1	1	1	0	0	1	2
Myc	3	0	3	0	4	0	2	0
MybSt1	1	3	7	3	3	3	3	3
Phytocrome regulation	0	0	0	0	0	0	0	2
Plastid regulation	2	3	4	0	8	1	1	3
Cell proliferation/growth	0	0	0	0	0	0	1	3
Sporamine	0	0	0	2	0	1	0	1
Wounding /jasmonate	6	1	4	6	17	3	16	4
Hypo osmolarity	1	1	1	0	0	1	1	1
Giberellin/sugar repression	1	4	3	4	2	5	3	3
Sugar starvation	0	1	2	0	0	0	1	3
Binding amilase	1	1	0	0	0	0	0	1
Etilene	2	1	2	0	0	0	1	2
Light	6	17	12	3	4	6	1	5
Circadian expresssion/light	2	3	2	2	7	2	1	1
Sorlip	0	2	0	0	1	1	1	4

Since regulation of anthoyanin biosynthesis is mediated by the formation of a regulatory complex between MYB proteins and bHLH partners, we performed a yeast two hybrid assay with a previously identified *StbHLH1* from potato in order to further investigate the regulatory function of *SmMyb1*. As shown in Figure 2, yeast cells co-transformed with *SmMyb1* and *StbHLH1* were capable of growing on selective media, indicating that a positive interaction between *SmMYB1* and *StbHLH1* does take place in yeast.

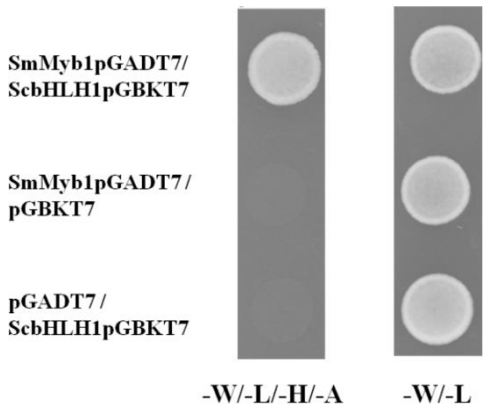

Figure 2
SmMyb1 interacts with the bHLH transcription factor ScbHLH1 in yeast two-hybrid assay. SmMyb1 was cloned in the prey plasmid pGADT7 and co-transformed with ScbHLH1pGBKT7 in the yeast strain AH109. The SmMyb1pGADT7/pGBKT7and pGADT7/ScbHLH1pGBKT7 combinations were used as negative controls. Yeast cells grown on synthetic complete media (-W/-L, Right) and on selective media (-W/-L/-H/-A, Left) are shown. Pictures were taken 3 days after incubation at 30°C.

Further insight into the role of *SmMyb1* TF in phenylpropanoid biosynthesis regulation was obtained by functional analysis. Transient over-expression of *SmMyb1*in *N. benthamiana* determined a red pigmentation of agro-infiltered leaves, which normally accumulate very low amounts of anthocyanins (Figure 3 a). The red leaf phenotype correlated with both a high expression level of the late anthocyanin biosynthetic gene *DFR* and with a higher content of the D3R pigment (Figure 3 b and c). These results confirmed that anthocyanin regulation by *SmMyb1* proceeds thorough the activation of *DFR* transcription, as it was shown for *SlANT1*and *SlAN2* (Kiferle et al., 2015). Interestingly, *SmMyb1* transformed leaves also showed higher expression of *CHS*, *HQT* and *ANS*, along with a doubled content of CGA. These results suggest that, similarly to*StAN1*, *SmMyb1*may have a direct involvement in CGA biosynthesis**.**

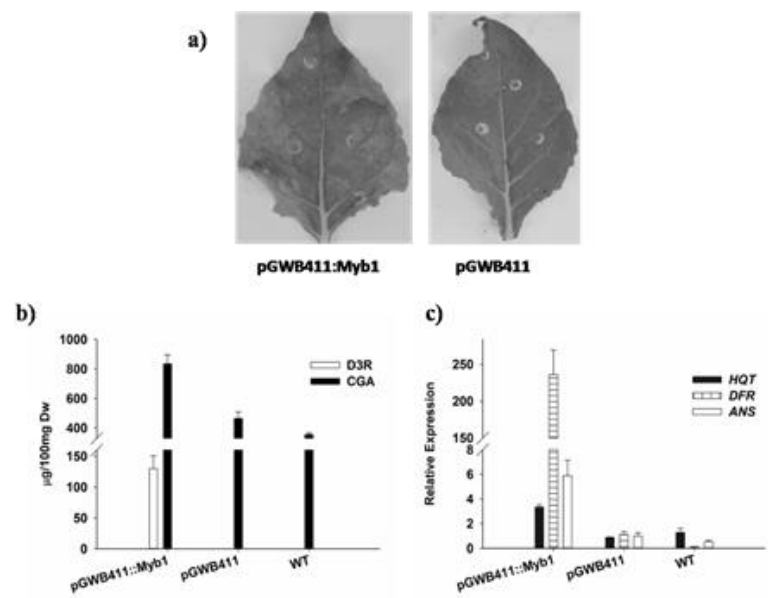

Figure 3
SmMyb 1 overexpression in N. benthamiana. a) Phenotype of SmMyb1 over-expressing pGWB411::Myb1) and mock-transformed (pGWB411) N. benthamiana leaves; b) LCMS analysis of CGA and D3R content in N. benthamiana leaves; and c) relative expression of key CGA and D3R biosynthetic genes.

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P4-10

Exogenous 6-benzylaminopurine application protects eggplant seedling against low temperature-induced oxidative stress

Xuexia Wu, Lian Yong

Horticultural Research Institute, Shanghai Academy of Agricultural Sciences, Shanghai, China

Abstract

Low temperature stress is one of primary constraints to plant production in many parts of the world. The objective of this study was to investigate the influence of 10 μM 6-benzylaminopurine (6-BA) on the plant growth, antioxidant system and osmoregulation responses of eggplant seedlings under low temperature stress. The 6-BA treatment ameliorated low temperature-induced decrease in plant growth and chlorophyll content compared with the control. Under low temperature stress, reactive oxygen species (ROS) levels and lipid peroxidation were remarkably increased, which were significantly inhibited by 6-BA application. The activities of antioxidative enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR), as well as the levels of ascorbic acid (AsA) and reduced glutathione (GSH) were increased during low temperature treatments, and these increases were more significant in the 6-BA applied eggplant. The 6-BA treatment also greatly enhanced the contents proline, soluble sugar and protein under low temperature stress. From these results, it can be concluded that 6-BA could play the positive roles in the alleviation of oxidative damage caused by ROS overproduction through enhancing antioxidant defense system, resulting in improving the tolerance of eggplant seedlings to low temperature stress.

SESSION 5

Molecular genetics and biotechnology

P5-01

Identification of *Capsicum* pentatricopeptide repeats via in-silico analysis provides insights into fertility restoration

Derek W. Barchenger, Joseph I. Said, Franchesca A. Ortega, Paul W. Bosland

Department of Plant and Environmental Sciences, New Mexico State University, Las Cruces, United States

Abstract

Pentatricopeptide-repeat (PPR) proteins are a large family of proteins that are prevalent in terrestrial plants, and have a range of regulatory functions primarily within the mitochondria and chloroplast. Restoration-of-fertility (*Rf*) genes can encode PPRs and act to edit the mitochondrial genes that confer sterility, thus restoring fertility. Molecular markers for *Rf* in *Capsicum* have had limited applicability in diverse *Capsicum* germplasm lines. We identified and mapped the PPR domains in the chile pepper genome, providing a map of PPR genes, for future work to further characterize the PPR encoding *Rf* genes in chile pepper. We queried the known *Arabidopsis* PPRs against the *C. annuum* genome using the software, MAKER2. Using the software R, we extracted the PPR domain coordinates and sequences. We identified 552 PPR domains in the chile pepper genome. Approximately 28% of the chile pepper PPR domains identified in this study were found to have structural similarity to previous reported PPRs. Chile pepper was found to have multiple domains with similarity to a previously reported PPR. We identified 14 PPR domains that encode proteins with similarity to previously reported *Rf* genes. On chromosome 1, two domains were found, and on chromosome 6, twelve domains were found. The potential *Rf* genes were characterized in individuals displaying different phenotypes for this trait. These 14 *Rf* gene copies provide a basis for developing more widely applicable molecular markers for this important trait. Additionally, the other PPR domains identified in this study provide a foundation for exploring the regulation of organelle gene expression in chile pepper.

Keywords: bioinformatics, PPR, cytoplasmic male sterility

1. Introduction

Over the past century, consumer demand for chile peppers globally has substantially increased. Recently, two independent chile pepper genome sequences were published (Kim et al., 2014; Qin et al., 2014), enabling genomic exploration to aid in the progress of breeding for important traits such as fruit size, pungency, disease and pest resistance, stress tolerance, and restorer of fertility (*Rf*) cytoplasmic male sterility (CMS), among other traits.

Pentatricopeptide repeat (PPR) proteins have evaded identification until recently, which has been largely enabled by the availability of complete genomic sequences (Giegé, 2013). PPR proteins are characterized by tandem 35 amino acid repeats (Small and Peeters, 2000). They are ubiquitous among eukaryotes (Giegé, 2013), and are one of the largest protein families in land plants, with often more than 400 PPR proteins in most species (Barkan and Small, 2014). PPR proteins are RNA binding proteins encoded in the nucleus and are largely targeted to the mitochondria or chloroplasts (Giegé, 2013)

CMS is the failure to produce functional pollen and is controlled by the plant mitochondrial genome. In most plant species, the mitochondrial genome and thus CMS is maternally inherited

(Ji et al., 2014). Conversely, *Rf* genes are encoded in the nucleus, and act to mask the mitochondrial genes that determine CMS, thus restoring fertility. The *Rf* genes function through cleavage or degradation of the CMS-associated mRNA (Wang et al., 2006). Genes that restore fertility are known to encode PPRs (Dahan and Mireau, 2013). A CMS-*Rf* system is useful for efficient F_1 hybrid seed production.

To date, 441 PPRs have been identified in *Arabidopsis thaliana*, as well as the expression, localization, and general function of many of the PPR family members (Lurin et al., 2004). Conversely in chile pepper, PPR identification has been limited. Jo et al. (2010) utilized the homologous petunia *Rf* gene (Bentolila et al., 2002), to identify a single PPR (*PePPR1*) gene in chile pepper. Previous work in other crops has largely focused on identifying a single or a few candidate PPRs. Our objective was to utilize in-silico techniques to identify and map the chile pepper PPR domains and to characterize the PPR encoding *Rf* genes in chile pepper.

2. Materials and Methods

Using MAKER2, an amalgamation of bioinformatics software the chile pepper genome was analyzed. MAKER2 utilizes BLAST (Basic Local Alignment Search Tool), and other alignment algorithms to match known query proteins against a genome by translating each of the six reading frames to find the best match for the protein on the genome. BLOSUM (Block Substitution Matrix) matched the PPRs to the genome, and a BLAST score was assigned to each match. The program gives each of the punitive genes in FASTA format that can then be further analyzed by motif scanners to confirm the identity of the punitive gene. We queried known *Arabidopsis* PPR proteins obtained from NCBI against the *C. annuum* genome. MAKER2 produces general feature files (GFF) for each chromosome. We then data mined the nucleotide (nt) coordinates for each candidate PPR domain. Candidate PPR domain sequences were extracted using script written within the BioStrings package in R (Version 3.1.2; R Foundation for Statistical Computing, Vienna, Austria).

3. Results and Discussion

We identified 552 PPR domains in the chile pepper genome. The majority of the PPR domains were found to be distally clustered. Approximately 28% of the chile pepper PPR domains identified in this study were found to have structural similarity to previous reported PPRs. As expected, chile pepper was found to have multiple domains with similarity to a previously reported PPR. One extreme example of this is chromosome 11, which has seven domains that encode proteins with similarity to the PPR protein Organelle Transcript Processing 82 (OTP82). OTP82 is known to have chloroplast RNA editing capacity, with two target sites in *Arabidopsis* (Hammani et al., 2009).

Interestingly, we identified 14 PPR domains that encode proteins with similarity to previously reported *Rf* genes. Two of these domains were on chromosome 1 (CaPPR1_20 and CaPPR1_21), and twelve were found on chromosome 6 (CaPPR6_34, CaPPR6_35, CaPPR6_40 through CaPPR6_46, and CaPPR6_48) (Table 1). The PPR CaPPR6_38 has been previously identified as *PePPR1* by Jo et al. (2010), based on similarity to the *P.* x *hybridia Rf* gene. The *Rf* candidate genes in chile pepper were generally more similar to each other than they were to other *Rf* genes; however, CaPPR1_21 was most similar to the *Rf* gene of *Zea mays* (Fig. 1).

Several molecular markers linked to *Rf* in chile pepper have been identified including OPP13-CAPS (Kim, 2005), AFRF8-CAPS (Kim et al., 2006), PR-CAPS (Lee et al., 2008), and CRF-SCAR (Gulyas et al., 2006). These markers linked to *Rf* have limited applications due to the lack of agreement between the phenotype and the marker (Jiang, 2015; Min et al., 2008),

resulting in no broadly applicable molecular markers. We propose that this lack of marker agreement is due to the multiplicity of genes for the restoration of fertility in chile pepper.

We characterized the *Rf* candidate genes in individuals with differing phenotypes. We found single nucleotide polymorphisms (SNPs) in most of the candidate *Rf* genes. This candidate gene approach provides a basis for *Rf* systems in other species as well as for the development of molecular markers for rapid identification of *Rf* plants in chile pepper.

Table 1
Location and length of C. annuum PPR domain identified to have similarity to restorer of fertility (Rf) genes.

PPR	Chromosome	Length (bp)[z]
CaPPR1.20	1	564
CaPPR1.21	1	285
CaPPR6.34	6	180
CaPPR6.35	6	213
CaPPR6.38	6	1731
CaPPR6.39	6	1062
CaPPR6.40	6	423
CaPPR6.41	6	468
CaPPR6.42	6	648
CaPPR6.43	6	603
CaPPR6.44	6	537
CaPPR6.45	6	273
CaPPR6.46	6	2259
CaPPR6.48	6	507

[z]*base pairs*

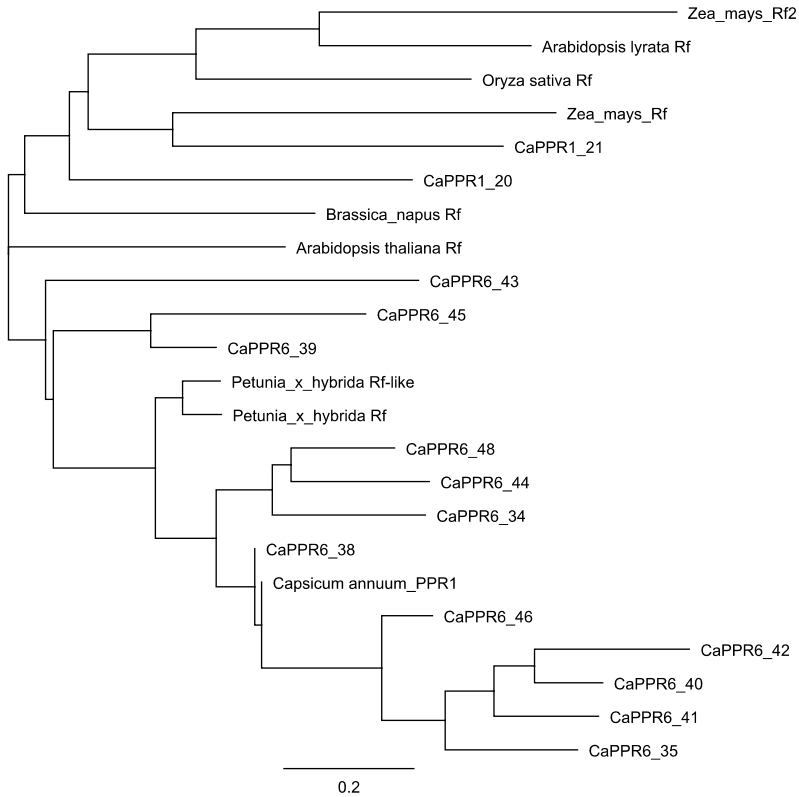

Figure 1
Dendrogram of the candidate Rf genes identified in this study (those that begin with CaPPR) with Rf and Rf-like genes from several species. Zea mays Rf-2 was used as the root of the tree because it does not encode a PPR protein. The dendrogram was made with protein sequences using ClustalW for multiple sequence alignment.

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P5-02

Fine mapping of the *Me7* gene controlling resistance to Root-Knot Nematode (*Meloidogyne incognita*) in chili pepper

Amornrat Changkwian[1], Ji-Woong Han[1], Joung-Ho Lee[1], Gyung-Ja Choi[2] and Byoung-Cheorl Kang[1]

[1] *Department of Plant Science, Plant Genomics and Breeding Institute, and Vegetable Breeding Research Center, College of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea*

[2] *Research Center for Biobased Chemistry, Korea Research Institute of Chemical Technology, Daejoen, Republic of Korea*

Meloidogyne incognita is a root-knot nematode causing severe yield reduction to pepper (*Capsicum annuum)* cultivation worldwide. The *Me7* gene has been reported as a resistance gene localized on long arm of the chromosome P9. The objective of this study was to fine map the *Me7* gene using 503 F2 individuals derived from a cross between ECW30R and CM334. Phenotype screening was performed by inoculating 1,000 second-stage juveniles per individual. The disease was scored 45 days of post inoculation using gall index system. The screening results depicted 391 resistant and 112 susceptible plants, which fit into 3:1 ratio confirming the resistance is controlled by a single dominant gene. The fine mapping was initiated using previously published PCR-based markers that are located at 4.3 and 2.7 cM from the *Me7* gene. To further narrow down the candidate gene interval, additional SNP markers were developed using CM334 reference genome information, yet no closer marker has identified. Therefore, Ren-Seq analysis was performed to locate the NB-LRR candidates that clustered between and nearby the flanking markers. At present, 22 NB-LRR candidates were identified in the flanking region. In addition, an F5 recombinant inbred lines carrying resistant gene are being screened to obtain a solid phenotype inheritance pattern. The candidate gene discovery will facilitate the breeding programs for the crop improvement against root-knot nematode in pepper.

Keywords: root-knot nematode, resistance gene, fine map, SNP marker, NB-LRR candidate

P5-03

Plant genetic background increasing the efficiency and durability of major resistance genes to root knot nematodes can be resolved into a few resistance QTLs

A. Barbary[1,2,3], C. Djian-Caporalino[1,2,3], P. Castagnone-Sereno[1,2,3], N. Marteu[1,2,3], A. Fazari[1,2,3], B. Caromel[4], A. Palloix[4]

[1] *INRA, UMR 1355 Institut Sophia Agrobiotech, Sophia Antipolis, France*

[2] *Univ. Nice Sophia Antipolis, UMR 7254 Institut Sophia Agrobiotech, Sophia Antipolis, France*

[3] *CNRS, UMR 7254 Institut Sophia Agrobiotech, Sophia Antipolis, France*

[4] *INRA, UR1052, Génétique et Amélioration des Fruits et Légumes, CS 60094, Montfavet, France*

Abstract

With the banning of most chemical nematicides, the control of root knot nematodes (RKNs) in vegetable crops is mainly based on the deployment of single-major resistance genes (*R*-genes). However, these genes are rare and their efficiency is threatened by RKNs capacities of adaptation. In pepper, several dominant *R*-genes are efficient against RKNs, but their efficiency and durability were shown to be increased in partially resistant genetic background. A QTL analysis was performed in such a genetic background, using a $F_{2:3}$ population from the cross between Yolo Wonder, a partially resistant to resistant accession depending on RKN species, and Doux Long des Landes, a susceptible one. The genetic linkage map was constructed from 130 F_2 individuals and the 130 F_3 families were tested for resistance to the three main RKNs species, *M. incognita*, *M. arenaria* and *M. javanica*. Four new major QTLs were mapped into two clusters. The cluster on chromosome P1 includes three tightly linked QTLs with specific effects against each RKN species. The fourth QTL, specific of resistance to *M. javanica*, mapped on the pepper chromosome P9, which is known to carry multiple NBS-LRR repeats with major resistance genes to nematodes and other pathogens. The newly discovered cluster on chromosome P1, displays a broad spectrum of action with major additive effects on resistance. Therefore, it provides innovative potential for breeding new cultivars or rootstocks combining quantitative resistance and major resistance genes and increasing the efficiency as well as the durability of RKNs genetic control.

Keywords: *Capsicum annuum*, *Meloidogyne* spp., quantitative resistance, major resistance, resistance durability

1. Introduction

Root-knot nematodes (RKNs), *Meloidogyne* spp., are major plant pathogens worldwide. They are extremely polyphagous endoparasites able to infest more than 5,500 plant species, among which many field and greenhouse crops *[1]*. Since the use of most chemical nematicides is being prohibited, due to environmental and public health issues, one alternative way to protect crops against these pests is based on the use of resistant cultivars. This method is efficient to control RKNs populations, economically sustainable, health safe and environmentally friendly.

Major resistance genes (*R*-genes) are extensively used in breeding RKN resistant cultivars and/or rootstocks. However, their efficiency is threatened by the capacities of adaptation of RKNs. Indeed, *R*-genes apply a selective pressure on nematode populations and cause a risk of emergence of virulent nematode populations *[2]*, which constitutes a severe limitation to their use.

A previous study showed that a partially resistant genetic background increases R-gene efficiency compared to a susceptible one *[3]*. The major role of the plant genetic background in preventing R-gene from overcoming was reported in other pathosystems *[4, 5, 6]*. In pepper/virus parthosystem, the reason of R-gene increased durability was shown to result from quantitative trait loci (QTLs) which slow down the selection of *R*-gene virulent variants and decrease the pathogen population (e.g., *[7]*). To date, no QTLs were found against RKN in pepper. In that respect, a QTL analysis was performed, as we strongly supposed that the protective effect of the plant genetic background on R-genes agaisnt RKNs is provided by such quantitative resistance factors as well.

2. Material and methods

In this study, a classical QTL analysis was conducted to determine the genetic factors, within the plant genetic background, that may explain the discrepancies in resistance level from a pepper genotype to another. A population of 130 $F_{2:3}$ families, derived from a cross between the resistant to partially resistant (Yolo Wonder) and the highly susceptible (Doux Long des Landes) pepper inbred lines (figure 1), was tested for quantitative resistance to the three main RKNs species (*M. incognita, M. arenaria and M. javanica*).

Genotyping data were collected on the F_2 population from 326 markers, among wich sequence Charaterized Amplified Region (SCAR), Simple Sequence Repeat (SSR) and Single Nucleotide Polymorphism (SNP). A genetic linkage map was constructed with a LOD score threshold of 3.0 and a maximum recombination fraction of 0.3

Assessement of resistance was performed on the F3 progenies for each RKN species according to a common experimental procedure previously described *[3]*. To estimate the phenotypic value of each F2, the number of egg masses (EMs) was counted for sixteen F3 plants derived from each F2.

QTL detection was performed using Composite Intervalle Mapping. (CIM). A permutation test with 1,000 replicates allowed to empirically determine the genome-wide LOD threshold at the 5% probability level for each phenotypic trait individually. The LOD threshold was estimated at 3.6 for the three traits. For each QTL, the confidence interval (CI) was defined as a 2-LOD drop-off around the maximum LOD score.

Figure 1

The susceptible and resistant pepper cultivars Doux Long des Landes (A) and Yolo Wonder (C) and their respective root systems (B, D). Arrows indicate egg masses.

3. Results

The genetic linkage map constructed with 326 markers provided a new saturated pepper map, among wich one SCAR, 13 SSR and 312 SNP. It comprised 12 linkage groups (LGs), which were assigned to the 12 pepper chromosomes, with an overall length of 1436 cM (table 1).

Table 1

Caracteritics of the map from the cross between YW x DLL

Chromosome	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	total
Number of markers	35	22	35	16	21	19	32	22	33	25	38	28	326
Lenght (cM)	205.5	94.9	154.3	93.5	125.3	106.6	130.4	70.3	108.6	106.5	108.4	132.1	1436.4
Mean distance between two disjointed markers (cM)	6.4	5.0	5.1	6.2	6.3	5.6	4.5	3.9	6.0	4.4	3.7	5.3	5.2
Maximum distance between two consecutive markers	25.9	15.7	17.6	14.9	18.6	19.6	14.1	12.3	23.6	14.8	15.6	21.0	

One major resistance QTL, named *Minc-P1*, was detected on pepper chromosome P1 for *M. incognita*. On this chromosome, in the vicinity of *Minc-P1* was detected a QTL for resistance to *M. arenaria* named *Mare-P1*. Regarding *M. javanica*, two QTL were detected. The first one, named *Mjav-P1*, was located on chromosome P1 as well, close to *Minc-P1* and *Mare-P1*. The confidence interval of these three QTLs overlapped. The second QTL, named *Mjav-P9*, was detected at the distal part of the chromosome P9. For all four QTLs, the resistance allele originated from YW. Their positions and effects are resumed in figure 2 and table 2.

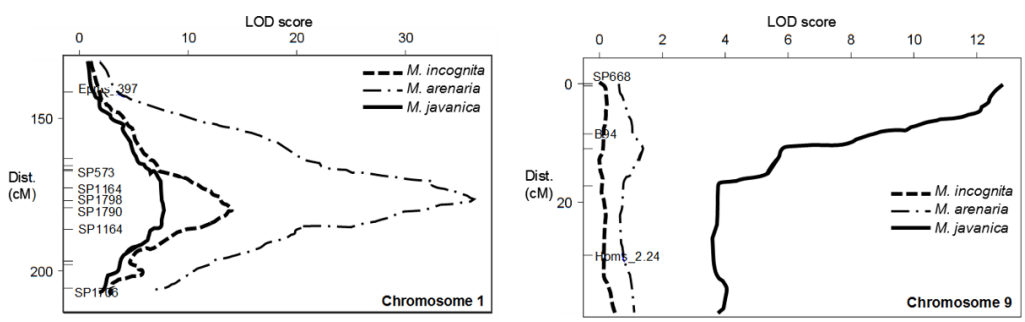

Figure 2

Quantitative trait loci (QTL) for M. incognita, M. arenaria and M. javanica resistance, on pepper chromosome 1 (left, box 1) and chromosome 9 (right, box 2). The log of the likelihood ratio (LOD) score is shown on the x axis. Scaled distances, flanking markers and markers in the confidence interval of the QTLs are given on the y axis.

Table 2
QTL for resistance to the different RKN species in the pepper F2:3 progeny
CI : Confidence interval, defined as a LOD-2 drop-off around the maximum LOD score

RKN species	QTL	Chromosome	Closest marker	Location (cM)	CI	LOD score	R^2
M. incognita	*Minc-P1*	1	SP1790	179.2	173.9 – 188.0	14.1	40.9
M.arenaria	*Mare-P1*	1	SP1798	177.0	168.2 – 182.0	36.5	73.9
M. javanica	*Mjav-P1*	1	SP1790	179.2	164.0 – 192.5	7.7	48.0
	Mjav-P9	9	SP668	1.0	0.0 – 9.8	12.9	52.2

4. Discussion

Albeit broad spectrum *R*-genes are often preferentially used in breeding programs, it was shown that using *R*-genes in an inappropriate pepper genetic background may reduce their efficiency, which may further affect their durability. Indeed, YW proved to be a better genetic background than DLL to reinforce *R-genes* efficiency *[3]* and also to reduce the frequency of *Me3* resistance breakdown compared to DLL *[9]*. The strategy which consists in combining an *R*-gene with a partially resistant genetic background (i.e., carrying QTLs) in order to increase its durability was validated in other pathosystems *[4, 5, 6]*. Quenouille *et al. [8]* suggested that this effect is mainly due to the additional resistance because of QTLs from the genetic background, which decrease the pathogen population and thus the risk of emergence as well as further selection of virulent variants. From that point of view, the new QTLs identified in this study are good candidates for pyramiding with *R-genes* against RKNs. Moreover, it was demonstrated that, under suitable agronomic practice conditions, *R*-gene pyramiding was the best alternative to increase *R-genes* against RKNs durability *[9]*. From our results, *Minc-P1*, *Mare-P1, Mjav-P1* and *Mjav-P9,* thus offer new opportunities for combining major and partial resistance factors together.

From a breeding point of view, the localization of new resistance factors on the pepper chromosome P1 will facilitate their introgression by MAS, in addition to current *R*-genes. Indeed, all the efficient genes against RKNs, mapped until now, are closely linked on the pepper chromosome P9 *[10]*. However, they are in repulsion phases, i.e. the different genes are carried by distinct pepper accessions. *Minc-P1*, *Mare-P1 and Mjav-P1* are independent from this cluster and all carried by the same accession (Yolo Wonder), which should make it easier to generate homozygous plant genotypes harbouring resistance factors from both the P9 and P1 clusters.

Full article is in press in Frontiers in Plant Sciences.

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P5-04

Highly efficient genome doubling method for haploid paprika (*Capsicum annuum* L.) plants

Anikó Gémesné Juhász[1], Zoltán Kristóf[2]

[1] *Medimat Kft., Budapest, Hungary*

[2] *Eötvös Loránd University, Dep.of Botany, Budapest, Hungary*

Abstract

The production of doubled haploid plants from microspores is an important technique used in pepper breeding programmes. The purpose of the study was to improve the effectiveness of fertile pepper doubled haploid (DH) induction from haploid plants *in vitro* by optimizing culture parameters.

A highly efficient, reproducible and economic method was elaborated for the *in vitro* genome duplication of pepper haploids originated from anther culture.

The colchicine treatment of 0,5% *in vitro* for six days resulted in conversion of 95% from haploid to doubled haploid, which were determined by flow cytometry. Survival rate of treated haploid plants was 96,2%. Diploidised plants developed similarly to their untreated, spontaneous diploid counterparts and no fertility problems were recorded in the past years.

Keywords: anther culture, haploids, flow cytometry, genome doubling doubled haploids, colchicine treatment

1. Introduction

Over the last twenty years one of the most intensive fields of research in plant biotechnology has been the widespread application of the *in vitro* haploid methods based on the artificial sporophytic development from gametes. The majority of the plant regenerants arising from *in vitro* andro- or gynogenesis will be haploid with regard to their genetic background, but plants with other ploidy levels may also develop spontaneously. Due to their sterility, haploid plants are of no value in practical plant breeding, but if their genome is doubled, valuable induced doubled haploids may be obtained for breeding purposes (Segui-Simarro *et al.*, 2011).

Breeding programs require large numbers of genetically stable, homozygote plants, so the efficient genome duplication methods need to produce large quantities of fertile breeding material. *In vitro* anther culture is generally used to produce homozygote paprika plants. The flow cytometer analysis of the ploidy level of paprika plants regenerated from anther culture showed the existence of haploid, diploid and a negligible amount tetraploid plants. The level of spontaneous diploidisation depended on the genotypes (Mitykó *et al.*, 1995) and the methods have been applied (Dolcet-Sanjuan *et al.*, 1997).

Colchicine is still the most effective chromosome doubling agent, although the frequency of diploidization varies with the concentration used, plant parts treated and developmental stage of the tissue (Rao and Suprasanna 1996). Colchicine inhibits the polimerization of tubulin so the chromosomes arrest at methaphase. This paper describes the results of colchicine treatment to *in vitro* grown haploid paprika plants.

2. Material and methods

2.1. Material

We investigated 2276 haploid paprika plants originated from *in vitro* anther culture of Hungarian sweet pepper types.

2.2. Methods

The induction and regeneration medium was prepared as reported by Gémes Juhász *et al.*, (1998; 2004). In order to improve the efficiency of microspore induction we use maltose in the induction medium.

2.3. Flow cytometry analysis of the regenerated plants

The determination of ploidy levels was carried out with a PARTEC Ploidy Analyser, PA-I (Partec Münster, Germany) equipped with a high-pressure lamp (Osram HBO-100W/2) according to the method of Dolezel *et al*., (1989) prior to sample preparation. Leaves (50 mg) from each young *in vitro* regenerants were chopped and macerated in LBO1 (1 ml) lysis buffer to release intact nuclei. The supernatant was filtered through a Cell Trics/TM type nylon filter with a pore size of 30 μm to eliminate cell debris. The cell nuclei were labelled with 1 ml DAPI (4',6'-diamino-2-phenylidole) solution (Partec High Resolution Kit Type P, Solution A) for 30 second. Each sample from each treatment was measured in three replications using diploid paprika plants as the control.

2.4. Colchicine treatment to the regenerants

Anther derived pepper plants in the 2-3- leaf stage, which were detected by flow cytometric analysis as haploids, were placed on V_3 (Dumas de Vaulx *et al.*, 1981) medium containing filter-sterilized 0,5% colchicine in concentration for two, four and six days. Before the treated plants were potted in greenhouses for seed production their roots were washed for twenty minutes. Two months later the genome change was screened on the new raising of the young leaves by flow cytometry.

3. Results

3.1. Colchicine treatment on pepper haploids

Statistical analysis of the results of two- and four-day colchicine treatments on haploids showed significant difference between the treatments. Although the four-day treatment significantly increased the number of dihaploids compared with the two-day treatment, only 24.22 % of the 161 treated plants were diploidised even in this case so two- or four-day treatment with colchicine did not appear to be sufficient for genome duplication Table 1). The high frequency of mixoploids (24.20 %), which contained both haploid and diploid cells, indicated that genome duplication was not yet complete. In order to achieve complete genome duplication it was thus necessary to increase either the concentration of colchicine or the length of the treatment.

In our trial the duration of the colchicine treatment was increased to six days to determine what effect this had on the efficiency of genome duplication.

Comparative statistical analyses on the four- and six-day colchicine treatments also showed a significant difference between the treatments. The six-day treatment led to a significant rise in the ratio of doubled haploid regenerants, resulting in 67–99% rediploidisation depending on the genotype. Diploidisation was achieved in 95.13% in average of the 2054 treated plants originated from 20 different genotypes. The six-day treatment also led to a substantial reduction in the ratio of haploid-diploid genome mosaics. Few of the mixoploids arising due to the six-day treatment were genome mosaics containing diploid and tetraploid cells. The six-day colchicine treatment had no negative effect on the survival of the plants.

No of treated haploid plants	Treatment days	Induced doubled haploids (%)	Induced tetraploids (%)	Induced mixoploids (%)	Rest of haploids (%)	Survival rate (%)
61	2	3.27 (2) a	-	8.19 (5)	88.52 (54)	100%
161	4	24.22 (39)b**	1.24 (2)	24.20 (39)	50.31 (81)	94%
2054	6	95.13 (1954)c**	0.62 (11)	1,32 (10)	4.6 (82)	96.2%

*(**; p< 0.01)*

Table 1
Results of 2-4-6 days treatment with colchicine (500 mg/l) on genome size in anther derived haploid paprika regenerants

4. Discussion

The successful utilization of haploids in breeding process depends not only on the production of large numbers of haploids, but also on the efficient doubling of chromosomes to reach homozigosity and genetic stability (Rao and Suprasanna 1996).

Of several chemical agent so far have been used, colchicine proved to be one of the most efficient (Campion *et al.*,1995).

Miyoshi and Asakura (1996) found that the doubling of chromosomas of 24.2-34.1% gerbera haploids was achived by treatment with 0.05% colchicine for 2-6 days

In the case of summer squash Caglar and Abak (1997) tested the effect of various colchicine concentrations and the treatment times on the doubling process of haploids and found that 0.5% colchicine treatment for 4 hours was the most effective, leading to a doubling of the chromosome number in 60% of the treated plants.

Gürel *et al.,* (2000) described a method of treating young haploid sugarbeet shoots with colchicine and trifluralin. Their results showed that, although colchicine was more effective (25.3%) than trifluralin (18.2%). The treatment duration of 48 h proved to be more effective than 12 h, but it was not different from 24 h or 36 h.

The pepper genotypes tested differed from each other in the ratio of spontaneous doubled haploids formed, ranging from 0 – to 62% (Gémes Juhász unpublished results). So far considerable progress has not been made in haploid paprika chromosome doubling as the treatment of decapitated haploid regenerants has not resulted proper a solution. The present technique offer an efficient, reproducible and economic method for in vitro genome duplication of pepper haploids. Since survival rate of treated regenerants is about 96 % and colchicine treated plants developed similarly to their untreated, spontaneous diploid counterparts and since no fertility problems were recorded, it would seem that this genome duplication technique will be suitable for wild application in pepper.

5. Acknowledgements

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P5-05

Isolation and characterization of pepper genes involved in CMV-P1 infection

Yeaseong Ha, Joung-Ho Lee, Yoomi Choi, Min-Young Kang, JeeNa Hwang, Won-Hee Kang, Jin-Kyung Kwon and Byoung-Cheorl Kang

Department of Plant Science, Plant Genomics and Breeding Institute and Vegetable Breeding Research Center, College of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea

Abstract

Capsicum annuum 'Bukang' is aresistant variety to *Cucumber mosaic virus* isolate-P0 (CMV-P0), CMV-P1 can overcome the CMV resistance of 'Bukang' due to mutations in Helicase (Hel) domain of CMV RNA1. To identify host factors involved in CMV-P1 infection, a yeast two-hybrid system derived from *C. annuum* 'Bukang' cDNA library was used. A total of 156 potential clones interacting with the CMV-P1 RNA helicase domain were isolated. These clones were confirmed by β-galactosidase filter lift assay, PCR screening and sequence analysis. Then, we narrowed the ten candidate host genes which are related to virus infection, replication or virus movement. To elucidate functions of these candidate genes, each gene was silenced by virus induced gene silencing in *Nicotiana benthamiana*. The silenced plants were then inoculated with green fluorescent protein (GFP) tagged CMV-P1. Virus accumulations in silenced plants were assessed by monitoring GFP fluorescence and enzyme-linked immunosorbent assay (ELISA). Among ten genes, silencing of *formate dehydrogenase* (FDH) or *calreticulin-3* (CRT3) resulted in weak GFP signals of CMV-P1 in the inoculated or upper leaves. These results suggested that FDH and CRT3 are essential for CMV infection in plants. The importance of FDH and CRT3 in CMV-P1 accumulation was also validated by the accumulation level of CMV coat protein confirmed by ELISA.

Altogether, these results demonstrate that FDHand CRT3are required for CMV-P1 infectionin plants.

Keywords: *Capsicum annuum*, *Cucumber mosaic virus*, host factor, virus resistance, *formate dehydrogenase*, *calreticulin-3*

P5-06

Genetic mapping of the *Powdery Mildew Resistance* (*PMR1*) gene in pepper (*Capsicum annuum* L.)

Jinkwan Jo[1], Wonhee Kang, Gyung JaChoi[2], Jin-Kyung Kwon[1] and Byoung-Cheorl Kang[1]

[1] *Department of Plant Science, Plant Genomics and Breeding Institute, and Vegetable Breeding Research Center, College of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea*

[2] *Research Center for Biobased Chemistry, Korea Research Instituteof Chemical Technology, Daejeon, Republic of Korea*

Abstract

Powdery mildew disease caused by *Leveillula taurica* is a serious fungal threat to greenhouse pepper production. In contrast to most epiphytic powdery mildew species, *L. taurica* is an endophytic fungus which colonizes in the mesophyll tissues of the leaf. In the genus *Capsicum*, several studies have been conducted to identify resistance sources to *L. taurica*. In the previous studies, five quantitative trait loci (QTLs) for powdery mildew resistance have been identified. An F_2:F_3 population derived from self-pollination of the *Capsicum annuum* commercial cultivar 'PM Singang' resistant to *Leveillula taurica* was used for genetic analysis of powdery mildew resistance. Resistance of the F_2:F_3 families were tested under the natural environmental conditions.White powder observed on infected leaves was used as a disease scale to determine resistance of plants. A total of 86 F_2: F_3 families were evaluated for resistance. The results showed that 16 F_2 plants were homozygous resistant, 50 F_2 were heterozygous resistant, and 20 F_2 were susceptible. The segregation ratio fitted to a single dominant resistance gene model and we named the resistance *Powdery Mildew Resistance 1* (*PMR1)*. We developed two closely linked markers to the *PMR1* gene andrevealed that this gene is located on the chromosome 4. These developed markers will be used to fine mapping the *PMR1* locus and identify underlying resistance gene.

Keywords: powdery mildew, *Capsicum annuum*, *PMR1*, *Leveillula taurica*

P5-07

Development of genetic markers linked to TSWV resistance for marker assisted selection in pepper

Lilla Hajnik[1], Zoltán Szabó[1], Apor Jeney[1], Zoltán Tóth[1], Ágota Domonkos[1], Nikolett Szabadi[1], György B. Kiss[1,2], Péter Kaló[1]

[1] *NARIC, Agricultural Biotechnology Institute, Gödöllő, Hungary*

[2] *AMBIS Biotechnology and Research Ltd, Szeged, Hungary*

Abstract

Tomato spotted wilt virus (TSWV) is one of the most widespread and polyphagous plant virus infecting several important crop species including pepper (*Capsicum annuum* L.). The most efficient way to control virus disease is growing resistant plants. Developing genetic markers linked to the resistance locus facilitates and accelerates the breeding programmes of TSWV resistant cultivars.

The aim of our study was to develop closely linked markers to the *Tsw* locus, and in long-term to identify the *Tsw* gene which confers monogenic dominant resistance against TSWV. The genome walking initiated from the closest genetic marker to the *Tsw* gene using a TSWV susceptible BAC library determined a 600 kb region covered by four overlapping BAC clones wherein the *Tsw* locus resides. Here, we describe two closely linked markers which tightly linked to the *Tsw* locus in our population.

Keywords: *tsw*, MAS, SNPs

1. Introduction

Pepper is an economically important and traditionally grown plant worldwide. *Capsicum* genus has more than 30 members from which *Capsicum annuum* varieties are grown for the fresh market and other four species *C. chinense*, *C. baccatum*, *C. frutescens*, *C. pubescens* are used as spice or genetic resources in resistance breeding programmes [1].

Tospovirus infection is one of the major diseases of pepper caused by the Tomato spotted wilt virus (TSWV). The virus transmitted by several species of thrips is highly polyphagous and infects more than 1000 plant host species [2]. In Hungary, the disease caused by TSWV was first described in 1972 [3] and had been widely spread with its most important and efficient vector, the Western flower thrips (*Frankliniella occidentalis*) [4]. The symptoms of the disease are chlorosis, necrosis, stunting, mottling, ring patterns and local lesions on leaves, stems and fruits, depending on the host plant and environmental conditions [5].

TSWV belongs to the genus *Tospovirus*, the only plant pathogen genus in *Bunyaviridae* family. Its genome consists of three single stranded RNA. The L RNA encodes an RNA dependent RNA polymerase, the M RNA encodes the precursor of two glycoproteins which are involved in thrips transmission and a movement protein, and the S RNA encodes the nucleocapsid protein and a non-structural protein which had been identified as the avirulence factor and predicted to be the silencing suppressor of the virus [6, 7].

Due to difficult management of the transmitting vector the most efficient way to control the TSWV infection is growing resistant pepper plants. Wild-type *Capsicum chinense* accessions (e.g. PI159236, PI152225) contain the *Tsw* gene that confers monogenic dominant resistance against the non-resistance breaking isolates of TSWV [8]. The characteristics of the resistant hypersensitive phenotype are necrotic local lesions and abscission of the infected organ [9]. The resistance might be overcome by high temperature (28–33 C°) or inadequate mechanical inoculation (2-4 true leaf stage) [10]. The TSWV resistance locus has been introgressed into *C. annuum* cultivars by cross breeding [11] and genetic mapping located the *Tsw* gene on chromosome 10 [12]. Although the gene identity has not been confirmed up to now the closely linked markers have been developed for marker assisted selection (MAS) [12, 13].

2. Material and methods

2.1. Plant material

950 F2 plants of the segregating population derived from a cross between two *C. annuum* breeding lines were used for genetic mapping. The male parent (CP-555) contained the *Tsw* resistance gene derived from the *C. chinense* PI 159236 wild pepper accession and a TSWV sensitive double haploid derivative *C. annuum* "HD 322" was used as a maternal parent.

2.2. TSWV inoculation

The cotyledon of 2-week-old seedlings grown at 23-26 ºC were dusted with carborundum powder and were mechanically inoculated with TSWV WT (TSWV-Ca1) isolate (P0 pathotype). Fresh or 80°C deep frozen fruits from TSWV infected susceptible pepper plants were used as inoculum. The presence of TSWV and the absence of other viruses were examined by polymerase chain reaction (PCR). Infected fruits were ground on ice in a turmix blender and the filtered juice was used as inoculum. Hypersensitive reaction (HR) of the resistant plants was observed 5-6 days after inoculation, in case of susceptible ones the virus spread systemically causing chlorosis on the inoculated cotyledons and on younger leaves.

2.3. Marker development and scoring genotypes

Total genomic DNA was isolated from fresh leaves (100 mg/sample) of pepper plants using ZenoGene purification kit according to user's manual [www.zenonbio.hu]. Available molecular markers Q6 and Scac were tested on the parental lines and the segregating population. The PCR conditions were optimized for each newly developed marker by gradient PCR. PCR reactions were carried out in 12 µl final volume containing 1xPCR buffer (included 2 mM $MgCl_2$), 0,2 mM dNTPs, 0,5 µM of each primer, 1 U Taq polymerase and 10 ng template DNA. Thermal cycling conditions consisted of one cycle of initial denaturation for 4 min at 94 ºC, followed by 35 cycles of 94 ºC for 40 s, 60 ºC for 40 s, 72 ºC for 40 s, with a final extension step of 7 min at 72 ºC. Amplification products were separated by electrophoresis on agarose gels (1-2.5%) or on 10% denaturing polyacrylamide gels. The genotype of the F2 individuals were identified and used for colormap genetic mapping [14].

3. Results and discussion

Classical Mendelian segregation ratio of 3:1 for the resistance against the TSWV-Ca1 isolate was detected following the phenotypic characterization of the F2 population (709 resistant and 241 sensitive plants) for resistance. The genotypes of F2 individuals for adapted and newly developed markers are presented in the colormap (Figure 1). Previously published markers (Q6

and Scac) were successfully adapted and used in our population derived from CP-555 (the source of *Tsw* resistance gene) and "HD 322" (TSWV sensitive parent) to define a region of the *Tsw* locus in ~ 30 cM genetic distance [12, 13]. Newly developed codominant and adapted markers from other pepper genetic maps were used to narrow down the Tsw region. Nine dominant AFLP (amplified fragment length polymorphism) markers were developed using the DNA pools of 10-10 TSWV resistant and susceptible F2 plants in PCR amplifications (BSA - bulk segregant analysis). AFLP1 and AFLP2 markers detected 3 and 23 recombination events to the tsw phenotype in our population. Adapted markers T1, T3 were mapped 6,7 cM and 5,2 cM, however T2 marker was tightly linked to the *Tsw* gene.

GENETIC MARKER	GENOTYPE CATEGORIES																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Scac*	2	3	3	3	2	1	1	1	1	1	1	1	1	2	2	2	2	2	3	3	3	3	3	2
T3	2	3	3	3	2	1	1	1	1	1	1	1	1	2	2	2	2	2	3	3	3	3	2	2
B1	2	3	3	2	2	1	1	1	1	1	1	2	2	2	2	2	2	3	3	3	3	2	2	2
B2	2	3	2	2	2	1	1	1	1	1	1	2	2	2	2	2	3	3	3	3	3	2	2	2
T2	2	3	1	1	1	1	1	1	1	1	2	2	2	2	2	2	3	3	3	3	3	2	2	2
Tsw phenotype	2	3	1	1	1	1	1	1	1	1	2	2	2	2	2	2	3	3	3	3	3	2	2	2
B3	2	3	1	1	1	1	1	1	1	1	2	2	2	2	2	2	3	3	3	3	3	2	2	2
B4	2	3	1	1	1	1	1	1	1	1	2	2	2	2	2	3	3	3	3	3	3	2	2	2
AFLP1	4	4	1	1	1	1	1	1	1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
AFLP2	4	1	1	1	1	1	1	1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
T1	2	1	1	1	1	1	2	2	2	2	2	2	2	2	3	3	3	3	3	3	2	2	2	2
Q6**	1	1	1	1	1	1	2	2	2	2	2	2	3	3	3	3	3	3	3	2	2	2	2	2
NUMBER OF PLANTS	59	18	5	1	1	182	32	15	2	3	4	6	3	33	13	1	3	5	172	18	12	22	24	315

1 Maternal homozygous
2 Paternal homozygous
3 Heterozygous
4 Non maternal homozygous

∑ 950 F2

Figure 1:
The colormap of the Tsw region, showing 24 genotype categories prepared from the genotype data of 950 F2 individuals for each marker. The most distant markers were the publicly available ones, Q6 and SCAC. The fine mapping defined the Tsw region between markers B2 and B4. No recombinations could be detected between the Tsw phenotype and the markers T2 and B3.

Genome walking initiated from the closest marker to the *Tsw* gene using a TSWV susceptible BAC library determined a 600 kb region covered by 4 overlapping BAC clones wherein the *Tsw* locus was located (Figure 2). Subcloning of each BAC clones followed by SOLID and Illumina sequencing resulted in a continuous 600 kb genomic sequence which could have been aligned and compared to the reference pepper genome sequences (CM334 1.55, Zunla-1, PI159236 scaffolds). Sequence analysis (BlastX) and gene prediction (FGeneSH (http://www.softberry.com/) revealed that the Tsw region contains at least 10 candidate genes encoding NBS LRR proteins. Our goal in the near future is to develop gene specific molecular markers for tracking the *Tsw* gene in MAS. Furthermore we will analyse the gene content of the region and search for single nucleotide polymorphisms (SNPs) or other genetic rearrangements to identify the *Tsw* resistance gene.

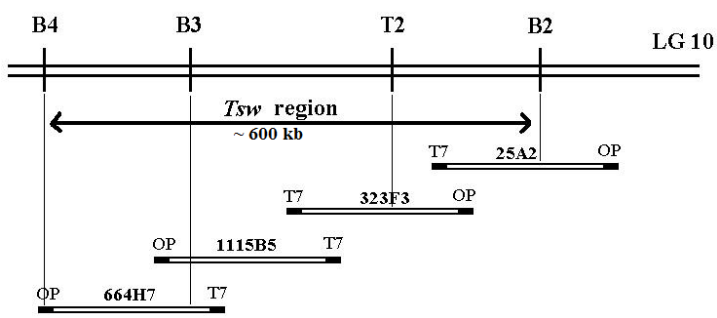

Figure 2
The Tsw locus located on pepper chromosome 10 covered with 4 overlapping BAC clones. B4 and B2 codominant markers determine the 600 kb Tsw region wherein the Tsw gene resides.

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P5-08

Phenotypical and Transcriptomic analysis of Rfo-sa1 resistant eggplant interaction with two fungal wilt pathogens

L. Bassolino[1], L. Toppino[1], V. Barbierato[1], T. Sala[1], G. Valè[2,3], A. Ferrarini[4], M. Delledonne[4], P. Bagnaresi[2], G. L. Rotino[1]

[1] *CREA-ORL, Unità di ricerca per l'orticoltura, Montanaso Lombardo, Italy*

[2] *CREA-GPG, Centro di ricerca per la genomica vegetale, Fiorenzuola d'Arda, Italy*

[3] *CREA-RIS, Unità di ricerca per la risicoltura, Vercelli, Italy*

[4] *Dipartimento di Biotecnologie, Università degli studi di Verona, Italy*

Abstract

Eggplant is susceptible to fungal wilts caused by *Fusarium oxysporum* f. sp. *melongenae* (*Fom*) and *Verticillium dahliae* (*Vd*). Wild relatives represent a good source of resistance and ILs have been obtained through introgression of the *Rfo-sa1* locus conferring resistance to *Fom* from the allied species *S. aethiopicum* into cultivated eggplant although the gene identity is still unknown. In order to investigate the defence mechanisms triggered by the resistance locus *Rfo-sa1*, we established a fine characterization of the plant-pathogen interactions which occur when *Rfo-sa1* lines resistant to Fusarium interact with *Fom* and/or *Vd*. Indeed, our empirical observations suggested that an improved tolerance to *Vd* occurs in IL lines when the *Rfo-sa1* resistance locus is actively responding to Fusarium attack. In this study, phenotypic and transcriptomic analyses were performed on three *Rfo-sa1* ILs and their correspondent susceptible recurrent lines using three different types of inoculation, Fom, Vd and a mixture of both fungi. The phenotypical characterization showed that Fom-resistant ILs carrying the introgression of the *Rfo-sa1* locus displayed significantly improved tolerance to Verticillium attack after a preliminary inoculation with *F. oxysporum*. This positive effect was particularly evident when Verticillium was inoculated in roots simultaneously or after Fusarium. To investigate at molecular level the genes involved in the early phase of defence responses, qRT-PCR analysis were conducted at different time points (0-4-8-h) after fungal inoculations and the results were also confirmed by measuring inoculated roots-derived mRNA transcripts from two independent experimental dataset. Expression data enabled the identification of DEGs and highlighted a number of candidate genes involved in different pathways like terpenoids and phenolic compounds biosynthesis, biotic stress responses, jasmonate signalling, cell wall metabolism, transport and translocation that might have role at the onset of infection. Interestingly, the expression profile of *Fom* inoculated roots is comparable to *Fom+Vd* but not to *Vd* infection, corroborating the hypothesis that a common defence mechanism is induced by *Rfo-sa1* locus which mediates the response to both the two wilt pathogens

1. Introduction

One of the biggest challenges for the eggplant (*Solanum melongena* L.) cultivation is to reduce yield losses both in greenhouse and in open field resulting from infection by soil-borne pathogens (e.g. bacterial and fungal wilts, nematodes) and insects. In fact, the two fungal wilts caused by *Verticillium dahliae* (*Vd*) Kleb. (Fradin & Thomma, 2006) and *Fusarium oxysporum* f. sp. *melongenae* (*Fom*) (Cappelli *et al*., 1995) are among the most serious diseases of eggplant. Partial resistance/tolerance to most pathogens was found within the eggplant gene pool, but the

resistance levels are often insufficient for effective utilization in breeding programs. *S. melongena* progenitors, allied and wild relatives are a valuable source of potential genetic variability for the traits underlying many agronomic and qualitative features of the plant and fruit as well as for valuable resistances to diseases and pests. For this reason, many progenitors have been employed to introgress traits of interest into the gene pool of cultivated eggplant (Rotino et al., 2014). In a previous work, the resistance to *Fom* was introgressed from the allied species *S. aethiopicum* through somatic hybridization followed by anther culture of the tetraploid somatic hybrid and subsequent steps to obtain advanced Introgression Lines (ILs) (Rotino et al., 2014). The ILs molecular characterization enabled to demonstrate that the introgressed resistance trait is controlled by a single dominant locus (named *Rfo-sa*1, Resistance to *Fusarium oxysporum* f. sp. melongenae from *Solanum aethiopicum* 1). The locus *Rfo-sa1* is strictly linked to the resistance to *Fusarium* wilt, and ILs introgressed with that locus do not gain tolerance traits against *Verticillium* fungal wilt. Despite the availability of genomic and genetic resources, there are very few reports on the defence responses and signalling pathways activated upon the interaction of eggplant either with *Fom*, or *Vd*, or of a combination of these two pathogens. With the purpose to investigate the defence mechanism triggered by the Rfo-sa1 locus, both phenotypic and molecular analyses were carried out on three Rfo-sa1 ILs and their correspondent susceptible recurrent lines using three different types of inoculation, Fusarium (F), Verticillium (V) and a mixture (M) of both fungi at different time points (0-4-8 hours) after fungal inoculations.

2. Materials and Methods

As plant material, seed-derived plantlets of three advanced ILs resistant to Fusarium oxysporum (All96/6, All96/6x1F5(9) and 305E40), and of two recurrent lines of eggplant, susceptible to Fom (1F5(9) and TAL1/1) were used. In addition to the inoculation with Fom and Vd, five different types of combined inoculations, either simultaneously (Fom+Vd) or organized in a two-steps inoculation (Fom+24hVd, Fom+48hVd, Vd+24hFom, Vd+48hFom), were carried out and both phenotypic and molecular analysis as well as bioinformatics analysis and functional annotations were performed according to Barbierato *et al*., (2016).

3. Results and Discussion

The phenotypic characterization of the Rfo-sa1 ILs infected with Fom+Vd showed a relevant reduction in disease symptoms with respect to lines inoculated with Vd alone. These different behaviour corroborate the hypothesis that in ILs plants a Rfo-sa1-mediated defence mechanism is induced by Fom infection and somehow determines even a protective effect towards the Vd attack thus leading to a significantly improved tolerance of the plants to both fungi (Table 1) For microarray analysis, since no ready-to-use chips were available for eggplant, we constructed a 4x2K customized CombiMatrix chip as reported in Barbierato *et al.*, (2016). As a result of expression analysis, almost 25% of probes (164 genes) resulted significantly differentially expressed at least in one contrast. Several DEGs displayed a shared expression pattern in Fom and Mixta, different from Vd inoculation. In the Vd library, most of the DEG sequences resulted associated to primary and secondary metabolism, with only few sequences belonging to the defence response or stress induced groups. Conversely, in the libraries from Fom and Mixta inoculated roots of *Rfo-sa1* ILs, the most representative categories were those of defence responses, cell wall modification and composition, transport, signal transduction and stress induced, suggesting that a specific resistance reaction is triggered in *Fom* resistant plants.

The microarray transcriptomic data were valdated through qRT-PCR analysis of 10 selected DEGs from inoculated and mock-inoculated roots of the IL All96/6x1F5(9) harvested at

different time points. The expression analysis highlighted some common behaviours. Genes encoding for lipoxygenase, proteinase inhibitor, STH21, LTP, PR1 and osmotin-like are induced at 4 and 8 HAI after Fom and Mixta inoculation in accordance with microarray data. Even the expression pattern of sesquiterpene synthase, PR5 and miraculin fits with the transcriptomic results although their induction is more remarkable after Fom inoculation.

Table 1

IESF values at 30 DAI of the five genotypes tested for seven types of inoculations. Values are reported as means ± SD and, for each column, values with different letters are significantly different. Mixed stands for Fom+Vd simultaneous inoculation.

Inoculation	1F5(9)		All96		All96 X 1F5(9)		TAL 1-1		305 E40	
Fom	100 ± 0	a	43,93 ± 1,92	d	25 ± 3,42	e	100 ± 0	a	13,33 ± 2,38	f
Mixed	100 ± 0	a	56,67 ± 3,36	cd	66,67 ± 0	cd	100 ± 0	a	48,33 ± 0,96	cd
Vd	100 ± 0	a	100 ± 0	a	95 ± 3,74	ab	100 ± 0	a	90 ± 1,86	a
Fom + 24h *Vd*	100 ± 0	a	41,67 ± 0,96	d	65 ± 3,01	cd	100 ± 0	a	20 ± 3,27	ef
Fom + 48h *Vd*	100 ± 0	a	45,00 ± 2,45	d	55 ± 3,31	d	100 ± 0	a	30 ± 2,72	de
Vd + 24h *Fom*	100 ± 0	a	65,00 ± 0,99	c	83,33 ± 1,49	bc	100 ± 0	a	61,67 ± 3,37	bc
Vd + 48h *Fom*	100 ± 0	a	86,67 ± 3,81	b	98,33 ± 3,74	a	100 ± 0	a	76,67 ± 2,17	ab

Here we report qRT-PCR of *Protease Inhibitor type II*, *N-hydroxycinnamoyl transferase*, *Extensin like*, and *Trehalose phosphate phosphatase* genes which were analyzed at 0, 4, and 8 HAI (Figure 1). Also this latter expression analyses confirmed differences in gene expression after inoculation with Fom and Mixta with respect to Vd and suggest that the coordinated expression of these genes may have a role in conferring full resistance to Fom and partial resistance Vd. Indeed, these genes can be considered as putative candidates involved in the resistance mechanism to Fom.

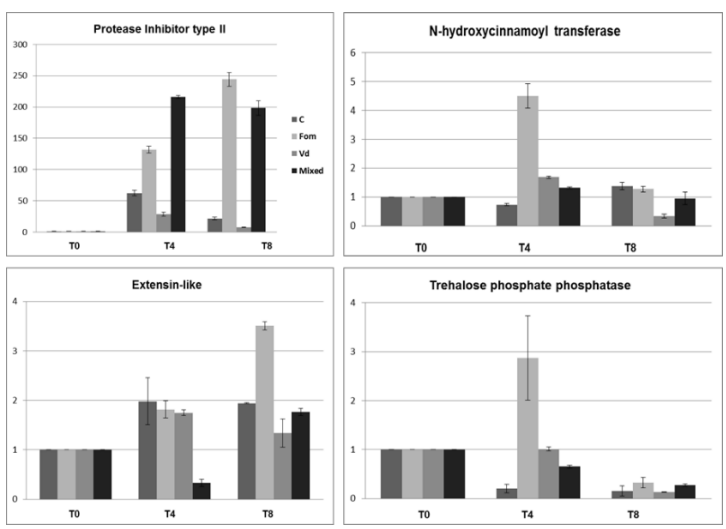

Figure 1

qRT-PCR of candidate genes involved in the early phases of response to Fom infection. Quantitative analysis of transcript levels in root extracts inoculated with mock control (C), Fom (F), Vd (V), and Mixed (M). Expression levels are shown as units relative to GADPH expression and data are expressed as means of three technical replicates ± SD.

The availability of candidate genes specifically involved either in the resistance to *Fusarium oxysporum* f. sp. *melongenae* or in the improved tolerance to *Verticillium dahliae* may have an impact both in the eggplant basic research studies as well as in the practical breeding activity. In fact, much remains to be elcuidated on how these candidate genes are co-regulated once the infection occurs and on the possible metabolic pathway(s) or networks underlying the resistance trait. For practical breeding, these genes might be exploited both for the identification of superior allelic variants and to develop molecular marker to assist the selection of improved cultivars.

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P5-09

Genetic mapping and identification of the *Me1* gene conferring resistance to root-knot nematodes in pepper (*Capsicum annuum* L.)

Zoltán Tóth, Zoltán Szabó, Tímea Földi, Nikolett Szabadi, Lilla Hajnik, Apor Jeney, György B. Kiss, Péter Kaló

NARIC, Agricultural Biotechnology Institute, Gödöllő, Hungary

Abstract

Root-knot nematodes (RKN) are widespread and polyphagous endoparasites of plants that can cause serious losses to agriculture. Breeding of disease resistant varieties is the most efficient strategy to manage RKN infection. Some pepper (*Capsicum annuum* L.) lines and accessions have several genes conferring resistance against root-knot nematodes (*Meloidogyne* sp). Application of linked DNA markers in breeding programs facilitates the tracking of the inheritance of beneficial qualities such as the nematode resistance and helps the selection of breeding materials. The aim of our study was to identify molecular markers linked to the pepper *Me1* dominant resistance gene that confers resistance against wide variety of root-knot nematode species (*Meloidogyne* spp.).

1. Introduction

Root-knot nematodes, *Meloidogyne* spp., are cosmopolitan plant parasitic nematodes widespread in many different climates. Nutrient losses and damage inflicted by the nematodes reduce root growth, compromise shoot growth and photosynthetic capacity of infected plants [1]. More than 98 *Meloidogyne* species have been identified [2] and three of them (*M. incognita*, *M. javanica*, *M. arenaria*) are widely distributed, have broad host ranges and cause significant yield losses in a range of crop species. Second-stage nematode juveniles (J2) invade the roots in the elongation zone and then migrate intercellularly and sedentarise into the differentiation zone of the vascular cylinder. Here, responding to stimulation from the parasite, few root cells located around the head of the nematode develop into specific feeding structures known as giant cells [3]. Each of these giant cells becomes multinucleated by synchronous mitosis in the absence of cytokinesis. Moreover, mature giant cells are metabolically very active, with unusual high DNA content per nucleus and increased numbers of organelles.

Although soil fumigation is one of the possibilities to control these pests, the use of many nematicides has been prohibited due to environmental and health concerns. Plant resistance is actually the most efficient method of controlling root-knot nematodes, especially in solaneceous crops including tomato (*Solanum lycopersicum*) and pepper (*Capsicum annuum*), for which several resistance genes have been identified in cultivated and wild species and subsequently used in breeding programs to develop nematode-resistant cultivars.

Major resistance genes against RKN are available in a number of plant species [4], and their application provides a safe and economically relevant strategy to control RKN. These resistance (R) genes generally harbour NBS–LRR motifs and are often located in syntenic clusters in the genomes of solanaceous plants. In *Solanaceae*, the manifestation of plant resistance to

Meloidogyne spp. is characterized by hypersensitive reaction (HR) developing localized cell necrosis around the nematode head [5]. In pepper, a complex system of resistance mechanism to root-knot nematodes, involving at least five genes, has been described [6]. Two of these genes (*Me1* and *Me3*) originally identified in two independent breeding lines that confer the similar resistance to the three main species of RKNs, *M. arenaria*, *M. incognita* and *M. javanica*. The three other genes (*Me2*, *Me4* and *Me5*) confer a more restricted resistance response, being specific against either a single nematode species or some populations of a species [7]. Six of the *Me* genes (*Me1*, *Me3*, *Me4*, *Me7*, *Mech1*, *Mech2*) are mapped to a 28 cM region of pepper chromosome 9 [6]. Introgression of *Me1* and *Me3* is the primary aim of pepper breeding programs because each of these genes provides resistance to the three major species of RKNs, *M incognita*, *M. arenaria* and *M. javanica.*

The aim of our study was to identify molecular markers linked to the *Me1* dominant resistance gene in pepper that confers resistance against wide variety of RKN species (*Meloidogyne* spp.) and delimit the genomic region wherein the *Me1* is located in order to identify the *Me1* gene in the future.

2. Materials and methods

2.1. Tests of Nematode susceptibility

The mixed culture of *Meloidogyne* species (*M. arenaria, M. incognita and M. javanica*) were maintained on the susceptible fresh market pepper line. Pepper seeds were germinated in steam-sterilized sandy soil in seed trays and 2-week-old seedlings were transplanted individually into 250 ml plastic pots and grown to the fourth true leaf stage prior to inoculation. Egg masses and J2 juveniles were collected from infected roots and soil and then used to inoculate the pepper plants. Plants were grown in a 25 °C growth chamber, egg masses were harvested 6-8 weeks after inoculation (wpi) and the susceptibility of the pepper plant to RKN infection was scored based on counting the egg masses on the roots.

2.2. Plant material

A resistant descendant of the pepper inbred line of *C. annuum* „HD322" derived from the Central American accession carrying the *Me1* gene conferring resistance to wide range of RKNs and a RKN susceptible pepper plant of the *C. annuum* „CP-555" cultivar were crossed to generate F1 progenies. The F1 plants were self-pollinated to generate the F2 segregating population for genetic mapping [8].

2.3. Marker development and scoring for genotypes

Total DNA was isolated from fresh leaf tissue (100 mg / sample) of the parental genotypes and more than 900 F2 individuals using a Zenogene DNA purification kit. Several publicly available SSR, AFLP and CAPS pepper markers [6, 8] were tested on the parental lines to detect polymorphism. Fragments of SSR markers were amplified in 12 μL reaction mixtures containing 1.2 μL 10x PCR buffer, 0.5 U Taq polymerase, 0.2 mM dNTP, 0.5 μM of each primers and 10 ng template DNA. Thermal cycling conditions consisted of one cycle of initial denaturation for 4 min at 94 °C, followed by 35 cycles of 94 °C for 40 s, 60 °C for 40 s, 72 °C for 40 s, with a final extension step of 7 min at 72 °C. Amplification products were separated by electrophoresis in agarose gels (1 – 2.5 %) or on 10 % denaturing polyacrylamide gels. The genotypes of the F2 individuals were identified based on the polymorphism and used for genetic mapping using the color map method [9].

3. Results and discussion

3.1. Phenotypic characterization of the F2 population for resistance against M. arenaria, M. incognita and M. javanica.

The individuals of the F2 population and the parental plants were tested for their response to the mixture of inoculants of *Meloidogyne* species. The *C. annuum* cv. „CP-555" displayed a susceptible phenotype when inoculated with *Meloidogyne* isolates and the average number of observed egg masses was more than 100/plant. The parent „HD322" was resistant to all *Meloidogyne* races and only up to six egg masses (0–6)/plant could be found on its roots. We counted the number of egg masses on the roots of F2 plants at 8 wpi and 73% of the F2 plants exhibited resistant phenotype indicating that the *Me1* resistance gene was inherited as a single dominant trait showing the classical Mendelian segregation ratio of 3:1.

3.2. Genetic mapping of the Me1 resistance locus

In earlier studies it was demonstrated that the *Me1* gene is located on linkage group 9 of the pepper genome [8]. Using the genetic markers of other linkage maps of pepper, the rough map position of the resistant locus was determined between two AFLP genetic markers spanning approximately 2.2 cM genetic distance of the pepper genome. In order to position the *Me1* gene more precisely, additional F2 and F3 plants were genotyped and analyzed for their resistance to RKN infection. The fine mapping narrowed down of the Me1 region into a genomic region of about 300 kbp between two codominant microsatellite (SSR) markers 971E and 741A (Fig. 1). The sequence of this genomic region was obtained from the sequence database of the cultivated pepper Zunla and CM334 (http://peppergenome.snu.ac.kr/download.php) and used to analyze the gene content and generate additional markers for further genetic mapping. The novel genetic markers 375A and 226B were used to delimit the region of the *Me1* locus into a 160 kbp region (Fig. 1). The genome sequence of Zunla and CM334 pepper plants was analyzed to reveal candidate genes, primarily R-genes for *Me1*. The tightly linked markers 375A and 226B are appropriate to use in marker assisted selection (MAS) that can facilitate breeding programs to develop RKN resistant pepper lines.

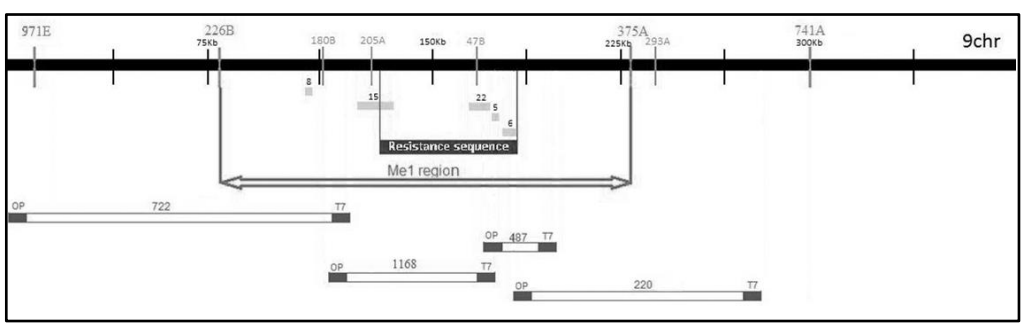

Figure 1

The black horizontal line represents the contig of the sensitive C. annuum BAC clones corresponding to the genomic region containing the Me1 gene. Genetic markers are shown above the black line. The five annotated NBS-LRR genes found in the contig corresponding to the Me1 region are presented with gray bars and figures (no. 5, 6, 8, 15, 22).

3.3. Analysis of the Me1 region

Within the region flanked by the newly developed markers 375A and 226B, the genome sequence of Zunla, has been analyzed by using the online software FGENESH (http://www.softberry.com/). The analysis identified 12 genes including housekeeping genes and other ones predicted to function in general biological processes. In addition we found five genes predicted to encode potential Nucleotide Binding Site–Leucine-Rich Repeat resistance proteins and therefore these genes are the best candidate for *Me1* (Fig. 1).

The genomic fragments used to construct the BAC (Bacterial Artificial Chromosome) library was derived from a susceptible *C. annuum* plant and thus the library does not contain the resistant allele of the *Me1* gene. In order to obtain the genomic sequence from RKN resistant genomic background we designed oligonucleotide primers to amplify the alleles of the candidate genes and the Me1 region. The sequence of the amplified products were determined and used to assemble the sequence of the Me1 region from the RKN resistant genomic background. The analysis to identify SNPs and smaller or larger genetic rearrangements in the five NBS-LRR-type R genes between the sensitive and resistant background or to identify additional genes in the parent „HD322" is in progress.

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P5-10

A transcriptomic study of the interaction between pepper and *Phytophthora capsici*

Gaëtan Maillot[1], Emmanuel Szadkowski[1], Anne Massire[1], Jean-Paul Bouchet[1], Véronique Brunaud[2], Marie-Laure Martin-Magniette[2], Sandrine Balzergue[2], Guillem Rigaill[2], Kurt Lamour[3], Véronique Lefebvre[1]

[1] *INRA, UR1052 GAFL, Fruit and Vegetable Genetics and Breeding Research Unit, Domaine St Maurice, Montfavet, France*

[2] *Institute of Plant Sciences - Paris-Saclay (IPS2), UMR 9213/UMR1403, CNRS, INRA, Université Paris-Sud, Université d'Evry, Université Paris-Diderot, Sorbonne Paris-Cité, Orsay, France*

[3] *Department of Entomology and Plant Pathology, the University of Tennessee, Knoxville, TN, United States*

Abstract

The Oomycete *Phytophthora capsici* is a polyphagic pathogen which attacks various plant species of agronomic interest. It is notably known to cause important damages on pepper for which a few partially resistant pepper genitors have been described. To identify the effect of the pepper resistance on the gene expression of *P. capsici*, a RNA-Seq analysis was performed. Two pepper accessions (one resistant and one susceptible) were inoculated with two *P. capsici* isolates separately. Infected plant tissues were collected at two times after inoculation giving 8 distinct conditions (2 hosts x 2 isolates x 2 time-points). Each condition was collected in 3 independent replicates generating 24 biological samples. The independent expression analyses in the 8 conditions of interaction highlighted between 3,014 and 11,110 genes expressed by *P. capsici* and between 13,677 and 39,928 genes expressed buy the pepper host. Comparison analysis between samples showed genes with significant differential expression patterns according to the level of resistance of the pepper genitors and the aggressiveness of the *P. capsici* isolates. These included genes associated with pathogenicity. Here we focus on the analysis of the pepper transcriptome contigs in order to identify the differentially expressed genes. Our main objective is to identify pepper genes involved in resistance. The completion of this study should new markers to aid the development of genetic resistance in pepper.

1. Introduction

Phytophthora capsici Leonian is an important plant pathogen causing economic losses in various agronomic crops. It infects roots, crowns, stems and leaves from the Solanaceae, Cucubitaceae and Fabaceae plant families (1), including pepper (*Capsicum annuum*). *P. capsici* is a pathogen with a hemibiotrophic life cycle. Its reproduction involves both asexual and sexual reproduction producing millions of sporangia that spread the disease in the fields.

Its fast rate of evolution, its broad host range, and the limited effectiveness of chemical treatment explains the difficulty in controlling this pathogen.

The use of naturally genetic resistances appears thus as a good alternative to control the disease (1, 2).

In pepper, few resistant genitors have been discovered with mostly quantitative resistances. Many studies have focused on the determination of the molecular determinants of the resistance to *P. capsici* (2).

Using RNA-Seq and microarrays, recent studies investigated the transcriptomic changes occurring in *P. capsici* in different conditions going from the pre-infective spores to biotrophic and necrotrophic stages of infection. More than a thousand genes of *P. capsici* showed important changes of expression including genes associated with pathogenicity (3, 4). More recently, another transcriptomic study focused on the changes occurring in pepper, according to its resistance level, after infection by *P. capsici* (5). Authors identified 211 candidate genes, associated with defence responses, with significant expression changes between resistant and susceptible accessions at different times after infection.

Although gene-expression studies already investigated the transcriptomic changes in pepper - *P. capsici* interaction, their primary focus was the infection kinetics of the interaction. Here we propose to dissect the expression changes occurring in pepper during the interaction with isolates differing for their level of aggressiveness, with regard to the resistance and susceptibility of the pepper host.

To respond to this objective, we designed a two time-point multi-factor RNA-Seq analysis on pepper accessions inoculated with *P. capsici* isolates. RNA samples from 8 conditions of interaction going from A to H, two pepper accessions differing in their resistance level (Criollo de Morelos 334 or CM334 and Yolo Wonder or YW) interacting with two isolates differing in their aggressiveness level (Pc107 and Pc273), at two times (24 and 72 hours post infection), were deeply sequenced (Table 1: Summary of the factors of each condition).

2. Materials & Methods

2.1. Pepper and P. capsici material

Two accessions of *C. annuum* were used in our study: the susceptible Yolo wonder (YW) and the partially resistant Criollo de morelos 334 (CM334).

The accessions were inoculated with two isolates differing by their original host and their aggressiveness on pepper. Pc273 was collected on pumpkin in the USA; it is lowly aggressive on pepper accessions with the artificial test previously described (6). Pc107, was collected on pepper in the south of France; it is highly aggressive on pepper accessions.

2.2. P. capsici inoculation and tissue preparation

Each accession was inoculated with the two isolates separately as previously described (6). The assay was conducted in a growth chamber with a 12/12H light/dark cyle and 24/22°C temperature cycle. 8 x 6 of CM334 and 8 x 4 of YW apexless plants of 6 week old were inoculated with 3 mm mycelium plugs of V8.

2.3. RNA-Seq design and library preparation

5 mm of stem samples under the necrosed tissues were collected at 24 and 72 hours post inoculation (hpi) for the 4 conditions of interaction: CM334 with Pc107 (A at 24 hpi, E at 72

hpi), YW with Pc107 (B at 24 hpi, F at 72 hpi), CM334 with (C at 24 hpi, G at 72 hpi) and YW with Pc273 (D at 24 hpi, H at T2). Stem samples were collected on 6 and 4 plants of CM334 and YW respectively for each condition of interaction at the 2 times. They were frozen in liquid-nitrogen and stored at -80ºC until further use. 6 and 4 stem samples were pooled in triplicate for CM334 and YW respectively and total RNA was extracted using a QIAGEN Rneasy Plant Mini Kit. The libraries preparation and the Illumina sequencing were done at the Institute of Plant Sciences Paris-Saclay (IPS2, France).

2.4. Transcriptomic data processing and mapping

After removing low quality and ribosomal reads, the retained paired-end reads were mapped to a dataset composed of sequences from pepper and *P. capsici*. We used a local assembled transcriptome of the accession Perennial for *C. annuum* composed of 42,000 contigs (from INRA lab). To complete the dataset, 496 sequences of genes associated with pathogenicity (from Lamour's lab) were added to the 19,805 transcript models of the *P. capsici* genome from the JGI (http://genome.jgi.doe.gov/Phyca11/Phyca11.home.html). The paired-end reads were mapped to the *P. capsici* and pepper dataset with Bowtie2 using the default parameters of the mode end-to-end (7). The raw reads were processed with a home script that removed not concordant reads in Pairs and ambiguous hits for a Paired-end.

2.5. Expression and differential analysis

The number of genes expressed was calculated using HTSfilter (8) for each condition separately. The normalization of the paired-end read counts and the differential expression analysis were done with edgeR (9, 10). Genes with an adjusted P value < 0.01 and an absolute logFoldChange > 2 were considered to be differentially expressed.

Condition	Pepper accession	*Pc* Isolate	Time (hpi)	No. of samples	Average number of paired-end reads	Average number of paired-end reads mapped	Pepper %
A	CM334	Pc107	24	3	37 020 880	28 806 747	99,79
B	YW			3	34 089 528	26 852 185	95,05
C	CM334	Pc273		3	34 909 207	27 211 802	99,46
D	YW			3	35 335 428	27 428 622	99,07
E	CM334	Pc107	72	3	42 204 121	33 546 033	99,84
F	YW			3	39 289 323	29 935 027	95,85
G	CM334	Pc273		3	35 582 491	28 383 607	99,95
H	YW			3	39 571 892	31 515 672	99,84

Table 1
Average RNA-Seq library size and mapping efficiencies for pepper accessions inoculated with P. capsici at 24 or 72 hours postr inoculation. CM334: cv Criollo de Morelos 334, YW: cv Yolo Wonder, Pc: P. capsici, hpi: hours post inoculation.

3. Results

3.1. Analysis of paired-end reads and the dual-mapping to the host and the pathogen

A summary of the total number of paired-end reads produced and the statistics of mapping is shown in Table 1, for the 8 conditions (2 hosts x 2 isolates x 2 time-points). On average, between 34 and 42 million paired-end reads were produced for the different conditions of interaction, and 74 to 81 % of those paired-end reads mapped to our dataset composed of 42 k pepper contigs and 20.3 k *P. capsici* transcripts models.

Among the mapped reads, pepper reads were the most abundant with 95.95% - 99.95% of the total amount of reads mapped per condition with the remaining sequences being *P. capsici* reads.

3.2. Expression analysis of pepper

We pre-filtered the 24 libraries to exclude non-expressed contigs in any conditions, and kept 41,903 pepper contigs for further analysis (see M&M). To study the gene expression in pepper during the interaction, we analysed each condition of interaction (A to H) independently, and detected expression vs non-expression for each.

A total of 41,481 contigs of pepper were detected in at least one condition of interaction. There are no big differences between the time-points in the number of contigs detected expressed, particularly for the paired conditions A-E, C-G and D-H. Strikingly, the conditions B and F show the lowest pepper contigs expressed, with 13,677 and 15,172 compared to the roughly 40,000 contigs of the other conditions (A: 39,469, C: 39,782, D: 38,999, E: 39,928, G: 39,899, H: 39,783).

3.3. Isolate effect on the expression of the genes

We compared the expression levels of the contigs of CM334 between those of YW at 24 and 72 hpi when infected by the aggressive Pc107 and the less aggressive PC273, giving four comparisons.

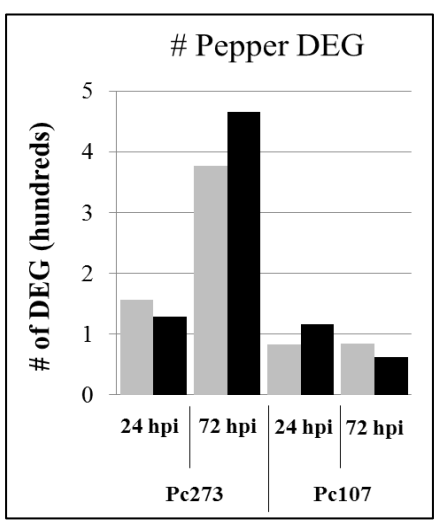

Figure 1
Number of significant (logFoldChange > 2, adj p < 0.01) differentially expressed contigs (DEGs) in 4 comparisons. The gray bar indicates the number of down-regulated DEGs and the black ones are for the up-regulated DEGs in CM334 compare to YW. T1: 24 hpi (hours post infection); T2: 72 hpi; Pc107: Highly aggressive and Pc273: Lowly aggressive.

In total, 1036 unique significant differentially expressed contigs (DEGs) were detected. The comparisons of the two accessions when they interact with Pc107 gave the highest DEG numbers with 157 down-regulated and 129 up-regulated DEGs in CM334 at 24 hpi, and 377 down-regulated and 466 up-regulated in CM334 at 72 hpi. For the comparison when both accessions interacted with Pc273, 83 down-regulated and 116 up-regulated DEGs at 24 hpi and 84 down-regulated and 62 up-regulated DEGs at 72 hpi were found in CM334.

4. Discussion

The goal of this study was to assess the expression changes occurring in pepper accessions when inoculated by *P. capsici* isolates with different aggressiveness level. Important reductions of the number of contigs expressed were observed when the aggressive isolate and the susceptible pepper interact compared to the other interactions. 1036 pepper DEGs were detected between the susceptible YW and the partially resistant CM334 highlighting a strong contrast in the response against *P. capsici* isolates. The contrast appeared to be stronger when the pepper accessions were inoculated by the highly aggressive isolate and when looking at the latest time of interaction. For the moment, we only describe the changes of expression patterns occurring in the pepper accessions when inoculated by isolates differing by the aggressiveness level. The Gene Ontology analysis of those DEGs should give us a better understanding of the molecular mechanisms used by pepper to resist infection by *P. capsici*.

5. Acknowledgment

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P5-11

Detailed mapping of semi-dominant loci controlling resistance to *Fusarium* wilt in cultivated eggplant (*Solanum melongena*).

Koji Miyatake, Takeo Saito, Eiji Yamamoto, Hirotaka Yamaguchi, Tsukasa Nunome, Akio Ohyama, Hiroyuki Fukuoka

NARO Institute of Vegetable and Tea Science (NIVTS), Mie, Japan

Abstract

Fusarium wilt is a worldwide serious soil-borne disease threatening stable production of eggplant (*Solanum melongena*). This disease is caused by a plant pathogen *Fusarium oxysporum* f. sp. *melongenae*. The three eggplant germplasms, LS1934, LS174 and LS2436 were reported as highly resistant materials to the pathogen. The objective of this study was to locate loci for *Fusarium* resistance in these cultivated eggplant germplasms. In this study, we performed quantitative trait locus (QTL) analyses using simple sequence repeat (SSR) markers and single nucleotide polymorphism (SNP) markers, and detected a semi-dominant resistance locus, *FM1* at the end of chromosome 2 with two alleles, $Fm1^L$ and $Fm1^E$ in the F_2 populations LWF2 (LS1934 × WCGR112-8 [susceptible]) and EWF2 (EPL-1 [derived from LS174] × WCGR112-8), respectively. The percentage of phenotypic variance explained by $Fm1^L$ derived from LS1934 was 75.0 % (LOD = 29.3) and that explained by $Fm1^E$ derived from EPL-1 was 92.2 % (LOD = 65.8). In addition, using backcrossed inbred lines (BILs) derived from LS1934 and AE-P03 [susceptible], we mapped *Fm1* between two SSR markers located ~4.9cM from each other. Comparing the location of the above locus to those of previously reported ones, the resistance locus *Rfo-sa1* from an eggplant ally (*Solanum aethiopicum* gr. *Gilo*) was mapped very close to *FM1*, whereas another resistance locus from LS2436 was mapped in the middle of chromosome 4. The detailed mapping of the resistance loci derived from the cultivated germplasms will permit the use of marker-assisted selection to bypass the problems posed by self-incompatibility and introduction of deleterious traits because of linkage drag, and will help us to understand genomic mechanisms of *Fusarium* resistance.

1. Introduction

Fusarium wilt, caused by the soil-borne plant pathogen *Fusarium oxysporum* f. sp. *melongenae*, is one of the most serious diseases of eggplant; the fungus can spread widely and it can persist for many years in the soil. Therefore, field idling or cultural control is not sufficient to eradicate the pathogen. In such a situation, developing a resistant cultivar is a radical solution for controlling the disease. The latest advances in molecular marker analyses can facilitate efficient breeding operations.

Previous studies have reported that three eggplant germplasms, LS1934, LS174, and LS2436, have been found to be completely resistant (Mochizuki et al. 1997; Monma et al. 1997; Sakata et al. 1996). Using two of these resistant germplasms, *Fusarium*-resistant rootstocks, Daitaro and Daizaburou derived from LS1934, and a cultivar Eggplant parental line 1 (EPL-1) derived from LS174, were developed at the NARO Institute of Vegetable and Tea Science (NIVTS), Japan (Mochizuki et al. 1997; Monma et al. 1997; Yoshida et al. 2004). In addition, a dominant single locus, *Rfo-sa1*, was mapped on chromosome 2 for *Fusarium* resistance in a somatic hybrid line 305E40 introgressed from an its ally (*Solanum aethiopicum* gr. *Gilo*) (Barchi et al. 2010; 2012; Portis et al. 2014) and sequence characterized amplified region (SCAR) markers

were developed linked to a *Fusarium* resistance locus in an eggplant line, LS2436, based on bulked segregant analysis (Mutlu et al. 2008).

In this study, we report (1) the precise mapping position of a *Fusarium* wilt resistance locus with two alleles derived from LS1934 and EPL-1, based on the mapping populations and linkage maps reported by Fukuoka et al. (2012) and Hirakawa et al. (2014); (2) the positional relationships of resistance loci reported here and in previous studies (Barchi et al. 2010; 2012; Mutlu et al. 2008; Portis et al. 2014); (3) the availability of resistance-linked markers that is suitable for marker-assisted selection (MAS).

2. Materials and Methods

2.1. Plant materials and linkage maps

Three F_2 mapping populations LWF2, EWF2 and ALF2, and corresponding linkage maps LW2012, EW2012 and AL2010, reported by Fukuoka et al. (2012) and Hirakawa et al. (2014), were used in this study. The LWF2 was derived from a cross between the lines LS1934 and WGCR112-8, and the EWF2 was derived from a cross between EPL-1 and WGCR112-8, and the ALF2 was derived from a cross between LS1934 and AE-P03. WCGR112-8 and AE-P03 are susceptible to *Fusarium* wilt (Nunome et al. 2001) whereas both LS1934 and EPL-1 are highly resistant (Mochizuki et al. 1997; Sakata et al. 1996). F_3 populations, LWF3 and EWF3, were developed by self-crossing each F_2 plants. A population of backcrossed inbred lines, ALBIL, was developed by single-seed descent from a cross between an F_1 plant (LS1934 × AE-P03) and LS1934.

2.2. Fusarium wilt resistance test

Fusarium oxysporum f. sp. *melongenae* strain SFU1267 was provided by NARO Kyushu Okinawa Agricultural Research Center (Yoshida et al. 2004). *Fusarium* wilt resistance tests were performed basically according to Saito et al. (2010). WCGR112-8, AE-P03, LS1934, EPL-1, F_1 plants (LS1934 × WCGR112-8, EPL-1 × WCGR112-8, and AE-P03 × LS1934), and the lines of LWF3 (n = 87), EWF3 (n = 120), and ALBIL (n = 182) were tested. A total of 15–20 seedlings per line were tested. The fungus was cultured at 28 °C in potato glucose medium supplemented with 0.1% dibasic potassium phosphate and 0.3% yeast extract. We used 10-day–old culture as the inoculum at 2.0×10^7 spores/ml. The inoculated seedlings were planted in a flat filled with sterilized soil and kept at about 28 °C in a greenhouse. Symptom index of each plant was scored on a scale of 0 to 2: 0 = no symptoms; 1 = half of the leaves wilted; and 2 = dead plant. The disease index (DI) was calculated as below.

2.3. Molecular mapping and QTL analyses

Linkage analyses were based on the maps LW2012, EW2012 (Hirakawa et al. 2014) and AL2010 (Fukuoka et al. 2012). Genetic distance was determined by using JoinMap software version 4.1 (Kyazma B.V., Wageningen, The Netherlands). QTL analyses were performed by using the DI values and the linkage maps LW2012 and EW2012 (Hirakawa et al. 2014). Composite interval mapping wasperformed according to Miyatake et al. (2012).

$$\text{Disease Index} = \frac{\sum(\text{Symptom index} \times \text{Number of plants with that symtom index})}{\text{The total number of plants}} \times 50$$

2.4. Development of markers in the vicinity of Fusarium resistance gene loci

To compare the precise positions of *Fusarium* resistance genes studied here (*Fm1*) and those reported previously (Barchi et al. 2012; Mutlu et al. 2008), restriction site associated DNA (RAD) markers linked to the *Rfo-sa1* gene and a sequence characterized amplified region (SCAR) marker linked to a resistance gene of LS2436 were converted to SSR and SNP markers. The scaffolds corresponding to the RAD or SCAR markers were detected by BLASTN against the open database of the scaffold sequences reported by Hirakawa et al. (2014). To search for SNP polymorphisms between the parents, we conducted direct sequencing with primer pairs covering the entire region of the selected scaffold sequence.

3. Results and Discussion

3.1. Evaluation of Fusarium resistance

The paternal parent of the F_2 populations WCGR112-8 showed severe symptoms (DI = 0.95), whereas the maternal parents LS1934 and EPL-1 and F_1 plants LWF1and EWF1 showed no symptoms (Fig. 1), suggesting dominant inheritance of this trait. Both F_2 populations (LWF2 and EWF2) showed a continuous distribution of the DI values (Fig. 1). The distribution was not normal according to the Shapiro–Wilks test, suggesting that resistance was likely controlled by a few genes.

3.2. QTL analyses

Using LWF2 and EWF2 populations, we detected QTLs for *Fusarium* resistance at the end of short arm of chromosome 2 (Table 1, Fig. 2). The LOD for the QTL detected in LWF2 was 29.3 (empirical threshold, 20.7; phenotypic variance explained, PVE = 75.0). The LOD for the QTL detected in EWF2 was 65.8 (empirical threshold, 20.4; PVE = 92.2). Detected QTLs play an important role as a single major gene controlling resistance to *Fusarium* wilt in each populations. We denoted them as $Fm1^L$ and $Fm1^{E,}$ respectively (Table 1). We grouped F_2 progenies according to genotypes of the markers nearest to the detected gene loci and calculated the mean DI values (Table 2). The F_2 progenies with susceptible parental homozygous genotypes at these markers showed remarkably high DI values (Table 2), therefore developed markers linked to $Fm1^L$ and $Fm1^E$ are a powerful tool for MAS for *Fusarium* resistance in eggplant.

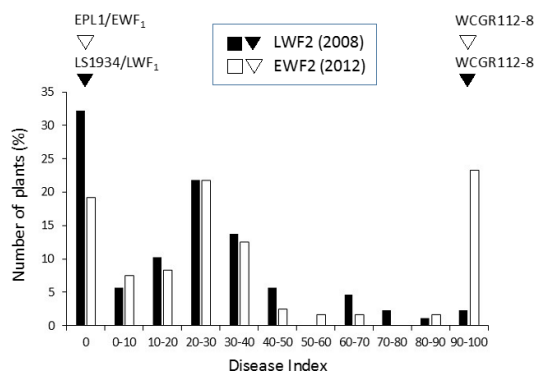

Figure 1

Frequency distribution of disease indices in two F_2 populations. Triangles show the mean values of the parents and F_1 plants in the respective populations.

Table 1
The results of QTL analyses for Fusarium resistance

Population	Flanking markers[a]	LOD	Additive effect[b]	Dominant effect[c]	PVE
LWF2	SOL8178 - emj01H11	29.3	-35.8	-12.9	75.0
EWF2	eme11C02 - SOL8178	65.8	-47.7	-22.6	92.2

[a] The nearest markers are underlined.
[b, c] Positive values indicate that higher-value alleles are from LS1934 or EPL-1 and negative values indicate that higher-value alleles are from WCGR112-8.

Table 2
Disease indices of F_2 progenies and genotypes of markers closest to FM1

Population	Nearest marker	Marker genotype[a]	Disease Index		n	
			Mean	SE[b]		
LW2012	emj01H11	R	0.7	0.5	25	a
		H	23.9	1.7	51	b
		S	72.5	5.0	11	c
EW2012	eme11C02	R	6.3	2.8	34	a
		H	28.5	1.6	56	b
		S	99.5	0.4	27	c

[a] R, homozygous for the resistant parent allele; H, heterozygous; S, homozygous for the suscestible parent allele
[b] SE: standard error
Within each population, values followed by different letters are significantly different at P = 0.05 by the Tukey–Kramer test.

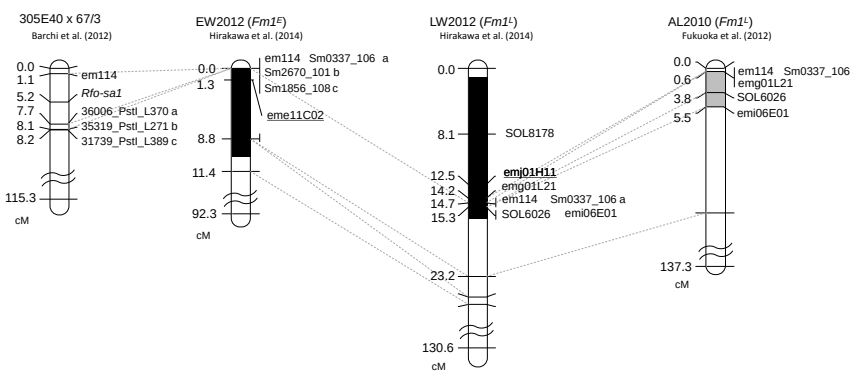

Figure 2
Comparison of the positions of Fusarium resistance loci on chromosome 2. Common markers are connected with dotted lines. Black bars show the genetic regions with an LOD score higher than threshold. Gray bars show the candidate region for FM1 detected by resistance test of ALBILs (Table 3). Markers followed by the same letter (a, b, c) are derived from the same scaffold

Table 3
Marker genotypes in the region around Fm1 and disease indices of ALBILs

Population	Marker position (cM)				No. of BILs	Disease Index Mean±SE
	em114	SOL6026	emi06E01	emh21J05		
ALF2	0.570	3.818	5.451	9.966		
LWF2	14.167	15.285	15.285	18.681		
A	■				3	2.1±2.1
B	■	■			2	100.0±0.0
C	■	■	■		5	96.2±3.8
D			■	■	3	12.5±9.5
E				■	4	6.3±3.6
F					125	4.9±0.9
G	■	■	■	■	40	94.5±2.7

White boxes represent marker genotypes of homozygous for LS1934.
Black boxes represent marker genotypes of homozygous

3.3. Detailed mapping of FM1 using the ALBIL population

Using four markers located close to *Fm1*^{L}, we mapped this locus between emg01L21 and emi06E01 markers; the distance between the two markers was 4.881 cM in AL2010 and 1.118 cM in LW2012 (Table 3).

3.4. Marker development for linkage map comparison

Rfo-sa1 was previously mapped at the end of chromosome 2 (Fig. 2; Barchi et al. 2010; 2012, Portis et al. 2014). To compare the position of *Rfo-sa1* with those of *Fm1*^{L} and *Fm1*^{E}, we developed three markers, that were derived from the original sequences of RAD markers, 36006_PstI_L370, 35319_PstI_L271 and 31739_PstI_L389, respectively (Fig. 2; Barchi et al. 2010; 2012). In addition, using primer pairs for the SCAR marker, Me8SCAR2/Em5SCAR1 (Mutlu et al. 2008), we obtained a 247-bp sequence based on direct sequencing. When we used the sequence as a query in a BLASTN search against a local database of eggplant scaffolds (Hirakawa et al. 2014), one scaffold, Sme2.5_14440.1 was detected. It's entire sequence was determined by using the developed 17 pairs of primers. One of the primer pairs, Sme2.5_14440_15 detected a SNP polymorphism.

3.5. Positions of Fusarium resistance loci of different origins

Comparison of the positions of the *Fusarium* resistance genes of different origins (305E40, LS1934 and EPL-1) showed that *Rfo-sa1* was located in exactly the same position in the maps of LWF2, ALF2 and EWF2 (Fig. 2). To check whether *Fm1*^{L}, *Fm1*^{E} and *Rfo-sa1* are allelic, additional large-scale allelic tests should be carried out. Meanwhile, the SNP marker Sme2.5_14440_15 derived from a SCAR marker linked to the resistance gene of LS2436 (Mutlu et al. 2008) was mapped in the middle of chromosome 4 (data not shown). It is interesting that the resistance genes that originated in different species collected in geographically distant areas were mapped to exactly the same linkage map position and that originated in the same species collected in the same country were mapped to the distinct map position.

3.6. Candidate gene search in the corresponding region of tomato genome

Hirakawa et al. (2014) reported putative orthologous pairs of eggplant and tomato genes deduced based on reciprocal-best-hit relationship; these data show synteny between the $Fm1^L$ and $Fm1^E$ regions in the two species. For $Fm1^L$, the candidate region was narrowed down to the region between two markers emg01L21 and emi06E01. Based on the synteny map, we selected a wider chromosome 2 region as candidate for $Fm1^L$ in tomato genome. Following the ITAG gene models v. 2.3 obtained from the Sol Genomics Network (http://solgenomics.net), 775 genes were identified in the candidate region; a search with the keywords "resistance" and "LRR (leucine-rich-repeat)", retrieved 25 genes.

4. Conclusion

The importance of this work are that it provided detailed mapping positions of the resistance loci from cultivated eggplant, and the developed markers could be easily used for eggplant breeding without any worries caused by wild relative genes. While, more important thing for the future is that the candidate *Fm1* locus was limited to the very narrow range and the tomato candidate genes were identified based on the genomic synteny. These findings could be used for high-performance forward and reverse genetic approach for efficient cloning of responsible sequence, and the expected results will act as a stepping stone for accumulating potential QTLs even if they are located near each other.

5. Acknowledgements

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P5-12

Two non-necrotic disease resistance types distinctly affect expression of key pathogenic determinants of *Xanthomonas euvesicatoria* in pepper

Dániel Krüzselyi[1], Zoltán Bozsó[1], Gábor Csilléry[2], János Szarka[3], Gábor F. Bognár[1], Péter G. Ott[1]

[1] *MTA ATK NÖVI, Budapest, Hungary*

[2] *Budakert Ltd, Budapest, Hungary*

[3] *Pirospaprika Ltd, Budapest, Hungary*

ott.peter@agrar.mta.hu

Abstract

gds is a recessive, monogenic and non-necrotic resistance trait of pepper that suppresses spot disease symptoms by *Xanthomonas euvesicatoria* bacterium (Xe) and has been used in a Hungarian commercial variety since 2003. An ancient disease resistance form, called pattern-triggered immunity (PTI) operates also in the absence of cell death. We set out to compare the transcription of key pathogenicity and stress response genes of Xe to these two seemingly similar resistance mechanisms in near isogenic pepper lines (with or without homozygous *gds*). Using a real-time quantitative PCR approach, we found that expression of an oxidative stress-response gene, *dpsA*, reflects the population growth pattern, suggesting a synergism between *gds* and PTI in inhibiting the bacteria. Transcription of structural genes of type III secretion system (T3SS), a protein complex essential for pathogenesis, was influenced oppositely by the two resistances, i.e. stimulation by *gds* and suppression by PTI. The same trend of influence was revealed on the expression of some T3SS controllers, but with a sensitivity strongly depending on the respective regulator. In summary, the antibacterial mechanism exerted by *gds* and PTI are at least partially different, as seen from the pathogen's point of view.

1. Introduction

Although resistance is the best way to protect plants from bacterial diseases, little is known about effector mechanisms that are deployed against these pathogens. General Defense System, attributable to the recessive *gds* gene, is a disease resistance form found in pepper around 20 years ago [1] and has been incorporated into a Hungarian bell pepper variety. The *gds* trait in fresh produce is desirable in that it resists disease in the absence of the hypersensitive reaction (HR). *gds* shares several characteristics with the well-known and ancient form of defense, pattern-triggered immunity (PTI [2]), for example it obviates cell death [3].

The usefulness of a disease resistance correlates with its ability to inhibit pathogen proliferation. We reasoned that plant resistance effectors may imprint a characteristic signature, e.g. of gene expression, within the pathogenic organism they interact with.

To explore this notion, the expression of several pathogenicity genes of the type III secretion system (T3SS) and some stress response genes were chosen for the following reasons: Proper

functioning of T3SS is critical for successful pathogenesis, because it implements translocation of important virulence factors called T3SS effector proteins, directly to the host cytoplasm. Previous studies have indicated that PTI has an impact on T3SS gene activation [4]. Recent papers also showed that PTI is able to inhibit T3SS effector translocation via an unknown mechanism [5].

2. Materials and Methods

We recorded bacterial growth and gene expression signatures *in planta* to compare the said resistance mechanisms.

Plants: double haploid, near isogenic bell pepper lines without (susceptible) and with the *gds* trait. Plants were raised in a glasshouse, and 2-2.5 month-old plants were put under controlled conditions (25 °C, high humidity, 16 h daily illumination) 1-2 days before treatments.

Bacteria, bacterial treatments: We chose the pathogenic bacterium *Xanthomonas euvesicatoria* (Xe) as the target of PTI and/or *gds* of pepper. It is a Hungarian isolate whose identity had previously been proved by PCR according to [6]. This strain was inoculated into leaves at 10^8 cells $cm^{3\,-1}$. Heat-killed cells of another plant pathogen, *Pseudomonas syringae* pv. *syringae* *hrcC* mutant was used at 10^8 cells $cm^{3\,-1}$ to activate PTI as a pre-treatment before Xe challenge.

Sampling: Leaf disks were taken at three time points, 0, 6 and 24 hours post inoculation (hpi), and either immediately frozen by liquid nitrogen for RNA isolation or ground in a buffer to estimate bacterial numbers as colony-forming units on agar plates. There were at least three biological replicates.

RNA processing: Leaf disks (100 mg) were ground in liquid nitrogen and total RNA isolated with RNeasy® Protect Bacteria Mini Kit (QIAGEN) and Total Plant RNA Mini Kit (GeneAid). Samples were additionally treated with DNAse (Ambion). cDNA synthesis was then performed using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems).

Quantitative PCR assays were used to measure gene expression. Target sequences for primer design (Primer3 4.0 and Oligo 6.31 softwares) were taken from *Xanthomonas campestris* pv. *vesicatoria* complete genome, GenBank: AM039952.1. For assessment of relative gene expression, 4 reference genes were used after thorough testing *in planta*. qPCR reactions were performed in a DNA Engine Opticon 2 Real-Time Cycler (MJ Research), with SensiFAST SYBR No-ROX Kit (Bioline). Expression data were maintained and processed with the softwares MySQL and Python.

3. Results and Discussion

3.1. Population studies, expression of stress-response genes:

Pathogenic bacterial survival within a resistant plant is a summation, reflecting multiple host factors acting in a complex way.

Xe grew in *gds* plants at a slower pace than in susceptible plants after 6 hpi. PTI was able to literally decrease bacterial numbers before 6 hpi, later some slow growth commenced. Of note, the lowest cell counts came from *gds* plus PTI treatments, and generally, *gds* and PTI appeared to have a synergistic effect.

The simplest interpretation would be that *gds* and PTI use at least partially different mechanisms to inhibit bacteria.

Expression of *dpsA*, a gene coding for a DNA-binding stress-responsive protein, appeared linked to the growth pattern, highlighting the only period when general stress on the bacteria was relieved (*dpsA* expression was lowest): in susceptible plant between 6 and 24 hpi, when even the stress exerted by growth medium and/or inoculum preparation was over and the pathogen started to thrive in its natural "medium" -- the intracellular space within pepper leaves. We could not detect appreciable activation of water stress-related genes.

These results implicate oxidative stress as the dominating inhibitory factor perceived - and seemingly mastered - by the pathogen.

3.2. Expression of T3SS regulators:

The T3SS system is controlled by both internal cues, e.g. quorum signal, cyclic di-GMP, and external, e.g. host cues. In *Xanthomonas campestris*, RpfC, RpfG and Clp regulators are more directly related to internal signals, while the HrpG-HrpX signalling axis responds more to the host environment, but the two pathways are not independent [7].

We found that *hrpG* and *hrpX* transcription responded to our treatments more characteristically than that of *rpfC, rpfG* or *clp*. Both *hrpG* and *hrpX* had a low basal expression level and were strongly induced within the plant. However, compared to the susceptible host, the *gds* trait stimulated and PTI only marginally affected *hrpG* (coding for a plant sensor). On the other hand, *hrpX* transcription was suppressed by PTI while *gds* had no influence.

Thus, expression of T3SS regulator genes responded to *gds* and PTI with different sensitivity.

3.3. Expression of T3SS structural genes (Hrp pilus) and T3SS effectors:

The T3SS machinery is a protein complex called Hrp pilus, consisting of a base that anchors in both inner and outer membranes, and a long hollow filament that can reach the host plasma membrane by growing through the plant cell wall. Effector proteins pass through the filament hole in an unfolded conformation.

Expression of genes coding for structural T3SS components, such as HcrU, HrcC, HrpE and HrpF, answered uniformly to the different *in planta* conditions: no transcription in culture medium, early and strong induction by any (susceptible/PTI/*gds*) host tissue, but reaching lower level in PTI and higher level in *gds*, relative to the susceptible plant tissue. The high demand for the filament monomer, HrpE, was also reflected by the data.

The *gds* trait also increased transcription of a "general" effector gene (PCR primers were designed to amplify a conserved motif of some effectors), but intriguingly, here was also a high basal level, a pattern resembling that of *hrpG*.

Thus, cues from PTI and *gds*, with opposite effect, leave a common, homogeneous signature in transcription of all examined T3SS structural genes.

4. Conclusion

We conclude that, despite their similarities, *gds* and PTI may culminate in a different antibacterial mechanism. PTI is inhibitory to *hrp* gene activation, but the question remains

whether this is enough to explain a block in effector translocation or some other virulence function. Even more intriguing is the fact that *gds* upregulates *hrp* gene transcription and studies are underway to see a wider picture that is suitable to explain the apparent discrepancy.

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P5-13

Genome-wide association mapping in eggplant

E. Portis[1], F. Cericola[1,2], L. Barchi[1], L. Toppino[2], N. Acciarri[3], L. Pulcini[3], T. Sala[2], S. Lanteri[1], G. L. Rotino[2]

[1] *DISAFA Plant Genetics and Breeding, University of Torino, Grugliasco, Italy*

[2] *CREA-ORL, Research Unit for Vegetable Crops, Montanaso Lombardo, Lodi, Italy*

[3] *CREA-ORA Research Unit for Vegetable Crops, Monsampolo del Tronto, Italy*

Abstract

An eggplant association panel was SNP genotyped and phenotyped for key breeding traits relating to either fruit morphology or plant and leaf morphology. A genome-wide association (GWA) analysis allowed to undercover 194 phenotype/genotype associations, relating to 30 of the 33 measured traits. These associations involved 79 SNP loci mapping to 39 distinct chromosomal regions distributed over all eggplant chromosomes. A comparison with results from conventional linkage mapping experiment showed that GWA analysis both validated many of the known controlling loci and detected a large number of new marker/trait associations. By exploiting established syntenic relationships between eggplant chromosomes and those of tomato/pepper, orthologous regions were recognized harbouring genes influencing key agronomical traits.

1. Introduction

The genome-wide association (GWA) mapping approach represents an alternative to biparental linkage mapping for determining the genetic basis of trait variation. Both approaches rely on recombination to re-arrange the genome, and seek to establish correlations between phenotype and genotype, based on the non-random association of alleles at two or more loci, termed *linkage disequilibrium* (LD). The major advantages of GWA lie in being able to sample a much wider range of the phenotypic and genotypic variation present in many different lineages, in exploiting multiple rounds of historical recombination, and in including multiple accessions of direct relevance to crop improvement (Yu et al. 2006). The genetic basis of certain fruit and plant morphology traits has been identified in eggplant by linkage mapping based on both intra-specific (Portis et al. 2014) and inter-specific (Doganlar et al. 2002, Frary et al. 2003, 2014) populations. In a pioneering attempt to apply a GWA approach in eggplant, Ge et al. (2013) were able to identify a number of phenotype/genotype associations related to eight fruit-related traits. Here we report on the analysis of a large association panel and SNP data set to identify and position marker/trait associations related to fruit, plant and leaf morphological traits relevant for eggplant breeding.

2. Materials and methods

2.1. Plant materials, phenotyping and SNPs assay

A previously selected eggplant association panel of 191 accessions (Cericola et al. 2013, 2014), comprising a mixture of breeding lines, old varieties and landrace selections originating

from Asia and the Mediterranean Basin, was SNP genotyped and phenotyped for key breeding traits (relating to either fruit morphology or plant and leaf morphology, and listed in Table 1) at two locations (Montanaso Lombardo - ML: 45 20'N, 9 26'E and Monsampolo del Tronto - MT: 42 53'N; 13 47'E, Italy) over two years. Genomic DNA was extracted from young leaves of three plants per accessions. Each accession was genotyped at 384 SNP loci, as reported by Barchi et al. (2011), 339 of thesebeing previously genetically mapped (Barchi et al. 2012).

2.2. GWA mapping and synteny

The GWA analysis was performed using Tassel v4.0.25 software using the mixed linear model (MLM) as described by Yu et al. (2006). Synteny between tomato and eggplant chromosomal regions was investigated by a BLAST search of RADtag sequences (Barchi et al. 2011) surrounding informative SNPs against the tomato SL2.40 genome sequence (http://solgenomics.net) and aligned using the Burrows-Wheeler alignment tool.

Table 1
Codes used to identify the traits measured (the full description of the traits has been reported in the paper by Portis et al. 2015).

Trait	Code	Trait	Code
Fruit weight	*fw*	Flesh color	*flcol*
Fruit length	*fl*	Flesh green ring	*gring*
Fruit diameter 1/4	*fd1/4*	Plant growth habit	*hab*
Fruit diameter 1/2	*fd1/2*	Number of branches	*br*
Fruit diameter 3/4	*fd3/4*	Leaf width	*lw*
Fruit diameter max	*fdmax*	Leaf length	*lle*
Fruit diameter max position	*fdmaxp*	Adaxial leaf central ven. prickl.	*adlcevepri*
Fruit shape	*fs*	Adaxial leaf lateral ven. prickl.	*adllavepri*
Fruit curvature	*fcur*	Abaxial leaf central ven. prickl.	*ablcevepri*
Fruit apex shape	*fas*	Abaxial leaf lateral ven. prickl.	*abllavepri*
Peduncle length	*pedl*	Stem prickliness (scale 0-5)	*stpri*
Fruit calyx prickliness	*fcpri*	Abaxial leaf prickles number	*ablprin*
Fruit calyx removal	*fcr*	Adaxial leaf prickles number	*adlprin*
Calyx coverage	*cacov*	Leaf hairiness	*lha*
Outer fruit firmness	*outfir*	Number of flowers / inflorescence	*flwin*
Inner fruit firmness	*intfir*	Flowering time	*flwt*
Number of locules	*slon*		

3. Results and Discussion

A total of 194 significant phenotype/genotype associations were detected. Regions carrying presumed genes/QTL affecting 30 of the 33 traits (with the exception of *slon*, *hab* and *flwin*) were identified on each of the 12 chromosomes, The number of associations per trait ranged from two (*fcr*, *cacov*, *outfir*, *br*, *lha* and *flwt*) to 17 (*intfir* and *stpri*). To correlate the associations with known QTL, SNP loci separated from another by <6.8 cM (double the global estimate for LD, Cericola et al. 2014) were considered as a unit, following the method proposed by Xu et al 2013, and their genomic location was obtained from the Barchi et al. (2012) map. Overall, 39 regions were defined, involving 1-7 SNP loci each. The most prominent trait clusters were found

on chromosomes E01, E06, E07, E08 and E10 (Fig. 1). The most important regions influencing variation in fruit morphology were E01.3 (*fw, fl, fs, fcur, intfir* and the fruit diameter traits) and E10.4 (*fw, fl, fd1/4, fdmax, fs, fcur, outfir, intfir* and *flcol*). Two regions, one on chromosome E01 (E01.1) and one in the distal part of E02 (E02.3) were associated with variation for fruit diameter and *fs*. E03.2 was associated with variation for fruit diameter, *fw* and *fas*, while the distally located segment E08.2 harboured genes affecting fruit diameter and *fas* as well as *fs*. Four regions of chromosome E06 were associated with variation for prickliness (*adllavepri, abllavepri, adlcevepri, ablcevepri, stpri, ablprin* and *adlprin*), as were E07.3, E08.1 and E08.4. Genes determining *fd1/4* were also located to E07.3 and those influencing *gring* on E08.1 and E08.4.

Comparative mapping has previously exposed the high degree of synteny retained between the tomato and eggplant genomes (Portis et al. 2014). Specifically, the gene content of a genomic region in eggplant harbouring a particular set of trait/marker associations has a good chance of being replicated in the orthologous segments of tomato and pepper. The presence of regions syntenic with either tomato or pepper was here identified on ten of the 12 eggplant chromosomes. For example, the syntenic regions on E01 and T01 both harbour genes/QTL associated with fruit size, weight and shape, and a similar relationship holds between E08.2 (fruit shape and size) and T08 (Fig 2). On the other hand, synteny-based comparisons between eggplant and tomato were not informative for the genetic basis of plant and leaf morphology, as these traits (e.g., prickliness) are of no relevance to either tomato or pepper.

4. Conclusions

The main objective of this study was to elucidate the genetic basis of some key breeding traits in eggplant. The genetic variability captured by the association germplasm panel, which includes contrasting morphology for most of the traits studied here, proved to be a great source of allelic variation. Our GWA approach successfully validated a number of previously detected QTL, thereby providing the potential for applying a marker assisted selection strategy for improving some key breeders' traits. At the same time, it identified the location of a number of as yet unknown genes/QTL (fully described by Portis et al. 2015). The study has also demonstrated that a comparative genetic approach, relying on the much larger knowledge base associated with tomato, provides a useful short cut for identifying candidate genes. The sequences of such genes can readily provide the materials necessary to develop marker assisted selection assays, while also advancing the understanding of synteny in the Solanaceae.

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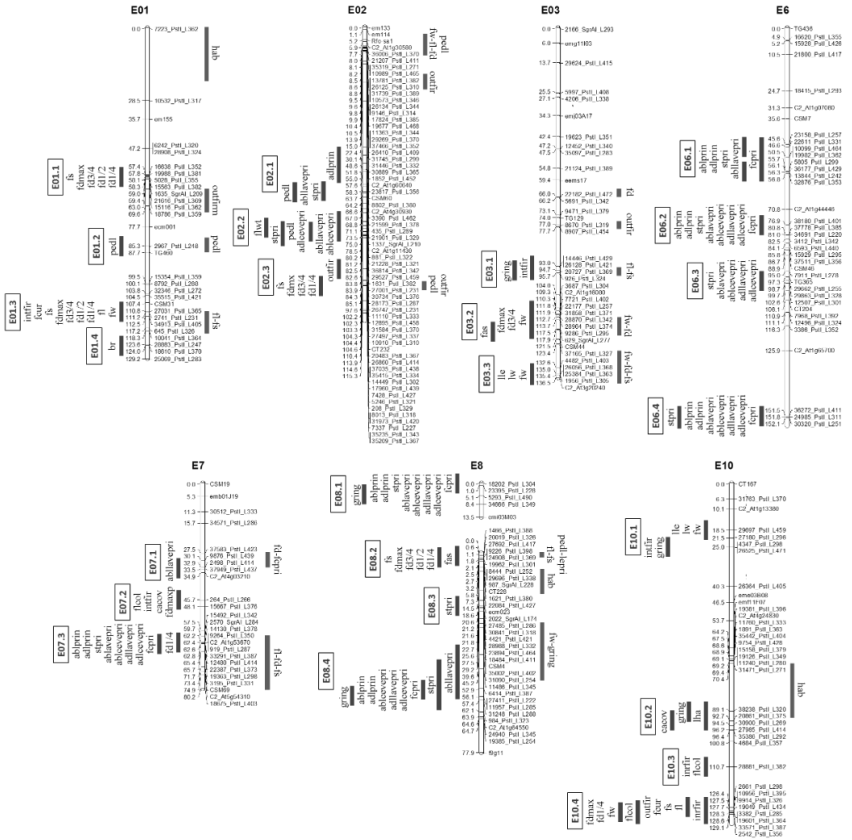

Figure 1
Regions identified by GWA (indicated on the left) in comparison to QTL locations (on the right) in seven eggplant chromosomes.

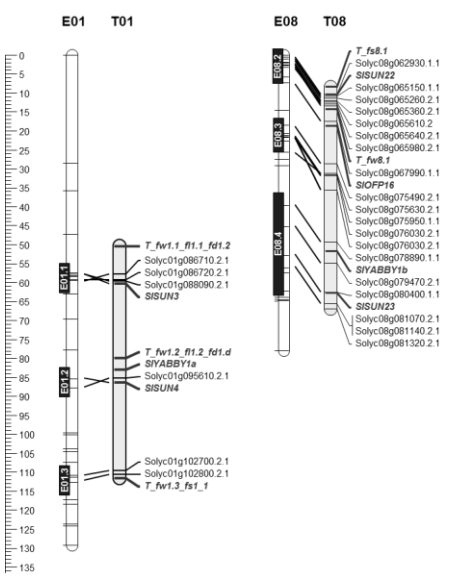

Figure 2: Synteny in the Solanaceae: eggplant chromosomes E01 and E08 are represented by white bars, and the site of QTL detected by GWA analysis is indicated; tomato chromosomes are represented on the right, along with the position of candidate genes.

P5-14

Scientific contributions to anther culture of pepper from Turkey

Hatıra Taşkin[1], Davut Keleş[2], Gökhan Baktemur[3], Saadet Büyükalaca[1]

[1]*Department of Horticulture, Faculty of Agriculture, Çukurova University, Adana, Turkey*

[2]*Alata Horticultural Research Institute, Erdemli, Mersin, Turkey*

[3]*Department of Biology, Faculty of Arts and Sciences, Osmaniye Korkut Ata University, Osmaniye, Turkey*

hatirataskin1@gmail.com

Abstract

Peppers are a commonly consumed vegetable in the world due to its high nutritional value such as vitamin C. After China and Mexico, Turkey is the third biggest pepper producing country with 2.1 million tons in 2013. Therefore, pepper breeding programs are gaining more importance. Haploid production techniques are important in vegetable breeding as they can shorten the breeding period. These techniques are species specific and promising results for pepper have been obtained from anther culture method. The findings obtained from anther culture studies to date in Turkey can be summarized as follows: The late uninucleate or early binucleate phase (the beginning of the first mitotic division) was found to be the most proper anther stage. At this step, the length of the corolla of flower buds should be equal to that of the calyx or slightly longer, and almost half of the anthers have anthocyanin. While the embryo formation frequency were calculated as 0.05-0.1% in 1980s in Turkey, this ratio has been increased to 66.36% using different nutrient media, different grow regulators, different seasons experiments in the recent years. In the studies carried out to determine the most effective nutrient media, different nutrient media combinations and grow regulators were tested and the positive effects of 6-benzylaminopurine (BAP) and naphthaleneacetic acid (NAA) in the majority of studies were reported. Likewise, the positive effects of silver nitrate ($AgNO_3$) and activated charcoal were determined in the studies carried out in 2000s. The effects of donor plant growing conditions on anther culture were evaluated and more embryos were obtained from the plants grown in greenhouse than open field conditions. Positive results were obtained from pre-treatment studies such as incubation of flower buds or anthers in hot or cold conditions. The studies for determining these conditions, incubation at 4°C and 35°C in the dark for 2 days were found to be the most suitable temperature degrees for cold and hot temperature conditions, respectively. The effects of different seasons on anther culture were tested and the anthers cultured in April, May and August produced the best results compared to the other periods in Mediterranean region of Turkey. Spontaneous doubled haploidy rate varied between 8.3-61.7% depending on the genotypes and different pepper types.

Keywords: *Capsicum annuum*, pepper, anther culture, Turkey

Anther culture studies of pepper in Turkey were initiated by Abak. Abak (1983a) investigated the effects of pre-treatments, growth regulators added to nutrient medium and proper microspore stage for anther culture on pepper. The successful results were obtained from the nutrient supplemented 5 mg L^{-1} kinetin, 5 mg L-1 2,4-Dichlorophenoxyacetic acid (2,4-D), 120 g L^{-1} sucrose, 37.3 mg L^{-1} Na_2EDTA+27.8 mg L^{-1} $FeSO_47H_2O$. The flower buds had 3.5-4

mm sizes were found to be the best proper stage for green long local pepper materials. As pre-treatment, the waiting of anthers at 25°C and 35°C did not show any positive effects.

Abak (1983b) reported that the number of embryo increased with increasing iron and sucrose doses (simple: 18.65 mg L^{-1} Na_2EDTA+13.90 mg L^{-1} $FeSO_47H_2O$, double: 37.30 mg L^{-1} Na_2EDTA+27.80 mg L^{-1} $FeSO_47H_2O$; 30 g L^{-1} simple and double iron and 60 g L^{-1} sucrose simple and double iron: 0 embryo; 90 g L^{-1} simple iron: 4.30 embryos per 100 anthers; 90 g L^{-1} double iron: 16.58 embryos per 100 anthers; 120 g L^{-1} simple iron: 9.22 embryos per 100 anthers; 120 g L^{-1} double iron: 18.58 embryos per 100 anthers).

Abak (1986) reported that the percentage of parthenogenetic haploid embryo formation was too low (It varied according to genotype usually 0.05%, in some genotypes increased to 0.1%). Also, the researcher noted that the growth conditions of donor plants affected the embryo regeneration. The number of haploid embryo was found to be high with average 5-10 haploid embryos per 100 anthers.

In a study conducted by Terzioğlu et al. (2000), the effects of different incubation conditions (first waiting at 35°C under dark photoperiod condition for 8 days and then transferring to 25°C-16/8 light/dark photoperiod condition, waiting at 29°C under continuous light photoperiod condition) and different nutrient media on anther culture of Kahramanmaraş local pepper population were investigated. The positive effects of waiting at 29°C under continuous light condition and adding activated charcoal to nutrient medium were determined.

The effect of $AgNO_3$ on obtaining haploid embryo was investigated by Comlekcioglu et al. (2001). As a nutrient medium, Murashige and Skoog (1962) (MS) containing 4 mg L^{-1} NAA, 0.1 mg L^{-1} BAP, 0.25% activated charcoal, 30 g L^{-1} sucrose and the same medium added 10 mg L^{-1} $AgNO_3$ were used. Researchers determined that the number of haploid embryo increased with the addition of $AgNO_3$: in Şanlıurfa local population 51.6%, in Kahramanmaraş local population 35.7%. Also, the most proper bud stage for anther culture of pepper was found to be the length of the corolla of flower buds nearly equal to the calyx or slightly longer.

In a study conducted by Ellialtıoğlu et al. (2001), the effects of different nutrient media and incubation conditions on Kahramanmaras pepper populations were determined. C nutrient medium described by Dumas de Vaulx et al. (1981) and supplemented 5 mg L^{-1} 2,4-Dichlorophenoxyacetic acid (2,4-D), 5 mg L^{-1} kinetin and MS nutrient medium including 4 mg L^{-1} NAA, 1 mg L^{-1} BA were used. Also, 1% activated charcoal alone and together with 200 ml L^{-1} carrot extract was added to nutrient medium except control groups. In the first incubation application, cultures were waited at 35°C for 8 days, then transferred to R medium contained 0.1 mg L^{-1} kinetin and incubated at 25°C. Another incubation condition was kept at 29°C under continuous light condition. The most embryo yield was obtained from MS medium without activated charcoal and carrot extract and waiting at 29°C under continuous light condition.

The effects of different incubation conditions (at 25°C under 16/8 light/dark photoperiod condition, waiting at 25°C for 1 week and at 35°C under dark photoperiod condition for 1 week) and cold pre-treatment applications (waiting at 4°C for 24 and 48 h) on anther culture of pepper were investigated by Biner et al. (2001). The results of cold applications varied according to the genotypes; however the positive effects were recorded. Waiting at 25°C for 1 week were found to be more successful than the other applications.

In a study conducted by Ercan et al. (2001) in pepper, the effects of nutrient medium (11 different MS nutrient media contained different doses of kinetin, BA, NAA, 2,4-D and activated charcoal) on five different pepper varieties were determined. The positive results were obtained from MS medium contained 1% activated charcoal, 5 mg L^{-1} 2,4-D, 5 mg L^{-1} kinetin and 1%

activated charcoal, 4 mg L^{-1} NAA, 0.1 mg L^{-1} BA.

In pepper, the effects of cold-shock treatments (at 4°C for 48 or 96 h) and the addition of activated charcoal (0.25%) were investigated by Özkum Çiner and Tıpırdamaz (2002). The highest number of embryos was obtained from the control anthers (the mean of androgenetic embryo production: 12.5%) and the growth regulators and activated charcoal showed a greater effect than a cold-shock treatment. Also, the proper microspore stage was investigated and the flower buds 5 mm in diameter and 7 mm in length were identified as the most proper bud size due to containing microspores at the uninucleate and 1st pollen mitosis stage. At this stage, the length of corolla was about the same as or slightly greater than that of the calyx.

The effects of growing season (summer and winter) and the age of donor plants (montly) on anther culture of Kekova and Sera-Demre 8 varieties were determined by Ayar (2003) and Ercan et al. (2006). As a result of the research, while Kekova variety with 4.97% gave the better results than Sera-Demre 8 variety (1.49%) in the summer season, the opposite findings were obtained from the winter season (Sera-Demre 8: 4.26%, Kekova: 2.69%). In the summer season, the highest embryo yield was recorded in Jun with 7.3% and August with 3.96% for Kekova and Sera-Demre 8 varieties, respectively. In the winter season, February gave the highest results for the both varieties (Kekova: 7.70%, Sera-Demre 8: 8.75%).

The effects of different nutrient media contained different concentrations of auxin-cytokinin, activated charcoal and $AgN0_3$ (2.0, 4.0, 6.0 mg L^{-1} NAA; 1.0, 2.0, 3.0, 4.0 mg L^{-1} 2,4-D; 0.1, 1.0, 2.0, 3.0 mg L^{-1} BAP; 0.1, 1.0, 5.0 mg L^{-1} kinetin; 0.25% activated charcoal; 10 mg L^{-1} $AgN0_3$) and different pre-treatments (at 4°C, 29°C and 35°C for a week) on anther culture of Kahramanmaraş red peppers were investigated by Çağlar et al. (2004). The best result was obtained from MS+0.1 mg L^{-1} BAP+4 mg L^{-1} NAA+0.2% activated charcoal+10 mg L^{-1} $AgNO_3$ with 2.8%.

The effects of different doses of $AgNO_3$ (5, 10, 15 and 20 mg L^{-1}) added to nutrient medium and donor plant growing conditions (in greenhouse and in open field) on anther culture were investigated by Buyukalaca et al. (2004). The results of study showed that embryo yield increased in the donor plants grown in the greenhouse. Also, 15 mg L^{-1} was found to be the most successful AgNO3 dose.

Sayılır and Özzambak (2005) tried to determine the most proper flower bud size, nutrient medium (six different MS and N (Nitcsh and Nitsch,1969) media contained 4 mg L^{-1} NAA+ 0.1 mg L^{-1} BA, activated charcoal and carrot extract) and cold pre-treatment time application for anther culture in six different pepper varieties. The best results were obtained from MS+4 mg L^{-1} NAA+0.1 mg L^{-1} BA with both activated charcoal and carrot extract, use of one of them or none of them. MS+4 mg L^{-1} NAA+0.1 mg L^{-1} BA gave the best results in Ç. Bağcı variety. While the use of activated charcoal alone could not show any positive effect, the better results were obtained from N+4 mg L^{-1} NAA+0.1 mg L^{-1} BA+0.1% activated choarcoal+200 ml carrot extract. Also, 5-6 mm found to be the most proper flower bud size.

Ercan and Ayar Şensoy (2011) studied on the effect of genotypes on anther culture. For this aim, 11 genotypes and MS medium contained 8 g L^{-1} agar and 30 g L^{-1} sucrose were used. Before anther culture, flower buds were waited in cold (+4°C) and dark conditions for 24 h. Incubation conditions were arranged to be waiting at 35°C for 8 days and then transferred to 25°C under 16/8 dark-light photoperiod condition with 3000 lux light intensity. Embryo formation frequency was calculated as 0.33%, 3.01%, 1.30%, 0.73%, 1.97%, 0.00%, 0.00%, 7.69%, 0.35%, 1.50% and 2.26% for the genotypes of Atris, Odesa, Demre, DRH-7118, Yağlık pepper, Yalova charleston, Kandil, Demre sivrisi, Sirena, Kekova and Sera-Demre 8, respectively.

In an anther culture study in pepper carried out by Taşkin et al. (2011), five pepper genotypes, four different culture media and different culture periods (from April to January) were tested. Also, the embryos that were unable to complete their growth in the culture medium studied were placed in a medium containing 0.5 mg L^{-1} abscisic acid for 10 days. The highest yield of embryos was obtained from A269, one of the genotypes tolerant to low temperature. The anthers cultured from April to May, gave the highest yields of embryos compared to anthers from the other periods. Most of the embryos were obtained from Medium III and the Medium IV. There was no positive effect of abscisic acid on the mature embryos.

The effects of different genotypes, different BAP doses (0.1 and 1 mg L^{-1}) added to nutrient medium and different culture periods on anther culture of pepper were studied by Ata (2011). The highest embryo yield was recorded in April and August. The best results were obtained from MS+30 g L^{-1} sucrose+0.25% activated charcoal+15 mg L^{-1} $AgNO_3$+4 mg L^{-1} NAA+0.1 mg L^{-1} BAP in November, December, January, April and September; MS+30 g L^{-1} sucrose+0.25% activated charcoal+15 mg L^{-1} $AgNO_3$+4 mg L^{-1} NAA+0.5 mg L^{-1} BAP in October, March, May, July, August, respectively. The highest plant regeneration observed in April. The percentage of embryo formation increased to over 60% in the some genotypes and nutrient media. In addition, the anthers taken on 1^{st}, 2^{nd}, 3^{rd},4^{th}, 8^{th}, and 14^{th} days of February, May, August and October fixed with Carnoy's solution and the development of microspores were examined staining with 4'-6-diamidino-2-phenylindol-2HCl (DAPI) and acetocarmine.

Three different pepper genotypes and one pepper variety, 16 different nutrient media were tested to determine the effects of nutrient medium and genotype on anther culture of pepper by Alremi et al. (2014). For Alfajer variety, no embryos could be obtained from the B series nutrient media. Embryo and plants formation frequency for C series varied between 0-11.24% and 1.12-8.99%, respectively. The highest values were obtained from C5 nutrient medium with 11.24% of embryo formation (8.99% plant formation). In the genotype B, the highest embryo formation frequency was obtained from C1 medium with 8.9% and 7.70% of these converted to plants. Embryo formation in the genotype 171 was observed only in B2 and B8 nutrient media (1.11 and 1.90%). In genotype 151, embryo formation varied between 0-3.33%. B5 nutrient medium without $AgNO_3$ gave the highest results in terms of embryo number and this medium was followed by C6, B3, B2, B7 nutrient media. All of the obtained embryos (except embryos obtained from B3 medium) converted to plants. In terms of genotypes, Alfajer variety and genotype B gave more successful results than genotypes 151 and 171. It was found that 94% of plants had haploid chromosome number.

In a study carried out by Keleş et al. (2015), the rates of spontaneous doubled haploidy of different pepper types (charleston, bell, capia and green) were compared. Different spontaneous doubled haploidy rates were obtained from different types and the highest rate was recorded in bell pepper types with 53.4%.

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P5-15

Comparison of wild type and resistance-breaking isolates of *Tomato spotted wilt virus* and searching for resistance on pepper

Asztéria Almási[1], Gábor Csilléry[2], Katalin Salánki[1], Katalin Nemes[1], László Palkovics[3], István Tóbiás[1]

[1] *Plant Protection Institute, Centre for Agricultural Research, HAS, Budapest, Hungary*

[2] *Budakert Ltd, Budapest, Hungary*

[3] *Corvinus University of Budapest, Department of Plant Pathology, Budapest, Hungary*

Abstract

In Hungary resurgence of *Tomato spotted wilt virus* (TSWV) frequently causes heavy crop losses in pepper production since the mid nineties. Management of TSWV control was first directed against the thrips (using different insecticides or plastic traps), and against weeds as host plants of the virus and the thrips. Later on *Tsw* resistant gene was introduced into different types of pepper. In 2010 and 2011 sporadically, but in 2012 more frequently a resistance breaking strain of TSWV on resistant pepper cultivars was observed in the Szentes region (South-East Hungary). The presence of a new resistance breaking strain was demonstrated by virological (test-plant, serological and RT-PCR) methods.

Previously, the non-structural protein (NSs) encoded by small RNA (S RNA) of TSWV was verified as the avirulence factor for *Tsw* resistance, therefore we analyzed the S RNA of the Hungarian RB and wild type (WT) isolates and compared to previously analyzed TSWV strains with RB properties from different geographical origins. Phylogenetic analysis demonstrated that the different RB strains had the closest relationship with the local WT isolates and there is no conserved mutation present in all the NSs genes of RB isolates from different geographical origins. According to these results, we concluded that the RB isolates evolved separately in geographic point of view, and also according to the RB mechanism.

In order to find new genetic sources of resistance in *Capsicum* species 89 lines from *Capsicum annuum, C. chinense, C. frutescens, C. chacoense, C. baccatum var. baccatum, C. baccatum var. pendulum* and *C. praetermissum* were tested with Hungarian TSWV-RB isolate.

1. Introduction

Tomato spotted wilt virus (TSWV) is the type member of the genus Tospovirus (family Bunyaviridae), causes an important disease of horticultural and agronomic crops. The virus distributed worldwide is having extremly broad host range and is now considered as one of the ten most economically destructive plant viruses (Tomlinson 1987). TSWV is transmitted by thrips in a persistant manner. The virion varies in size from 80 to 120 nm and has spherical enveloped character. The genome of TSWV consists of three ssRNA segments: small (S) and medium (M) RNAs have ambisense coding strategies, whereas the large (L) RNA is of negative polarity. In Hungary TSWV was described in 1972, but the virus was not considered as an

important pathogen. In 1995 very severe damage of TSWV infection was observed in tomato and pepper production in the Szentes vegetable growing region (Hungary). The introduction and spread of western flower thrips (*Frankliniella occidentalis*), an efficient TSWV vector, in that time certainly played an important role in TSWV emergence Gáborjányi et al. 1995).

Management of TSWV control was first directed against the thrips using different insecticides or plastic traps, and against weeds as host plants of the virus and the thrips. Later on *Tsw* resistance gene (Black et al.1996) was introduced into different types of pepper (conical white, long pale green hot and sweet, tomato shape, spice pepper and blocky types) (Csilléry unpublished). Pepper cultivars carrying *Tsw* resistance gene upon TSWV inoculation show necrotic local lesions on the leaves or other parts of the plant without systemic infection. In 2010 and 2011 sporadically, but in 2012 more frequently systemic virus symptoms were observed on resistant pepper cultivars in Szentes region (Bese et al 2012, Csilléry et al 2012, Salamon et al 2010). The presence of new resistance breaking strain of TSWV was proved by virological (test-plant, serological and RTPCR) methods. It was demonstrated that TSWV can adapt very rapidly to plant resistance, and the *Tsw* resistance gene was broken down only a few years after its deployment in pepper crops (Margaria et al 2004, Roggero et al 2002, Sharman and Persley 2006).©

2. Materials and methods

Virus isolates. TSWV isolates originated from pepper cultivars susceptible and resistant against TSWV from Szentes region (South-east Hungary). Fruit samples were collected from plants exhibiting typical symptoms of virus infection such as stunting, mosaic, chlorotic and/or necrotic spots, rings and distortion on the leaves and fruits. The isolates were used for ELISA serological tests, RT-PCR and maintained by mechanical inoculation on *Nicotiana tabacum* cv. Xanthi-nc test plants.

2.1. RNA extraction

*RT-PCR.*Total RNA was extracted from leaves of *N. tabacum* cv. Xanthi-nc plants systemically infected by TSWV or from infected pepper fruits using the Spectrum Plant Total RNA Kit (Sigma) following the manufacturer's instructions. RT-PCR reactions for synthesis of first-strand cDNA were performed with Revert Aid H Minus First Strand cDNA Synthesis Kit (Thermo Science). Specific primers amplified 1720 bp fragment of N gene and the non-coding genomregions. PCR reaction was performed in 25µl – 50 µl final volume. PCR products were electrophoresed in 1% agarose and stained with ethidium bromide.

2.2. Phylogenetic and sequence analysis

The nucleotide homology of the Hungarian and other TSWV strains retrieved from the GenBank was analyzed/examined by the BLAST program of NCBI. The nucleotide and deduced amino acid sequenceswere aligned by the ClustalW algorithm of the MEGA 6.06 program. Phylogenetic trees were composed by the Neighbor-Joining method with 1,000 bootstrap replications (MEGA 6.06 program) with the entire viral proteins. The *Groundnut ringspot virus* (GRSV) were incorporated into the phylogenetic trees as outgroup.

In the resistance test to TSWV-RB strain 89 Capsicum items *[Capsicum annuum* (8), *C. chinense* (50), *C. frutescens* (8), *C. chacoense* (2), *C. baccatum var. baccatum* (4), *C. baccatum var. pendulum* (11) és *C. praetermissum* (6)*]* were inoculated at cotyledos stage with TSWV-RB strain.

3. Results

3.1. Virus isolation and pathological characterisation

Fruit samples with typical TSWV symptoms showing positive reaction only to TSWV and negative result to all other examined viruses (TMV, CMV, and PVY) in the DAS-ELISA (data not shown) were selected for further characterization. TSWV isolates were tested on TSWV-susceptible pepper cultivars (‘Carma’, ‘Century’, ‘Dimentio’, ‘Skytia’), and pepper cultivars carrying *Tsw* resistance gene (‘Celtic’, ‘Censor’, ‘Karakter’, ‘Brendon’, ‘Bronson’, ‘Bravia’). TSWV isolates causing necrotic local lesions (HR) on resistant papper cultivars belonged to wild type (TSWV-WT) strain, and isolates causing systemic symptoms (chlorotic mosaic and ringspot pattern on the leaves, stunting) on all pepper cultivars belonged to resistance breaking (TSWV-RB) strain.

Tree TSWV isolates were selected (HUP1-2012-RB, HUP2-2012-RB and HUP4-2012-WT) for further study. All the three virus isolates induced systemic symptoms (chlorotic or necrotic ringspot) on the inoculated leaves of N. tabacum cv. Xanthi-nc plants.

Sequence similarities of the NSs genes were compared to the sequences of WT and RB isolates, originated from pepper from distinct geographical locations. Nucleotide sequence identity among the Hungarian isolates was 99 %, while compared to other isolates this value varied between 95 and 99 %.

Amino acid (aa) sequences of the NSs protein (467 aa) were compared among the WT and RB isolates several mutations/changes were present only in the three Hungarian isolates at positions 122 (A to D), 137 (T to K), 174 (M to T), 450 (G to R), and 459 (P to S). The Hungarian RB isolates (HUP1-2012-RB, HUP2-2012-RB) had two aa substitutions compared to the WT Hungarian isolate (HUP4-2012-WT) at positions 104 and 461 (A instead of T). Substitution at position 104 has occurred only in case of the Hungarian RB isolates.Phylogenetic tree was constructed based on the deduced amino acid sequences of the NSs genes of the Hungarian and the selected isolates from the GenBank (Figs. 1).

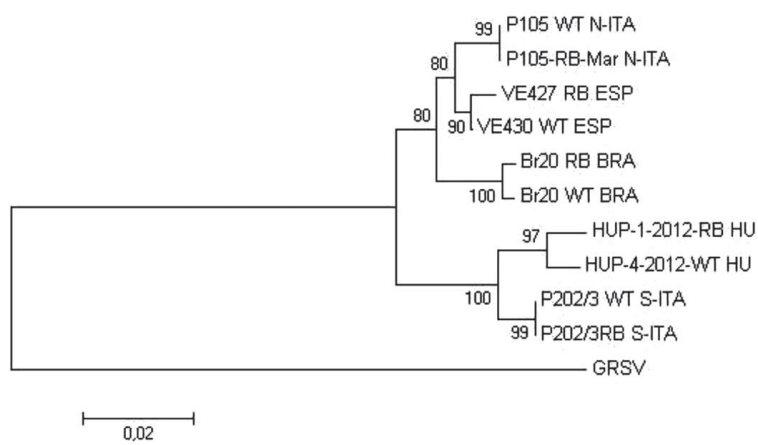

Figure 1
Phylogenetic tree based on the deduced amino acid sequences of the NSs protein of TSWV.

One of the two main clusters consists of Spanish, the northern Italian, and the two Brazil strains (further divided into different subgroups) regardless of the strain type, i.e., RB or WT. The other main branch contains the Hungarian and Italian strains from Sicily. The phylogenetic analysis supported the hypothesis that TSWV RB strains has been developed locally, and the worldwide trade and transport of plant propagating material seem not to contribute to the expansion of RB strains.

Searching for resistance to TSWV-RB strain was carried out testing 89 Capsicum items *[Capsicum annuum* (8), *C. chinense* (50), *C. frutescens* (8), *C. chacoense* (2), *C. baccatum var. baccatum* (4), *C. baccatum var. pendulum* (11) és *C. praetermissum* (6)*]* 85 items were susceptible and 4 *C. baccatum var. pendulum* items showed HR-like symptoms (Fig.2). Further study is necessary to clear the genetic background and the possibility to use these items in resistance breeding.

Figure 2
HR-like symptoms on C. baccatum var. pendulum items after inoculation with TSWV-RB strain.

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P5-16

Identification of QTL associated to biochemical components and fruit quality traits in eggplant

L. Toppino[1], L. Barchi[2], G. Mennella[3], R. Lo Scalzo[4], G. Francese[3], A. D'Alessandro[3], M. Fibiani[4], V. Papa[4], E. Palazzolo[5], V. A. Laudicina[5], L. Sabatino[5], T. Sala[1], E. Portis[2], S. Lanteri[2], G. L. Rotino[1]

[1] *CREA-ORL, Unità di ricerca per l'orticoltura, Montanaso Lombardo, Italy*

[2] *DISAFA Plant Genetics and Breeding, University of Torino, Grugliasco, Torino, Italy*

[3] *CREA-ORT, Centro di ricerca per l'orticoltura, Pontecagnano-Faiano, Italy*

[4] *CREA-IAA, Unità di ricerca per i processi dell'industria agroalimentare, Milano, Italy*

[5] *Dipartimento di Scienze Agrarie e Forestali, Università degli Studi di Palermo, Palermo, Italy*

Abstract

Here we report on the identification of some QTL for the fruit metabolic content in an F2 intraspecific mapping population of 156 individuals, obtained by crossing the same population previously used for the development of a RAD-tag based linkage map and the identification of QTLs associated to morphological, agronomical and physiological traits. The mapping population was biochemically characterized for both fruit basic qualitative data, like dry matter, soluble solid content, sugars and organic acids, as well as for health-related compounds such chlorogenic acid, (the main flesh monomeric phenol), the two peel anthocyanins [i.e. delphinidin-3-rutinoside (D3R) and delphinidin-3-(*p*-coumaroylrutinoside)-5-glucoside (Nasunin)] and the two main steroidal glycoalkaloids, Solasonine and Solamargine. For most of the traits, one major QTL (PVE ≥ 10%) was spotted and putative orthologies with other Solanaceae crops discussed. The present results supply valuable information to eggplant breeders on the inheritance of key fruit quality traits, thus providing potential tools to assist future breeding programs.

1. Introduction

Eggplant (*Solanum melongena* L.) berries are a source of health-promoting metabolites including antioxidant and nutraceutical compounds, mainly anthocyanins and chlorogenic acid with anti-diabetic, hypotensive, cardioprotective, and hepato-protective properties. Soluble solid content and dry matter, together with sugars and acids, are also key components of the fruit quality as they determine the nutritional values as well as organoleptic properties. On the other hand, eggplant fruits are also characterized by the presence of some anti-nutritional compounds such as saponins and steroidal glycoalkaloids (*SGA*), responsible of the fruit bitter taste and with potential toxic effect on humans. Up to now, Quantitative Trait Loci (QTL) for the metabolic content are far from being characterized in eggplant, thus hampering the application of breeding programs aimed at improving its fruit quality. Inter-specific genetic maps (Wu et al., 2009; Gramazio et al., 2014) were developed and employed to spot QTL

underpinning several morphological traits (Frary et al., 2014) and genes involved in the chlorogenic acid biosynthetic pathway (Gramazio et al., 2014).

Further genetic maps were also obtained from intra-specific crosses (Barchi et al., 2012; Fukuoka et al., 2012) The inter-specific mapping population developed by Fukuoka was the basis for mapping two QTL underpinning parthenocarpy and resistance to *Fusarium oxysporum* (Miyatake et al., 2012 and 2015). Information about position of the genomic region involved in these biochemical and qualitative fruit traits are rather limited. Here, an F_2 intraspecific population of 156 individuals, obtained by crossing the eggplant (*Solanum melongena* L.) breeding lines '305E40' and '67/3', and previously used for developing a RAD-tag-based intraspecific linkage map and the localization of QTL related to key eggplant morphological, agronomical and physiological traits (Barchi et al., 2012; Portis et al., 2014; Toppino et al., 2016a, b), was employed to identify genomic regions underlying simple sugars, soluble solid content (*SSC*), organic acids, fatty acids, chlorogenic acid (*CGA*), anthocyanins and glycoalkaloids accumulation together with the fruit skin color. QTL for the considered traits were identified, located in the genetic map, and syntenic relationships with other Solanaceae species highlighted.

2. Materials and methods

The parental lines '305E40' and '67/3', the hybrid and 8 replicates of each F_2 individual of the mapping population were grown at two sites [ML-Montanaso Lombardo (LO) and MT-Monsampolo del Tronto (AP)] and characterized for several agronomic traits. Samplings for biochemical analyses were carried out at two fruit ripening stages: commercial and unripe. The former [stage B, approximately 38 DAF (days after flowering)] was used for anthocyanins analysis after the phenotypical evaluation, the latter (stage A, approximately 21 DAF) was used for all the remaining biochemical characterization as, in a preliminary study, it was the stage in which the parental lines showed the highest significant differences for the majority of compounds in study. Phenotypical evaluation of the fruit skin color (*frucol*, *undcal* and *pncc*), as well as sampling, extraction and analysis of all biochemical compounds were performed as described in Toppino et al. (2016). PCA analysis was performed using PAST software. DNA extraction, molecular analysis of the mapping population, statistical analysis and QTL detection were performed according to Barchi et al. (2012). The syntenic regions of the available tomato genome sequence (http://solgenomics.net/organism/Solanumlycopersicum/genome) were investigated for identifying candidate genes and transcription factors co-localizing with the eggplant identified QTL.

3. Results and Discussion

The parental lines differ each other for most of the traits. In fact, '305E40' produces dark purple fruits *(Frucol*) with the same peel color under (*Undcal*) and next (*Pncc*) to the calyx, contains exclusively the D3R pigment and has a greater organic acids content than '67/3'. The latter is characterized by a violet colour given by the exclusive presence of the Nasunin pigment, and absence of pigmentation under calyx, together with a higher amount of sugars and *CGA*. At both sites, the F_1 hybrid was intermediate for the fruit colour, *SSC*, *Solasonine* and *CGA.* For several traits, F_1 performance was significantly superior to the better performing parent, as for *Undcal* and sugar contents (both environments); on the contrary, as an example, *Citric* and *Quinic Acid* (*CA- QI*) in MT and *Dry Matter* (*DM)* in ML contents were lower to the worst performing parent.

3.1. PCA analysis

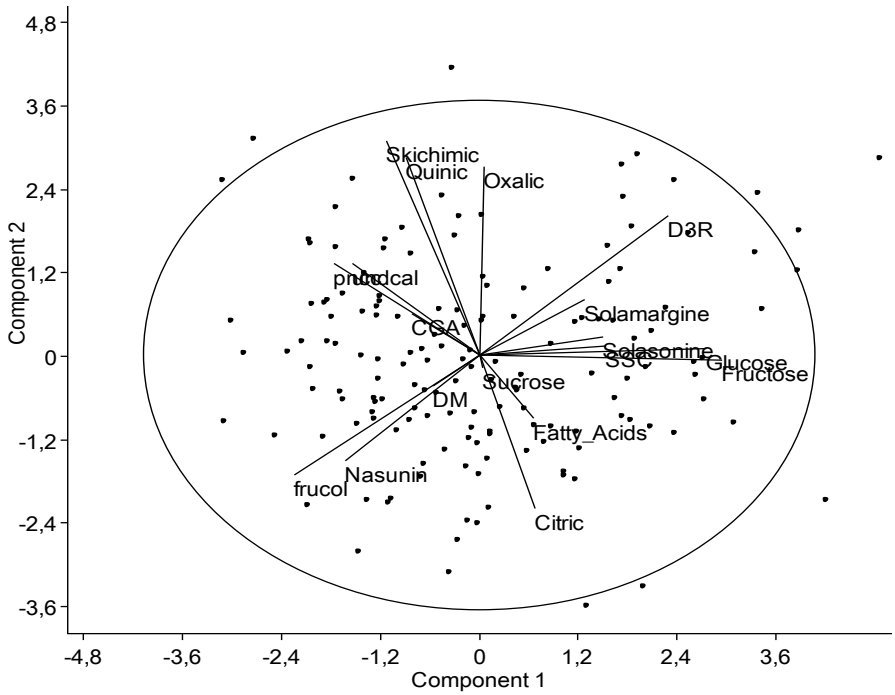

Figure 1
Plot of the distribution of traits and F2 progeny according to the first two PCA components

The first 3 components accounted for 40% of the total variance: considering the correlation coefficients of each trait with respect to each component, it can be observed that:

- 1st component (17%Var) is related to all the traits for sugars, colors, anthocyanins, *CGA* and glycoalkaloids: it is positively correlated with *Glucose*, *Fructose*, *SSC*, *Solamargine*, *Solasonine* and *D3R*, while negatively correlated with *Nasunin*, *frucol*, *pncc*, *undcal*.
- 2nd component (12%Var) is positively correlated with *oxalic, quinic and shikimic acids* while negatively correlated with *citric acid, fatty acids* and *Sucrose*.
- 3rd component (11%Var) represents sugars, organic acids, colors and fatty acids: it is positively correlated with fatty acids and colors, while it is negatively correlated with *SSC*, sugars and *CGA*. All the individuals of the F2 population fitted in one single group (Fig. 1) and could be discriminated principally considering traits related to color, anthocyanins, glycoalkaloids and organic acids.

3.2. QTL identification

Table 1
List of traits and QTL detected in the mapping population. For each trait, code, name of QTL detected, position, LOD and the percentage of variation explained (PVE) are indicated

Trait	code	CH	Montanaso L.			Monsampolo T.		
			QTL	LOD	PVE	QTL	LOD	PVE
Fruit colour	*Frucol*	5	FrucolE05.ML	32.0	56.3	frucolE05.MT	40.6	69.9
		8	FrucolE08.ML	4.9	5.5			
Undercalyx colour	*Undcal*	5	UndcalE05.ML	20.6	13.8	UndcalE05.M	27.4	13.8
		10	UndcalE10.ML	58.8	77.0	UndcalE10.M	72.4	82.5
Peel next to colour	*Pncc*	10	PnccE10.ML	68.9	86.9	PnccE10.MT	58.7	82.3
Dry matter	*DM*	2	DME02.ML	4.3	11.9			
Soluble Solid Content	*SSC*	3				SSCE03.MT	6.7	14.7
		4	SSCE04.ML	3.9	10.7	SSCE04.MT	4.6	10.1
		11				SSCE11.MT	3.7	7.8
D3R	*D3R*	5	D3RE05.ML	21.9	49.7	D3RE05.MT	24.7	52.0
Nasunin	*Nas*	5	NasE05.ML	10.5	28.0	NasE05.MT	11.2	28.4
Solamargine	*SM*	6	SME06.ML	5.0	13.9			
Chlorogenic acid	*CGA*	4	CGAE04.ML	2.8	7.3	CGAE04.MT	4.1	9.5
Fructose	*Fru*	4				FruE04.MT	3.8	10.7
Glucose	*Glc*	4				GlcE04.MT	3.9	10.7
Quinic acid	*QA*	1				QAE01.MT	6.6	16.0
		9				QAE09.MT	3.8	8.9
Shikimic acid	*SA*	2	SAE02.ML	4.7	12.1			
		9	SAE09.ML	3.6	8.2			

For almost all the traits in study (Tab. 1), one major QTL (PVE ≥ 10%) was spotted in at least one location; moreover, putative orthologous QTL with other Solanaceae were also found (Toppino et al., 2016). In all, 27 QTL (21 major, explaining at least 10% of the phenotypic variance) were identified and mapped onto ten of the 12 eggplant chromosomes (no QTL were identified in E07 and E12). At ML, 13 QTL (10 major) were identified, while at MT there were 14 QTL (11 major). Seven major QTL and one minor were expressed at both sites, three major (+ 2 minor) were only detectable in ML while four (+ 3 minor) only detectable in MT. The LOD score ranged from 2.78 (*CGAE04.ML*) to 72.35 (*undcalE10.MT*), and the PVE varied from 7.3% (*CGAE04.ML*) to ~82.5% (*undcalE10.MT*). The 8 conserved QTL between the two environments (together with their high PVE) suggest that they play a key role in controlling the production of several biochemical compound in eggplant. Finally, the QTL cluster on chromosome E05 and controlling *frucol, undcal, pncc, D3R* and *Nas* (responsible of the peel color), as well as the inter-trait correlation detected, suggests the presence of a putative pleiotropic locus.

4. Conclusions

QTL controlling basic qualitative traits (like SSC, simple sugars, organic acids and DM) and health-related metabolites in eggplant have been not extensively exploited for breeding purposes aimed at the improvement of the fruit quality. Molecular breeding strategies would be of great

utility for developing eggplant cultivars with improved phenolics, antioxidant and other health-related compounds, as well as with reduced amount of anti-nutritional compounds. The stability of many of the QTL here detected is promising in the context of using them for marker-assisted selection, considering that biochemical traits are known in literature to be highly influenced by the environment. The present results supply valuable information to eggplant breeders on the inheritance of important traits, and provide potential tools to assist selection.

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P5-17

Fine mapping of Anthracnose (*Colletotrichum acutatum*) resistance in pepper (*Capsicum* spp.)

Liu Yiwei, Zhang Zhenghai, Cao Yacong, Zhang Baoxi, Wang Lihao

Institute of Vegetable & Flowers, Chinese Academy of Agricultural Sciences, Beijing, China

Abstract

Pepper （*Capsicum* spp.） is an important vegetable in China. Recently pepper anthracnose, mainly causing pre- and post-harvest fruit rot, has become one of the main diseases affecting the yield and quality of pepper, which can lead to huge economic losses. Pepper anthracnose resistance is controlled by major genes and minor genes, influenced by environmental factors. Using interspecific backcross segregation BC1 populations, derived from an interspecific cross of inbred line 77013 (*C. annuum* L.) (recurrent parent susceptible to *C. acutatum*) and PBC932 (*C. chinense* Jack.) (male parent with strong resistance to *C. acutatum*), we preliminarily located the main effect QTLs of anthracnose resistance in the green and red ripe stage. The main QTLs of the two periods were located at the same region on the end of chromosome 5, they are all between InDel marker and SSR marker, and the InDel marker is nearest to the major QTL.

This research used KASPar markers to fine mapping the major anthracnose resistance genes of the interspecific backcross segregated groups BC3S1 and BC4S1 (77013×PBC932) at green ripe stage and red ripe stage based on the preliminary positioning, to further explore the molecular markers which linked to the major QTL more closely, and thus accelerate the breeding process of resistant varieties.

Anthracnose resistance evaluation was performed using the microinjection method. Mature green and red pods of BC3 (26 individuals) population and BC4 (18 individuals) population selected by InDel markers and SSR markers, were evaluated with C. acutatum. Individuals, BC3-1 and BC4-17, were selected according to their high and significant resistance. Progeny of the two individuals were used further for fine mapping. In the population, selected by InDel markers and SSR markers, 297 individuals were found exchanging between the two markers arranged the major QTL in the P5 chromosome. We used these recombination individuals for resistance identification, to discover the molecular markers more closely linked to the major genes in the green and red ripe stage by the newly developed KASPar markers and to complete the fine mapping.

Keywords: Anthracnose, *Capsicum*, Major QTL

Authors's index

E

F

G

H

I

J

K

L

M

N

O

P

R

S

T

V

W

Y

Z

The Faculty of Horticultural Science of the Szent István University is pleased to welcome you at the XVIth EUCARPIA Capsicum and Eggplant Working Group Meeting in Hungary.

Hungary is a founding member of the Capsicum and Eggplant Working Group and gets an opportunity to organize the Working Group Meeting thirdly since 1971. More internationally acknowledged leading experts accepted our invitation to participate in the work of the Scientific Committee concerning five topics as: genetic resources, molecular genetics and biotechnology, breeding strategies, growing and seed production, physiology and nutrional value. These topics may provide a good occasion for exchange of experience and useful professional discussion and debates.

We wish you a pleasant and fruitful event,

Attila Hegedűs, PhD, DSc
Dean, Faculty of Horticultural Science,
Szent István University

Katalin Ertsey-Peregi, PhD
Chair, Capsicum and Eggplant
Working Group