# FOREWORD

Starting with this issue, we have a new member on our Scientific Committee: Dr. Sergio Lanteri, who works at Turin University (DIVAPRA Plant Genetics and Breeding). In this way we trust that the review of submitted contributions will be quicker, enabling us to be more punctual in publishing the Newsletter.

Other important news concerns the Internet connection of "Capsicum and Eggplant Newsletter". **Please take note that both the Email address and the Internet Home Page have changed**. The new Email address is **capsicum@unito.it**, while the Website Home Page where you can find all the information about the Newsletter can be reached by linking to **www.capsicum.unito.it.**

Starting with the next issue, No. 22, (published summer of 2003) **it will be possible to have an on-line copy of the Newsletter**. The main advantage will be the possibility of reading the journal as soon as it is ready, saving the time requested for printing, mailing, and so on. If you want to have the next issue of the Newsletter on-line, please communicate your request to us as soon as possible.

The present issue of the journal includes an interesting invited paper. It was written by J.B. Baral and P.W. Bosland and deals with the updated synthesis of the *Capsicum* genus terminology. We thank these authors for their efforts to increase the scientific value of our publication. In addition, we would like to remind you that any suggestions on the topics and/or authors to be considered for invited papers in future issues of *Capsicum and Eggplant Newsletter* are welcomed.

As in the past, the papers have been printed as received and we have not modified the accepted contributions. Therefore, the authors, not CENL, are responsible for the scientific content of their reports.

Please remember that this Newsletter is highly dependent on the financial support of the recipients. Therefore, a subscription fee is appreciated. The subscription fee is the same as last year: 30 EURO for normal and 150 EURO for supporter subscribers. Remember that to make the payment less time-consuming and to reduce bank costs, we have introduced the option of a 3-year subscription. It is possible (and encouraged!) to order your own personal copy to hasten its delivery to you. Just fill in the order form on page 119 and send it to us, together with a copy of the payment order that must be made out to **Eucarpia**. In case you decide to pay by credit card, please use the voucher on page 121. Because **the cost to cash cheques is very high, you are kindly requested to avoid this method of payment: credit card payment is highly preferred**.

The deadline for submission of articles to be included in the next issue of the Newsletter (No. 22, 2003) is **February 28, 2003**. Please note that **contributions will be accepted only if submitted through electronic mail** (as attached file) **or on computer disk.** Suitable formats are as follows: operating system Windows 95-98-2000; word processing systems Word; floppy disk sizes 3½ inches or CD 650 Mb. **Please, note that the EUCARPIA Secretary is now in Vienna: you can find the new address and banking directions on page 3 of this volume.**

We regret to report that many papers had to be rejected because of inadequate scientific rigor. Therefore, we would like to remind everyone that submitted articles must deal with genetics and breeding of pepper or eggplant. Reports on cultural practices (fertilisation, space between plants, etc.) will not be accepted. Most of the rejected articles had poor English grammar and syntax. Please, before submitting a manuscript, have it proofed by someone capable of editing it in the English language. **It is imperative that you follow the submission instructions very carefully. Otherwise your contribution will not be accepted.**

Luciana Quagliotti
(Director)

Piero Belletti
(Editor)

Turin, 30 June 2002

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**Capsicum and Eggplant Newsletter 21 (2002): 11-21 (invited paper)**

# AN UPDATED SYNTHESIS OF THE *Capsicum* GENUS

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The history of *Capsicum* nomenclature is one of many iterations. Linneaus described two species under the genus *Capsicum,* namely *C. annuum* and *C. frutescens* in the Species Plantarum (1753) and two additional ones, *C. grossum* and *C. baccatum* in the Mantisa plantarum (1767). By the end of the 19th century, the list of binomials for *Capsicum* had reached well over 100 (Heiser, 1969). Early taxonomists were impressed with the variation in floral characters and fruit type both shape and size. Now it is accepted that the numereous fruit types (or pod types) can exist in a single *Capsicum* species (Bosland, 1994; Smith *et al.*, 1987). Pod types are intraspecefic categories, commonly used to distinguish among horticultural varieties. As taxonomists started to consider the underlying biological principle in demarcating taxa, more and more species of the genus *Capsicum* began to merge. At present, it is generally agreed that five domesticated and at least 25 wild species exist under the genus *Capsicum* (Bosland and Votava 2000; Eshbaugh, 1996). In addition, there are wild forms associated with domesticated species making the situation more complex. The generic limit for *Capsicum* is also not very definitive.

This synthesis of the *Capsicum* genus has been prepared from all major taxonomic databases. These include Gray Card Index (GCI), Index Kewensis (IK), Vascular Plants of Tropics (VasT) of Missouri Botanical Garden, and Genetic Resource Information Network (GRIN), USDA. Within this paper we have attempted to consolidate the species names for the genus *Capsicum* into a concise and readable form. The specific epithet or intraspecific taxa authored by different taxonomists have been placed together if they are synonymous or represent intraspecific taxa. Unfortunately, it was not possible to differentiate between taxonomic synonym and nomenclatural synonym. When possible, homonyms (same name given for two or more plants that belongs to different taxa) have been moved to their present taxa. Attempts have been made to associate each species or intraspecific entries with commonly accepted names. If an entry possesses a basionym (name given for the first time) it is also given in this list. These names are followed by "bsn." This list also includes the citation where the name was published.

A total of 227 binomial/trinomial (genus species/genus species variety or forma) names for *Capsicum* have been dealt within this compilation. There were 91 specific epithets, which have been either accepted or identified as synonym of an accepted name. In the following list, the number in parenthesis after each name represents the reference number for where that name was published. For instance ("1") after *C. annuum* refers in the reference to 'Species Plantarum' of Linnaeus where the name *Capsicum annuum* was published. Similarly the synonyms of a particular species or its intraspecific taxa are placed together in parenthesis. A few entries have been associated with accepted names (entries followed by "nr") for which no references are available. The basis for such association is merely similarity with the accepted name(s).

There are a few species that may no longer belong in the genus, e.g. *Capsicum geminifolium* (Dammer), *C. ciliatum* (H, B & K) Kuntze, and *C. testiculatum*

Dun. are morphologically so distinct that they may not be accommodated under the genus *Capsicum*. Our recent studies on molecular systematics suggest these species should be removed from the genus *Capsicum* (unpublished data). There are 57 names (species) that have neither been associated with any of the accepted names under the genus *Capsicum* nor been declared to belong to other genera. It is possible that some of them represent synonyms of the accepted species, some belong to the genus other than *Capsicum*, and others may represent truly new species of the genus *Capsicum*. We believe that a careful study of their type specimen could resolve part of the problem. There were 23 specific epithets that were once assigned to genus *Capsicum* but now these names have become nomenclatural synonyms for genera other than *Capsicum*.

**THE FOLLOWING SPECIES ARE CURRENTLY ACCEPTED UNDER THE GENUS *CAPSICUM*.**

***C. annuum*** L. (1) [*C. axi* Vell. (2); *C. conoideum* Mill. (3); *C. milleri* Roem. & Schult. (4); *C. olivaeforme* Mill. (3); *C. petenense* Stand. (5); *C. silvestre* Vell. (2); *C. sphaerium* Willd. (6); *C. tetragonum* Mill. (3); *C. cydoniforme* Roem. & Schult. (4); *C. tournefortii* Bess. (7)].

***C. annuum*** var. ***annuum*** [*C. annuum* var. *cerasiforme* Irish (8); *C. annuum* var. *conoides* Irish (8) bsn. *C. conoides* Mill. (3); *C. annuum* var. *fasciculatum* (Sturtev.) Irish (8) bsn. *C. fasciculatum* Sturtev.; *C. annuum* var. *globiferum* (Mey.) Voss (11) bsn. *C. globiferum* Mey. (12); *C. annuum* var. *grossum* Send.; *C. bicolor* Jacq. (13) nr; *C. cerasiforme* Mill.; *C. cerasiforme* Willd. (6); *C. chamaecerasus* Nees. (14); *C. cordiforme* Mill. (3); *C. frutescens* var. *cerasiforme* Bailey (15); *C. frutescens* var. *conoides* Bailey. (15); *C. frutescens* var. *longum* Bailey (15); *C. frutescens* var. *fasciculatum* (Sturtev.) Bailey (15); *C. frutescens* var. *grossum* Bailey (15); *C. globosum* Bess. (7); *C. grossum* L. (30); *C. longum* D.C. (16); *C. nigrum* Willd. (6) nr; *C. pomiferum* Steud. (17); *C. purpureum* Hornem. (18)].

***C. annuum*** var. ***glabriusculum*** (Dun.) Heiser & Pickersgill (19) [*C. annuum* var. *acuminatum* Fingerh (20); *C. annuum* var. *aviculare* (Dierb) D'Arcy and Eshbaugh (21); *C. annuum* var. *baccatum* Kuntze (22); *C. annuum* orma *leucocarpum* Kuntze (22); *C. annuum* var. *minimum* Heiser (23); *C. annuum* var. *minus* (Fingerh.) Shinners. (24); *C. chlorocladum* Dun. (25); *C. conoideum* var. *sulcatum* Fingerh. (20); *C. frutescens* var. *minus* Fingerh. (20); *C. indicum* var. *conoideum* (Mill.) Dierb. (26); *C. havanense* H. B. & K. (26); *C. minimum* Mill. (3); *C. hispidum* var. *glabriusculum* Dun. (25); *C. indicum* var. *aviculare* Dierb. (26); *C. microphyllum* Dun. (25); *C. pendulum* Willd. var. *minus* Fingerh. (20)].

***C. baccatum*** L. (30) [*C. angulosum* Mill. (3); *C. angustifolium* Dun. (25); *C. annuum* forma *chlorocarpum* Kuntze (22); *C. conicum* Lam. (10); *C. annuum* var. *microcarpum* (Cav.) Voss. (11); *C. microcarpum* Cav. (16); *C. annuum* forma *luteum* Kuntze (22); *C. baccatum* var. *umbilicatum* (Vell.) Hunz. and Barboza (28). bsn. *C. umbilicatum* Vell. (2); *C. conicum* Vell. (2); *C. cumanense* Fingerh. (20); *C. frutescens* var. *baccatum* Irish (8); *C. pulchellum* Salisb.(29)].

***C. baccatum*** var. ***baccatum*** Eshbaugh (31) [*C. microcarpnm* var. *tomentosum* Chodat and Hassl. (32); *C. ciliare* Willd. (6); *C. microcarpon* forma *fructicosum* Send. nr (33); *C. microcarpum* var. *glabrescens* Hassl.(34) nr.].

***C. baccatum*** var. ***pendulum*** (Willd.) Eshbaugh (30) [*C. pendulum* var. *majus* Dun. (25) nr.; *C. indicum* var. *pendulum* (Willd.) Dierb. (26).; *C. pendulum* Willd. (6); *C. frutescens* var. *pendulum* (Willd.) Besser(35)].

***C. breviflorum*** (Send.) Hunz. (43).

***C. buforum*** Hunz. (36).

***C. campylopodium*** Send. (33) [*C. campylopodium* forma *laurifolia* Chodat. (32)].

***C. cardenasii*** Heiser & Smith (37).

***C. chacoense*** Hunz. (38) [*C. chacoense* var. *tomentosum* Hunz. (38).

***C. chinense*** Jacq. (39) [*C. luteum* Lam. (10); *C. toxicarium* Fingerh. (20)].

***C. ciliatum*** (H.,B. &K) Kuntze (22) bsn. *Witheringia ciliata* Kunth [*C. diversifolium* (Klotzsch) Kuntze (22) bsn. *Witheringia diversifolia* (Klotzsch); *C. dumetorum* (Dun.) Kuntze (22) bsn. *Witheringia dumetora* Dun.; *C. haughtii* (Svenson) Macbr. (40) bsn. *Brachistus haughty* Svenson; *C. mendax* (Heurck & Muell.) Macbr. (41) bsn. *Solanum mendax* Van.; *C. molle* (H, B & K) Kuntze (22) bsn. *Witheringia mollis* H., B. & K.; *C. pringlei* (SWats.) Macbr. & Stand. (42) bsn. *Brachistus pringelei* SWats; C. *rhomboideum* (Dun.) Kuntze; *C. vargasii* Kuntze (22) bsn. *Fregirardia vargasii* Dun.].

***C. coccineum*** (Rusby) Hunz. (43) bsn. *Brachistus coccineus* Rusby.

***C. cornutum*** (Hiern) Hunz. (43) bsn. *Bassovia cornuta* Hiem.

***C. dimorphum*** (Miers) Kuntze (22) bsn. *Brachistus dimorphus* Miers.

***C. dusenii*** Bitter (44).

***C. eximium*** Hunz.(38) [*C. eximium* var. *tomentosum* Eshbaugh & Smith (45)].

***C. frutescens*** L. (1) [*C. annuum* var. *frutescens* Kuntze. (22); *C. baccatum* Vell. (2); *C. cereolum* Bertol. (46); *C. conoides* Roem. and Schult (4); *C. comarim* Vell. (2); *C. frutescens* var. *lanicaule* Green. (47) nr; *C. frutescens* var. *queenslandicum* Domin.(48) nr; *C. odoratum* Steud. (17); *C. odoriferum* Vell. (2); *C. queenslandicum* Domin. (48); *C. fastigiatum* Blume. (49)].

***C. galapagoense*** Hunz. (43) bsn. *Brachistus pubescens* Stewart.

***C. geminifolium*** (Dammer) Hunz.(43) bsn. *Acnistus geminifolius* Dammer.

***C. hookerianum*** (Miers) Kuntze (22) bsn. *Brachistus hookerianus* Miers. [*C. eggersii* Bitter(50); *C. brachypodum* (Dun.) Kuntze (22) bsn. *Bassovia brachypoda* Dun.].

***C. lanceolatum*** (Green.) Morton and Stand. (51) bsn. *Brachistus lancaefolium* Green.

***C. leptopodum*** (Dun.) Kuntze (22) bsn. *Bassovia leptopoda* Dun.

***C. minutiflorum*** (Rusby) Hunz. (46) bsn. *Bassovia minutiflora* Rusby.

***C. mirabile*** Mart (33) [*C. mirabile* var. *grandiflorum* Send. (33) nr].

***C. parvifolium*** Send.(33) [*C. parvifolium* var. *sellowianum* Dun.(25) nr].

***C. praetermissum*** Heiser & Smith. (37) [*C. baccatum* L. var. *praetermissum* (Heiser and Smith.) Hunz. (52)].

***C. pubescens*** Ruiz and Pav. (53) [*C. annuum* var. *violaceum* (Kunth) Voss (11); *C. guatemalense* Bitter (9); *C. lanceaefolium* (Miers) Kuntze (22) bsn. *Brachistus lanceaefolius* Miers. *C. quitense* Roem. and Schult.(4); *C. violaceum* Kunth(27) ].

***C. scolnikianum*** Hunz.(60).

***C. schottianum*** Send. (33) [*C. schottianum* var. *leptophyllum* Dun. (25); *C. schottianum* var. *flexuosum* (Send.) Hunz. (54); *C. flexuosum* var. *perrottettii* Dun. (25)].

***C. tovarii*** Eshbaugh, Smith & Nickrent (55).

***C. villosum*** Send. (33) [*C. villosum* forma *vimineum* Wawra. (56); *C. villosum* var. *muticum* Send. (33) nr; *C. villosum* var. *latifolium* Send. (33)nr ].

**FOLLOWING SPECIFIC EPITHETS ARE NEITHER ACCEPTED FOR THE GENUS *CAPSICUM* NOR ARE VERIFIED AS THE SYNONYM OF ANY ACCEPTED NAMES.**

***C. abyssinicum*** Rich.(57).
***C. aggregatum*** Roem. & Schult.(4).
***C. albescens*** (Britton) Kuntze (58) bsn. *Poecilochroma albescens* Britton.
***C. anthropophagorum*** Etudes (59).
***C. bauhini*** Dun. (25).
***C. caerulescens*** Bess. (7).
***C. cerasiflorum*** Link (76).
***C. ceratocarpum*** Fingerh. (20).
***C. crassiflorum*** (Dun.) Kuntze (22) bsn. *Bassovia crassiflora* Dun.
***C. crispum*** Dun.(25).
***C. curvipes*** Dun.(25).
***C. dicholomum*** Vell.(2).
***C. dunalii*** Kuntze (22).
***C. dulce*** Dun.(25).
***C. eriolarynx*** (Dun.) Kuntze(22) bsn. *Fregirardia eriolarynx* Dun.
***C. fuscoviolaceum*** (Cufod.) Morton & Stand (60) bsn. *Brachistus fuscoviolaceus* Cufod.
***C. glomuliflorum*** (Send.) Kuntze (22) bsn. *Aureliana glomuliflora*.Send.

***C. glandulosum*** Dun.(25).
***C. gracilipes*** Dun. (25).
***C. hebephyllum*** (Miers) Kuntze (22) bsn. *Brachistus hebephyllus* Miers.
***C. hebepodum*** Kuntze (22) bsn. *Bassovia hebepoda* Dun.
***C. hirsutum*** (Gardner) Kuntze (22) bsn. *Witheringia hirsuta* Gardner.
***C. hornemanni*** Dun. (25).
***C. inaequale*** Vell. (2).
***C. laurifolium*** Dun.(25).
***C. laeve*** (Dun.) Kuntze (22) bsn. *Bassovia laevis* Dun.
***C. leptoclada*** Kuntze (22).
***C. leptocladum*** (Dun.) Kuntze (22) bsn. *Fregirardia leptoclada* Dun.
***C. leucocarpon*** (Mill.) Fingerh. (20).
***C. ligustrinum*** (Dun.) Kuntze. (22) bsn. *Fregirardia leptoclada* Dun.
***C. lindenii*** (Dun.) Kuntze (22) bsn. *Fregirardia lindenii* Dun.
***C. lucidum*** (Moric.) Kuntze (22) bsn. *Solanum lucidum* Moric.
***C. lycianthoides*** Bitter (61).
***C. maximowiczii*** Regel and Rach (62).
***C. micranthum*** Link (76).
***C. mositicum*** Toledo (63).
***C. oblongifolium*** (Miers) Kuntze (22) bsn. *Brachistus oblongifolius* Miers.
***C. ovatum*** DC. (31).
***C. oxycarpum*** Dun. (25)
***C. pyramidale*** Mill.(3).
***C. pyraster*** (Dun.) Kuntze ( 22) bsn. Bassovia pyraster Dun.
***C. rabenii*** Send.(33).
***C. ramosissimum*** Witasek (64).
***C. recurvatum*** Witasek (64).
***C. salicifolium*** Dun. (25).
***C. sectio*** var. ***eucapsicum*** Wettst. (65).
***C. silvaticum*** Kuntze (22).
***C. spina-alba*** (Dun) Kuntze (22) bsn. *Fregirardia spina-alba* Dun.
***C. strictum*** Fingerh.(20 ).
***C. sylvaticum*** (Aubl.) Kuntze (22) bsn. *Bassovia sylvatica* Aubl.
***C. testiculatum*** Dun.(25).
***C. tomatiforme*** Steud.(17).
***C. torulosum*** Vell. (2).
***C. ustulatum*** Paxt. (66).
***C. velutinum*** (Send.) Kuntze (67).
***C. wildenowii*** Don (68).

**FOLLOWING ENTRIES WERE ONCE ASSIGNED TO THE GENUS *CAPSICUM* BUT NOW ARE CONSIDERED AS SYNONYM OF ACCEPTED NAME FOR OTHER GENERA.**

***C. anomalum*** Franch. and Sav. (69) synonym for the accepted name *Tubocapsicum anomalum* (Franch and Sav) Makino.

***C. asterotrichum*** Stand.(70) synonym for the accepted name *Witheringia asterotricha*
(Stand.) Hunz.

***C. escuintlense*** (Coult.) Stand. (71) synonym for the accepted name *Lycianthes heteroclita* (Send.) Bitter.

***C. lundellii*** Morton (72) synonym for the accepted name *Athenaea affinis* Morton.

***C. boninense*** Koidz and Sum. (73) synonym for the accepted name *Tubocapsicum boninense* (Koidz) Hara.

***C. costaricense*** Stand. and Morton (60) [*C. isothrix* Stand. (60); *C. malacophyllum* Stand. (70); *C. solanaceum* Kuntze (22); *C. solanaceum* var. *glabrescens* Kuntze (22) ; *C. solanaceum* var. *pubescens* Kuntze (22); *C. tetramerum* Stand and Morton (60)] synonym for the accepted name *Witheringia solanacea* var *solanacea* L'Her.

***C. grandiflorum*** Kuntze (58) synonym for the accepted name *assobia fasciculata* (Miers) Hunz.

***C. macranthum*** Stand. & Morton (60) accepted name for moved to *Witheringia macrantha* (Stand. & Morton) Hunz.

***C. macrophyllum*** Stand. (74) synonym for the accepted name *Witheringia solanacea* L'Hér.

***C. maculatum*** Stand & Morton(60) synonym for the accepted name *Witheringia maculatum* (Stand & Morton) Hunz.

***C. meianthum*** (Don) Stand. & Steyerm. (51) synonym for the accepted name Witheringia *meintha (*Don) Hunz.

***C. punctatum*** Kuntze (22) synonym for the accepted name *Saracha punctata* Ruiz. and Pav.

***C. riparium*** (Kunth) Kuntze (22) synonym for the accepted name *Cautresia riparia* var *riparia* Kunth.

***C. silvigaudens*** Stand. and Williams (75) synonym for the accepted name *Witheringia*
*meiantha* (Don) Hunz.

***C. solanaceum*** (L'HTr.) Kuntze(22) synonym for the accepted name *Witheringia solanacea* var. *solanacea* L'Her.

***C. stenophyllum*** Morton & Stand.(60) synonym for the accepted name *Witheringia meiantha* (Don Sm.) Hunz.

***C. stramonifolium*** (H., B. and K.) Kuntze(22) synonym for the accepted name *Witheringia stramonifolia* Kunth.

***C. subulatum*** Stand. and Morton (60) synonym for the accepted name *Brachistus stramoniifolius* (Kunth) Miers.

***C. viscidum*** Stand (5) synonym for the accepted name *Brachistus nelsonii* (Fernald) D'Arcy, Gentry and Averett.

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# CHARACTERISTICS OF SOME PEPPER CULTIVARS COMMONLY GROWN IN NIGERIA

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## Introduction

Peppers (*Capsicum annuum* L. and *C. frutescens* L.) are very important vegetable crops in Nigeria. They are annual (*Capsicum annuum*) or perennial (*C. frutescens*) herbaceous plants. Nigeria's annual production in 1991, 1992 and 1993 were 850,000 and 900,000 metric tons (Anonymous, 1993). The low yield obtained by farmers is due to lack of improved cultivars, and attack by pests and diseases (Alegbejo *et al.*, 1999). Consequently this trial was conducted to evaluate forty two pepper cultivars for six agronomic and physiological characteristics: days to flowering and maturity, plant height, fruit length and width, and fruit yield.

## Materials and Methods

The experiment was conducted at Samaru, Northern Guinea Savanna of Nigeria (latitude 11° 11'N, longitude 07° 38'E, elevation 686) in the 2000 wet season. The forty two pepper cultivars were obtained from Research Institutes and State Agricultural Development Projects (ADPs).

Seven week and old seedlings were transplanted into the field on 5th July 2000 at 45cm apart in two rows, 4.5 x 2.4m making up a plot. Adjacent plots were separated by a boarder of 120cm with maize (two seeds per hole, at 25cm apart) to avoid cross pollination. Treatments (pepper cultivars) were arranged in a randomize block design with three replicates. The field was weeded at 3,5,7 and 9 weeks after transplanting (WAT). Fertilization was carried out at 2 WAT with N.$P_2O_5$.$K_2O$ 15: 15: 15 and at 6 WAT with N.$P_2O_5$.$K_2O$ 27: 10: 10 at the rate of 250kg/ha in each case.

The following parameters were taken for each cultivar: days to flowering, maturity days, plant height, fruit length, fruit width and fruit yield/plot. Fruits were harvested weekly from 10 to 16 WAT. The data was analysed using the analysis of variance while the standard error of difference was used to separate means at the 50% level of probability.

## Results and Discussion

Days to flowering ranged between 65 to 70 (Table 1). Cultivars FNHVID, FNHVIF, Rodo (DT 95/907), Atarugu-M, Tasshi Kan Damo, Taffassai-KT and Chilli hot had the lowest (65) days to flowering while Dan Damasak 3, EX-Wandi Dass and Kancin-SA had the highest (70) days to flowering.

All the cultivars matured between 100 to 110 days. Cultivars FNHVIF matured in 100 days while cultivars Dan Damasak 3, EX-Wandi Dass and Hancin-SA matured in 110 days. All others cultivars were in between the two (Table 1). Plant height ranged from 42.2 (Tattasai-KD) to 83.6 cm (FNHVIC). Fruit length ranged from 1.5 (Dan Maunchr-KT) to 12.0 cm FNHVIG) while fruit width ranged from 0.8 (Tattasai-KT) to 2.3 cm (Cama padu). Yield per hectare ranged from 1.2 to 3.0 t/ha.

The study indicates that there are significant differences in the characteristics of the pepper cultivars evaluated at Samaru, Northern Guinea Savanna of Nigeria. The cultivars that had lower days to flowering and maturity might be preferred at Samaru because of the short wet season (June - October). Cultivars FNHVID, FNHVIF, Rodo (DT 95/70), Atarugu-M Tasshi, Kan Damo, Tattasai-KT and Chilli hot are therefore likely to be preferred by farmers because they produce branches and flowers early and therefore mature early even in years of low rainfall. Cultivars that flower and mature late (Dan Damasak, 3, EX-Wandi Dass and Hancin-SA) are likely to be preferred in the more southern ecologies with longer wet season (7-9 months).

Tall cultivars such as FNHVIC, Hancin Dunya and Ataragu-B are most likely to be preferred by farmers because of ease of picking. The high yielding cultivars such as Tca 14, known to be resistant to pepper venial mottle potyvirus (Alegbejo, 1999) are likely to be highly accepted by farmers. These cultivars are also very pungent.

Since many of the cultivars evaluated were obtained from local farmers through the ADPs, purification of the promising cultivars will be done so that only selected plants showing the desirable characters will be used in subsequent hybridization and selection program.

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Table 1: Pepper cultivars evaluated at Samaru in the 2000 wet season

| Pepper cultivar | Days to flowering | Maturity days | Plant height (cm) | Plant length (cm) | Fruit width (cm) | Fruit yield (kg/ha) |
|---|---|---|---|---|---|---|
| FNHVID | 65.00 | 106.00 | 70.81 | 9.30 | 0.90 | 2.42 |
| FNHVIC | 67.00 | 106.00 | 83.62 | 8.00 | 1.81 | 2.27 |
| FNVHVIB | 66.00 | 103.00 | 63.81 | 7.51 | 2.30 | 2.14 |
| FNHVIE | 68.00 | 104.00 | 47.61 | 5.52 | 1.07 | 2.23 |
| FNHVIF | 65.00 | 100.00 | 69.80 | 8.02 | 1.02 | 2.10 |
| FNHVIA | 66.00 | 103.00 | 56.31 | 7.01 | 1.12 | 2.20 |
| FNHVIG | 67.00 | 105.00 | 55.50 | 12.03 | 1.41 | 2.24 |
| Rodo (DT 95/907) | 65.00 | 106.00 | 54.42 | 3.33 | 1.50 | 1.93 |
| Sombo (DT 95/297) | 67.00 | 107.00 | 67.61 | 8.62 | 1.41 | 1.83 |
| NH 94/343 | 69.00 | 108.00 | 68.20 | 6.10 | 1.03 | 1.94 |
| Dan Damasak 1 | 66.00 | 105.00 | 61.42 | 4.62 | 1.81 | 1.80 |
| Dan Damasak 2 | 69.00 | 108.00 | 52.23 | 8.33 | 1.54 | 1.71 |
| Dan Damasak 3 | 70.00 | 110.00 | 74.21 | 9.82 | 1.53 | 1.72 |
| EX-Wandi Dass | 70.00 | 110.00 | 75.82 | 8.51 | 1.03 | 1.73 |
| EX-Ningi | 66.00 | 106.00 | 61.40 | 4.92 | 1.84 | 1.71 |
| EX-T/Wass Dass | 67.00 | 107.00 | 42.60 | 10.82 | 1.62 | 1.94 |
| Ex-Dara 90 | 67.00 | 106.00 | 53.81 | 5.64 | 0.92 | 1.91 |
| Zugande | 68.00 | 109.00 | 65.03 | 8.63 | 1.74 | 1.90 |
| Tsiduhu | 69.00 | 108.00 | 65.32 | 9.81 | 1.83 | 1.92 |
| Gama Pada | 67.00 | 105.00 | 63.84 | 9.42 | 2.33 | 1.93 |
| Tattasai-M | 67.00 | 103.00 | 49.42 | 9.03 | 1.04 | 1.90 |
| Tsichfi | 68.00 | 108.00 | 71.40 | 6.81 | 0.92 | 1.85 |
| Ataragu-M | 65.00 | 103.00 | 50.81 | 5.02 | 1.04 | 1.82 |
| Tca 14 | 67.00 | 108.00 | 73.20 | 8.51 | 1.74 | 2.96 |
| Tugande hancin Samuwa | 68.00 | 106.00 | 63.21 | 9.03 | 1.32 | 1.64 |
| Hancin Dunya | 69.00 | 108.00 | 81.43 | 7.32 | 0.91 | 1.55 |
| Atarugu Dan Garhi | 66.00 | 109.00 | 62.04 | 9.63 | 1.03 | 1.64 |
| Tasshi Kan Damo | 65.00 | 105.00 | 69.21 | 7.53 | 2.34 | 1.82 |
| Dan Damasa (Barliya) | 66.00 | 106.00 | 61.82 | 5.54 | 2.31 | 1.23 |
| Hancin-SA | 70.00 | 110.00 | 54.20 | 9.32 | 1.12 | 2.92 |
| Kenba | 69.00 | 109.00 | 76.31 | 8.63 | 1.82 | 2.80 |
| Borthono-DM | 68.00 | 107.00 | 57.84 | 6.83 | 1.13 | 2.40 |
| Ataragu-B | 67.00 | 106.00 | 81.53 | 8.52 | 2.04 | 2.20 |
| Dan Maunchi-KT | 66.00 | 107.00 | 53.84 | 1.53 | 0.92 | 2.02 |
| Tattasai-KT | 56.00 | 105.00 | 69.41 | 2.83 | 0.81 | 2.30 |
| Dan Damasa | 66.00 | 106.00 | 61.82 | 8.32 | 1.43 | 2.21 |
| Hancinsa-KD | 67.00 | 105.00 | 70.83 | 5.54 | 1.02 | 2.30 |
| Hansin burgu | 68.00 | 107.00 | 46.34 | 8.62 | 2.01 | 2.61 |
| Chili hot | 65.00 | 104.00 | 56.41 | 8.30 | 1.13 | 2.13 |
| Ataragu-KD | 67.00 | 107.00 | 66.43 | 1.93 | 0.94 | 2.21 |
| Tattasai-KG | 66.00 | 108.00 | 42.20 | 8.61 | 1.84 | 2.04 |
| Chilli-KD | 68.00 | 106.00 | 60.32 | 7.32 | 1.72 | 1.83 |
| **S.E.D. (P=0.05)** | **2.61** | **3.23** | **8.02** | **2.01** | **0.12** | **0.18** |

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# APPLICATION OF THE MICROSATELLITE-AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (M-AFLP) TECHNIQUE IN PEPPER (*Capsicum annuum* L.).

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**Key words**: AFLP, M-AFLP, microsatellite, *Capsicum annuum,* polymorphism.

## Introduction

Microsatellites (SSR) are arrays of simple sequence repeats (usually 2-4 bp in length) that are interspersed in all eukaryotic genomes (Toth *et al.,* 2000). The variability of such arrays is attributed to unequal crossover and slippage replication (Nagy *et al.,* 1998). They can find application in cultivar fingerprinting, phylogenetic studies as well as in saturating the developed inter-specific (Livingstone *et al.,* 1999; Kang *et al.*, 2001) and intra-specific (Lefebvre *et al.,* 1995; 1997) *Capsicum* genetic maps.

SSR markers for plant studies have generally been developed by three routes: (i) transfer from closely related species (Provan *et al.,* 1996; White and Powell, 1997), (ii) searching sequence databases (Smulders *et al.* 1997; Sanwen *et al.,* 2000) and (iii) by screening cDNA or smaller insert libraries with tandemly repeated oligonucleotides and sequencing candidate clones (Powell *et al.,* 1996).

Here we report on an ongoing research activity aimed at developing microsatellite markers in pepper by means of the M-AFLP (Microsatellite-AFLP) technique, which is a modification of AFLP (Amplification Fragment Length Polymorphism) (Vos *et al.,* 1995) and does not require construction of libraries (Van Eijk *et al.*, 2001).

## Material and methods

DNA from six pepper (*Capsicum annuum* L.) cultivars ('H3', 'Vania', 'Yolo Wonder' , 'CM334', 'PM687', 'Perennial') and four pepper F1 hybrids ('H3' x 'Vania', 'Perennial' x 'Yolo Wonder', 'Yolo Wonder' x 'CM334', 'Yolo Wonder' x 'PM687'), was kindly provided by INRA (Montfavet, France) and used in this study.

*M-AFLP.*

The preliminary AFLP step was performed as described by Vos *et al.* (1995) with minor modifications (Lanteri *et al.,* 2002). Restriction and ligation were done concurrently using DNA from all the above mentioned sources. Five µl DNA (400-500 ng DNA) were added to 45 µl buffer (10 mM Tris-HCl pH 7.5; 10 mM MgAc, 50 mM KAc) containing 5 units *Eco*RI, 5 units *Mse*I (Gibco BRL), 2 units T4 DNA ligase (Promega), 5 pmol *Eco*RI adapter, 50 pmol *Mse*I adapter (Lanteri *et al.* 2002) and 0.2 mM ATP. The mixture was then incubated at 37°C for 4h and diluted 10 times in 0.1x TE. A first selective pre-amplification of *Eco*RI-*Mse*I fragments was performed using 5 µl of the above mentioned diluted mixture, added to a 15 µl mixture giving a final concentration of 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2mM of each dNTPs, 40 ng of *Eco*RI and *Mse*I adapter-directed primers, each possessing a single selective base (E0 and M0, Table 1) and 1 unit of Taq polymerase (Promega). PCR reactions were performed with the following profile: 94°C for 60 s, 25 cycles of

30 s denaturing at 94°C, 30 s annealing at 55°C and 60 s extension at 72°C, ending with 10 min at 72°C to complete extension. After checking for the presence of a smear of fragments (100-1000 bp in length) by agarose electrophoresis, the amplification products were diluted 40 times in 0.1x TE.

A second selective amplification was carried out on the diluted pre-amplification products using 4 *Eco*RI or 4 *Mse*I selective primers, each of them in combination with four ISSR (Inter-SSR, Zietkiewicz *et al.*, 1994) 5' anchored primers as listed in Table 1. Selective PCR reactions were performed with the following profile: 94° C for 60 s, 36 cycles of 30 s denaturing at 94°C, 30 s annealing and 60 s extension at 72°C, ending with 10 min at 72°C to complete extension. Annealing was initiated at a temperature of 65°C, which was then reduced by 0.7°C for the next 12 cycles and maintained at 56°C for the subsequent 23 cycles. The products of the amplifications were separated by electrophoresis on 5% denaturating polyacrylamide sequencing gels and silver stained (Bassam *et al.*, 1991) in order to detect microsatellite-enriched fingerprints. Bands showing polymorphism among different DNA sources, and thus, presumably, including putative microsatellites, were excised, eluted and re-amplified with the same primer combinations used in the amplification step and under the same conditions. In order to avoid sequencing of co-migrating bands, the PCR products were cloned into PCRII cloning vector (Invitrogen) and transformed in *E.coli* competent cells InvF'α (Invitrogen). Positive clones were sequenced (MWG-Biotech) and primers were designed on the flanking region, using Primer3 software (www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi).

The M-AFLP markers were converted into conventional SSR assays based on amplification with the specific flanking primer and the 5' anchored ISSR primer. They were tested at first on the pre-amplification products and then on the genomic DNA (30 ng) from the six pepper cultivars and four pepper F1 hybrids above reported. Amplifications, electrophoresis conditions and staining procedures were the same as reported above.

**Results and Discussion**

Among the 32 M-AFLP primer combinations tested, only 7 originated clear electrophoretic patterns (Table 2). A total of 57 polymorphic bands, containing putative microsatellites, were detected (Table 2). All of them were eluted, re-amplified and observed as pure fragments on the agarose gels.

Four bands obtained from E1/ISSR1 primer combination were cloned and sequenced. They were found to include repetitive motifs and primers reported in Table 3 were designed on one of the flanking regions.

When each of the designed primer, together with the ISSR1 primer, were tested on the pre-amplification products, single bands of the expected size (Table 3) were originated. However, when tested on genomic DNA from pepper cultivars or F1 hybrids, complex patterns were observed which made it difficult to resolve polymorphism.

Extra bands were, presumably, originated by competitive ISSR fragment amplification. An ISSR primer, in fact, can amplify genomic segments flanked by an inversely oriented, closely spaced repeated sequences (Zietkiewicz *et al.*, 1994). As reported by Albertini *et al.* (2001), extra band detection can be avoided when radioactive or fluorescent specific flanking primers are used.

In order to avoid the use of labelled primers and convert the M-AFLP markers into conventional SSR assay, we are planning to obtain the second specific flanking microsatellite primer. The restricted-ligated DNA will be amplified with the *Mse*I not

selective primer (M0, Table 1) in combination with each of our designed specific primer. The amplification products will be sequenced and the second microsatellite flanking primer designed.

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**Table 1 –** Sequences of the AFLP and ISSR (5' anchored) primers used.

| AFLP primers | | | |
|---|---|---|---|
| **Primer** | **Sequence (5'–3')** | **Primer** | **Sequence (5'–3')** |
| *Eco*RI-Core | GACTGCGTACCAATTC | *Mse*I-Core | GATGAGTCCTGAGTAA |
| E0 | + A | M0 | + C |
| E1-selective | + AC | M1-Selective | + CA |
| E2-selective | + AT | M2-Selective | + CC |
| E3-selective | + AA | M3-Selective | + CG |
| E4-selective | + AG | M4-Selective | + CT |

| ISSR primers | |
|---|---|
| **Primer** | **Sequence (5'–3')** |
| ISSR1 | $CAGC(TC)_7$ |
| ISSR2 | $CGCAA(CA)_9$ |
| ISSR3 | $GCCAC(GCT)_6$ |
| ISSR4 | $CGCAC(AAG)_5$ |

**Table 2 –** Number of polymorphic bands observed for each of the seven M-AFLP best primer combinations

| Primer combination | Number of bands |
|---|---|
| E1/ISSR1 | 16 |
| E4/ISSR1 | 14 |
| E4/ISSR2 | 5 |
| E4/ISSR4 | 7 |
| M1/ISSR1 | 6 |
| M3/ISSR4 | 4 |
| M4/ISSR1 | 5 |
| total | 57 |

**Table 3 –** Oligonucleotide sequences and associated information for the four specific microsatellite primers designed and tested with the ISSR 1 primer.

| Specific primer | Sequence (5'–3') | Length (bp) | Tm (°C) | Product (bp) |
|---|---|---|---|---|
| M-AFLP1 | 5'-GATCAATGGGTTGTTGTCAG | 20 | 56 | 93 |
| M-AFLP2 | 5'-CCTCGCTAGAGTTCCAAAC | 19 | 55 | 119 |
| M-AFLP3 | 5'-GCCTTTACCCTACTATTGTGC | 21 | 56 | 247 |
| M-AFLP4 | 5'-CTGACTCAGTCTGCGAAATC | 20 | 56 | 228 |

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# DETERMINATION OF HOT PEPPER "YUEJIAO NO. 1" $F_1$ HYBRID SEED PURITY BY RAPD MARKERS

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**Abstract**

Determination of hot pepper "Yuejiao No. 1" $F_1$ hybrid seed purity by RAPD markers was studied under the direction of the theoretical and technical systems of vegetable crop seed purity tested by DNA molecular markers. The results showed that specific primer P1 and P2 could be applied in RAPD test of "Yuejiao No.1" hot pepper variety seed purity. RAPD test result of one random sample using these primers was the same as that of the grow-out test.

**Key words** *Capsicum annuum*, $F_1$ hybrid, seed purity, RAPDs

DNA molecular markers have been applied in the studies of vegetable crop $F_1$ hybrid seed purity test in recent years (Ballester *et al.*, 1998; Paran *et al.*, 1995; Livneh *et al.*, 1990). The results usually have been doubtful because an integrated system of molecular marker assay hasn't been established. So this method is difficult to be acted as an independent assay of seed purity test, thus deeply influencing its application in commercial practice and its industrialization development.

Recently the authors established the theoretical systems of vegetable crop seed purity tested by DNA molecular markers (Wang *et al*, 1999b, 1999c; Yin *et al*, 1999). The system consisted of four parts, 1) selection of molecular marker systems, 2) test principles, 3) the experimental design and 4) statistical evaluation. On the basis of this system , the technical systems were analyzed (Wang *et al*, 1999a, 1999d, 1999e), which contained 1) seed sampling, 2) DNA extraction, 3) selection of molecular markers and 4) RAPD analysis. The present work reports the results of applying RAPD markers to assess the genetic purity of hot pepper (*Capsicum annuum* ) "Yuejiao No.1" $F_1$ hybrid seeds under the direction of the theoretical and technical systems.

## Material and methods

Hot pepper "Yuejiao No.1" $F_1$ hybrid , its female parent line and male parent line were analyzed in this study.

Genomic DNA was isolated from the young leafs samples using a modified SDS method (Wang et al., 1998). DNA concentrations in the extractions were measured using a uv-spectrometer. RAPD reaction were performed in a 25-μl volume consisting of 20 ng of template DNA, 0.2 μM of a decamer primer (Operon Technologies, Almeda, California, USA), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 2 mM of each dNTPs, and 1.5 units of Taq DNA polymerase (Sino-America Biotechnology Co., Luoyang, Henan, P. R. China). Amplification was conducted in a GeneAmp PCR System 9600 thermal cycler (Perkin Elmer) programmed for 45 cycles of 1 min at 94℃, 1 min at 36℃ and 2 min at 72℃, with an initial denaturation step at 94℃ for 5 min and a final extension step at 72℃ for 10 min. RAPD products were analyzed by electrophoresis in 1.5% agarose gels (TAE buffer, pH 8.0) and DNA bands were visualized by ethidium bromide staining.

**Results**

RAPD markers

A total of 200 10-mer primers were used to screen for polymorphisms between the parents. In the RAPD analysis, the female parent DNAs were the mixture of ten individual plant DNA, similar for the male parent DNAs. In the primers tested, 24 produced polymorphisms between the parent lines, and the number of DNA bands in the RAPD pattern ranged from 2 to 6. This result also proved that the cultivated pepper was known as possessing very little genetic diversity (Ballester *et al.*, 1998), although the female and male parent line of "Yuejiao No. 1" $F_1$ hybrid belongs to *Capsicum annuum* var. var. *longum* and var. *grossum*, respectively.

Data on these primers were used to select further for the presence of bands specific to the male and female parents of "Yuejiao No. 1" $F_1$ hybrid, respectively. Those primers producing the same RAPD pattern in the three replications were chosen , and then were called ideal RAPD markers according to the following principles: 1) 1-4 DNA bands in the patterns, 2) certain specific DNA marker was clear and intensive, 3) the specific marker was easily distinguished from the contiguous bands in the gel.

The authors found 2 primers useful in determining the hot pepper "Yuejiao No. 1" $F_1$ hybrid seed purity. Primer P1 generated one marker specific to the female parent (Fig 1 left), the marker size was approximately 550 bp. In this pattern, the band number of female parent, male parent and $F_1$ hybrid was 1, 2, 2, respectively. Primer P2 gave one useful marker specific to the male parent (Fig 1 right), approx. 1000 bp marker was very clear and intensive in the pattern.

*Fig 1 - RAPD patterns amplified with the primer P1(left) and primer P2 (right). Both in left and right pattern: Lane 1: 100 bp DNA Ladder , Lane 2: female parent, Lane 3: male parent, Lane 4: $F_1$ hybrid.*

Determination of seed purity by RAPD markers

Seed purity of a random sample was tested using these RAPD markers. According to the theoretical systems and practical systems, two replications were undertaken with 50 seeds per replication in the seed purity test practice. Genomic DNA of every individual plant was prepared using the above mentioned method. Then RAPD reactions containing every individual plant DNA were amplified with specific primer P1 and P2, respectively.

In the first replication, the patterns of 50 seeds, amplified with the primer P1, were completely identical (Fig 2, others not shown), and that amplified with the primer P2 were almost the same. (Fig 3, others not shown), reflecting that these 50 seeds were true $F_1$ hybrids. The same was the second replication (data patterns not shown). This indicated that the seed purity of this random sample was 95%. The grow-out test also proved this sample seed purity was 95%. This demonstrated that RAPD test result of one random sample using these primers was the same as that of the grow-out test.

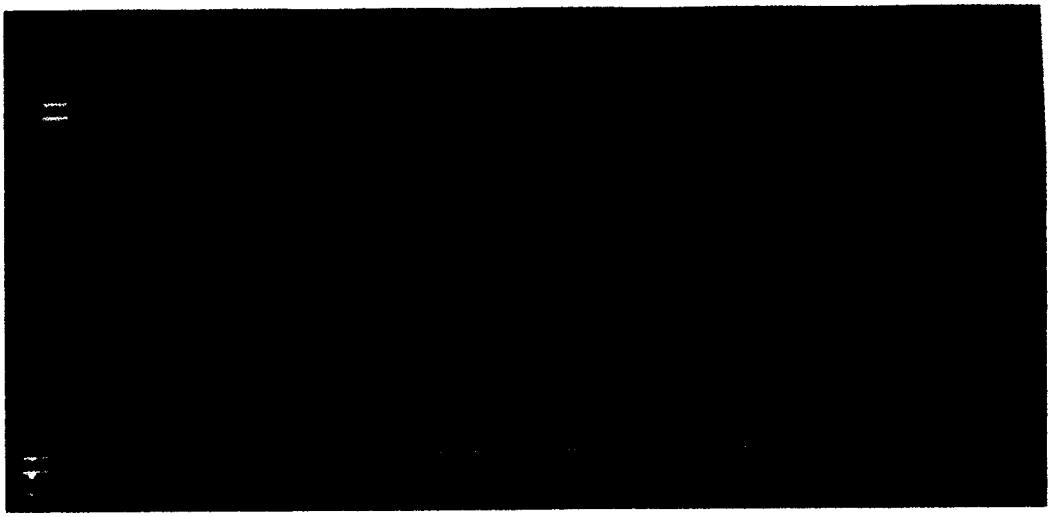

*Fig 2 - RAPD patterns of one sample of "Yuejiao No. 1" hot pepper generated by with the primer P1. From left to right in both the upper and lower row: Lane 1: 100 bp DNA Ladder, Lanes 2-4: male parent, Lanes 5-7: female parent, others : one random sample of 'Yuejiao No. 1' hot pepper*

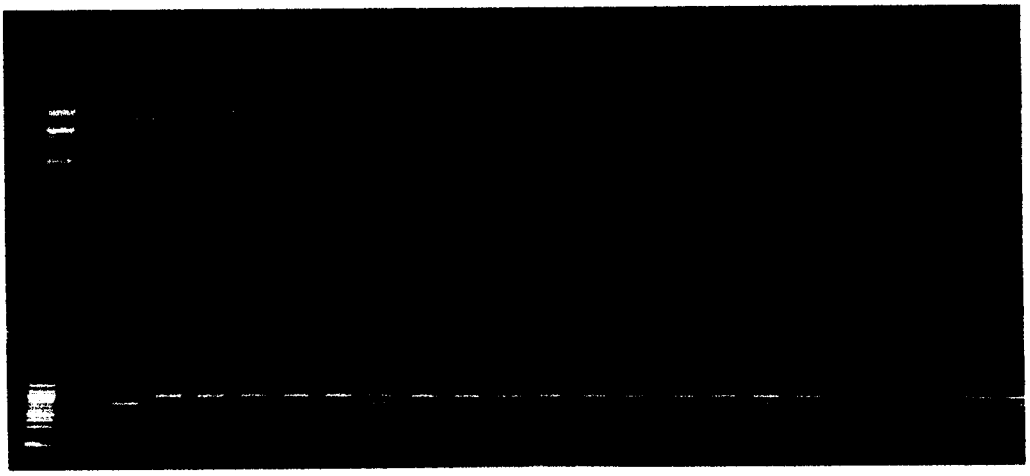

*Fig 3 - RAPD patterns of one sample of "Yuejiao No. 1" hot pepper generated by the primer P2. From left to right in both the upper and lower row: Lane 1: 100 bp DNA Ladder, Lane 2: female parent, Lane 3: male parent, others: one random sample of 'Yuejiao No. 1' hot pepper*

**Discussion**

In the practical test, failure of amplification may occur, i.e. no DNA bands appeared in the RAPD reaction of some individual plant DNAs. For the trouble-shooting, the authors' methods were that RAPD amplifications were done once more for these individual DNAs, then the final result was determined.

It was pointed out in the theoretical systems (Wang *et al.*, 1999b), that the DNA bands present in both the female parent and $F_1$ and absent in that of male parent, and that present in both the male parent and $F_1$ and absent in female parent should be selected as RAPD markers. These two kinds of RAPD markers must be combined to apply in the practical $F_1$ hybrid purity

test. Hence, usually two RAPD markers were needed. To reduce the cost of seed purity tests, one marker specific to both the female and male parent should be the ideal.

Under the direction of the theoretical and technical systems, hot pepper "Yuejiao No. 1" $F_1$ hybrid seed purity tested by RAPD markers was studied. The results showed that RAPD test result of one random sample using these primers was the same as that of the grow-out test. The results suggested that RAPD markers could be used with effectiveness in very reduced time for $F_1$ hybrid seed purity determination.

**Acknowledgements**

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# MULTIPOLAR SPINDLE ABNORMALITY IN *CAPSICUM ANNUUM L.*

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**Summary**

Among 20 KR treated line of a local cultivar of *Capsicum annuum* L meiotic spindle abnormality was observed in one plant. It is likely that gamma-rays may have disturbed the spindle organiser thereby causing abnormal spindle behaviour. The genetic basis for this abnormality could not be studied because of very high pollen sterility encountered in the aberrant plant.

**Key words:** *Capsicum annuum*, gamma-rays, multipolar spindle

## Introduction

Abnormal mitosis and meiosis induced by various agents such as chemical treatments, temperature, irradiation and dehydration has been reported in plants (Giles 1939; Peto 1935; Puza and Srb 1964). Sax (1937) described the effects of changes in temperature in *Tradescantia* and reviewed abnormalities of nuclear and cell divisions. The phenomenon of cell division whether mitotic or meiotic is a highly complex and delicately balanced one-a composite of several separate but integrated processes.

*Capsicum annuum* L. a member of the family Solanaceae is an important condiment and vegetable. In view of it's importance mutagenic experiments were being conducted in our laboratory on some cultivars of this taxon to create genetic variability to obtain agronomically superior plants. In one treated line (20KR) of a local cultivar, one plant looked abnormal-stunted growth and wrinkled leaves and spindle abnormalities were pronounced in the meiocytes and the findings are reported in this paper.

## Materials and Methods

Dry dormant seeds of a local cultivar of *Capsicum annuum* L were exposed to gamma-rays from 10-50 K rads at a dose interval of 5 KR per minute. The treated and untreated seeds were raised in pots separately. One plant in 20 KR treated line was morphologically different from the rest of the line. The anthers of that plant were squashed with 2.0% acetocarmine to study the PMC meiosis.

## Results

*Morphology*: The plant exhibiting abnormal spindle behaviour was different from other treated plants and the control in having stunted growth, wrinkled leaves, and reduced internodal length so that the leaves appeared to be grouped at the top of the plant. The flower buds were slightly irregular in size and shape and often possessed more than five stamens. The stigamatic surface was flattened instead of the normal capitate condition which ultimately lead to the premature disintegration of the stigma.

*Cytology*: All the meiocytes of the plant with abnormal spindle behaviour were found to be uninucleate. Chromosome pairing at diakinesis showed normal bivalent formation without any apparent abnormality. The mean chaisma frequency per cell in the abnormal plant was slightly less than that of the control plant (15.56 in abnormal and 16.60 in the control). At metaphase I quadripolar, tripolar and dipolar spindles were observed in 30.80, 51.90 and 17.30 per cent of meiocytes respectively. As a result of this, formation of three or four groups at anaphase I and telophase I was discernible in a large proportion of the PMC's. Irregularities were noticed in the second meiotic division as well. The irregular segregation of chromosomes and laggards etc., at anaphase II in a large proportion of the PMC's was reflected by the production of variable number of nuclei at telophase II (Table–1) and polysporic condition where the spores were of different sizes (2-8 spores) and shapes at the pollen quartet stage. Pollen stainability was very low (5.05%) indicating high pollen abortion. For proper understanding of the mode of inheritance of the abnormal spindle behaviour, crosses were made between this plant and the sib plants in the $M_1$ 20 KR treated line and also with the control which were reasonably pollen fertile. No seed was obtained either upon selfing or crossing upon or open pollination inferring that this plant was completely female sterile. Therefore, the genetic basis for this trait (abnormal spindles) could not be established.

**Table1.** Frequency of Telophase II groupings in gamma ray treated plant of *C. annuum* L. showing abnormal spindle behaviour. (Percentage indicated in parenthesis)

| Sl. No. | Plant | No. of Groups | | | | | Micro nuclei | Total |
|---|---|---|---|---|---|---|---|---|
| | | 2 | 3 | 4 | 5 | More than 5 | | |
| 1. | Control | - | - | 46 (100) | - | | - | 46 |
| 2. | Treated | 24 (13.9) | 33 (19.2) | 39 (22.7) | 27 (15.7) | 23 (13.4) | 26 (15.1) | 172 |

**Discussion**

The presence of tripolar and quadripolar spindles in a very large proportion of the meiocytes at metaphase I, were due to the meiotic spindle abnormalities and these have been primarily reported in induced autotetraploids, amphidiploids, species hybrids and rarely in natural diploids. Some of these spindle abnormalities were inherited as a simple (Mendalian) recessive genes. This is the first report on spindle abnormality either induced or under natural conditions in *Capsicum annuum* L.

The spindle is probably a composite structure in most organisms and the normal spindle appears to be the product of spindle fibre organiser located on the chromosome plus the pole determinants co-operating with each other, synchronized in time and space although either can produce the spindle unaided in some organisms. The structural abnormalities described here can be attributed to variations in the behaviour of pole determinants. The spindle organiser of Walters (1958) and Tai (1970) and the pole determinants of Swanson and Nelson (1942) are one and the same. According to Tai (1970) the multipolar spindles are the products as a result of random breakage of a unit spindle organiser. Multipolar spindles were recorded in *Triticum aestivum* when treated with acetone (Kabarity,1966When the seedlings of Allium cepa were subjected to low temperatures multipolar spindle abnormalities were pronounced in the PMC's (Huskins and Cheng, 1950). Spontaneous breakage of spindle organiser as a result of gene mutation resulted in the occurrence of multipolar spindles in the meiocytes of wheat grass

(Tai, 1970). When 0.2% of aqueous Colchicine was injected into the young flower buds of wheat which had not entered the meiosis it resulted in either achiasmatic meiosis (Dover and Riley, 1973) or the induction of multipolar spindles (Dover and Riley, 1977). Multipolar spindle abnormality was also reported in Colchicine induced autotetraploids of *Physalis pubescens* (Lydia and Raja Rao, 1982). In all these cases Colchicine or other experimental agents mostly seem to act directly on the PMC's inducing spindle abnormality except in *P.pubescens* where Colchicine was applied to the seeds (before initiation of the flowering). The abnormal behaviour of the spindle encountered in the present study could possibly be due to the damage caused by gamma-rays in the premeiotic mitosis instead of acting on the pollen mother cells and this could have altered the stability of pole determinants in the early seedling stage at the time of the treatment and this abnormality could have persisted over many cell generations and subsequently expressed in the PMC's, a view also shared by Lydia and Raja Rao (1982), in the Colchiploid *P.pubescens*. The split spindle observed in the present study is also attributed to the altered stability of pole determinants as seeds were exposed to gamma-rays.

The wrinkled leaves and other morphogical abnormalities are presumably the result of similar abnormal spindle behaviour in the somatic tissue.

**Acknowledgements**

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# FRUIT MORPHOLOGY OF TETRAPLOID PEPPER

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## Abstract

Tetraploid plants of *Capsicum annuum* L. 'Shishitoh no.562', 'Chigusa' and 'Jalapeno' were obtained by colchicine treatment (1.0%) of the seeds. In these cultivars, the seed number of tetraploid fruits were average 18%, and the length of tetraploid mature fruits were average 74% of the diploid mature fruits. The diameter and the weight were increased by 10% in 'Chigusa'. These differences between diploid and tetraploid fruits were almost the same in colchicine- treated generation and subsequent generation.

## Introduction

We have investigated the characteristics of tetraploid pepper for breeding. In the previous report, we established the method for obtaining tetraploid pepper (Ishikawa *et al.*, 1997), and then reported that the mature fruits of tetraploid 'Shishitoh' of colchicine treated generation, had reduced length and seed number (Ishikawa *et al.*, 2000).

In this paper, we examined the fruit morphology using subsequent generation of tetraploid 'Shishitoh' in order to eliminate the possible side effects of colchicine treatment. We also compared fruit morphology of tetraploid pepper using different types of fruit shape: 'Shishitoh' (elongated bell), 'Chigusa' (bell type) and 'Jalapeno' in order to generalize the characteristics of tetraploid fruits of pepper.

## Materials and Methods

Tetraploid plants of *Capsicum annuum* L. 'Shishitoh no.562', 'Chigusa' and 'Jalapeno' (Nihon Horticultural Production Institute, Japan) were obtained by colchicine treatment (1%) of the seeds, and the polyploidy was analyzed by flow cytometry (Partec Cell analyzer CAII, Germany) (Ishikawa et al., 1997). Both colchicine treated and non-treated plants were grown in the greenhouse with maximum/minimum temperatures of 37/18°C, day/night, at natural light conditions, and the seeds were harvested.

Five seeds from one pod of each line were sown on August 15th 2000 in the greenhouse and grown for 1 month in pots, with soil temperature of 30°C, at natural light

conditions. The plantlets were then transplanted on the ground in the greenhouse under the same condition as described above.

Naturally pollinated mature fruits of diploids and tetraploids were harvested in March and April, and the number of seeds, length, diameter, weight of the fruits were investigated.

**Result and Discussion**

In comparison to the diploid counter parts, the number of the seeds of tetraploid fruits of 'Shishitoh no.562', 'Chigusa' and 'Jalapeno' were 19, 12 and 22% (mean 18%), respectively, and the fruit length of tetraploids were 77, 82 and 64 % (mean 74%), respectively (Table 1,2 and 3). The fruit weight and diameter of tetraploid 'Chigusa' was increased by 10 % comparing to the diploid fruits (Table 1,2 and 3). But in 'Shishitoh no.562' and 'Japaleno' the fruit weight and diameter were equal or reduced. In general, tetraploid pepper had reduced seed number and fruit length but the weight and the diameter of the fruits were not reduced in all cultivars. These differences between diploid and tetraploid fruits were almost the same in colchicine treated generation and subsequent generation.

The fruit shape was different also. The fruit apex of tetraploid 'Shishitoh no.562' was pointed (Fig.1 A), the shape of tetraploid 'Jalapeno' was slightly deformed (Fig.1 B) and the tetraploid fruits of 'Chigusa' was round (Fig.1 C and D).

The reasons for these changes are still to be investigated. Marcelis and Baan Hofman-Eijer showed a linear increase in individual fruit weight with seed number (1997). Thus, the seed number may be reduced by low fertility of the pollen grains, and this may lead to the decrease in the fruit length. The effect of pollination may also influence the growth or elongation of the fruits.

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**Table 1. Morphology of mature fruits of diploid and tetraploid 'Shishitoh no. 562'.**

| Polyploidy of the plants | Weight (g) | Length (mm) | Diameter (mm) | No. of seeds |
|---|---|---|---|---|
| Diploid* | 11.3 ± 1.0 | 70.2 ± 2.1 | 26.5 ± 0.9 | 89 ± 18 |
| Tetraploid** | 9.6 ± 0.8 | 54.1 ± 4.0 | 26.0 ± 0.6 | 17 ± 4 |

Mean±SE. *: n = 11. Two lines gave the same results. **: n = 10. Four lines gave the same results. The data from one line of diploids and tetraploids were shown here.

**Table 2. Morphology of mature fruits of diploid and tetraploid 'Chigusa'.**

| Polyploidy of the plants | Weight (g) | Length (mm) | Diameter (mm) | No. of seeds |
|---|---|---|---|---|
| Diploid* | 130.3 ± 6.2 | 90.1 ± 0.8 | 73.0 ± 1.6 | 196 ± 9 |
| Tetraploid** | 143.1 ± 8.3 | 73.7 ± 2.5 | 80.0 ± 3.1 | 24 ± 7 |

Mean±SE. *: n = 8. Two fruits from each 4 lines were examined. **: n = 6. Two fruits from each 3 lines were examined.

**Table 3. Morphology of mature fruits of diploid and tetraploid 'Jalapeno'.**

| Polyploidy of the plants | Weight (g) | Length (mm) | Diameter (mm) | No. of seeds |
|---|---|---|---|---|
| Diploid | 24.5 ± 0.8 | 61.5 ± 1.3 | 32.7 ± 0.6 | 116 ± 9 |
| Tetraploid | 11.2 ± 1.0 | 39.2 ± 1.8 | 25.4 ± 0.7 | 26 ± 4 |

Mean±SE. n = 7. Three lines of diploids and tetraploids gave the same results. The data from one line of diploids and tetraploids were shown here.

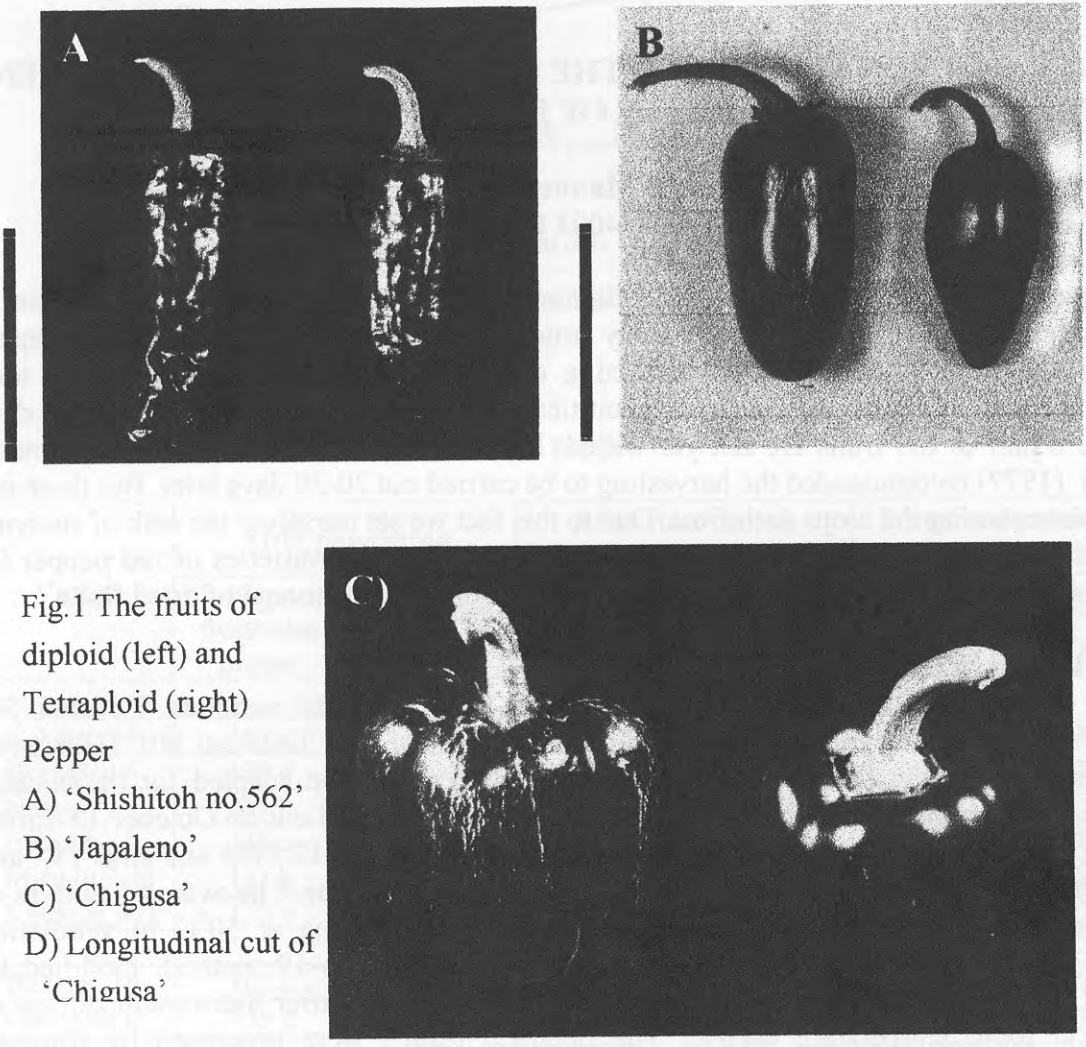


Fig.1 The fruits of diploid (left) and Tetraploid (right) Pepper
A) 'Shishitoh no.562'
B) 'Japaleno'
C) 'Chigusa'
D) Longitudinal cut of 'Chigusa'

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# INFLUENCE OF THE VARIETY AND THE FROST ON THE TOTAL PIGMENT CONTENT AND COLOUR STABILITY OF RED PEPPER (PAPRIKA)[1]

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Frequently under South Bulgaria conditions the first autumn frosts fall by October 15 (Kyuchukova *et al.*, 1983). In the applied variety structure and growing technology of red pepper for grinding the fruits have not often reached a complete technological maturity and total biological potential of maximum pigment quantities has not been realized prior to this date. Furthermore a part of the fruits are not yet wilted. In order to be accumulated more pigments Hristov et al. (1977) recommended the harvesting to be carried out 20-30 days later. But there is a certain risk for covering the crops with frost. Due to this fact we set ourselves the task of studying the effect of the frost on total pigment content in different by origin varieties of red pepper for grinding, analyzed immediately after harvesting and after four-mount storage of dried fruits.

## MATERIAL AND METHODS

The experiment was performed in 1996 and 1998 with the varieties 'Buketen 50', 'Gorogled 6' (Bulgaria), 'Negral', 'Belrubi' (Spain), 'Myhaliteleki' and 'Kalocsai 801' (Hungary). The plants were grown in four replications in block method and the adapted for the country technology was used. The non-frostbitten fruits harvesting was carried out on October 13 during 1996 and on October 28 during 1998. The first frost fell on November 12 1996 and on $6^{th}$, $9^{th}$ and $10^{th}$ November 1998 and the harvestings were carried out 3-4 days later. The average samples of 0.500 kg from each replication were dried at once after harvesting at $50^{0}$C in ventilation evaporator. The total pigments content was determined after ASTA-19 method, modified by Manuelyan (1979). The analysis was performed after harvesting and after four-month storage of dried fruits at room temperature 6-10°C. The obtained results were processed by two-way analysis of variance (Lidanski, 1988) and Duncan's multiple range test (1955).

## RESULTS AND DISCUSSION

The first experimental year is more favorable in climatic condition for growing of red pepper for grinding (Figure 1). There is a month between the non-frostbitten fruit harvesting and having the frost. The varieties, which have not reached their maximum possibilities for pigment accumulation, continue to synthesize new one during this period. This can be observed in the varieties 'Buketen 50', 'Gorogled 6' and 'Negral' (table 1). In the variety 'Negral' even after the frost there is a considerable amount of chlorophyll in comparatively ripe red fruits. The variety 'Belrubi' is typically indeterminate with the longest period of formation, ripening and pigment accumulation. In view of this a month after the first harvesting the fruits have lower pigment content because the average sample includes a great number of light-red fruits, decreasing the total carotenoids content. The Hungarian varieties are comparatively earlier ripening and they have reached the maximum pigments accumulation in the first harvesting.

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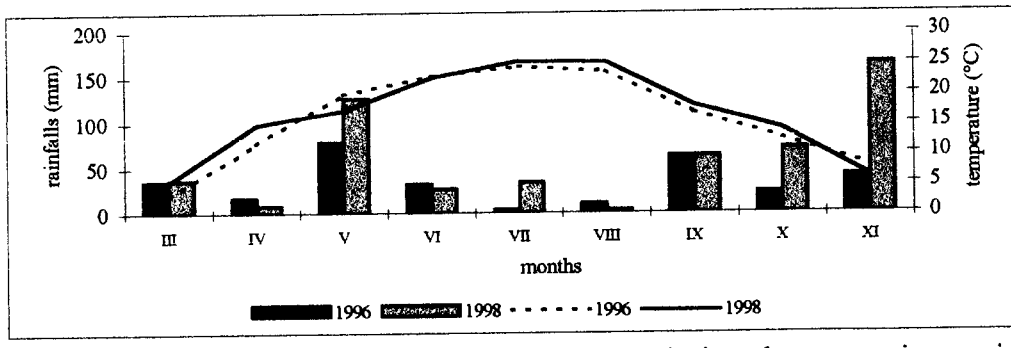



Figure 1. Average month temperature and rainfall quantity during the vegetation period of red pepper for grinding

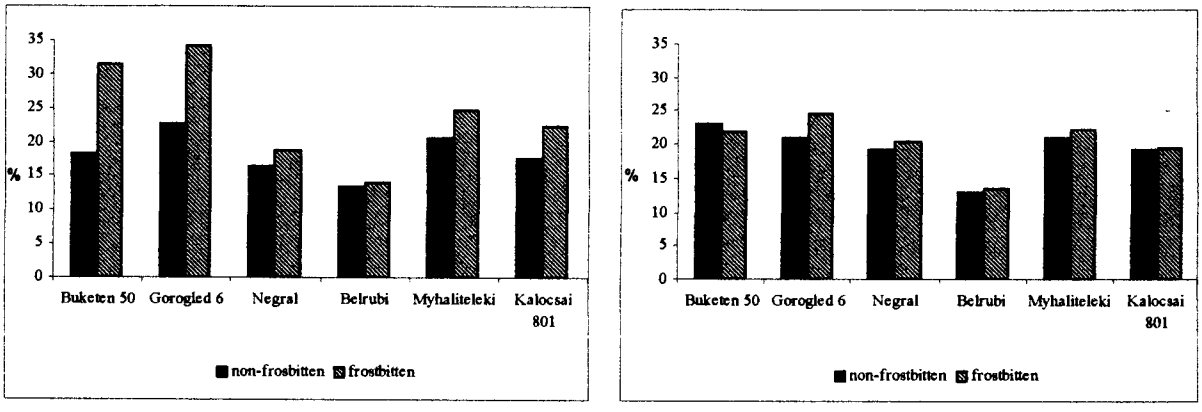
Table 1. Total pigment content of studied red pepper varieties

| Variety | After harvesting | | After four-month storage (ASTA) | | Remained colour after storage (%) | |
|---|---|---|---|---|---|---|
| | Non-frostbitten pepper | Frostbitten pepper | Non-frostbitten pepper | Frostbitten pepper | Non-frostbitten pepper | Frostbitten pepper |
| | 1996 | | | | | |
| Buketen 50 | 181 a | 190 a | 170 a | 157 a | 93.92 | 82.63 |
| Gorogled 6 | 138 d | 146 b | 132 c | 129 b | 95.65 | 88.36 |
| Negral | 65 f | 85 f | 65 e | 80 d | 100.00 | 94.11 |
| Belrubi | 148 c | 131 d | 138 d | 117 c | 93.24 | 89.31 |
| Myhaliteleki | 163 b | 142 c | 160 b | 140 a | 98.16 | 98.59 |
| Kalocsai 801 | 133 e | 128 e | 130 c | 124 b | 97.74 | 96.88 |
| | 1998 | | | | | |
| Buketen 50 | 162 c | 160 a | 160 c | 147 a | 98.77 | 91.88 |
| Gorogled 6 | 135 e | 128 bc | 130 d | 120 bc | 96.30 | 93.75 |
| Negral | 139 e | 118 c | 132 d | 98 d | 94.96 | 83.05 |
| Belrubi | 194 b | 137 b | 190 b | 113 d | 97.94 | 82.48 |
| Myhaliteleki | 206 a | 153 a | 204 a | 130 b | 99.03 | 84.97 |
| Kalocsai 801 | 153 d | 136 b | 150 c | 119 bc | 98.04 | 87.50 |

The Bulgarian variety 'Gorogled 6' takes the first place according to dry matter content in the raw material from non-frostbitten and frostbitten pepper as it accumulates 22.5% and 34.0% respectively (Figure 2). 'Buketen 50', 'Myhaliteleki' and 'Kalocsai 801' also show good results. The remained colour in the non-frostbitten fruits varies from 93.24% to 100.00% after four-month storage (Table 1). The remained colour in the frostbitten pepper is lower. The difference between the remained colour of non-frostbitten and frostbitten fruits is the greatest one in the Bulgarian varieties, followed by the Spanish varieties and in the Hungarian varieties the pigment change is smallest (Figure 3).

During the second experimental year the yields are considerably lower compared to these of the first one (Todorova, 2001). As a result of the unsuitable climatic conditions due mainly to the increased rainfall amount the non-frostbitten fruit harvesting moves by two weeks later. The best adaptability to these conditions show the Hungarian varieties as the pigment content in 'Myhaliteleki' reaches 206 ASTA (table 1). There is a good reaction of the Spanish varieties too. The frost on November 6, that is with a week earlier than in the first year did not allow the

additional fruit ripening. In all studied varieties the total pigment content of the frostbitten fruits is lower.




a) b)

Figure 2. Dry matter content during 1996 (a) and 1998 (b) of red pepper after harvesting

The unsuitable climatic conditions prevent the dry matter content accumulation as in the frostbitten fruits it reaches only 24.4% in variety 'Gorogled 6' (Figure 2). By analogy with the first year the remained colour after storage of non-frostbitten fruits is with high values and varies from 94.96% to 99.03% (Table 1). In all varieties the total pigments are lower in the frostbitten pepper after four-month storage compared to the non-frostbitten one. In the Bulgarian varieties the difference between remained colour of the frostbitten and non-frostbitten fruits decreases in comparison to the first year, while in the foreign varieties the difference increases considerably and reaches to 15.46% in variety 'Belrubi' (Figure 3).


Figure 3. Difference between remained colour of non-frostbitten and frostbitten red pepper fruits after four-month storage

The results from the two-way analysis of variance show, that during the first experimental year the influence of the variety (factor A) on the ASTA units in red pepper is 95.08% after the harvesting and 97.75% after the storage (table 2). The influence of the frost (factor B) is slight. However the combination of the unsuitable climatic conditions during the second year with frost fall on a few consecutive days reflects considerably on the strength of factor B influence. The frost effect is two times longer than the variety effect immediately after harvesting, but after the storage factor B exceeds factor A with 18.20%. This shows that in spite of the quick drying of the raw material after harvesting, the frost plays important role for pigment stability during red pepper storage. Taking into consideration that the experiment is carried out under laboratory

conditions, we can suggest that in production where the stay before drying of raw material is not impossible, the negative effect of the frost on the pepper remained colour will be greater.

Table 2. Two-way analysis of variance for total pigment content of red pepper depending on variety (factor A) and frost (factor B)

| Experimental year | Treatment | Factor influences (%) | | | | |
|---|---|---|---|---|---|---|
| | | Total | Error | Variety (A) | Frost (B) | A x B |
| 1996 | After harvesting | 99.65*** | 0.38 | 95.08*** | 0.03 | 4.54*** |
| | After storage | 99.75*** | 0.25 | 97.75*** | 1.79* | 0.20 |
| 1998 | After harvesting | 94.47*** | 5.53 | 51.44*** | 25.91*** | 17.12*** |
| | After storage | 93.56*** | 6.44 | 30.04*** | 48.24*** | 15.27*** |

The variety 'Gorogled 6' reacts weaker to the frost as regards to the pigment saving compared to variety 'Buketen 50' during the two years. The results for foreign varieties are not in one direction and most probably it is due to the different strength of the frost during the experimental period.

**The following more important conclusions can be made as a result of this study.** The frost decreases remained colour during red pepper storage performs as dried fruits. The decrease depends on the frost degree combined with the climatic conditions before frost fall. In order to avoid the unfavourable effects, caused from the frost, it is necessary to bred uniform ripening varieties of red pepper for grinding with shorter vegetation period, which allows maximum realization of the biological potential for the conditions of Bulgaria.

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# STUDIES ON THE CAPSAICIN CONTENT IN CHILLI HYBRIDS

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**Abstract**

An investigation was carried out to select superior hybrid in chilli with high capsaicin content at Tamil Nadu Agricultural University, Coimbatore during June, 2000. The parents chosen were Pusa Sadabahar, Arka Lohit, PKM 1, CHD 8, Ujwala, Punjab Lal, CF 53, KDC 1, CC 3 and CC 4. The synthesis of the capsaicin content starts form the early phase of the fruit growth in all the parents and hybrids. It was non - significant at 15$^{th}$ days after flowering in both the parents and hybrids. At 30$^{th}$ days after flowering the difference in the synthesis was significant. During the later stages of the fruit growth, the capsaicin content starts declining. Dry fruit analysis revealed that the hybrid, Arka Lohit X CF 53 recorded the highest capsaicin content (0.875 %).

**Key words:** Chilli- hybrids - capsaicin

## Introduction

Chilli is one of the important spice vegetable crop grown in the world, which has two important commercial qualities. Some varieties are famous for its red colour (capsanthin); others are known for its biting pungency (capsaicinoids). India is the only country rich in many varieties with different quality factors. Among the capsaicinoids, capsaicin, also known as n - vanillyl -8 - methyl - 6 - (e) - nonenamide, is the most pungent one. It is sparingly soluble in water, but highly soluble in fats, oils and alcohol (Hoffman *et al.*, 1983). The other compounds in the capsaicinoid groups are Dihydrocapsaicin, Nordihydrocapsaicin, Homocapsaicin and Homodihydrocapsaicin. The fraction of pungency level in the fruit was studied by many workers such as Iwai *et al.* (1979), Suzuki *et. al.* (1980) and Garciglia and Alejo (1990). The pericarp contains almost all the fractions, where as the chilli seeds contain only the traces of pungency with a capsaicin content of 0.005 per cent. Iwai *et. al.* (1979) examined the fluctuation of pungent principles of hot pepper fruits of *Capsicum annuum* var. *annuum* cv. Karyastzubusta at different growth stages after flowering. The success of any breeding programme depends on the correct choice of parents. Gilbert (1958) opined that parent with higher order of mean performance would be useful in producing better genotypes. With this background an investigation was carried out to select a superior cross combination for high capsaicin content in chilli.

## Materials and Methods

The parents chosen for this study were drawn for the germplasm maintained at the Department of Vegetable Crops, Tamil Nadu Agricultural University, Coimbatore. The parent appeared to be high in capsaicin content were selected by means of biting test (Anan *et al.*, 1996). The selected parents are Pusa Sadabahar (PS), Arka Lohit (AL), PKM 1, CHD 8, Ujwala, Punjab Lal (PL), CF 53, KDC 1, CC 3 and CC 4. In total, 30 cross combinations were

made and they were raised in a randomized block design in three replications with 20 plants per replication during June, 2000. The capsaicin content at 15 DAF, 30 DAF, 45 DAF and at mature green fruit and in the dry fruit were estimated adopting the procedure given by Collins *et al.* (1995). Capsaicin content was also estimated in the fruit parts *viz.*, pericarp, placenta and seed.

**Results and Discussion**

The data recorded were statistically analysed and presented in the table. Synthesis of the capsaicin content starts form the early phase of the fruit growth in all the parents and hybrids. The synthesis was non - significant at 15$^{th}$ DAF in both the parents and hybrids. At 30$^{th}$ DAF the difference in the synthesis of capsaicin between the genotypes was significant, the highest capsaicin content was recorded in the parent Ujwala (0.384 %). The hybrid, which involves either Ujwala or CHD 8 as one of its parents exhibited highest synthesis of capsaicin [Ujwala X CHD (0.461 %) and Ujwala X Arka Lohit (0.438 %)]. The increasing trend in capsaicin content was noticed until the maturity of the green fruit. At 45$^{th}$ DAF and in the mature green fruit. The parent CF 53 was adjudged the best (0.798 % and 0.956 % respectively) . Among the hybrids, Ujwala X CF 53 recorded the highest content of capsaicin at 45$^{th}$ DAF (0.925 %) followed by Ujwala X CHD 8 (0.905 %). During the later stages of the fruit growth, the capsaicin content starts declining. Dry fruit analysis revealed that the hybrid, Arka Lohit X CF 53 recorded the highest capsaicin content (0.875 %). The surpassing of the Ujwlaa X CHD 8 (0.819 %) by hybrid Arka Lohit X CF 53 capsaicin content in the dry fruit might probably due to the lowest moisture content of the latter. The result is in accordance with Iwai *et. al.* (1979) and Fujiwake *et. al.* (1982), who opined that the capsaicin progressively accumulated during the development of the fruit, revealed maximum after 45-50 days after fruit set and then started to decline gradually.

When the fruit capsaicin content was partitioned to different parts as pericarp, placenta and seed in mature green fruit, the difference among the genotypes were non-significant except in placenta. This was in conformity with the findings of Narayanan *et al.*, (1979). In placenta, the highest capsaicin content was recorded in the hybrid Ujwala X CHD 8 (0.854 %). Ohta (1963) stated that the capsaicin accumulation specifically in secreting organs localized in the placenta and the intercellular septum of the fruit. The seeds were not the main source of the pungency, they occasionally absorb the capsaicin because of their proximity to placenta. Bosland (1995) suggested that no other plant parts except placenta synthesis capsaicin. The highest capsaicin content in placenta was acquired in the hybrid Ujwala X CHD 8 (0.854 %) followed by Arka Lohit X CF 543 (0.784 %).

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**Mean performance of parents and hybrids for capsaicin content**

| Parents and hybrids | Capsaicin content (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 15th DAF | 30th DAF | 45th DAF | Mature green fruit | Dry fruit | Pericarp | Placenta | seed |
| PS | 0.005 | 0.338 | 0.744 | 0.811 | 0.685 | 0.032 | 0.701 | 0.005 |
| AL | 0.014 | 0.374 | 0.796 | 0.844 | 0.714 | 0.025 | 0.744 | 0.006 |
| PKM 1 | 0.007 | 0.251 | 0.600 | 0.675 | 0.511 | 0.038 | 0.531 | 0.005 |
| CHD 8 | 0.013 | 0.313 | 0.720 | 0.785 | 0.580 | 0.041 | 0.662 | 0.004 |
| Ujwala | 0.004 | 0.384 | 0.715 | 0.781 | 0.636 | 0.025 | 0.698 | 0.002 |
| PL | 0.002 | 0.345 | 0.725 | 0.825 | 0.735 | 0.025 | 0.712 | 0.005 |
| CF 53 | 0.005 | 0.369 | 0.798 | 0.956 | 0.825 | 0.038 | 0.736 | 0.006 |
| KDC 1 | 0.007 | 0.342 | 0.658 | 0.721 | 0.635 | 0.065 | 0.621 | 0.004 |
| CC 3 | 0.006 | 0.336 | 0.695 | 0.792 | 0.710 | 0.052 | 0.637 | 0.007 |
| CC 4 | 0.008 | 0.345 | 0.698 | 0.836 | 0.723 | 0.062 | 0.712 | 0.006 |
| PS X AL | 0.010 | 0.304 | 0.662 | 0.712 | 0.575 | 0.039 | 0.757 | 0.005 |
| PS X PKM 1 | 0.008 | 0.302 | 0.626 | 0.706 | 0.553 | 0.035 | 0.584 | 0.007 |
| PS X CHD 8 | 0.009 | 0.336 | 0.754 | 0.827 | 0.693 | 0.046 | 0.704 | 0.007 |
| PS X Ujwala | 0.005 | 0.340 | 0.738 | 0.807 | 0.653 | 0.028 | 0.693 | 0.005 |
| PS X CF 53 | 0.006 | 0.365 | 0.765 | 0.905 | 0.814 | 0.064 | 0.758 | 0.009 |
| AL X PS | 0.012 | 0.384 | 0.817 | 0.863 | 0.733 | 0.048 | 0.757 | 0.008 |
| AL X PKM 1 | 0.007 | 0.411 | 0.814 | 0.866 | 0.746 | 0.034 | 0.768 | 0.015 |
| AL X CHD 8 | 0.009 | 0.413 | 0.816 | 0.874 | 0.745 | 0.057 | 0.773 | 0.008 |
| AL X Ujwala | 0.005 | 0.366 | 0.778 | 0.856 | 0.693 | 0.025 | 0.731 | 0.012 |
| AL X CF 53 | 0.005 | 0.425 | 0.825 | 0.987 | 0.875 | 0.036 | 0.784 | 0.015 |
| PKM 1 X PS | 0.008 | 0.353 | 0.784 | 0.842 | 0.711 | 0.074 | 0.730 | 0.003 |
| PKM 1 X AL | 0.006 | 0.413 | 0.818 | 0.866 | 0.736 | 0.039 | 0.774 | 0.004 |
| PKM 1 X CHD 8 | 0.006 | 0.297 | 0.752 | 0.809 | 0.640 | 0.047 | 0.698 | 0.002 |
| PKM 1 X Ujwala | 0.013 | 0.295 | 0.685 | 0.745 | 0.607 | 0.042 | 0.635 | 0.003 |
| CHD 8 X PS | 0.018 | 0.414 | 0.809 | 0.847 | 0.603 | 0.062 | 0.737 | 0.004 |
| CHD 8 X AL | 0.010 | 0.287 | 0.735 | 0.835 | 0.718 | 0.032 | 0.687 | 0.008 |
| CHD 8 X PKM 1 | 0.005 | 0.254 | 0.646 | 0.729 | 0.678 | 0.023 | 0.597 | 0.006 |
| CHD 8 X Ujwala | 0.018 | 0.422 | 0.812 | 0.869 | 0.641 | 0.041 | 0.756 | 0.008 |
| Ujwala X PS | 0.011 | 0.421 | 0.815 | 0.877 | 0.721 | 0.027 | 0.769 | 0.015 |
| Ujwala X AL | 0.020 | 0.438 | 0.827 | 0.877 | 0.742 | 0.058 | 0.783 | 0.005 |
| Ujwala X PKM 1 | 0.009 | 0.413 | 0.817 | 0.869 | 0.743 | 0.045 | 0.770 | 0.009 |
| Ujwala X CHD 8 | 0.014 | 0.461 | 0.905 | 0.973 | 0.819 | 0.055 | 0.854 | 0.010 |
| Ujwala X CF 53 | 0.005 | 0.425 | 0.925 | 1.021 | 0.712 | 0.042 | 0.725 | 0.005 |
| KDC 1 X CHD 8 | 0.008 | 0.358 | 0.857 | 0.952 | 0.658 | 0.035 | 0.742 | 0.004 |
| KDC 1 X CF 53 | 0.003 | 0.354 | 0.864 | 0.946 | 0.666 | 0.049 | 0.769 | 0.004 |
| PL X CC 3 | 0.004 | 0.399 | 0.875 | 0.995 | 0.789 | 0.026 | 0.785 | 0.003 |
| PL X CC 4 | 0.006 | 0.412 | 0.854 | 0.925 | 0.721 | 0.038 | 0.714 | 0.012 |
| CC 3 X AL | 0.005 | 0.356 | 0.856 | 0.956 | 0.698 | 0.061 | 0.736 | 0.006 |
| CC 3 X CHD 8 | 0.008 | 0.374 | 0.789 | 0.925 | 0.649 | 0.052 | 0.698 | 0.004 |
| CC 4 X PS | 0.006 | 0.325 | 0.769 | 0.958 | 0.645 | 0.034 | 0.640 | 0.006 |
| SE d | 0.001 | 0.005 | 0.011 | 0.014 | 0.016 | 0.014 | 0.016 | 0.023 |
| CD (0.05) | NS | 0.010 | 0.023 | 0.003 | 0.033 | NS | 0.033 | NS |

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# CONSIDERABLE AMOUNT OF NATURAL CROSS POLLINATION ON MALE STERILE LINES OF CHILLI (*CAPSICUM ANNUUM* L.)

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## Abstract

During two consecutive seasons, utilizing male sterile lines, experiments were conducted to examine extent of natural cross pollination and to compare effects of manual and natural pollination on $F_1$ seed yield of chilli (*Capsicum annuum* L.). The formation of normal amount of fruits and seeds on male sterile plants of 'MS-12' (nuclear male sterility), 'CCA-4261' (nuclear-cytoplasmic male sterility; cms) lines and their isogenic male fertile plants ('MS-12' fertile and 'PBC-534', respectively) revealed considerable amount of natural cross pollination. The results also revealed non-significant difference between natural pollination and manual pollination on number of fruit and seed set on 'MS-12' line and better effect of natural pollination on seed yield on 'CCA-4261' line because upon natural pollination significantly increased number of seeds were obtained from the fruits developed on 'CCA-4261'. Thus it may be suggested that during cms ('CCA-4261') based chilli hybrid seed production, expenditure on manual pollination can be saved, without losing the yield of hybrid seeds.

## Introduction

Hot pepper (chilli) hybrid seed production based on male sterility system although provide scope to reduce expenditure on hand emasculation, investment on manual pollination is often required (Zhao *et al.*, 1995) or would depend on extent of natural cross pollination on male sterile plants. In chilli, up to 91 % of cross pollination has been reported (Tanksley, 1984). The extent of natural out crossing in *Capsicum* is determined by several factors including genotype (Nowaczyk and Nowaczyk, 1999). At this institute, work is under progress to develop and evaluate cytoplasmic-nuclear male sterility (cms) based experimental crosses in order to identify potential male inbred(s).

Therefore, a comparative study on effects of manual and natural pollination on amount of fruit and seed set on cms line was needed in order to decide appropriate mode of pollination (manual or natural) during cms based hybrid seed production. Hence this study was conducted to examine extent of natural cross pollination on two male sterile lines and suggest possible scope to reduce expenditure on manual pollination during cms based hybrid seed production.

## Materials and Methods

### Male sterile lines

Nuclear male sterile line ('MS-12'), cytoplasmic-nuclear male sterile line ('CCA-4261') and its maintainer line ('PBC-534') were utilized during this study. The expression of male sterility in both these lines have been found to be complete and stable (Kumar *et al.*, 2001, Dash *et al.*, 2001).

### Raising of plants

During winter season of 2000-2001 and 2001-2002, 30 days old seedlings of 'MS-12' line (segregating for 50% male sterile and 50% male fertile plants), 'CCA-4261' (A line) and 'PBC-534' (B line) were transplanted inside the chilli-breeding field at the distance of 60 x 45 cm. Since planting was done inside the chilli field, male sterile plants were also apart from

others breeding materials at the distance of 60 cm. During both the seasons, manual pollination (without supplementary pollination) was carried out on the plants of 'MS-12 and 'CCA-4261' utilizing pollen from plants of 'MS-12' (fertile) and 'PBC-534' lines, respectively.

During 2001-02, single fruit progenies were raised from the seeds obtained from natural cross fruits developed on 'CCA-4261' line with unknown pollen parent(s).

**Recording of observations**

**Season 2000-2001**

Five mature fruits from one randomly selected plant of 'CCA-4261' and 'PBC-534' lines were harvested in the last week of November 2000. The observations on fruit length, fruit width, number of seeds per fruit were recorded. Similar observations were also recorded for MS-12 line utilizing 10 fruits each of both male sterile and fertile plants. Observations on fruit length, fruit width, number of seeds per fruit were also recorded on five fruits developed on 'MS-12' (sterile) and 'CCA-4261' plants through hand pollination using pollen from plants of MS-12 (fertile) and "PBC-534' lines, respectively.

**Season 2001-2002**

Total number of fruits developed through natural pollination on 'MS-12' sterile, 'MS-12' fertile, 'CCA-4261' and 'PBC-534' lines were recorded on five randomly selected plants of each line. Ten naturally pollinated fruits from plants of 'MS-12' sterile, 'MS-12' fertile, 'CCA-4261' and 'PBC-534' were harvested during last week of November 2001 and observations on fruit length, fruit width and number of seeds per fruit were recorded. Similar observations were also recorded on 10 hand pollinated crossed fruits developed using pollen from 'PBC-534' and 'MS-12' (fertile) on 'CCA-4261' and 'MS-12' (sterile) plants, respectively.

Two single fruit families derived from 'CCA-4261' and unknown pollen parent(s) were visually examined and observations on plant height, fruit length, fruit width and number of fruits per plant were recorded on 50 plants of each family.

**Statistical analysis**

For the comparison of two mean values, t tests for equal or unequal variance were applied using Microsoft Excel program.

## Results and Discussion

**Natural vs. manual pollination**

On male sterile plants of 'MS-12' line, average seed count in fruits developed through natural pollination were 64.1 and 44.5 during 2000-01 and 01-02, respectively, while 47.78 and 26.4 seeds, respectively were obtained from the fruits developed through manual pollination. During both the seasons, plants of 'CCA-4261' line also had more number of seeds from the fruits developed through natural pollination (61.6 and 105.6) than the fruits developed through manual pollination (49.8 and 27.0) (Table 1). During 2001-02, upon natural pollination, plants of 'MS-12' (sterile), 'MS-12' (fertile), 'CCA-4261' and 'PBC-534' lines produced 42.8, 62.0, 65.6 and 50.6 fruits, respectively. The difference between average fruits per plant developed on male sterile plants of 'MS-12' (42.8) and its male fertile sister plant (62.0) was statistically non-significant. Likewise, difference between average fruits developed through natural pollination on plants of 'CCA-4261' line (65.6) and 'PBC-534' line (50.6) was also found to be non-significant (Table 1). Similarly, non-significant differences between pollination treatments (manual vs. natural) were also observed for average seeds (over the season) per fruit, except on the plants of 'CCA-4261', where natural pollination produced significantly increased number of seeds per fruit (Table 1).

The comparison of average number of fruits and seeds formation following natural and manual pollination on the plants of male sterile lines ('MS-12' and 'CCA-4261') and their isogenic male fertile lines ('MS-12' fertile and 'PBC-534', respectively; Table 1) suggest considerable amount of natural cross pollination. These results also revealed non-significant difference between natural pollination and manual pollination on number of fruit and seed set on 'MS-12' line and better effect of natural pollination on seed yield on 'CCA-4261' line. This

is because, upon natural pollination significantly increased numbers of seeds were obtained from the plants of 'CCA-4261', as compared to its isogenic line ('PBC-534') (Table 1). In a similar result, natural pollination was found to be more effective than the hand pollination on seed yield of sweet pepper male sterile line (Korzeniewska and Niemirowicz, 1998). In contrast, Meshram and Mukewar (1985) and Gill and Gill (1995) reported that manual pollination gives much better result than the insect or natural pollination on seed yield. These discrepancies are expected, as a number of factors like genotype, environment, activities of pollinating insects etc. determine the hybrid seed yield in *Capsicum* (Nowaczyk and Nowaczyk, 1999). More differences for number of seed/fruit between the seasons following manual pollination (47.78 and 26.40 for 'MS-12' and 49.80 and 27.0 for 'CCA-4261') is seems to be due to the error during the manual pollination because number of seed set on MS-12 and CCA-4261 lines is comparable within the seasons. Despite of laborious exercise on the identification and removal of male fertile plants from hybrid seed production field, farmers in Punjab province are utilizing 'MS-12' line to produce natural cross pollination dependent hybrid seeds (Dash *et al.,* 2001). However, now in the light of availability of stable cms line like 'CCA-4261' and frequent occurrence of restorer gene in chilli genotypes, exploitation of cms system should be encouraged for hybrid seed production.

**Table 1. Effect of pollination treatments on fruit size, amount of fruit and seed set on male sterile and their isogenic lines of chilli**

| Line/methods of pollination | Season | Fruit length (cm) | Fruit width (cm) | Fruits/ Plant*$^{\Psi}$ | Seeds/fruit | Mean$^{\Psi}$ |
|---|---|---|---|---|---|---|
| 'MS-12' sterile (ST) | 2000-01 | 4.68 ± 0.13 | 0.95 ± 0.05 | 42.8a ± 3.93 | 64.1 ± 5.13 | 55.5a |
| Natural pollination | 2001-02 | 3.28 ± 0.04 | 0.84 ± 0.05 | | 44.5 ± 4.80 | |
| 'MS-12' fertile (FT) | 2000-01 | 3.96 ± 0.12 | 0.98 ± 0.03 | 62.0a ± 8.90 | 55.70 ± 4.88 | 51.5a |
| Natural pollination | 2001-02 | 4.78 ± 0.20 | 1.14 ± 0.06 | | 52.00 ± 3.55 | |
| 'MS-12' ST x FT | 2000-01 | 2.88 ± 0.21 | 0.75 ± 0.05 | | 47.78 ± 7.83 | 37.9a |
| Manual pollination | 2001-02 | 3.18 ± 0.10 | 0.80 ± 0.06 | | 26.40 ± 7.00 | |
| 'CCA-4261' ('A' line) | 2000-01 | 8.47 ± 0.37 | 1.28 ± 0.03 | 65.6a ± 2.14 | 61.60 ± 6.33 | 83.6b |
| Natural pollination | 2001-02 | 10.7 ± 0.53 | 1.70 ± 0.39 | | 105.6 ± 14.1 | |
| 'PBC-534' ('B' line) | 2000-01 | 9.42 ± 0.04 | 1.04 ± 0.04 | 50.6a ± 6.99 | 33.67 ± 12.33 | 57.9a |
| Natural Pollination | 2001-02 | 11.5 ± 0.56 | 1.14 ± 0.06 | | 82.20 ± 10.78 | |
| 'A 'x 'B' line | 2000-01 | 8.35 ± 0.37 | 1.40 ± 0.02 | | 49.80 ± 8.46 | 38.4a |
| Manual pollination | 2001-02 | 8.00 ± 1.80 | 1.00 ± 0.08 | | 27.00 ± 7.71 | |
| 'CCA-4261' Self pollination | 2000-01 | 2.74 ± 0.35 | 1.66 ± 0.05 | | Seedless (Parthenocarpic) | |

* Observations of 2001-2002 (i. e. fruit set during October-November, 2001); $\Psi$ same letter indicate non-significant differences between the means

## Variability in natural crosses

Several single fruit families derived from 'CCA-4261' and unknown pollen parent(s) were visually examined and within family less variability was noticed. In order to validate this visual observation, two such families were randomly selected and observations were recorded on 50 plants of each family. The differences between mean plant height of family # 1 (43.68 ± 0.97 cm) and family # 2 (68.84 ± 1.5 cm) was found to be significant. However, when means of 25 plants of family # 1 (42.71 ± 1.32 & 43.46 ± 0.57) and family # 2 (69.79 ± 1.37 & 67.89 ± 1.65) were compared among themselves, they were non-significant. Similar trends were also obtained for fruit size and number (data not shown). Thus the progenies (natural hybrids with unknown pollen parent) derived from the single fruit (harvested from 'CCA-4261') were unexpectedly less variable in terms of plant height, fruit size, shape and

number. In contrast, between families more variability was recorded. The less variability observed in the natural $F_1$ plants derived from single fruit may be explained on the basis of collection of sufficient amount of pollen from the flower of one plant and their release on flower of male sterile plant by the honey bees in single visit.

## Conclusion

The formation of normal amount of fruits and seeds on male sterile plants during the month of October-November in two consecutive seasons suggest that honeybees frequently visited flowers of both male sterile lines and released sufficient amount of pollen on stigma in single visit during October-November, which is the ideal time to produce quality seeds in Varanasi region. Thus it may be suggested that considerable amount of natural cross pollination takes place on male sterile plants of chilli and during 'CCA-4261' (cms) based chilli hybrid seed production, expenditure on manual pollination can be saved, without losing yield of hybrid seeds.

## Acknowledgements

Authors thank Dr. J.S. Hundal (PAU, Ludhiana) and Dr. Terry Berke (then at AVRDC, Taiwan) for providing seeds of 'MS-12' and 'CCA-4261' lines. We are grateful to Prof. G. Kalloo, DDG (Hort.) for providing all the facilities, as Director of this institute.

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# A NEW STABLE AND AVAILABLE CYTOPLASMIC MALE STERILE LINE OF CAPSICUM

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## INTRODUCTION

$F_1$ hybrid seed production of pepper (*Capsicum annuum*) is almost all dependent on hand pollination and makes great demands on the producer. Cytoplasmic male-sterility is useful mean that produces $F_1$ hybrid seed of many crops and horticultural plants. Male-sterility of pepper has been documented by Peterson (1958). Cytoplasmic male-sterility is dominated by a recessive rf gene interacting with S cytoplasm. Expression of this trait is sensitive to temperature and fertile pollen can be produced under cool conditions (Peterson 1958; Shifriss and Guri 1979; Shifriss 1997). Expression of male-sterility is influenced by environmental condition so that cytoplasmic male-sterility of pepper has not been utilized for $F_1$ seed production. Recently Yazawa succeeded in breeding a more stable cytoplasmic male-sterile pepper, 'P-MS' (Fig.1, 2), from Peterson's material. In this experiment we investigated stability of progeny of 'P-MS' and assessed maintainers of male-sterility or restorers of pollen fertility among commercial cultivars when crossed with 'P-MS'.

## MATERIALS AND METHODS

Yazawa produced the original male-sterile pepper by selfing and selection from Peterson's male-sterile material (MS4-11×223026-1-1) and named it 'P-MS'.

In order to confirm stability of 'P-MS' and 'Murasaki-MS' (Fig. 3), which is the progeny of 'P-MS' backcrossed 'Murasaki' several times, the following experiment was carried out. Self-pollination of 'P-MS' and 'Murasaki-MS', crosses of 'P-MS' and 'Murasaki-MS' with male-fertile pepper, and crosses of 'Shishitoh' with 'P-MS' and 'Murasaki-MS' were made in glasshouse with insect control network in July, August, and September, 2000. Stability of male-sterility was evaluated on fruit set number on the pollinated plant.

'Murasaki-MS' was spontaneously pollinated with 'CH-19 Sweet' and 'FM8' in open fields. The color of cotyledons of 'Murasaki-MS' is purple, whereas those of 'CH-19 Sweet' and 'FM8' are greenish. Progeny with purple cotyledons were considered to be produced by self-pollination and the cotyledon color of 'Murasaki-MS'×'CH-19 Sweet' and 'Murasaki-MS'×'FM8' is greenish. 'Murasaki-MS' were planted in the neighboring row to 'CH-19 Sweet' in Kyoto Univ. Kyoto experimental farm in the beginning of June. 'Murasaki-MS' and 'FM8' were planted in Kyoto Univ. Takatsuki experimental farm in the middle of June. From October to November fruits of 'Murasaki-MS' were harvested at random. We sowed the seeds obtained from 'Murasaki-MS' and checked cotyledon color. 'Murasaki-MS' was also spontaneously pollinated with 'Murasaki' to maintain male-sterility and when these progeny began to flower, we checked for the existence of pollen.

Capsaicinoid content of pepper fruit was determined using a HPLC. Fruit was freeze-dried and ground to a powder using a coffee mill (MK-51M, MATSUSHITA). 0.2 mg of powder was extracted with acetone and ethyl acetate. An aliquot was then

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injected into a HITACHI HPLC and the samples developed on C-18 reverse phase columns detected at 280 nm.

In order to investigate the distribution of maintainer or restorer lines among peppers, 'P-MS' was crossed with 'Murasaki', 'Takagamine', 'Supinoza', 'CH-19 Sweet' , 'Shishitoh', and 'California Wonder' and 'Murasaki-MS' was crossed with 'Kyonami', 'Sampoh-amanaga'. After the resulting progeny began to flower, we checked them for the existence of pollen and classified them whether maintainer or restorer lines (sweet type: 'Murasaki', 'Takagamine', 'CH-19 Sweet' , 'Shishitoh', 'California Wonder', 'Kyonami' and 'Sampoh-amanaga', pungent type: 'Supinoza').

## RESULTS AND DISCUSSION

We investigated stability of male-sterility of 'P-MS' and 'Murasaki-MS'. Expression of male-sterility 'P-MS' and 'Murasaki-MS' was stable from June to September. Fruit set was not observed during the experiment. Even in September when the temperature not so high, 'P-MS' and Murasaki-MS' failed to self-fertilize in spite of hand pollination (Table 1). When 'Shishitoh' was crossed with 'P-MS' or 'Murasaki-MS', no fruit set was observed. From these results, it is clear that no seed is obtained by using 'P-MS' and 'Murasaki-MS' as a male parent. The pistils of 'P-MS' and 'Murasaki-MS' were normal because when 'P-MS' and 'Murasaki-MS' were crossed with fertile pollen, fruit setting was more than 50% (Table 1). Furthermore, 'Murasaki-MS' was spontaneously pollinated with 'CH-19 Sweet' and 'FM8' in the open fields. Color of cotyledons of all resulting progeny was greenish (Table 2). Thus, the result of cotyledon color, the progenies were hybrids. Progeny of 'Murasaki-MS' crossed with 'Mursaki' by natural pollination all had purple cotyledons and no pollen. We can therefore maintain male-sterile peppers by natural pollination. From these results, it is clear that 'Murasaki-MS' is male-sterile during growing period and is available for $F_1$ hybrid seed production as a female plant. According to Peterson(1958), cytoplasmic male-sterility of pepper is unstable under cool conditions. But in this experiment self-pollinated progeny of 'P-MS' and 'Murasaki-MS' were not obtained during the growth period. We conclude that 'P-MS' and 'Murasaki-MS' are stable cytoplasmic male-sterile materials and available for $F_1$ seed production.

Many pepper breeders in USA, Japan, etc. want to use non-pungent(sweet) cytoplasmic male-sterile lines. Capsaicinoid content in the fruits of 'P-MS', 'Murasaki', and 'P-MS'×'Murasaki' was 2339, 0, 1070 μg/gDW, respectively. The progeny of 'P-MS' backcrossed with 'Murasaki' four times had no pungency (Table 3). Pungency of 'P-MS' can be eliminated by backcrossing non-pungent pepper and maintainer, 'Murasaki'. We can use 'Murasaki-MS' as the male-sterile line for $F_1$ hybrid seed production of non-pungent cultivar.

Secondly, we investigated distribution of maintainer or restorer line among some cultivated peppers. Some commercial varieties are either maintainers (rfrf) or restorers (RfRf) (Table 4). 'California Wonder' is considered to be a maintainer, but in this experiment the progeny of 'P-MS' crossed with 'California Wonder' sometimes produced fertile pollen. 'California Wonder' may be heterozygous.

'P-MS' and 'Murasaki-MS' were stable male-sterile through their growing period and 'Murasaki-MS' is non-pungent. 'Murasaki-MS' is considered to be a material that produces $F_1$ hybrid seed. From the results of the experiment, restorer lines of sweet cultivars and maintainer lines of hot cultivars have been found, $F_1$ seed production of peppers may be greatly enhanced by using these materials.

*Table 1 – Fruit set number of male sterile peppers by pollination through the growing period*

| | June | | August | | September | |
|---|---|---|---|---|---|---|
| | Number of crossing flower | Number of fruit setting | Number of crossing flower | Number of fruit setting | Number of crossing flower | Number of fruit setting |
| 'P-MS' x 'P-MS' | 30 | 0 | 30 | 0 | - | - |
| 'Murasaki-MS' x 'Murasaki-MS' | 43 | 0 | 41 | 0 | 30 | 0 |
| 'Shishitoh' x 'P-MS' | 30 | 0 | 30 | 0 | - | - |
| 'Shishitoh' x 'Murasaki-MS' | 30 | 0 | 30 | 0 | 30 | 0 |
| 'P-MS' x 'Shishitoh' | 49 | 27 | - | - | - | - |
| 'Murasaki-MS' x 'Murasaki' | 49 | 27 | - | - | 30 | 19 |

- Crossing was not carried out in August and September 2000

*Table 2 – Hybridization rate of the cytoplasmic male-sterile as female and restorer lines as male in the open fields.*

| Crosses | Total plants | Hybridization rate (%) |
|---|---|---|
| 'Murasaki-MS' x 'FM8' | 400 | 100 |
| 'Murasaki-MS' x 'CH-19 Sweet' | 3.900 | 100 |

Hybridization was checked with the color of cotyledons

*Table 3 – Capsaicinoids content in the fruit of 'P-MS', 'Murasaki', 'P-MS' x 'Murasaki' and 'Murasaki-MS'.*

| | Capsaicin | Dihydrocapsaicin | Total capsaicinoid |
|---|---|---|---|
| 'P-MS' | 1.356 | 1.043 | 2.399 |
| 'Murasaki' | 0 | 0 | 0 |
| 'P-MS' x 'Murasaki' | 616 | 454 | 1.070 |
| 'Murasaki-MS'* | 0 | 0 | 0 |

* The progeny of 'P-MS' backcrossed with 'Murasaki' Four times.

*Table 4 – Distribution of maintainer or restorer cultivars among peppers.*

| Maintainer | Restorer | Unstable |
|---|---|---|
| 'Murasaki' | 'CH-19 Sweet' | 'California Wonder' |
| 'Takagamine' | 'Shishitoh' | |
| 'Supinoza' | | |
| 'Kyohnami' | | |
| 'Sampoh-amanaga' | | |

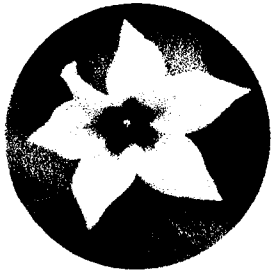

Fig. 1 – A flower of 'P-MS'

Fig. 2 – A fruit of 'P-MS'

Fig. 3 – Fruits of 'Murasaki-MS' (backcrossed with 'Murasaki-MS four times)

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# CORRELATION AND PATH COEFFICIENT ANALYSIS IN CHILLI (*CAPSICUM ANNUUM* L.)

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## INTRODUCTION

Chilli (*Capsicum annuum* L.) is an important vegetable as well as spice crop, widely grown throughout India. As India is the secondary centre of origin, a lot of natural variability exists in this crop. Chilli is a facultative cross pollinated crop with high natural cross pollination and this also contributes to its variability. Before venturing in to a breeding programme through selection it is essential to ascertain the importance and inter-association of various components and their association with yield. Estimation of inter relationship of yield with other traits and correlation studies would facilitate effective selection for simultaneous improvement of one or many yield contributing characters. Assessing the direct and indirect effects of each component towards yield through path coefficient analysis would help in identifying the reliable characters contributing to yield. With this objective in view the present investigation was undertaken.

## MATERIALS AND METHODS

Present investigation was carried out at the College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, India. The materials for the study consisted of 37 genotypes of chilli collected from different agro-climatic regions of the country. The experiment was conducted in Randomized Block Design (RBD) with three replications. Plot size was 2.25 m x 0.90 m with a spacing of 45 cm x 45 cm. Ten plants were maintained in each plot. Seeds were sown on raised nursery beds during October 2000. The seedlings were transplanted during November 2000 when they were one month old; with one seedling per pit. All recommended cultivation practices were followed to raise the crop under irrigated conditions. In each genotype, five plants were selected at random excluding the border plants for recording the observations on 12 characters viz. average fruit weight (g), number of fruits per plant, fruit yield per plant (g), plant height (cm), 100-seed weight (g), fruit length (cm), fruit girth (cm), number of secondary branches, number days to first flower, number of flowers per plant, duration of flowering/fruiting span (days) and duration of crop (days). Mean values of the data were used for statistical analysis. Correlation coefficients were computed using the method according to Al-Jibouri *et al.* (1958). The direct and indirect effects of component characters on yield were estimated through path analysis technique (Wright, 1954).

## RESULTS AND DISCUSSION

Yield is a complex character influenced by a number of other component characters. The extent of relationship between yield and its component traits as well as among the component traits is revealed through correlation analysis. Improvement of characters with high correlation to yield can lead to significant increase in yield.

The genotypic correlations were higher than the phenotypic correlations (Table 1) for most of the characters indicating that phenotypic expression for the correlation is reduced by the influence of environment despite inherent association between various characters. Similar

observations were made by Sundaram and Ranganathan (1978), Rao and Chhonkar (1981) and Choudhary *et al.* (1985). The genotypic correlation of yield per plant was positive with average fruit weight, number of fruits per plant, number of flowers per plant, number of primary branches, number of secondary branches, plant height, 100-seed weight, fruit length, fruit girth, fruiting span and crop duration. Sundaram and Ranganathan (1978), Choudhary *et al.* (1985), Gopalakrishnan *et al.* (1985), Kaul and Sharma (1989), Bhagyalakshmi *et al.* (1990), Ali (1994), and Rani (1995) also reported such positive and significant association of above characters with yield per plant. Yield per plant was negatively correlated with days to first flower indicating that selection for earliness can lead to an increase in yield. Similar view was expressed by Singh and Singh (1970) and Bhagyalakshmi *et al.* (1990). However, positive correlation was reported by Sundaram and Ranganathan (1978). Days to first flower had high negative correlation with fruiting span and crop duration suggesting that the early flowering genotypes had longer duration of fruit production and life span. The correlation of fruiting span and crop duration with yield was high and positive suggesting that increased fruiting span and life span can lead to increased yield.

The genotypic correlation can at times be misleading because it may not indicate the actual effect of one character upon another. Path analysis provides information on the real nature of association of several yield related characters contributing to yield, by separating the genotypic correlation into direct and indirect effects.

The direct effects of average fruit weight, number of fruits per plant and crop duration were high and positive. The direct effect of average fruit weight was positive and much higher than its genotypic correlation with yield. Its indirect effect through crop duration was high and positive indicating that direct selection for average fruit weight and indirect selection for crop duration can increase yield. Rao and Chhonkar (1981) also observed direct effect of fruit weight. Number of fruits had high and positive direct effect, very close to its genotypic correlation with yield indicating that the correlation represents a true relationship between the two traits. It exerted positive indirect effect through days to first flower and crop duration while its contribution through fruiting span and average fruit weight was negative. Positive direct effect of number of fruits was supported by Gill *et al.* (1977), Sundaram and Ranganathan (1978) and Munshi *et al.* (2000). Korla and Rastogi (1977) found negative indirect effect through average fruit weight. Crop duration exerted high positive direct effect on yield. Its indirect effect through fruiting span was high and negative, leading to a lower genotypic correlation with yield. The indirect effect through days to first flower and average fruit weight was positive.

Days to first flower showed a very high negative direct effect on yield though its correlation with yield was much smaller and negative. This strong negative direct effect might have been subdued by its strong positive indirect effect through fruiting span. This led to the conclusion that early flowering varieties produced higher yields. The negative direct effect of days to first flower was supported by the findings of Gill *et al.* (1977) and Sundaram and Ranganathan (1978). Fruiting span exerted a strong negative direct effect, though its correlation with yield was positive. The high negative direct effect was nullified by the strong positive indirect effects through days to first flower and crop duration. This was supported by Pandian and Sivasubramanian (1978) who obtained negative correlation for flowers produced in later stage with total number of fruits per plant. The early yield (from first two harvests) was an important factor contributing to total yield and this might have undermined the importance of fruiting span. So the genotypes producing higher fruit yield within the shortest period appeared better than that with a long fruiting span. The residual value was low (0.0810) indicating that most of the important component characters contributing to yield were included in the study. Rao and Chhonkar (1981) and Munshi *et al.* (2000) also observed low residual value in their study. Based on correlation and path analysis studies, it could be

concluded that selection for average fruit weight, number of fruits per plant, crop duration, early flowering and yielding types might lead to increase in yield.

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| Characters | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | $X_{11}$ | $X_{12}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Average fruit weight ($X_1$) | 1.0000 | **-0.1694** | **0.3615** | **0.4216** | **0.7896** | **0.5665** | **0.0440** | **-0.0962** | **-0.2945** | **0.0542** | **0.5667** | **0.6436** |
| No. of fruits per plant ($X_2$) | -0.1644 | 1.0000 | **0.1589** | **0.2576** | **-0.2108** | **0.6593** | **0.3644** | **0.7173** | **-0.1528** | **0.5969** | **0.2950** | **0.3498** |
| 100-seed weight ($X_3$) | 0.3300 | 0.1538 | 1.0000 | **0.2920** | **0.1281** | **0.3411** | **0.4117** | **0.0881** | **0.1489** | **0.1535** | **0.0628** | **0.2869** |
| Fruit length ($X_4$) | 0.4172 | 0.2409 | 0.2718 | 1.0000 | **-0.0138** | **0.4767** | **0.5520** | **0.3695** | **-0.0930** | **0.4985** | **0.3021** | **0.4024** |
| Fruit girth ($X_5$) | 0.7619 | -0.1870 | 0.1257 | 0.0002 | 1.0000 | **0.4458** | **-0.3120** | **-0.1484** | **-0.3345** | **-0.0444** | **0.4921** | **0.4800** |
| Yield/plant ($X_6$) | 0.5572 | 0.6640 | 0.3076 | 0.4630 | 0.4280 | 1.0000 | **0.2179** | **0.5666** | **-0.3764** | **0.5314** | **0.6516** | **0.7017** |
| Plant height ($X_7$) | 0.0461 | 0.3193 | 0.3761 | 0.4890 | -0.2436 | 0.1985 | 1.0000 | **0.2806** | **0.2517** | **0.5103** | **-0.0129** | **0.2556** |
| No. of Secondary branches ($X_8$) | -0.0686 | 0.5965 | 0.0718 | 0.2913 | -0.1100 | 0.4813 | 0.2503 | 1.0000 | **-0.0821** | **0.7212** | **0.2413** | **0.3319** |
| Days to first flower ($X_9$) | -0.2813 | -0.1557 | 0.1427 | -0.0931 | -0.3239 | -0.3669 | 0.2170 | -0.0898 | 1.0000 | **-0.0452** | **-0.8331** | **-0.3320** |
| No. of flowers per plant($X_{10}$) | 0.0604 | 0.5706 | 0.1421 | 0.4802 | -0.0430 | 0.5159 | 0.4506 | 0.5865 | -0.0418 | 1.0000 | **0.2670** | **0.3905** |
| Fruiting span ($X_{11}$) | 0.5489 | 0.2741 | 0.0674 | 0.2919 | 0.4819 | 0.6174 | -0.0075 | 0.1830 | -0.8080 | 0.2578 | 1.0000 | **0.7993** |
| Crop duration ($X_{12}$) | 0.5945 | 0.2982 | 0.2672 | 0.3703 | 0.4439 | 0.6214 | 0.2043 | 0.2037 | -0.2521 | 0.3648 | 0.7663 | 1.0000 |

**Table 1. Genotypic and phenotypic correlation coefficients**
(Genotypic correlation coefficients in bold & phenotypic correlation coefficients in normal figures)

| Characters | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | $X_{10}$ | Genotypic correlation coefficient |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Average fruit weight ($X_1$) | **0.6581** | -0.1119 | -0.0142 | 0.0023 | 0.0818 | -0.0118 | 0.5712 | 0.0041 | -1.6773 | 1.0642 | 0.5665 |
| Number of fruits ($X_2$) | -0.1115 | **0.6608** | -0.0062 | 0.0014 | -0.0218 | 0.0880 | 0.2964 | 0.0455 | -0.8716 | 0.5784 | 0.6593 |
| 100-seed weight ($X_3$) | 0.2379 | 0.1050 | **-0.0392** | 0.0016 | 0.0133 | 0.0108 | -0.2888 | 0.0117 | -0.1855 | 0.4744 | 0.3411 |
| Fruit length ($X_4$) | 0.2775 | 0.1702 | -0.0114 | **0.0054** | -0.0014 | 0.0453 | 0.1804 | 0.0380 | -0.8925 | 0.6653 | 0.4767 |
| Fruit girth ($X_5$) | 0.5196 | -0.1393 | -0.0050 | -0.0001 | **0.1036** | -0.0182 | 0.6488 | -0.0034 | -1.4539 | 0.7937 | 0.4458 |
| No. of secondary branches ($X_6$) | -0.0633 | 0.4740 | -0.0035 | 0.0020 | -0.0154 | **0.1226** | 0.1592 | 0.0550 | -0.7129 | 0.5488 | 0.5666 |
| Days to first flower ($X_7$) | -0.1938 | -0.1010 | -0.0058 | -0.0005 | -0.0347 | -0.0101 | **-1.9396** | -0.0034 | 2.4614 | -0.5489 | -0.3764 |
| Number of flowers ($X_8$) | 0.0357 | 0.3944 | -0.0060 | 0.0027 | -0.0046 | 0.0884 | 0.0877 | **0.0763** | -0.7888 | 0.6457 | 0.5314 |
| Fruiting span ($X_9$) | 0.3736 | 0.1949 | -0.0025 | 0.0016 | 0.0510 | 0.0296 | 1.6158 | 0.0204 | **-2.9545** | 1.3216 | 0.6516 |
| Crop duration ($X_{10}$) | 0.4236 | 0.2311 | -0.0112 | 0.0022 | 0.0497 | 0.0407 | 0.6439 | 0.0298 | -2.3615 | **1.6534** | 0.7017 |

(Residual, R = 0.0810 Figures in bold are the direct effects)

**Table 2. Path coefficient analysis**

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# STUDIES ON VARIABILITY AND CHARACTER ASSOCIATION FOR DIFFERENT TRAITS IN SIX GENERATIONS OF THE CROSS 'LCA 301 X PUNJAB LAL' (*Capsicum annuum* L.) UNDER TWO ENVIRONMENTS WITH RESPECT TO LEAF CURL COMPLEX

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## Abstract

A crossing programme involving LCA -301, susceptible to leaf curl complex and Punjab Lal, resistant to leaf curl was initiated to raise six generations namely $P_1$, $P_2$, $F_1$, $F_2$, $BC_1$ and $BC_2$.Two experiments (under epidemic and non epidemic environment) were set up in " compact family block design" and observations pertaining to morphological traits, yield traits and qualitative traits were recorded. High heritability coupled with high genetic advance was reported in total fresh yield per plant, under both the environments. Total fresh yield per plant was having positive and significant correlation with total dry yield per plant under control environment. Under epidemic, fruit diameter, total dry yield per plant and chlorophyll content in leaf showed positive and significant association.

**Key Words**: - Variability, Correlation, *Capsicum annuum* L , Leaf Curl.

## Introduction

'Chilli' is affected by a conglomeration of parasitic and non-parasitic diseases causing considerable economic loss. Among the diseases, reported in pepper, leaf curl complex have become increasingly important in recent years. Most of the reported resistant sources are becoming susceptible with the rapid development of new pathogenic races. Keeping the importance of chillies as spice, vegetable and pickle and in view of the dearth of information on genetic architecture of breeding population and character association, the present investigation was undertaken.

## Materials and Methods

On the basis of the reaction of forty genotypes of chilli to leaf curl complex resistance, two genotypes namely LCA-301 and Punjab Lal from susceptible resistant category respectively, having good yield traits were chosen for the inheritance studies. A crossing programme involving the mentioned parents was initiated to raise six generations namely $P_1$ ,$P_2$ , $F_1$, $F_2$ ,$BC_1$and $BC_2$ generations at the vegetable research farm, Department of Horticulture, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India. In the ensuing cropping season (June/July,1996) the susceptible line i.e. LCA 301 was taken as female parent and crossed with Punjab Lal, the resistant line. During mid February (1997), the $F_1$ plants were raised and transplanted to the well prepared crossing block at the vegetable research farm during mid March 1997. All recommended package of practices were carried out to raise a healthy crop. The $F_1$ was backcrossed to their corresponding parents to generate $BC_1$ and $BC_2$ progenies. Some of the $F_1$ s were selfed to produce $F_2$ seeds. In addition, fresh $F_1$'s were produced for the final trial.

During June-July 1997, two experiments (under epidemic and non-epidemic environment) each having three replications were set up in "compact family block design". Experiment 'A' comprised of six progenies i.e., $P_1$, $P_2$, $F_1$, $F_2$, $BC_1$ and $BC_2$ of the family LCA 301x Punjab Lal. For $P_1$,$P_2$ and $F_1$ there were three rows, for $F_2$ , $BC_1$ and $BC_2$ there were ten rows in each block; each row having 15 plants, with a spacing of 60cm x 45cm. Spreader rows of a highly susceptible line (LCA-206) to leaf curl virus, were planted after three rows and also around the entire experimental plots on three separate dates to create a local epidemic. Mechanical inoculation was also carried out to facilitate disease development. Recommended standard agronomic practices were followed to raise a good crop. A similar experimental setup i.e 'B' was carried out in isolation, in absence of spreader rows and by controlling the vectors i,e white flies, mites etc. responsible for the spread of the disease. The main objective behind was to find out yield potentiality of the lines obtained in disease free condition.

Observations on various parameters were recorded on randomly selected plants viz. 20 from each of the parents $P_1$, $P_2$ and $F_1$ s; 50 from each back cross progenies ($BC_1$ and $BC_2$) and 100 from $F_2$ in each replication. Incidence of leaf curl complex was recorded in the entire plant population and intensity of disease was worked out as per reference of Banerjee and Kalloo (1987). The border plants from each row

were excluded from observation. The parameters of variability like grand mean, range, phenotypic and genotypic coefficient of variation (as per the method suggested by Burton and Devance, 1953), broad sense heritability (Burton and Devance,1953) and genetic advance (Johnson *et al,* 1955) were studied under both the environments. Correlation coefficients (pulled) were calculated at the phenotypic level.

**Results and Discussion**

Good amount of variability was observed for all the characters under study (Table: 1). Maximum range was observed for total fresh yield per plant under both the environments, while the minimum was noticed for fruit diameter (under control) and hundred seed weight (under epidemic). In control as well as under epidemic, the highest coefficients were obtained for leaf curl incidence while lowest values were observed for chlorophyll content in leaf (under control) and ascorbic acid content (under epidemic). Narrow difference between GCVand PCV values for most of the traits, indicated their low sensitivity to environment. Over all, the findings were in agreement with those of Barai and Roy (1989) and Pitchaimuthu and Pappiah (1992). Heritability estimates were high for all the characters under study, the highest being observed in leaf curl incidence under both the environments. Enhanced genetic advance with high heritability was reported in total fresh yield per plant, leaf curl incidence and number of pods per plant under both the environments. Rest of the characters showed high heritability with low to moderate genetic advance. High heritability coupled with high genetic advance is indicative of greater proportion of additive genetic variance and consequently a high genetic gain is expected from selection under such a situation (Singh and Rai, 1981). The characters which exhibited high heritability with moderate or low genetic advance can be improved by intermating the superior genotypes in the segregating populations developed from multiple crosses and the desirable genes can be accumulated in the lines (Gopalakrishnan *et al*, 1987; Jiang *et al*., 1987; Bhagyalakshmi *et al*., 1990). In the present investigation, an attempt was made to study the inter relationships of morphological traits with quantitative traits. Correlation coefficients at the phenotypic level were worked out by pulling all the progenies together under the cross combination for various traits (Table:2). Total fresh yield per plant was having positive and significant correlation with total dry yield per plant under control environment. Under epidemic, fruit diameter, total dry yield per plant and chlorophyll content in leaf showed positive and significant association, whereas days to 50 percent flowering and number of primary branches exhibited negative and significant correlation with total fresh yield per plant. Similar findings were reported by Despetre *et al* (1986) and Bhagyalakshmi *et al* (1990). A perusal of the data revealed that larger number of characters were found to be positively and significantly associated with total fresh yield per plant under epidemic environment than control. This may be due to the presence of greater number of desirable segregants under diseased situation. The genotypes used included improved strains evolved through direct selection and thus had fortification of desirable genes in a specific manner. When favourable and unfavourable genes are linked together, breakage of linkage in $F_2$ or in further segregating generations, would tend to reduce the magnitude of the associations.

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**Table1: Grand mean, range, genotypic and phenotypic coefficient of variation, heritability ($h^2$%) and genetic advance for different traits in six generations of the cross LCA 301 x Punjab Lal.**

| Scale | Env. | Morphological traits | | | | | | | | | Yield traits | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Days taken for germination | Plant height (cm) | No. of primary Branches | Leaf area ($cm^2$) | Days to 50% flowering | Days to fr. set to fr. maturity | Pedicel length (cm) | Chlorophyl content (leaf) | Chlorophy content (fruit) | No. of fr./plant | Fruit Length (cm) | Fruit dia. (cm) |
| Grand mean | C | 11.41 | 63.61 | 3.77 | 10.69 | 61.67 | 33.48 | 2.91 | 52.38 | 20.58 | 96.91 | 4.16 | 0.78 |
| | E | - | 57.56 | 3.72 | 10.04 | 64.01 | 37.04 | 2.90 | 44.46 | 19.50 | 83.04 | 4.11 | 0.77 |
| Range | C | 10.74-12.85 | 54.32-76.15 | 3.60-4.13 | 8.11-14.39 | 58.87-66.18 | 31.42-37.15 | 2.66-3.20 | 51.56-53.00 | 18.29-23.98 | 68.88-110.25 | 3.56-4.78 | 0.76-0.83 |
| | E | - | 51.98-65.09 | 3.58-4.14 | 7.92-12.40 | 60.02-72.23 | 32.50-45.37 | 2.61-3.15 | 33.00-50.28 | 18.13-20.34 | 56.80-101.36 | 3.49-4.61 | 0.69-0.81 |
| GCV | C | 6.55 | 12.63 | 5.20 | 19.86 | 4.07 | 6.37 | 6.14 | 1.20 | 9.79 | 15.91 | 10.84 | 3.62 |
| | E | - | 9.00 | 5.72 | 15.38 | 7.02 | 12.36 | 6.73 | 13.63 | 4.67 | 20.45 | 10.47 | 5.78 |
| PCV | C | 6.57 | 12.70 | 5.26 | 19.90 | 4.23 | 6.51 | 6.39 | 1.40 | 9.99 | 16.91 | 10.87 | 3.85 |
| | E | - | 9.04 | 5.73 | 15.39 | 7.06 | 12.43 | 6.75 | 13.98 | 4.85 | 20.61 | 10.47 | 5.93 |
| ($h^2$)% | C | 99.40 | 98.80 | 97.70 | 99.50 | 92.60 | 95.80 | 92.30 | 85.71 | 96.00 | 88.50 | 99.40 | 88.00 |
| | E | - | 98.90 | 99.50 | 99.90 | 98.80 | 98.80 | 99.70 | 95.00 | 92.70 | 98.40 | 99.90 | 94.90 |
| GA | C | 1.54 | 16.45 | 0.40 | 4.36 | 4.97 | 4.30 | 0.35 | 0.39 | 4.07 | 29.88 | 0.93 | 0.05 |
| | E | - | 10.61 | 0.44 | 3.18 | 9.20 | 9.37 | 0.40 | 12.17 | 1.80 | 34.69 | 0.88 | 0.09 |
| GA as % of mean | C | 13.50 | 25.86 | 10.61 | 40.79 | 8.06 | 12.84 | 12.03 | 2.74 | 19.78 | 30.83 | 22.36 | 6.41 |
| | E | - | 18.43 | 11.83 | 31.67 | 14.37 | 25.30 | 13.79 | 27.37 | 9.23 | 41.78 | 21.41 | 11.69 |

| Scale | Env. | Yield traits | | | | | Quality traits | | | | | Disease Incidence |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Fresh wt. Of fruit (g) | Total fresh Yield/pl. (g) | Dry weight of fruit (g) | Total dry Yield/pl. (g) | No. of seeds/fr. | L/D ratio of fruit | Seed wt./fruit (g) | 100 seed weight (g) | Capsaicin content(%) | Ascorbic acid (mg/100g) | Leaf curl Incidence(C.I) |
| Grand mean | C | 2.88 | 268.71 | 0.69 | 64.74 | 80.45 | 5.34 | 0.30 | 0.36 | 0.67 | 228.48 | 9.18 |
| - | E | 2.88 | 231 .54 | 0.66 | 52.40 | 76.60 | 5.40 | 0.27 | 0.36 | 0.61 | 220.08 | 18.37 |
| Range | C | 2.22-3.54 | 240.91-297.98 | 0.53-0.84 | 58.55-72.28 | 75.78-85.79 | 4.27-6.31 | 0.28-0.32 | 0.34-0.40 | 0.56-0.93 | 217.74-245.12 | 0.35-31.45 |
| - | E | 2.25-3.34 | 184.49-263.58 | 0.51-0.77 | 41.61-61.89 | 66.12-85.90 | 4.35-6.72 | 0.25-0.30 | 0.34-0.38 | 0.48-0.91 | 208.77-230.10 | 3.70-60.45 |
| GCV- | C | 16.03 | 7. 90 | 16 .16 | 7. 96 | 4. 96 | 13 . 93 | 3.66 | 6.02 | 19.90 | 4.61 | 122.06 |
| - | E | 13.55 | 12.46 | 13.85 | 13.88 | 10.57 | 15.49 | 7.89 | 3.77 | 25.76 | 3.18 | 115.50 |
| PCV | C | 16.09 | 9.39 | 16.12 | 9.29 | 5.38 | 14.00 | 4.10 | 6.05 | 20.14 | 4.70 | 122.17 |
| - | E | 13.57 | 12.59 | 13.90 | 14.22 | 10.62 | 15.55 | 7.93 | 3.81 | 25.99 | 3.45 | 115.65 |
| ($h^2$)% | C | 99.30 | 70.60 | 99.20 | 85.68 | 85.20 | 99.00 | 79.50 | 99.20 | 97.70 | 96.50 | 99.80 |
| - | E | 99.60 | 98.00 | 99.30 | 95.20 | 99.10 | 99.20 | 98.80 | 97.70 | 98.20 | 85.00 | 99.70 |
| GA | C | 0.95 | 36.73 | 0.23 | 6.96 | 7.59 | 1.53 | 0.02 | 0.04 | 0.27 | 21.33 | 23.05 |
| - | E | 0.80 | 58.85 | 0.19 | 14.62 | 16.61 | 1.71 | 0.04 | 0.03 | 0.32 | 13.29 | 43.64 |
| GA as % of mean | C | 32.99 | 13.67 | 33.33 | 10.75 | 9.43 | 28.65 | 6.67 | 11.11 | 40.06 | 9.34 | 251.23 |
| | E | 27.78 | 25.42 | 28.79 | 27.90 | 21.68 | 31.67 | 14.81 | 8.33 | 52.46 | 6.04 | 237.56 |

**Table 2: Phenotypic correlation coefficient between different pairs of characters in six generations of the cross "LCA 301 x Punjab Lal" (*Capsicum* sp.)**

CONTROL

EPIDEMIC

| | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.960** | 0.709 | 0.853* | -0.827* | 0.597 | 0.608 | 0.508 | -0.292 | -0.497 | 0.783 | 0.606 | -0.727 | -0.680 | 0.763 | 0.239 | 0.743 | 0.731 | 0.489 | 0.584 |
| 2 | | 0.630 | 0.762 | -0.765 | 0.482 | 0.490 | 0.413 | -0.206 | -0.484 | 0.687 | 0.498 | -0.789 | -0.743 | 0.684 | 0.182 | 0.655 | 0.614 | 0.397 | 0.494 |
| 3 | 0.462 | | 0.956** | -0.938** | 0.955** | 0.951** | 0.904* | -0.801 | -0.853* | 0.967** | 0.928** | -0.251 | -0.294 | 0.953** | 0.394 | 0.969** | 0.956** | 0.910* | 0.420 |
| 4 | 0.958** | 0.647 | | -0.941** | 0.898* | 0.898 | 0.839* | -0.663 | -0.745 | 0.974** | 0.879* | -0.377 | -0.401 | 0.954** | 0.418 | 0.948** | 0.957** | 0.829* | 0.579 |
| 5 | -0.907* | -0.493 | -0.899* | | -0.877* | -0.875* | -0.813* | 0.695 | 0.814* | -0.946** | -0.861* | 0.523 | 0.554 | -0.924** | -0.384 | -0.927** | -0.906* | -0.819* | -0.442 |
| 6 | 0.658 | 0.536 | 0.731 | -0.874* | | 0.998** | 0.874* | -0.807 | -0.772 | 0.931** | 0.903* | -0.076 | -0.120 | 0.935** | 0.510 | 0.956** | 0.943** | 0.882* | 0.450 |
| 7 | 0.631 | 0.526 | 0.708 | -0.862* | 0.998** | | 0.861* | -0.792 | -0.753 | 0.929** | 0.894* | -0.086 | -0.120 | 0.939** | 0.501 | 0.958** | 0.942** | 0.868* | 0.463 |
| 8 | 0.656 | 0.699 | 0.716 | -0.856* | 0.852* | 0.849* | | -0.859* | -0.826* | 0.917** | 0.946** | 0.065 | -0.110 | 0.872* | 0.497 | 0.877* | 0.928** | 0.993** | 0.442 |
| 9 | -0.952** | -0.507 | -0.941** | 0.907* | -0.724 | -0.706 | -0.682 | | 0.875* | -0.746 | -0.850* | -0.090 | 0.023 | -0.672 | -0.499 | -0.724 | -0.772 | -0.911* | -0.058 |
| 10 | -0.862* | -0.401 | -0.794 | 0.923** | -0.750 | -0.738 | -0.794 | 0.891* | | -0.800 | -0.845* | 0.221 | 0.330 | -0.738 | -0.434 | -0.763 | -0.769 | -0.869* | 0.001 |
| 11 | 0.802 | 0.759 | 0.887* | -0.910* | 0.899* | 0.886* | 0.922** | -0.808 | -0.786 | | 0.938** | -0.312 | -0.334 | 0.970** | 0.495 | 0.971** | 0.990** | 0.908* | 0.566 |
| 12 | 0.757 | 0.569 | 0.790 | -0.949** | 0.951** | 0.947** | 0.946** | -0.800 | -0.880* | 0.931** | | -0.133 | -0.143 | 0.888* | 0.445 | 0.924** | 0.952** | 0.952** | 0.400 |
| 13 | -0.932** | -0.275 | -0.844* | 0.786 | -0.409 | -0.381 | -0.495 | 0.831* | 0.749 | -0.610 | -0.571 | | 0.924** | -0.299 | 0.159 | -0.262 | -0.206 | -0.056 | -0.216 |
| 14 | -0.871* | -0.207 | -0.774 | 0.743 | -0.340 | -0.317 | -0.466 | 0.763 | 0.705 | -0.548 | -0.524 | 0.985** | | -0.293 | 0.071 | -0.269 | -0.228 | -0.118 | -0.118 |
| 15 | 0.948** | 0.616 | 0.974** | -0.958** | 0.828* | 0.811* | 0.805 | -0.957** | -0.884* | 0.930** | 0.890* | -0.803 | -0.737 | | 0.448 | 0.976** | 0.962** | 0.857* | 0.593 |
| 16 | -0.955** | -0.507 | -0.953** | 0.881* | -0.646 | -0.625 | -0.678 | 0.901* | 0.771 | -0.824** | -0.742 | 0.911* | 0.871* | -0.935** | | 0.390 | 0.518 | 0.508 | 0.320 |
| 17 | 0.110 | 0.382 | 0.277 | -0.342 | 0.710 | 0.721 | 0.365 | -0.207 | -0.149 | 0.498 | 0.481 | 0.188 | 0.267 | 0.339 | -0.114 | | 0.968** | 0.870* | 0.541 |
| 18 | 0.780 | 0.668 | 0.863* | -0.924** | 0.963** | 0.954** | 0.901* | -0.808 | -0.784 | 0.974** | 0.959** | -0.568 | -0.498 | 0.920** | -0.795 | 0.588 | | 0.920** | 0.581 |
| 19 | 0.852* | 0.690 | 0.890* | -0.952** | 0.863* | 0.852* | 0.944** | -0.883* | -0.897* | 0.957** | 0.958** | -0.698 | -0.649 | 0.948** | -0.850 | 0.323 | 0.940** | | 0.363 |
| 20 | -0.791 | -0.181 | -0.661 | 0.808 | -0.571 | -0.560 | -0.617 | 0.819* | 0.960** | -0.584 | -0.729 | 0.737 | 0.707 | -0.754 | 0.665 | 0.040 | -0.589 | -0.746 | |

**Note: -** 1 - Days taken for germination; 2 - Days to 50% flowering; 3 - Plant height; 4 - No. of fruits per plant; 6 - Fresh weight of fruit; 7 - Dry weight of fruit; 8 - Fruit length; 9 - Fruit diameter; 10 - Number of seeds per fruit; 11 - Weight of 100 seeds; 12 - Pedicel length; 13 - Total fresh yield per plant; 14 - Total dry yield per plant; 15 - Days to fruit maturity; 16 - Chlorophyll content (leaf); 17 - Chlorophyll content (fruit); 18 - Leaf area; 19 - Fruit L/D ratio; 20 - Seed weight per fruit.

* and ** are significant at 5% and 1% levels of significance respectively.

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# SOURCES OF SALT TOLERANCE IN *CAPSICUM*

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## Introduction

High soil salinity can damage or kill young furrow irrigated chile plants (Miyamoto *et al.*, 1986). Seedlings can be killed via girdling at the soil line, or they can die when light rains move salt to the roots (Bosland and Votava, 2000). Plants can also be affected by guttation-salt injury through the adaxial surface of leaves following water splashing of salt sediments onto the leaves resulting in large necrotic areas with water-soaked borders (Bosland and Votava, 2000).

In 2000, chile seedlings in the New Mexico State University Chile Pepper Institute Teaching and Demonstration Garden suffered salt damage. Damage from salt generally occured on the leaves, petioles, and stem. Likewise, it was noted that several accessions showed few or no symptoms of salt damage. The pattern of damage did not follow any particular location within the field, but did vary depending on genotype. The information presented here is observational data that may prove useful to reseach concerned with salt tolerance in *Capsicum*.

## Materials and Methods

The 35 chile accessions evaluated contained between 4 and 12 plants. The majority of cultivars sampled belong to the species *Capsicum annuum,* but *Capsicum chinense* and *Capsicum frutescens* were also surveyed (Table 1). A scoring system was used to characterize the amount of salt damage sustained by each plant within an accession. The plants were scored as follows: 1 = 0% damage, 2 = detectable damage to 24% damage to the plant, 3 = 25% to 49% damage to the plant, 4 = 50% to 74% damage to the plant, and 5 = 75% to 100% damage to the plant. The percent susceptibility of an accession was derived by dividing the total number of plants that received a score of 2, 3, 4, and/or 5 by the total number of plants and multiplying by 100. If an accession had no plants with a score of 1, that accession was defined as 100% susceptible to salt damage. Three evaluators characterized the damage to the accessions. Comparisons of the values scored by the evaluators were made to determine if there were any differences in the way in which the plants were scored.

## Results

No differences were observed in the way the evaluators scored the plants. The results are presented in Table 1. Plants that received a score of 5, generally succumbed to salt damage. Plants that received scores of 2, 3, and 4 had variable survival rates, but the chances of survival were greater in those individuals that had lower scores (i.e., scores of 2) One cultivar, 'Balada', showed no damage to any of the plants within the cultivar. The cultivar, 'Japones', on the other hand, had no surviving plants.

## Discussion

Salt tolerance in *Capsicum* appears to vary according to genotype, but does not appear in this study to be affected by species. This may provides researchers a list *Capsicum* cultivars that could be evalulated for salt tolerance under controlled conditions and used in a breeding program.

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Table 1. Percent of plants per cultivar exhibiting salt damage and the number of plants within a cultivar receiving a particular damage score.

| **Cultivar Name** | % exhibiting symptoms | # plants 0% damage | # plants 0%-24% damage | # plants 25-49% damage | # plants 50-74% damage | #plants 75-100% damage |
|---|---|---|---|---|---|---|
| Balada* | 0 | 12 | | | | |
| Tequila Sunrise* | < 1 | 11 | | 1 | | |
| Aji Cito◆ | 17 | 5 | 1 | | | |
| Golden Treasure* | 20 | 8 | 2 | | | |
| Tepin* | 20 | 8 | | | | 2 |
| Tears of Fire* | 25 | 6 | 2 | | | |
| Equador Hot+ | 25 | 3 | | | | 1 |
| Antohi Romanian* | 25 | 9 | 3 | | | |
| Andy* | 42 | 7 | 5 | | | |
| Serrano del Sol* | 42 | 7 | 3 | | | 2 |
| NuMex Sweet* | 44 | 5 | 1 | | 2 | 1 |
| NuMex Bailey Pequin* | 71 | 2 | 3 | | | 2 |
| Habanero+ | 75 | 1 | 3 | | | 2 |
| Malagueta~ | 77 | 2 | 5 | | 1 | 1 |
| Chinese Multicolor* | 80 | 1 | 4 | | | |
| Chocolate Beauty* | 83 | 2 | 10 | | | |
| Grif 9238+ | 83 | 2 | 10 | | | |
| NuMex Big Jim* | 92 | 1 | 11 | | | |
| Peter Pepper* | 100 | | 9 | | | |
| Park's Whopper* | 100 | | 6 | | | |
| Valencia* | 100 | | 5 | | 4 | 1 |
| Sweet Ivory* | 100 | | | 1 | 1 | 7 |
| Perfection* | 100 | | 10 | 2 | | |
| Labrador* | 100 | | 4 | | | 8 |
| Lilac Hybrid* | 100 | | 5 | | | |
| Jingle Bells* | 100 | | | 1 | 8 | 1 |
| Chile Mayo* | 100 | | 1 | | | 4 |
| Blushing Beauty* | 100 | | 2 | 4 | 5 | |
| NuMex Joe E. Parker* | 100 | | 5 | 1 | | 2 |
| Granny Smith Pepper* | 100 | | 3 | | | 6 |
| Sheepnose Pimiento* | 100 | | 10 | | | 1 |
| Cobra* | 100 | | 8 | | 1 | 3 |
| Onza* | 100 | | 2 | | 1 | 5 |
| Tabasco~ | 100 | | 6 | 2 | 1 | 3 |
| Japones* | 100 | | | | | 4 |

* *Capsicum annuum.* ◆*Capsicum baccatum.*
\+ *Capsicum chinense.* ~ *Capsicum frutescens.*

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# NOVEL SOURCES OF NON-PUNGENCY IN *CAPSICUM* SPECIES

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## Introduction

Published studies concerning the inheritance of pungency in *Capsicum* species have shown its presence to be conferred by a single dominant gene, and that the lack of pungency is controlled by a single recessive gene (*pun1)* (Greenleaf, 1986; Daskalov and Poulos, 1994). About 15 years ago, scientists presented evidence that a second locus (*pun2*) for non-pungency in *Capsicum* had been found (Loaiza-Figueroa and Tanksley,1988). After this discovery, research groups around the world have unsuccessfully attempted to duplicate the results with the same accession. Remnant seed of BG 3547, the sole accession known to contain the *pun2* gene was received from S. D. Tanksley and grown under greenhouse conditions at New Mexico State University in 1994. Unfortunately, all plants produced pungent fruit, and no other sources of this accession are known. Because no source for *pun2* is available, it is impossible to determine if new sources of non-pungency contain the *pun2* gene, and because the existence of the *pun2* gene itself can no longer be verified, it must be considered non-existent.

Novel sources of non-pungency in two accessions of *Capsicum chinense*, one accession of *C. chacoense,* and one accession of *C. frutescens* are described. We suggest a mechanism for the action of at least two of these novel non-pungent sources.

## Materials and Methods

*Capsicum* accessions that come into the New Mexico State University Chile Pepper Breeding program are routinely characterized for a variety of traits, including the presence or absence of pungency. Two accessions of *C. chinense* (NMCA 30036 and PI 543190), one accession of *C. chacoense* (PI 260433), and one accession of *C. frutescens* (CATIE 9838 ) were noted as being non-pungent or containing non-pungent individuals.

The inheritance of non-pungency in the two *C. chinense* accessions was examined by planting seed of NMCA 30036, PI 543190 and seeds of a known non-pungent source - the bell pepper cultivar Keystone Resistant Giant. Plants were grown in a controlled greenhouse at the Fabian Garcia Science Center, Las Cruces, NM. After germination, seedlings were fertilized with approximately 1.5 g of slow release fertilizer. When seedlings were at the 8 to 10 true leaf stage, they were transplanted into a 1:1:1 mixture of sand:soil:peatmoss in 1.5L plastic pots. Upon flowering, reciprocal interspecific hybridizations were made with NMCA 30036 and PI 543190 to 'Keystone Resitant Giant'. Additionally, NMCA 30036 and PI 543190 were hybridized to one another to determine if the alleles conferring non-pungency in these two accessions were identical. $F_1$ seed from these hybridizations were planted as described above to produce $F_2$ populations.

Evaluation for pungency was made in the $F_1$, and $F_2$ generations. Pungency was determined by tasting mature fruit, using a panel of trained tasters. If the fruit of a plant was pungent, the taster was not allowed to sample any additional fruit that day. If the fruit was non-pungent, additional fruit from that plant were tested by at least two more tasters to confirm non-pungency.

One accession of *C. chacoense*, PI 260433 likewise was observed to contain pungent and non-pungent individuals. The non-pungent *C. chacoense* plants were hybridized to the non-pungent *C. annuum* bell pepper cultivar, CalWonder. The $F_1$ plants were evaluated for the presence or absence of pungency as described above. When the $F_2$ segregating population was examined, distortion of gene ratios occurred due to the fact that the individuals arose from the interspecific hybrid. Currently, lines are being fixed for non-pungency to re-test the novel source of non-pungency in a compatible genetic background.

In addition to *C. chinense* and *C. chacoense,* a non-pungent *C. frutescens* accession has been identified; CATIE 9838. To date, no analyses of this source of non-pungency have been accomplished.

**Results**

Only two seeds germinated from the hybridization of NMCA 30036 X 'Keystone Resistant Giant'. Plants produced from this hybridization were pungent, indicating a different source of non-pungency has been identified. Unfortunately this interspecific hybridization did not produce viable seed to evaluate the $F_2$ generation. Additional hybridizations are currently being performed to produce $F_1$, $F_2$, and test cross populations. Hybridization to a pungent *C. chinense* cultivar - 'Habanero' (PetoSeed) resulted in $F_1$ progeny that were 100% pungent, indicating that non-pungency is a recessive trait in NMCA 30036. The $F_2$ segregating population and testcross progeny are currently being evaluated.

All $F_1$ progeny of the PI 543190 X 'Keystone Resistant Giant' hybridization were pungent indicating PI 543190 is also a different source of non-pungency. $F_2$ populations and testcross populations are currently being developed. Hybridizations to known pungent *C. chinense* accessions are also being performed to further elucidate the genetic inheritance of this novel source of non-pungency.

The $F_1$ plants derived from NMCA 30036 X PI 543190 hybridizations were 100% pungent, indicating that these two novel sources of non-pungency are not the same allele and are thus two new and separate sources of non-pungency. Additional $F_2$ and testcross populations are being developed.

The *C. chacoense* accession produced pungent $F_1$ progeny, indicating a novel allele or gene producing non-pungency was present. Further genetic analyses of this non-pungent source will be made pending stabilization of the genetic background derived from the $F_2$ population.

**Discussion**

Non-pungency in *Capsicum* may result as a loss of function in the genes involved in the biochemical pathway for the production of capsaicinoids. Two non-pungent parents may produce a pungent $F_1$ via complementation of differing alleles or genes.

The two novel non-pungent *C. chinense* accessions described here have been observed as exhibiting very smooth placental walls. In pungent accessions, vesicles along the placental walls can be clearly identified and have been shown to be the site of capsaicinoid accumulation (Rowland, et al., 1983). Vesicles are also present in non-pungent varieties that contain the *pun1* allele, but these vesicles are fewer in number and less turgid than in pungent varieties. Mutations that prevent the glandular vesicles from filling may be a possible means by which the two novel non-pungent *C. chinense* exhibit non-pungency, and would explain why a hybridization with another non-pungent cultivar containing the *pun1* gene would result in pungent $F_1$ progeny via complementation. We are testing the hypothesis that alleles for what is tentatively called a loss of vesicle (*lov*) gene is responsible for non-pungency in the two accessions of *C. chinense*.

Seed of all novel non-pungent accessions will be maintained by the New Mexico State University Chile Pepper Breeding Program. Small seed samples have been and will continue to be made available to interested researchers through the Chile Pepper Institute.

Non-pungency in *C. annuum* is limited to only one known gene; *pun1*. We have demonstrated that novel sources of non-pungency exist in *C. chinense*, *C. chacoense,* and *C. frutescens*. The existence of these new sources of non-pungency add to the body of knowledge concerning pungency in *Capsicum*.

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# *IN VITRO* SHOOT AND ROOT MORPHOGENESIS FROM COTYLEDON AND HYPOCOTYL EXPLANTS OF HOT PEPPER CULTIVARS BYADAGI DABBI AND ARKA LOHIT.

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**Abstract**

Complete plant regeneration from hypocotyl and cotyledon explants of *Capsicum annuum* L. cvs. Byadagi Dabbi and Arka Lohit was obtained. Shoots and root hairs were induced 21-25 and 9-12 days after culture respectively on half strength MS medium supplemented with BAP alone or BAP in combination with IAA. Best shoot morphogenesis response was recorded on medium supplemented with 2 mg/l BAP and 1 mg/l IAA. Kinetin at all levels (0-5mg/l) failed to induce shoot regeneration. Average frequency of shoot regeneration among treatments was 1.43.Cultivar Byadagi Dabbi has shown a better regeneration response and cotyledon was proved to be a better explant with respect to *in vitro* regeneration ability.

## Introduction

Genus *Capsicum* includes group of plants, which are commercially used as spices, vegetables and colouring agents. Polyphyletic origin of capsicum and differential crossability among the wild and cultivated species has lead to high level genetic variability among capsicum lines. The production of hot and sweet peppers throughout the world are far below the actual demand (Peter, 200). Maintenance of homozygous lines is a pro-breeding requisite in any crop. Tissue culture acts as an alternative for the conventional method of raising crop in isolation (400m for foundation seed), selfing and covering flowers using cotton. Cultivar Byadagi Dabbi is less pungent, highly coloured and mainly export oriented while Arka Lohit is highly pungent and less coloured. Many studies have proved that *in vitro* regeneration in peppers is varietal (Arroyo and Revilla, 1991; Ochoa-Alejo and Moreno, 1990; Philips and Hubstenberger, 1985; Gunay and Rao, 1978; Agrawal *et al.*, 1989; Sripichitt et al; 1987; Szasz *et al.*, 1995; Christopher and Rajam, 1997; Ramirez-Malagon and Ochoa-Alejo, 1996; Ezura *et al.*, 1993) and explant (Arroyo and Revilla, 1991; Ediba and Hu, 1993; Buyukalaca and Mavituna, 1996; Christopher and Rajam, 1996) specific. Hence the present study was carried out to standardize regeneration strategies in specified pepper cultivars.

## Materials and Methods

Seeds obtained from Vegetable Breeding Unit, Indian Institute of Horticultural Research, Bangalore, India were surface sterilized by immersing

in 0.1 percent mercuric chloride for 2 minutes followed by three 5 minute washes in sterile double distilled water. Seeds were sown on the surface of hormone free Murashige and Skoog's medium (pH 5.8) solidified with 0.8 percent (w/v) of agar (Sigma) and germinated under 16 hours of photoperiod at 25°C. Cotyledon and hypocotyl explants of 2 weeks old seedlings were excised in approximately 1 cm length segments. Proximal and distal parts of cotyledon, the lower surface in contact with the medium and lower most region of hypocotyl (white in colour) devoid of root region in horizontal position were separately cultured on solidified MS medium supplemented with BAP or Kinetin (0.5, 1.0, 2.0 and 5.0 mg/l) in combination with IAA (0,0.5 and 1.0 mg/l). Concentration of cytokinins was always kept higher than that of auxins and thus a total of 16 growth regulator combinations were tried.

Cultures were maintained at 25°C under 16 hours photoperiod (fluorescent tubes from Mysore lamps at 25μ mol/$m^2$/s). Each treatment consisted of a minimum of 10 replications laid under completely randomized design. Regenerated shoots isolated along with roots from mother cultures were further grown upto 2-cm length on MS medium with no growth regulators. Plants were carefully taken out, agar rests were removed and complete plants were hardened in earthen pots with a mixture of peat and perlite (2:1) under green house conditions. High relative humidity for the seedlings was given by covering the pots with polythene bags.

**Results and discussion**

*In vitro* shoot morphogenesis response from cotyledon and hypocotyl explants of two hot pepper cultivars (Byadagi Dabbi and Arka Lohit) is presented in Table-1.

*Effect of media on shoot and root morphogenesis*

Shoot morphogenesis was observed on MS medium supplemented with a combination of BAP and IAA. At all levels (0.5, 1, 2 and 5 mg/l) in combination with IAA (0.0, 0.5 and 1.0), kinetin failed to induce any shoot morphogenesis. But abundant rooting from cut ends of hypocotyl and cotyledon even with low (0.5 mg/l) concentration of IAA was observed. BAP at a high concentration (5.0 mg/l) induced low frequency shoot regeneration only from cotyledon explants of both varieties (0.60 and 0.30 shoots/explant in Byadagi Dabbi and Arka Lohit respectively). Among different combinations of BAP and IAA tried, 2 mg/l BAP with 1 mg/l IAA induced maximum average number of shoots per explant (2.70) which was significantly superior than the next best combination of 5mg/l BAP and 1 mg/l IAA (2.05). Other two combinations (1.0 and 0.5 mg/l BAP in combination with 0.5 mg/l IAA) were commentably inferior (1.625 and 0.550) in its shoot morphogenesis capacity. Enhanced regeneration of *Capsicum annum* L. on medium containing BAP and IAA was previously appreciated by many workers (Arroyo and Revilla, 1991; Ramirez-Malagon and Ochoa – Alejo, 1996; Valera – Montero and Ochoa – Alejo, 1992; Szasz *et al.*, 1995; Zhu *et al.*, 1996; Christopher and Rajam, 1996). Average frequency of shoot regeneration was low (1.43), which justifies Steinitz *et al.* (1999) `s observation that genus *Capsicum* is highly recalcitrant for regeneration.

Long and whitish roots were induced on all explants when IAA was used in regeneration medium. The regenerated shoot tips with 1 or 2 roots when separated transferred to MS medium with no growth regulators further induced profuse rooting.

*Effect of cultivars*

Significant cultivar difference was observed in the regeneration capacity of two cultivars experimented. Cultivar Byadagi Dabbi has shown a superior regeneration (1.58 shoots per explant) compared to Arka Lohit (1.28). Varietal difference among different cultivars of *Capsicum annuum* is already reported by many workers as discussed previously.

*Effect of explants*

Superiority of cotyledon in its regeneration potential over hypocotyl is already shown (Subhash and Christopher, 1988; Fari *et al.*, 1990; Shen *et al*; 1994; Sripichitt *et al.*, 1987). In this study also, cotyledon was superior in regeneration (1.67) compared to hypocotyl (1.19).

**Conclusion**

The repetitive search in laboratories worldwide reflects a persisting dissatisfaction over regeneration response of *Capsicum* (Steinitz *et al.*,1999). This study also shows a highly recalcitrant nature of hot peppers towards regeneration but rooting was rather a ready response. Cultivars showed significant variation in their response emphasizing the need to standardize the protocols in each variety of commercial or breeding importance.

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**TABLE 1: AVERAGE NUMBER OF SHOOTS INDUCED FROM COTYLEDON AND HYPOCOTYL EXPLANTS OF HOT PEPPER CVS. BYADAGI DABBI AND ARKA LOHIT.**

| Sl. No | MS medium with growth regulators (mg/l) | Byadagi Dabbi | | Arka Lohit | |
|---|---|---|---|---|---|
| | | Cotyledon | Hypocotyl | Cotyledon | Hypocotyl |
| 1 | 5.0 BAP | $0.60\pm0.6^{kl}$ | $0.00^{m}$ | $0.30\pm0.70^{lm}$ | $0.00^{m}$ |
| 2 | 0.5 BAP+0.5 IAA | $0.80\pm0.80^{jk}$ | $0.00^{m}$ | $1.10\pm1.10^{ij}$ | $0.30\pm0.70^{lm}$ |
| 3 | 1.0 BAP+0.5 IAA | $2.40\pm0.60^{c}$ | $1.30\pm0.70^{hi}$ | $1.90\pm0.90^{def}$ | $0.90\pm1.10^{jk}$ |
| 4 | 2.0 BAP+1.0 IAA | $3.30\pm1.70^{a}$ | $2.60\pm0.60^{be}$ | $2.70\pm0.70^{bc}$ | $2.20\pm1.80^{ed}$ |
| 5 | 5.0 BAP+1.0 IAA | $2.00\pm1.00^{de}$ | $2.80\pm1.20^{b}$ | $1.60\pm0.60^{fgh}$ | $1.80\pm1.80^{efg}$ |

Number of replications - 10
Design - Completely randomized design.
a-m means within each column followed by same script are not significantly different by CRD analysis at 0.05 probability level.

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# REACTION OF DIFFERENT CHILLI (*CAPSICUM ANNUUM*) GENOTYPES TO MOSAIC VIRUS

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**Abstract**

Fifty three chilli accessions collected from various state Agricultural Universities and abroad were screened against mosaic virus. Chilli accessions widely vary in their reaction to mosaic virus. Out of 53 accessions tested only nine were resistant. Another 12 accessions showed moderate resistance and the remaining 33 accessions were susceptible.

## Introduction

Chilli (*Capsicum annuum*) is an important commercial crop, grown throughout the tropics. Chilli is known to be affected by several diseases and pests. Among them mosaic is a major constraint in chilli cultivation. The seriousness of mosaic infection stems from the fact that there is no cure for the plant, once it has become infected, and the infection can result in loss of all saleable produce from that plant. The mosaic viruses affecting the chilli are efficiently transmitted in nature by insects which are often difficult to control. Further the complication is added by the capability of significant pathogenic variation between strains of a given mosaic virus. So chilli cultivation is profitable only when the lines were resistant to mosaic. So it was decided to screen several indigenous, and exotic chilli accessions for resistant to mosaic.

## Materials and Methods

This experiment was carried out in the vegetable research farm of Department of Olericulture, College of Horticulture, Kerala Agricultural University, Vellanikkara during the period from 1996-1998. Fifty three chilli accessions, collected from various state agricultural universities and abroad were added to the germ plasm maintained at Department of Olericulture, College of Horticulture, KAU were screened against the mosaic. The seedlings were raised in sterilized soil in pots under insect proof conditions. Twenty days old seedlings were selected for the mechanical inoculation. The inoculum was prepared by grinding the young chilli leaves showing symptoms of mosaic in a mortar by adding 0.05 M phosphate buffer (pH 7.2) and 0.5 per cent sodium sulphite per gram of leaf tissue. The resultant homogenate was filtered through double layer of muslin cloth and used for inoculation. Twenty plants were inoculated in each variety and in each plant 4-5 leaves were inoculated. Plants were scored for leaf symptom expression 30 days after inoculation, and rated as completely resistant, moderately resistant, and susceptible as per George (1989).

Plants which did not show symptoms were reinoculated with virus and scored again twenty days after inoculation. The resistant genotypes were further subjected to confirmation studies like inoculation on indicator plants, back inoculation and graft transmission.

**Results and Discussion**

Results of screening 53 genotypes for mosaic resistance presented in the Table 1. Out of the 53 genotypes tested only nine viz. CA 703 (Hisar Vijay), CA 337 (Punjab Lal), CA 731 (Perennial), CA 730 (Lorai), CA 644 (Pusa sadabahar), CA 737 (PBC 148), CA 738 (PBC 204), CA 739 (PBC 375) and CA 744 (PBC 518) were free from mosaic, with the mean disease score of 1.00, indicating that the genotypes were either symptomless carriers or resistant ones. But as their sap failed to produce local lesions on *Chenopodium amaranticolor* and back inoculation failed to index back, the mosaic virus these genotypes were rated as resistant. Holmes (1954) reported that resistance may be absolute constituting natural immunity or it may involve a tendency to escape infection despite artificial infection and thus genotypes may be considered as highly resistant. Resistance of Punjab Lal and Perennial to mosaic has already been reported by Bansal *et al.* (1992). Dhawan *et al.* (1996) reported Hisar vijay as multiple disease resistant variety including mosaic. Resistance of accessions namely CA 737, CA 738, CA 739 and CA 744 also confirmed (personal communication). The chilli lines showing resistance to mosaic were originated from different geographical regions. It is possible that each of this resistant lines carry different genes for mosaic resistance. If that is the case, the genes for resistance can be combined in one line to obtain a more durable resistance.

A set of twelve accessions showed moderate infection under artificial inoculation with mean disease score between 1 and 2. They were CA 696 (CH-1), CA 710 (PBC 717), CA 715 (PBC 385), CA 734 (Arka Lohit), CA 725 (Punjab Guchedar), CA 53 (Pant C-1), CA 733 (Suryamukhi), CA 33 (Manjari), CA 219 (Ujawala), CA 740 (PBC 384), CA 746 (PBC 535) and CA (PBC 716). Pant C-1 was rated as moderately resistant by Bansal *et al.* (1992). Singh (1993) Rathaiah (1983) found the chilli line Suryamukhi as tolerant to all diseases including mosaic. Holmes (1954) has made revelation that the genotypes have a sufficient degree of tolerance which may be either due to partial suppression of viral multiplication or suppression of systematic spread or both. Remaining thirty two genotypes were susceptible to mosaic with a disease score of above 2. Susceptible ones showed the symptoms with in seven to ten days after inoculation, as slight vein clearing of expanding leaves followed by mosaic mottling. Upward cupping of leaves were observed. Some leaves showed irregular expansion of lamina along with green blisters. The internodes were shortened giving the plant a stunted appearance.

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Table 1. Reaction of 53 chilli genotypes to mosaic

| Sl.No. | Accession Number | Mean disease score | Sl.No. | Accession Number | Mean disease score |
|---|---|---|---|---|---|
| 1 | CA 33 | 1.25 | 28 | CA 728 | 2.20 |
| 2 | CA 53 | 2.00 | 29 | CA 729 | 2.50 |
| 3 | CA 67 | 2.45 | 30 | CA 730 | 1.00 |
| 4 | CA 87 | 2.50 | 31 | CA 731 | 1.00 |
| 5 | CA 94 | 2.80 | 32 | CA 733 | 2.00 |
| 6 | CA 153 | 2.10 | 33 | CA 734 | 1.64 |
| 7 | CA 186 | 2.80 | 34 | CA 737 | 1.00 |
| 8 | CA 219 | 1.20 | 35 | CA 738 | 1.00 |
| 9 | CA 337 | 1.00 | 36 | CA 739 | 1.00 |
| 10 | CA 451 | 2.60 | 37 | CA 740 | 1.05 |
| 11 | CA 452 | 2.55 | 38 | CA 744 | 1.00 |
| 12 | CA 517 | 2.50 | 39 | CA 745 | 1.10 |
| 13 | CA 591 | 2.32 | 40 | CA 746 | 1.20 |
| 14 | CA 644 | 1.00 | 41 | CA 747 | 2.90 |
| 15 | CA 695 | 2.50 | 42 | CA 748 | 2.68 |
| 16 | CA 696 | 2.00 | 43 | CA 750 | 3.00 |
| 17 | CA 698 | 2.55 | 44 | CA 751 | 2.80 |
| 18 | CA 699 | 2.45 | 45 | CA 752 | 2.62 |
| 19 | CA 701 | 3.00 | 46 | CA 753 | 2.75 |
| 20 | CA 702 | 2.25 | 47 | CA 754 | 3.00 |
| 21 | CA 703 | 1.00 | 48 | CA 755 | 2.80 |
| 22 | CA 710 | 1.40 | 49 | CA 756 | 2.25 |
| 23 | CA 714 | 2.23 | 50 | CA 757 | 2.47 |
| 24 | CA 715 | 1.35 | 51 | CA 758 | 2.20 |
| 25 | CA 716 | 2.32 | 52 | CA 759 | 2.40 |
| 26 | CA 725 | 2.00 | 53 | CA 760 | 2.20 |
| 27 | CA 727 | 2.05 | | | |

Score: 1 Completely resistant, >1 to 2 moderate infection, >2 to 3 susceptible (George, 1989)

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# CAPSICUM LEAF CURL DISEASE IN RELATION TO WEATHER VARIABLES AND ITS ECO-FRIENDLY MANAGEMENT

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## Introduction

*Capsicum* (*Capsicum annum* L.) is an important vegetable and spice crop in India which is attacked by a number of viral diseases and thus suffers heavy yield losses. Leaf curl virus disease is caused by a geminivirus, which is transmitted by whitefly (*Bemisia tabaci*) and is grouped with begomovirus diseases. Tropical climate provides ideal conditions for the perpetuation of whitefly vector. Breeding for disease resistance is time consuming and use of insecticide has not been found helpful because of non persistent nature of the virus, high cost of chemicals and residual toxicity. Therefore, the present studies were undertaken to develop a better disease management strategy through IPM by studying the functional relationship of whitefly population with weather variables and its effect on disease progress.

## Material and Methods

Four genotypes of *Capsicum* namely AVRDC 183, 'Hisar Vijay', 'Hisar Shakti' and 'Pusa Jwala' were grown under field conditions for three years (1996-99). 'Pusa Jwala', a highly susceptible cultivar was used as infector row. Observations on whitefly population and disease incidence were recorded at 10 days intervals. Whitefly population was counted on randomly selected plants using cages with one glass pane. Data on weather parameters were obtained from the observatory of the university. Regression equation for prediction of leaf curl disease was developed.

The experiments on management of disease were conducted using *cv.* 'Pusa Jwala'. The *Capsicum* seedlings in the nursery were divided into three beds and one bed each was sprayed with Malathion, Neem based insect repellent Achook and water (control) twice before transplanting in the field. Pearl millet and sesamum were grown as barrier crops on one meter area around the main *Capsicum* crop three weeks prior to the transplantation of *Capsicum* seedlings according to the field plan 'C' used earlier (Dhawan and Rishi, 1999). The barrier crops were sprayed three times at 15 days interval with the recommended doses of Malathion or neem-based repellent Achook or water, respectively as was earlier on the *Capsicum* seedlings at nursery stage. Three replications for each treatment were taken. Data on whitefly population and disease incidence was recorded at 10 days intervals. Yield was calculated on the basis of four pickings of green mature fruits. The data was analysed using standard statistical methods after proper transformations.

## Results and Discussion

The percent disease incidence differed significantly over the years with highest disease incidence recorded in 'Pusa Jwala'. The whitefly population started to build up during August reaching its peak in September and then declined gradually to almost nil in December during all the three years. The leaf curl incidence also picked up in August and touched its maximum in September when the whitefly population was also highest. The disease incidence was positively and significantly correlated with whitefly population and negatively and significantly with average temperature. A high positive correlation between whitefly population and geminivirus disease incidence in okra (Bhagabati and Goswami, 1992; Nath *et*

*al.*, 1992 and Nath and Saikia, 1994) and cotton (Khan *et al.*, 1998) has also been reported. Variability in disease can be explained between 71 & 93% using whitefly population and average temperature as independent variables in different cultivars (Table 1).

In the experiment on disease management, lowest whitefly population build up and disease incidence was recorded in *Capsicum* crop where sesamum barrier was used which was sprayed with neem based insect repellent Achook, followed by sesamum barrier sprayed with Malathion (Table 2, Fig. 1). This provided an eco-friendly management of the disease to a good extent. The yield also improved significantly where the disease incidence was less (Table 2, Fig. 2). However, the crops grown with sesamum barrier, which were not sprayed, also gave good control of the disease. Maize crop sprayed with Metasystox was found to reduce mosaic disease complex in pepper upto 25% and increased the yield (Handa *et al.*, 2000). A number of studies have shown positive effect of barrier and intercrop and insecticidal sprays including neem products in checking the incidence of viral diseases in pepper by reducing whitely population (Coudriel *et al.*, 1985; Walter, 1999; Fajinma and Oladiran, 2000; Jayashree *et al.*, 2000).

**Table 1. Regression equation for prediction of leaf curl disease in different cultivars of *Capsicum.***

| Cultivar | Regression equation | $R^2$ |
|---|---|---|
| AVRDC-183 | $26.11+ (-) 1.11X_1 0.216X_2+0.185X_3$ | 0.93 |
| Pusa Jwala | $38.044+ (-) 0.999X_1+0.266X_2$ | 0.86 |
| Hisar Vijay | $12.587+ (-) 0.701X_1+0.183X_2+0.229X_3$ | 0.80 |
| Hisar Shakti | $27.4+ (-) 0.836X_1+0.406X_2$ | 0.71 |

Where $X_1$=Av. Temp., $X_2$ = whitefly population, $X_3$= Av. RH.

**Fig.1: Effect of barrier crops and insect repellent on whitefly populations and leaf curl incidence.**

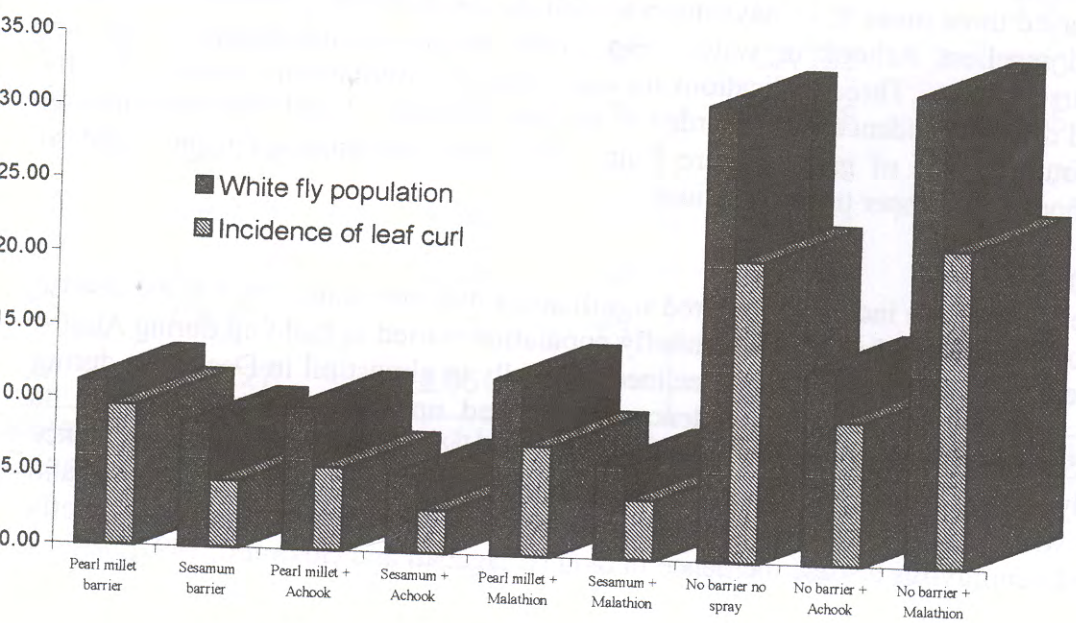
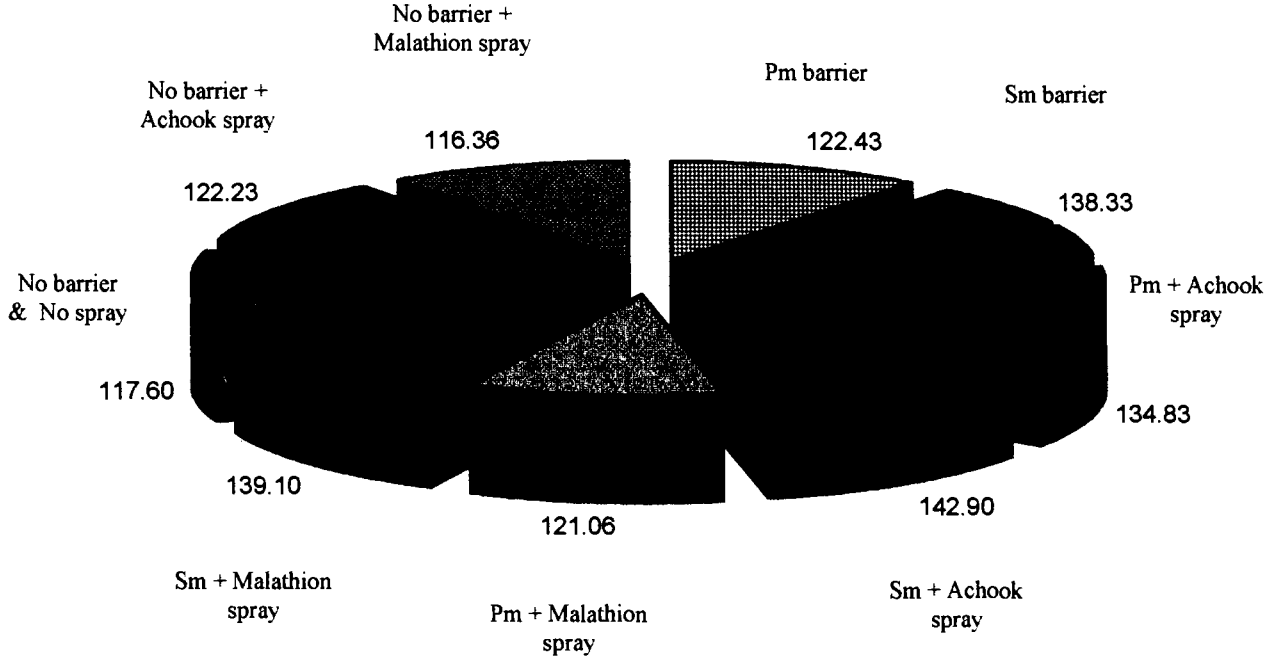


**Table 2. Effect of barrier crops and insect repellent sprays on leaf curl incidence, whitefly population and yield in *Capsicum*.**

| Treatment* | % leaf curl incidence | Whitefly population/plant | Yield (q/ha) |
|---|---|---|---|
| Pm barrier | 9.56 | 11.16 | 122.43 |
| Sm barrier | 4.56 | 8.43 | 138.33 |
| Pm + Achook spray | 5.66 | 9.66 | 134.83 |
| Sm + Achook spray | 2.93 | 5.26 | 142.90 |
| Pm + Malathion spray | 7.50 | 12.0 | 121.06 |
| Sm + Malathion spray | 4.06 | 6.43 | 139.10 |
| No barrier no spray | 20.53 | 34.06 | 117.60 |
| No barrier + Achook | 9.80 | 13.20 | 122.23 |
| No barrier + Malathion | 21.70 | 32.13 | 116.36 |
| C.D. At 5% | 2.402 | 2.555 | 3.361 |

Pm=pearl millet; Sm=sesamum.

**Fig.2: Effect of barrier crops and insect repellents on yield (q/ha) of *Capsicum*.**



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# INVESTIGATIONS ON LEAF CURL OF SWEET PEPPER IN KONKAN (WESTERN INDIA)

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**ABSTRACT:** *A survey conducted to study the leaf curl incidence on sweet pepper in Thane district of Maharashtra State, India, revealed that the disease was a major set back in the commercial cultivation of the crop. The disease was found to occur in the range of 11.28 to 44.50 per cent in different sweet pepper growing regions of Thane district depending upon the cultivars/hybrids under cultivation. In symptomatological studies, the sequential trend observed was slight curling, rolling, deforming of leaves, followed by puckering smalling of leaves, plant stunting and bushy appearance which resulted in almost no flower and fruit formation. Relative humidity, minimum and maximum temperatures had direct correlation with the percent leaf curl incidence and the correlation coefficients between these factors and leaf curl incidence were found to be +0.364, +0.857 and + 0.932, respectively.*

## INTRODUCTION

The sweet pepper (*Capsicum annum* var. *grossum* L. Sendt.) also called as bell pepper or capsicum is a quite popular fruit vegetable grown in most of the states of India mainly for commercial purpose. In Thane district of Maharashtra State, India, the sweet pepper is gaining importance by virtue of its high export potential. However, the leaf curl disease seems to be a major hurdle in commercial cultivation of this crop. So far no studies of any kind have been conducted on this threatening disease in this part of Western India. It was therefore, decided to conduct preliminary studies on disease symptomalogy in the field, survey of leaf curl incidence in major capsicum growing areas of the district and the weather factors affecting the disease.

## MATERIALS AND METHODS

**Disease Survey** – A rowing survey was conducted in sweet pepper growing areas during the Rabi season (January to March) 2000-2001 in Thane district. Sweet pepper growing pockets were identified from the records available at the office of Sub- divisional Agricultural Officer, Dist. Thane. From these records Wada, Palghar, Bhiwandi, Dhahanu and Murbad were found to be major sweet pepper growing areas (tahsils). Two villages in each of the above mentioned areas were chosen. Five fields under sweet pepper cultivation from each village were visited for recording leaf curl incidence. Per cent disease incidence was recorded based on total plant population in the field.

**Field symptomalogy** – For studying field symptomalogy and effect of weather factors, the sweet pepper var. 'California Wonder' was grown at Central Experiment Station, Wakawali, on an area of 37.5 m × 3.7 m. The field was

daily inspected and incidence of the disease and expression of symptoms were recorded. One hundred plants were selected and tagged at random and the observations were recorded right from the seedling stage till the harvest of the crop at weekly interval. The disease incidence was computed based on standard 0-4 scale adopted by Datar (1980) to cover each symptom.

**Weather factors** – To study the effect of weather parameters on the severity of the disease, the data pertaining to temperature and relative humidity was obtained from meteorological observatory and side by side observations on disease development were made in the field. The weather factors were correlated with the severity of disease.

The correlation coefficient between the weather factors and per cent disease incidence was calculated by the following formula.

$$\vartheta \times Y = \frac{\Sigma \times - \frac{1}{n}(\Sigma X)(\Sigma Y)}{\sqrt{\Sigma X^2 - \frac{(\Sigma X)^2}{n}}\sqrt{\Sigma Y^2 - \frac{(\Sigma Y)^2}{n}}}$$

Where, $\vartheta \times Y$ = Correlation coefficient  x = Weather factor
Y = per unit disease incidence  n = number of observations.

## RESULTS AND DISCUSSION

It is evident from the table 1 that leaf curl incidence in different villages in Thane district ranged from 11.28 to 44.50 per cent. Incidence of leaf curl was relatively higher in Met village of Wada tahsil (44.50%). Where as the least disease incidence was observed in Vangaon village of Dhahanu tahsil (11.28%). It was observed that in Met, Khaniwali, Myhade, Sonawale, Ambadi and Angaon, the majority of the cultivators had grown Indo-American cultivar of sweet pepper namely, 'California Wonder'. The leaf curl incidence in these places ranged from 24.22 to 44.50 per cent. While in areas of Palghar and Dhahanu the farmers used commercially accepted hybrids like 'Indra', 'Lario', 'Portos', 'Manhurt', 'Bharat', etc. The hybrids of sweet pepper reported relatively lower incidence ranging from 11.28 to 22.18 per cent in areas of Dhahanu and Palghar which produce export quality sweet pepper. From the survey report, the variety 'California Wonder' seemed to be comparatively more susceptible to leaf curl than other commercial hybrids grown in Thane district.

Symptomatological studies revealed that the symptoms appeared 57 days after transplanting (DAT) in the field. Mild curling of occasional few leaves was noticed on young tender, apex leaves. The plants at this stage were shifting from vegetative growth to the flowering stage. In the second week of observations i.e. 67 DAT curling of the leaves was predominant accompanied with smalling and slight rolling of leaves towards mid-rib. In third week the originally infected plants showed severe curling smalling, puckering paling and rolling of leaves towards mid-rib. In the fourth week the severely infected plants showed no flower formation while healthy plants produced flowers and fruits. In the fifth

week the majority of the infected plants remained stunted and smalling of the leaves and severe clustering was noted. The overall symptoms produced, gave the plants a bushy appearance. During the sixth week i.e. around 100 DAT, some of the infected plants which had produced flowers, dropped many of their flowers prematurely. The pollen grains were found to be absent in stamens of such flowers. Some of the infected plants produced a few fruits, which remained deformed and there was heavy reduction in yield of such plants. More or less the similar type of symptoms were reported by Moghe (1977) and Hassan (1995) in chilli crop infected with leaf curl virus.

In the studies related to effect of weather parameters on incidence of disease, it was observed that three factors viz., minimum temperature, maximum temperature and relative humidity had positive correlation with per cent leaf curl incidence. (Table 2). The correlation coefficients between maximum temperature, minimum temperature, relative humidity and leaf curl incidence were +0.364, +0.857 and + 0.932, respectively. Similar correlation between weather factors and per cent disease incidence was reported by a number of workers while working on white fly transmitted virus diseases (Nour – Eldin *et. al.* 1969: Dengel, 1981, Hayati and Verma, 1984).

The severity of the disease was directly related to the time of infection. Early infected plants produced more severe symptoms as compared to those infected later. Disease was severe in the plants affected at the flowering stage of the crop. Disease symptoms were not observed during the lower temperatures in the month of December and January, while temperatures of 31-38 $^{0}$C during flowering and fruiting stage of the crop produced severe disease symptoms. This might be due to the fact that leaf curl vector *Bemisia tabaci* becomes most effective and virulent at temperatures between 33-39 $^{0}$C as suggested by Butter and Rataul (1978).

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**Table 1: Incidence of leaf curl of Sweet pepper in different villages of Thane District.**

| Area | Village | Total area (ha) surveyed | Percent leaf curl incidence |
|---|---|---|---|
| Wada | Met | 3.20 | 44.50 |
| | Khaniwali | 3.60 | 38.06 |
| Murbad | Myhade | 3.75 | 30.82 |
| | Sonawale | 2.80 | 24.22 |
| Bhiwandi | Ambadi | 3.20 | 39.82 |
| | Angaon | 3.80 | 32.00 |
| Palghar | Manor | 3.75 | 22.18 |
| | Palghar | 2.80 | 18.18 |
| | Bhoisar | 2.50 | 20.90 |
| | Kolawade | 2.80 | 21.12 |
| | Kelwe | 2.60 | 18.24 |
| Dhahanu | Vangaon | 3.75 | 11.28 |
| | Golwad | 3.00 | 12.14 |
| | Bordi | 3.80 | 15.16 |

**Table 2: Correlation coefficients between max. min. temperature relative humidity and per cent disease incidence.**

| Met. Week | Date | Temperature $^0$C | | Relative humidity (%) | % leaf curl (average) |
|---|---|---|---|---|---|
| | | Maximum | Minimum | | |
| 5 | 29-1 to 4-2-2000 | 31.50 | 14.42 | 82.14 | 7.00 |
| 6 | 5-2 to 11-2-2000 | 34.21 | 14.36 | 88.24 | 14.00 |
| 7 | 12-2 to 18-2-2000 | 35.39 | 15.00 | 89.92 | 35.90 |
| 8 | 19-2 to 25-2-2000 | 36.32 | 15.28 | 84.12 | 36.00 |
| 9 | 26-2 to 4-3-2000 | 37.14 | 16.20 | 85.16 | 36.11 |
| 10 | 5-3 to 11-3-2000 | 37.25 | 16.30 | 85.88 | 37.08 |
| 11 | 12-3 to 18-3-2000 | 38.10 | 16.32 | 86.17 | 38.90 |
| 12 | 19-3 to 25-3-2000 | 38.25 | 16.80 | 87.72 | 45.50 |

Correlation coefficient between:

a) Max. temperature and per cent leaf curl incidence: +0.932

b) Min. temperature and per cent leaf curl incidence: +0.859

c) Relative humidity and per cent leaf curl incidence: +0.364

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# Inheritance of Resistance to Pepper Anthracnose Caused by *Colletotrichum capsici*

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## Abstract

Inheritance of the resistance to anthracnose, caused by *Colletotrichum capsici*, was studied in *Capsicum annuum* L. populations established from a cross between accession '83-168', and cv. KKU-Cluster; and their progenies in $F_1$s, $F_2$s and $BC_1$s. The resistance was evaluated by inoculating mature green fruits with a 6-µl drop of $10^6$conidia/ml suspension after making a wound with a 0.1-cm length needle. Reciprocal effects were not present. Two phenotypic classes, resistant and susceptible, were determined based on numbers of fruits infected at four days after inoculation (DAI). The segregation of resistance to susceptibility appeared to be 3:1 in the $F_2$s and 1:1 in the $BC_1$ ($F_1$ x KKU-Cluster). This indicated that one dominant gene was responsible for the resistance to *C. capsici* in the breeding line "83-168" at 4 DAI.

**Key words**: anthracnose, *Capsicum annuum* L., *Colletotrichum capsici*, resistance to anthracnose, pepper

## Introduction

Anthracnose is a major disease of pepper. It occurs worldwide wherever pepper is grown under warm temperatures and overhead irrigation or rain-fed conditions (AVRDC, 2000). Anthracnose is mainly a problem on mature fruits causing severe losses due to both pre- and postharvest fruit decay (Hadden and Black, 1989). Two significant causal pathogens found in Thailand are *Colletotrichum capsici* (Syd.) E.J. Butler& Bisby and *C. gloeosporioides* (Penz. & Sacc. in Penz.; Sangchote *et al.*, 1998; Sangchote, 1999). They are also the main causal agents in the tropical Asia (Chang and Chung, 1985; Manandhar *et al.*, 1995), Korea (Kim *et al.*, 1989), Louisiana (Hadden and Black, 1989) and Mississippi (Roy *et al.*, 1997). *C. capsici* generally infects ripe red fruit, while *C. gloeosporioides* infects both green and ripe fruits (Kim *et al.*, 1989; Sangchote *et al.*, 1998; Sangchote, 1999).

Several varieties resistant to *Colletotrichum* spp. have been reported (Kim *et al.* 1989; AVRDC, 1998; AVRDC, 2000). Inheritance of resistance to *Colletotrichum* spp. in pepper has been studied in these varieties. Resistance to *C. dematium*, a synonym of *C. capsici* according to Verma (1982), in $F_2$ and BC *Capsicum annuum* populations segregated in a Mendelian fashion and was likely to be controlled by a single dominant gene (Park *et al.*, 1990b), while resistance to *C. gloeosporioides* was partially dominant or overdominant (Park *et al.*, 1990a). Fernandes (1998) inoculated seedlings of progeny from a cross between three resistant varieties and one susceptible variety and found that resistance to *C. gloeosporioides* in one variety was controlled by a single dominant gene, and in two others was controlled by a pair of dominant genes. The purpose of this study was to determine the inheritance of resistance to pepper anthracnose caused by *C. capsici* in the breeding line '83-168' in order to enhance the utility of disease resistance in pepper breeding programs.

## Materials and Methods

### Plant materials

Crosses were reciprocally made between an anthracnose resistant variety '83-168' and a susceptible cv. 'KKU-Cluster'. The parents, $F_1$-8K ('83-168' x 'KKU-Cluster'), $F_1$-K8 ('KKU-Cluster' x '83-168'), $F_2$s (selfed from each $F_1$), and backcross ($BC_1$) populations which $F_1$s were backcrossed to both parents were grown in the field at the Tropical Vegetable Research Center (TVRC), Kasetsart University, Kamphaengsaen Campus, Thailand. Five mature green fruits per plant were harvested from 10 plants of each parent, 15 plants of each $F_1$ and every individual plant of each $F_2$ and $BC_1$ generations. Calyx was removed from the harvested fruit to avoid contamination. Fruits were surface sterile by soaking in 0.6% sodium hypochlorite solution for 1 minute, washed in distilled water twice and dried with paper towels. They were subsequently placed on a piece of polystyrene (16x21x1cm) floating in a plastic box (20x30x10cm) filled with 500ml water before inoculation.

### Inoculation

The experiment was conducted at the Pathology Laboratory of ARC-AVRDC. The *Colletotrichum capsici* isolate 158ci, which was collected from Thailand by Dr. Somsiri Sangchote (Department of Plant Pathology, Kasetsart University), was used in this study. A piece of mycelia was grown on potato-dextrose agar (PDA) at 27°C under continuous fluorescent light for 5-7 days. Conidia were harvested by adding 5-10 ml of sterilized distilled water to culture dishes and gently swirling the liquid to dislodge the spores. The conidia concentration was adjusted to $10^6$ spores/ml.

Inoculation method was modified from the drop procedure developed by the AVRDC (1998) and Manandhar *et al.* (1995). Fruits were inoculated by placing a 6-µl drop of spore suspension on the wound previously made with a 1-mm long needle on the fruit surface. One inoculation site was used because of the small fruit size. Inoculated fruits were incubated in the dark during the first 24 hours, then moved to12 /12 h light/dark cycle at 26-27°C, nearly 100% RH. Number of infected fruits showing a necrotic area of at least 4 mm diameter was recorded at 4, 5 and 7 days after inoculation (DAI).

### Genetic analysis of the tolerance to anthracnose

The frequency distribution of number of infected fruits at 4, 5 and 7 DAI from each individual in each population was analyzed. Separation of phenotypic classes was based on the number of fruits infected and the difference between the parents. Segregation of the phenotypes was determined to fit a Mendelian ratio using Chi-square goodness-of-fit test.

## Results and Discussion

### Disease development in the parents

Percent infected fruits of susceptible parent 'KKU-Cluster' reached 100% at 4 DAI, while that of resistant parent '83-168' was 24% at 4 DAI, 60% at 5 DAI, and 91.1% at 7 DAI (Figure 1). Student's t-test revealed that anthracnose incidences on the two parents was significantly different at 4 and 5 DAI, but not at 7 DAI. This result indicated that resistance in '83-168' could consist of delaying *C. capsici* infection.

### Inheritance of resistance to anthracnose in the $F_2$s and $BC_1$s populations

The distribution of the number of infected fruits in all populations was exhibited in Figure 2. The number of infected fruits at 4 DAI, whereby the parents showed the greatest difference, was used to determine the inheritance of the resistance to anthracnose in these populations. Number of infected fruits was 0-3 in the resistant parent '83-168', while it was 5 in the susceptible parent 'KKU-Cluster' (Figure 2). Based on the obvious break point between the parents, 0-3 and 4-5 infected fruit numbers were used to classify susceptible and resistant phenotypes.

All $F_1$ plants in both crosses appeared to be resistant (Figure 2). This indicated that the resistance to anthracnose derived from '83-168' was likely to be a dominant trait. The segregation of resistance and susceptibility in the $F_2$s was well fit to 3:1 Mendelian ratio (Table 1), without reciprocal effects. The $BC_1$ ($F_1$ x '83-168') progeny appeared to be resistant, and the

$BC_1$ ($F_1$ x 'KKU-Cluster') appeared to be 1:1 resistance and susceptibility segregating. Considering the segregation in all progenies derived from the crosses between '83-168' and 'KKU-Cluster', it was likely that a single dominant gene conferred the resistance to anthracnose caused by *C. capsici* evaluated at 4 DAI.

**Conclusions**

This study showed that resistance to anthracnose in the breeding line '83-168' was expressed by delaying fruit infection by *Colletotrichum capsici*. Genetic analysis indicated that one dominant gene was responsible for the resistance evaluated at 4 DAI.

**Acknowledgement**

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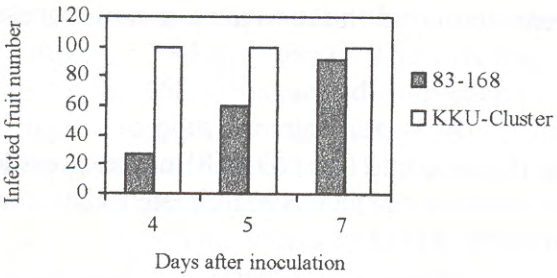


**Figure 1** Difference of disease development between the two parents, '83-168' and 'KKU-Cluster', from 4 to 7 DAI

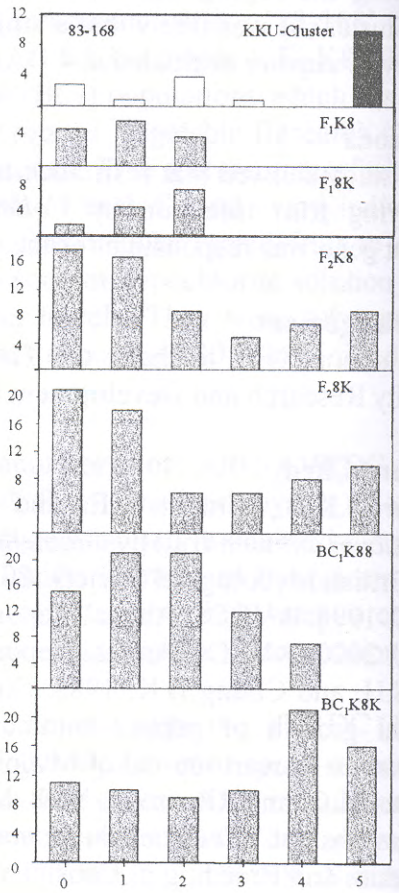


**Figure 2** Frequency distribution of infected fruits number of the parents, '83-168' and 'KKU-Cluster', and their progeny, $F_1$s, $F_2$s and $BC_1$s generations at 4 DAI

**Table 1** Goodness-of-fit test of the segregation for the resistance and susceptibility to *Colletotrichum capsici* based on the infected fruit number at 4 DAI in the progeny populations derived from a cross between '83-168' and 'KKU-Cluster'

| Population | Expected ratio (R:S)[1] | Plant observed | | Chi-square ($\chi^2$) | Probability (P) |
|---|---|---|---|---|---|
| | | R | S | | |
| $F_2$-K8 | 3:1 | 49 | 16 | 0.0051 | 0.945 |
| $F_2$-8K | 3:1 | 51 | 18 | 0.0435 | 0.875 |
| $BC_1$-K88 | 1:0 | 69 | 7 | na[2] | na[2] |
| $BC_1$-K8K | 1:1 | 40 | 37 | 0.1169 | 0.808 |

[1] R = resistant plant S = susceptible plant
[2] na = not available

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# WIDE HYBRIDIZATION OF EGGPLANT (*SOLANUM* SPS.)

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## Introduction

Eggplant (*Solanum melongena* L.) is an important vegetable crop of Indian origin holds a coveted position in Indian sub-continent in summer and rainy season. Due to good nutritional value and its suitability to grow under a wide range of growing conditions, it has been extensively cultivated in different parts of world. Immense variability exists in this crop, which provides an opportunity to the researchers to develop plants with good quality and productivity. Due to greater susceptibility of this crop towards insects and diseases, it becomes hard to achieve the maximum yield potential. There are reports that wild relatives of *Solanum melongena* i.e. *S. sisymbriifolium, S. viarum, S. khasianum, S. incanum* and *S. nigrum* are potential source of resistance to several biotic and abiotic stresses like Phomopsis blight, little leaf disease and borer problem ( Kalda *et al* 1976, Lal *et al.* 1976 ). Transfer of resistance from wild species to *S. melongena* is difficult due to cross incompatibility between the two. A cross-ability study was conducted at this institute, which indicated that only few wild species were crossable with the cultivated species (*S. melongena*) (Table-1). Thus in present study the possibility of embryo rescue technique was investigated to overcome the crossability barrier and to get interspecific hybrid.

## Materials and Method

Nine cultivated accessions of *Solanum melongena* L. and five wild accessions, one each of S. *sisymbriifolium*, S. *viarum*, *S. incanum, S. nigrum* and *S. torum* were utilized for this study. All the accessions were used as both male and female parent.

Sexual plants were hybridized in the green house. Flowers at anthesis were emasculated then pollinated and the crosses were protected using cotton. Flowers were collected starting from 5$^{th}$ day after pollination.

**Table 1**. The crosses used in the embryo rescue technique

| Cross | No. of days to dissect out embryo |
|---|---|
| CHBR-1 x *S. sisymbriifolium* | 5, 10, 16 and 21 days |
| CHBR-1 x *S. viarum* | 15, 23 and 32 days |
| CHBR-2 x *S. sisymbriifolium* | 5, 8 and15 days |
| DBR-8 x *S. sisymbriifolium* | 10 days |
| H-8 x *S. sisymbriifolium* | 15 days |
| KS-224 x *S. viarum* | 5 days |
| Saatha x *S. viarum* | 9 days |
| VRB-9 x *S. indicum* | 6 & 9 days |
| VRB-9 x *S. viarum* | 11, 23 and 32 days |
| VRB-9 x *S. sisymbriifolium* | 5, 15 and 21 days |

Firstly embryos at various developmental stages (5, 6, 8, 9, 10, 15,....DAP) were collected from selected crosses and tested for determining right stage of embryo to be cultured (Table 1). The number of days of zygotic embryo maturation stage of seed taken after crossing and date of collection from sources was also recorded. The immature small fruits were immersed in 70% ethanol for 1 minutes followed by surface sterilization with 0.1% $HgCl_2$ for 5 minutes and then rinse in sterile distilled water for 3-4 times.

**Table 2.** Culture media used in the embryo rescue technique

| Sl. No. | Medium | Supplements |
|---|---|---|
| 1. | MS Basal | 6% sucrose |
| 2. | MS Basal + $NH_4NO_3$ reduced to half | 6% sucrose + 200 mg/l Glucamine |
| 3. | MS Basal + $NH_4NO_3$ reduced to half | 6% sucrose + 40 mg/l Glucamine |
| 4. | MS Basal | 3% sucrose + 250 mg/l CHL |
| 5. | MS Basal | 3% sucrose + 500 mg/l CHL |
| 6. | MS Basal | 250 mg/l CHL + 0.1 mg/l Kinetin |
| 7. | MS Basal | 1.0 mg/l NAA |
| 8. | MS Basal | 0.5 mg/l BAP |
| 9. | MS Basal | 1.0 mg/l BAP |
| 10. | Nitsch (N6) medium | 250 mg/l CHL |
| 11. | Nitsch (N6) medium | 250 mg/l CHL + 0.5 mg/l NAA |
| 12 | Nitsch (N6) medium | 250 mg/l CHL + 1.20 mg/l NAA |
| 13. | Nitsch (N6) medium | 250 mg/l CHL + 1.0 mg/l BAP |
| 14. | Nitsch (N6) medium | 2.0 mg/l NAA + 0.5 mg/l BAP |

The immature embryos were dissected out from surface sterilized immature fruits, and were cultured on to Murashige & Skoog's and Nitsch medium with different concentrations of growth regulator and other supplements (Table 2). The cultured explants were incubated in a growth chamber for initial 20-25 days in dark at 25 ± 2°C and after callus induction transferred to light for 16 hour photoperiod.

**Results and Discussion**

The crossability study conducted at this Institute, show that the fruit setting was poor in the interspecific crosses and only few combinations, viz., JB-8 x *S. incanum*, Green Long Thick x *S. incanum* and Ram Nagar Giant x *S. incanum* produced fruits with seeds. The cross Punjab Sadabahar x *S. incanum* produced fruits but they were devoid of seeds. Some more crosses also produced fruits but all these crosses also the fruits were devoid of seeds (Table 3).

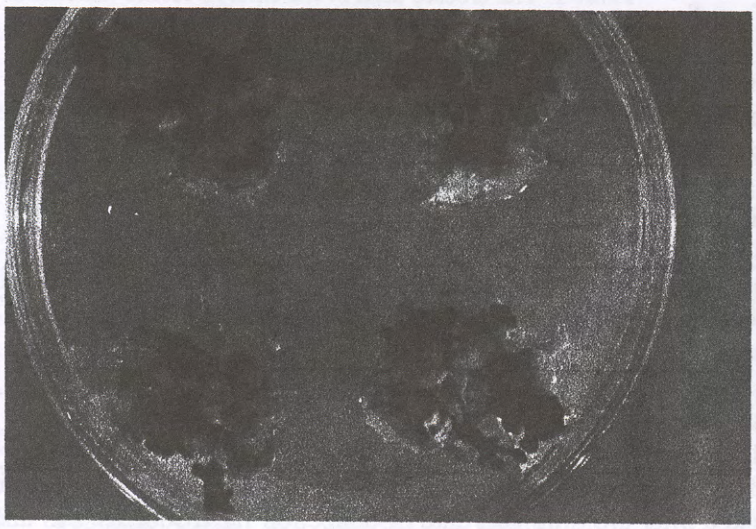

Fig. 1: ***Regeneration of shoots in embryo rescue of the cross S. symbriifolium and S. melongena (Green long thick)***

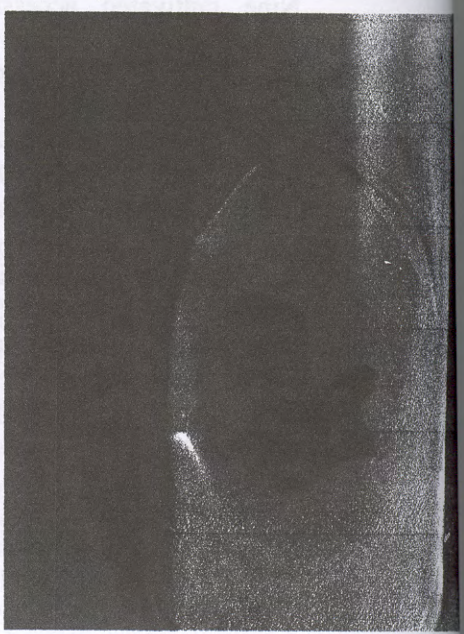

Fig.2. ***Shoot elongation in embryo rescue the cross S. symbriifolium and S. melongena (Green long thick)***

These results are in accordance with Sharma *et al.* 1984, where the cross, *S. melongena* x *S. khasianum*, *S. khasianum* x *S. melongena* and *S. melongena* x *S. sisymbriifolium* did produce some fruits with seeds but no viable seeds was obtained. From this experiment it was evident that *S. incanum* was the only species, which was easily crossable with *S. melongena* and produced fertile progeny. The other wild species i.e. *S. sisymbriifolium*, *S. viarum*, *S. khasianum*, *S. incanum* and *S. nigrum* were not crossable to *S. melongena*. Looking into the difficulty of obtaining hybrid, the embryo rescue was attempted. Embryo rescue can be a very useful tool, to overcome post zygotic incompatibilities in interspecific hybridization/hybridization between distantly related species or between species belonging to different agronomic complexes (Otubo *et al.* 2000). Among the medium tested for callus induction the immature embryo did not show any development on medium devoid of growth regulators. Among all the media tested for initial study the medium supplemented with NAA show some swelling of embryos with enlargement and callus initiation from margins. Out of the embryos of selected cross cultured, only three crosses show some degree of development on culture medium.

**Table 3.** Hybridization among the wild and cultivated species of eggplant

| Female parent (*S. melongena*) | Male parent | Buds pollinated | Fruit setting | Seed obtained |
|---|---|---|---|---|
| Ram Nagar Giant | *Solanum incanum* | 60 | 13 | 13 |
| Ram Nagar Giant | *S. indicum* | 45 | Nil | Nil |
| Ram Nagar Giant | *S. sisymbriifolium* | 60 | Nil | Nil |
| Ram Nagar Giant | *S. nigrum* | 30 | Nil | Nil |
| Green Long Thick | *Solanum incanum* | 150 | 5 | 8 |
| Green Long Thick | *S. indicum* | 120 | Nil | Nil |
| Green Long Thick | *S. sisymbriifolium* | 180 | Nil | Nil |
| Green Long Thick | *S. nigrum* | 150 | Nil | Nil |
| Punjab Sadabahar | *Solanum incanum* | 45 | Nil | Nil |
| Punjab Sadabahar | *S. indicum* | 150 | Nil | Nil |
| Punjab Sadabahar | *S. torvum* | 150 | 6 | 6 |
| Punjab Sadabahar | *S. nigrum* | 180 | Nil | Nil |
| VRB-6 | *Solanum incanum* | 75 | 16 | Nil |
| VRB-6 | *S. indicum* | 165 | 4 | Nil |
| VRB-6 | *S. torvum* | 150 | 7 | Nil |
| VRB-6 | *S. nigrum* | 180 | 4 | Nil |
| JB-8 | *Solanum incanum* | 150 | 5 | 10 |
| JB-8 | *S. indicum* | 120 | Nil | Nil |
| JB-8 | *S. sisymbriifolium* | 120 | Nil | Nil |
| JB-8 | *S. nigrum* | 120 | Nil | Nil |

In case of cross H-8 x *S. Sisymbrifolium* the embryo turned green and enlarged to some extent. Later on these embryos transferred to MS medium supplemented with 250 mg/L CHL and 0.1 mg/l kinetin and MS basal with 1.0 mg/L NAA. However, these embryos did not show any further development on any media and turned brown. In previous studies with *S. melongena* x *S. sisymbriifolium* cross, it was reported that there is problem of degeneration of ovule in cross (Sharma et al. 1984). They also reported that few shriveled seeds could be obtained in this cross but they did not germinate. Some embryos of the cross CHBR-1 x *S. sisymbriifolium* were cultured on to Nitsch medium containing 250 mg/L CHL and different concentration of NAA and BAP.

In many interspecific crosses the hybrid embryos were formed but failed to develop mainly due to degeneration of endosperm and abortion of embryo at an early stage of development.

A remarkable observation was also recorded that when the embryos cultured alongwith placenta, they remained fresh for longer period, whereas those cultured after separation from placenta shriveled and turned brown very quickly. According to Raghavan and Sharma (1994) the placental cells serve to facilitate the absorption of metabolite from endosperm. So this may be a reason why the embryos without placenta turned brown. Many experiments of this kind have shown that the growth in callus of embryos of *Capsella* (Monnier,1984) enhanced in presence of attached placenta, then in its absence. The embryo of cross *S. melongena* x *S. viarum* attached to placenta did show enlargement and swelling, when transferred on to MS basal+ 3% sucrose+ 250 mg/l CHL. Further the callus initiation was observed on the Nitsch medium containing 1.0mg/l NAA and 250 mg/l CHL (figure.2). It was observed that the growth of the callus was too slow,

though many combinations of the media were tried to quicken the growth. After repeated transfer to different media shoots were generated gradually. The shoot elongation was too slow on all the media except MS basal supplemented with 0.5 mg/L BAP. Minute shoots were repeatedly cultured on the same medium. Higher concentration of BAP (>0.5 mg/l) promoted excessive callus formation. The work is still under progress and several media are still under test to promote root initiation.

In eggplant there are few reports on the successful use of embryo rescue technique for interspecific hybridization. Since immature embryo must be isolated in order to be cultured, the difficulty of extracting them from ovule could be one of the reason why them are few reports of successful culture of immature embryo. The present study would provide necessary information regarding stages of explant, the combination of media and the type of cross combination, which will be very helpful for further studies.

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# BIOCHEMICAL CHANGES DURING FRUIT DEVELOPMENT IN EGGPLANT (*Solanum melongena* L.)

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## Introduction

Brinjal or eggplant is one of the most important vegetables grown throughout the tropical and subtropical parts of the world. Like many other vegetables, fruit quality in brinjal is greatly influenced by stages of fruit development. Anthocyanin and total phenols are the two important chemicals which affect quality of fruits.The information on changes in these chemical constituents during fruit development is meagre. Therefore, a study was carried out with ten cultivars of brinjal released by the Indian Agricultural Research Institute (IARI), New Delhi, to observe the changes of these constituents during fruit development.

## Materials and method

Ten cultivars *viz.* Pusa Hybrid-5, Pusa Hybrid-6, Pusa Hybrid-9, Pusa Uttam, Pusa Upkar, Pusa Bindu, Pusa Ankur, Pusa Purple Long and Pusa Purple Cluster were taken for studies.All the cultivars had purple skin. The crop was raised at the Vegetable Research Farm of IARI during the year 1999-2000 and all the recommended cultural practices were followed. Sufficient number of fruits, just after set, were tagged and were harvested at different stages of fruit development *i.e.*10, 15,20,25and 30 days after fruit set (DAS) to estimate the anthocyanin and total phenols. The anthocyanin content of brinjal fruit peel was estimated by the method proposed by Nothman *et al.*(1976) while the total phenols were determined as per AOAC(1970) with slight modification. Data were analysed as per the procedure of completely randomized design.

## Results and discussion

The data in table-1 reveal that absorbance value (O.D.) for anthocyanin increased gradually up to a particular stage of fruit development in most of the cultivars. But in Pusa Ankur, Pusa Purple Long and Pusa Bindu it decreased gradually after 10 days of fruit set. This pattern may be attributed to the fact that in these cultivars peak value of absorbance might have reached before $10^{th}$ day when sampling for analysis did not start.

The general decline in anthocyanin content in the brinjal cultivars at later stage of growth might be due to rapid oxidation of phenolic compounds with the increased catalytic activity of polyphenol oxidase.This result is in conformity with those reported by Singh *et al.*(1990). Highest or maximum absorbance value was recorded in Pusa Bindu (0.878)at $10^{th}$ day of fruit set followed by Pusa Kranti and Pusa Upkar on $25^{th}$ day .

There was significant difference in total phenols content between different stages of fruit development (Table-2). All the cultivars also differed significantly with respect to this character. The highest quantity of total phenols was recorded in Pusa Hybrid –9 (72.65 mg/100g) in 15 days old fruits which was followed by Pusa Hybrid-6 on the same day. In Pusa Hybrid-6, Pusa Hybrid-9, Pusa Uttam, Pusa Bindu, and Pusa Purple Long, content of the total phenols increased up to $15^{th}$ day and decreased thereafter. In Pusa Upkar,Pusa Kranti and PusaPurple Cluster it increased up to $20^{th}$day after that decreased gradually.In Pusa Hybrid-5, it

increased up to 25 days. While in Pusa Ankur a gradual decrease in content of total phenols was recorded throughout. The variation among cultivars in total phenols during fruit development might be due to variation in growth rate of the fruits which is truly a genotypic character. The decrease in total phenols at later stage of growth may be attributed to increased oxidation of phenolic compounds *viz.* lignin, flavanoids and tannin due to high catalytic activity of polyphenol oxidase (Singh *et al.*, 1990).

It is concluded from the present investigation that much variation existed in the material taken for study with regard to anthocyanin and content of total phenols. Furthermore, the content varied considerably with the advancement of fruit maturity.

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Table 1 Changes in the level of anthocyanin (absorbance of extract at 550 nm)in different cultivars of brinjal at different stages of fruit development.

| Cultivar | Anthocyanin level (O.D.) on days after fruit set. | | | | | |
|---|---|---|---|---|---|---|
| | 10 | 15 | 20 | 25 | 30 | Mean |
| Pusa Hybrid-5 | 0.451 | 0.504 | 0.611 | 0.592 | 0.408 | 0.513 |
| | (5.77) | (5.25) | (14.11) | (26.98) | (23.57) | (15.10) |
| Pusa Hybrid-6 | 0.369 | 0.393 | 0.492 | 0.720 | 0.514 | 0.497 |
| | (4.72) | (4.09) | (11.60) | (32.19) | (29.69) | (16.46) |
| Pusa Hybrid-9 | 0.378 | 0.395 | 0.422 | 0.722 | 0.402 | 0.463 |
| | (4.83) | (4.12) | (9.96 | (32.31) | (23.22) | (14.89) |
| Pusa Uttam | 0.418 | 0.742 | 0.744 | 0.704 | 0.640 | 0.649 |
| | (5.35) | (7.73) | (17.50) | (31.48) | (36.95) | (19.81) |
| Pusa Upkar | 0.421 | 0.536 | 0.638 | 0.755 | 0.626 | 0.595 |
| | (5.39) | (5.58) | (15.05) | (33.76) | (36.23) | (19.20) |
| Pusa Ankur | 0.630 | 0.506 | 0.493 | 0.401 | 0.259 | 0.457 |
| | (8.06) | (5.27) | (11.63) | (17.94) | (14.95) | (11.57) |
| Pusa Purple Long | 0.660 | 0.632 | 0.463 | 0.402 | 0.286 | 0.488 |
| | (8.45) | (6.58) | (10.92) | (18.00) | (16.53) | (12.09) |
| Pusa Bindu | 0.878 | 0.523 | 0.405 | 0.347 | 0.322 | 0.490 |
| | (11.24) | (5.45) | (9.56) | (15.51) | (18.62) | (12.08) |
| Pusa Kranti | 0.478 | 0.511 | 0.613 | 0.792 | 0.450 | 0.568 |
| | (6.120 | (5.32) | (14.44) | (35.94) | (26.00) | (17.46) |
| Pusa Purple Cluster | 0.265 | 0.310 | 0.379 | 0.358 | 0.294 | 0.321 |
| | (3.40) | (3.23) | (8.93) | (16.10) | (16.99) | (9.71) |
| Mean | 0.494 | 0.505 | 0.523 | 0.579 | 0.420 | |
| | (6.34) | (5.27 | (12.41) | (25.92) | (24.28) | |

C.D. at 5 %; Cultivar = 0.744; Days = 0.526; Cultivar x Days = 1.65

**Note** : Figures in parenthesis pertain to transformed mean values i.e. actual mean divided by corresponding square root of EMS. Decrement in transformed value at any stage does not indicate actual decrease in character as it is influenced by EMS at that stage. Difference has been considered to observe the significant change with the help of critical difference of the transformed data. However, trend depends on the original mean of character.

Table 2 Changes in total phenols content (mg/100g) in different cultivars of brinjal at different stages of fruit development.

| Cultivar | Total phenols (mg/100g) on days after fruit set | | | | | |
|---|---|---|---|---|---|---|
| | 10 | 15 | 20 | 25 | 30 | Mean |
| Pusa Hybrid-5 | 36.10 | 44.80 | 52.75 | 58.75 | 52.40 | 48.96 |
| Pusa Hybrid-6 | 44.15 | 64.40 | 64.05 | 45.64 | 41.20 | 51.88 |
| Pusa Hybrid-9 | 39.40 | 72.65 | 60.60 | 45.78 | 38.98 | 51.47 |
| Pusa Uttam | 43.55 | 45.65 | 35.70 | 23.75 | 17.75 | 32.28 |
| Pusa Ankur | 34.25 | 43.65 | 51.59 | 41.65 | 17.55 | 37.81 |
| Pusa Purple Long | 41.45 | 50.70 | 47.35 | 29.55 | 14.60 | 36.73 |
| Pusa Bindu | 54.20 | 56.10 | 32.05 | 16.35 | 13.95 | 34.53 |
| Pusa Kranti | 44.80 | 47.64 | 51.65 | 48.88 | 31.60 | 44.91 |
| Pusa Purple Cluster | 46.20 | 56.60 | 60.95 | 27.55 | 23.75 | 43.01 |
| Mean | 44.32 | 54.16 | 50.36 | 37.98 | 28.08 | |

C.D. at 5 %; Cultivar = 2.82; Days = 1.99; Cultivar x Days = 6.30

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# A PRELIMINARY STUDY ON INHERITANCE OF HIGH TEMPERATURE TOLERANCE IN EGGPLANT (*Solanum melongena* L.)

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## ABSTRACT

The inheritance of high temperature tolerance (HT) on eggplant was studied with 4×4 half diallel crosses which derived from four parents with different tolerance to high temperature. Four parents and relevent $F_1$, $F_2$ and $BC_1$ involved in present experiment were simultaneously treated by high temperature in plastichouse and open field. HT index and ratios of fruit setting were respectively collected following Hayman's procedure. The results showed that inheritance of HT on eggplant fits non-complete dominant in a model of additive-dominance, and additive genes play more important roles on HT than dominant genes do. Furthermore, the appropriate methods for improving HT of eggplant were suggested.

## INTRODUCTION

Eggplant, *Solanum melongena*, L. (2n=24), is one of world-wide important vegetables, particularly in some Asia countries, such as China, India and Japan. Even more than 95% of total yield all over the world attributed to this region. But, the local high temperature in summer(often more than 35°C) severely degrade not only the quality of products but also yield. Hence, HT of eggplant is an important improving objective.

Injuries caused by high temperature can be showed in each growing stage: in seedling stage, abnormal leaves with irregular edge and wrinkle foliage appeared; in fruits setting stage, a retard pollen germinating and a slowly elongation of pollen tube in style were reported (Han *et al.* 1996; Khah *et al.* 1992), eventually, obviously reduction of fruit setting ratio can be observed. Thus, the aforementioned traits were employed to measure HT on eggplants.

This experiment aims to know the inheritance of HT on eggplant not only for understanding heriditary law but also for developing an effective way to enhance HT on eggplant.

## MATERIALS AND METHODS

Accordance with different performance of HT in previous observation, four accessions namely E9302, E9009-1-1-1, E9718-1 and E9010-J5-1 were selected and participated in a 0.5P (P-1) diallel cross, furthermore, E9302 and E9009-1-1-1 were grouped in susceptible parent (SP), whereas E9718-1 and E9010-J5-1 in tolerant parent (TP). Six $F_1$ hybrids derived from diallel crosses between S and T parents, $F_2$ and $BC_1$ were further obtained through $F_1$ selfing and backcross, respectively.

All generations were obtained in JAAS in China during 1997-1998. The present experiment was conducted in ARC-AVRDC, Kampaeng Sean, Thailand during Nov.1998 to Mar.1999. All seeds were sown in plastic plate in nursery on Nov. 14, 1998. Transplantation to experimental field was implemented on Dec. 11, 1998 in with RCBD

and four replications, 20 plants per replication. The ratios of fruit setting were investigated under an average temperature 34.4/23.1 °C (d/n). The other seedlings were transplanted into plastic pot ($10\times10cm^2$) which were arranged in plastichouse with RCBD. $F_1$ and parents, $F_2$ and $BC_1$ were planted 30 and 60 plants respectively with three replications. Five days after transplanting, an average temperature at 38.8/24.6 °C (d/n) was applied to all plants. Sequentially, injury degrees were investigated every 5 days since symptom appeared on plants, data in third investigation were employed in calculation of HT index. The symptoms on plants were graded in the following standard:

0- No symptoms;
1- leaves less than 25% showed symptoms;
2- leaves of 25 to 50% showed symptoms;
3- leaves of 50% to 75% showed symptoms;
4- leaves more than 75% showed symptoms;
5- Plant death

Thus, the HT index can be calculated in the formula:

$$\text{HT index} = (1 - \frac{\sum i \times Pi}{Maxi \times \sum Pi}) \times 100$$

where i= grade of HT, Maxi= 5, $P_i$= plants in grade i(i= 0,1,2,3,4, 5).

Hayman's method was employed to estimate genetic parameters. analyses on variance(V) and covariance (CoV) followed Gill's method (Gill *et al.* 1991).

## RESULTS AND DISCUSSION

### Performance of HT among different generations

Parents showed HT index with significant differences (F=137.03**). HT index of S parents involving E9302 and E9009-1-1-1 was significant lower than that of T parents involving E9718-1 and E9010-J5-1 (Data not shown). The results further proved that the grouping of T and S parents were reasonable.

$F_1$ hybrids displayed a moderate HT index between T and S parents, which is significant higher than S parents ($t_{0.10}$=2.123*) but no difference to T parents, as for Fig.1. This data inferred a partial dominant inheritance of HT on eggplant. In addition, correlation coefficient between MP and $F_1$ (r=0.902**) indicated that MP was a helpful target to predict the HT of $F_1$ hybrids.

$F_2$ and $BC_1$ revealed a progressively reduction but higher than S parents in HT index.

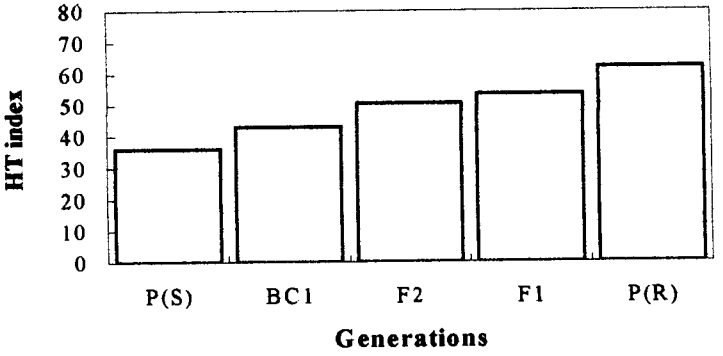


Fig.1, Performance of HT among generations

**Analyses on the inheritance of HT with diallel crosses**

Owing to existence of significant different HT index between $F_1$ hybrids and relevent parents, both general combined ability (GCA) and special combined ability (SCA) can be analyzed following Griffing's Method. Results showed that GCA and SCA played significant effects both in Model I and Model II. These conferred that both GCA and SCA are important to HT inheritance, moreover, the ratio of Gca/Sca>>1 inferring a more important role played by additive genes than that of dominant genes. Thus, conclusions can be safely drawn that odds for $F_1$ hybrid performing over-dominant are rare.

Variance(V) and covariance(CoV) among parents enable to work out a linear regression between V and CoV(Fig. 2.), in which the slope of the line(b=1.134, linear regression coefficient) showed no different to unit slope I (t=0.219) but significant to 0 (t=6.098**). Further analyses on (CoV+V) among replications showed significant difference (F=113.35**), but similar analyses on (CoV-V) among parents displayed no difference (F=2.78). From above analyses, we can draw conclusion that HT inherence on eggplant fits the additive-dominant model, epistasis effect can be omitted, and additive gene had more important effects. Additional, HT is controlled by dominant genes.

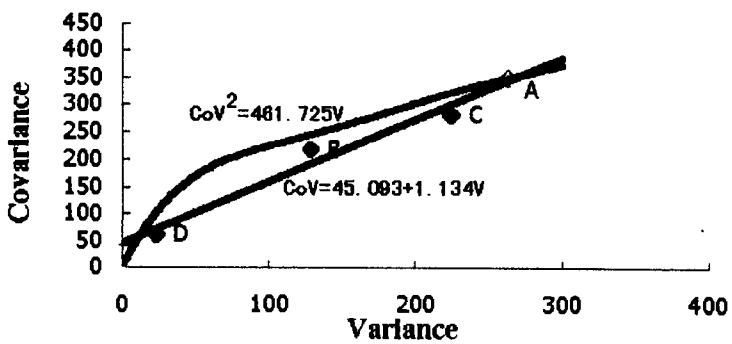


Fig. 2, Variance and Covariance of Parents. The regression equation was CoV=45.093+1.134V, the parabola equation (CoV2=461.725V) zoned all points (V, CoV). A, B, C and D respectivly represents four parents E9302, E9718-1, E9009-1-1-1 and E9010-J5-1.

Based on Hayman's hypothesis (Hayman, G., 1987), the intercept(a) involved in aforementioned regression equation can measure the average degree of dominance and the more number of dominant genes a parent contained, the shorter distance to innate point. Oberviously, in present experiment, a= 45.093 (D>H) indicated non-complete dominant inheritance on HT. Additonal, parents were ordered from D (E9010-J5-1), B (E9718-1), C (E9009-1-1-1) to A (E9302) with progressive reducing dominant genes, as well as a reducing HT index. This evidence confirmes that dominant genes give a positive effects on HT.

**Parameter Estimation**

Average degree of dominance ($\sqrt{H/D}$ = 0.567) means the inheritance of non-complete dominance in HT. Broad-sense heritability ($h_B^2$) and narrow-sense heritability ($h_N^2$) of $F_1$ hybrids came up 90.6% and 82.8%, respectively, as for table1. On the basis of fitting to quantitative hereditary pattern, minimum numbers of gene corresponding to HT were estimated by Mather's method: $K=(P_1-P_2)^2/8\delta d^2= 2.24$, it means more than 2 genes corresponding to HT inheritance on eggplant.

| Parameter | Value | Meanings |
|---|---|---|
| $\sqrt{H/D}$ | 0.567 | Average degree dominance |
| $r_{(CoV+V)/Y}$ | -0.799 | Correlation coefficient between (CoV+V) and relevant parents |
| $h^2_B$ | 90.60% | Broad-sense heritability |
| $h^2_N$ | 82.80% | Narrow-sense heritability |

Table 1, Estimation of Hereditary Parameter

**Efficiency of HT index and proper method for improvement of it on eggplant**

The significant correlation coefficient (r=0.868**) between HT index and ratios of fruit setting collected in open field indicated that the higher HT index appeared on seedlings, the higher ratio of fruit setting given by mature plants. So it is reasonable to measure by HT index in this experiment.(Data not shown).

Finally, additive effects are essential to enhance HT on eggplant, but the average degree of dominance and $h_B^2$ came up to 0.6 and 90%, respectively. It means that dominant effects shouldn't be neglect. As a result, an effective way to enhance HT on eggplant may begin with accumulations of HT genes on favorite inbred lines through recurrent selections, once available, combining them for target hybrids (Yi *et al.* 1995).

**ACKNOWLEDGEMENT**

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# SCREENING OF EGGPLANT GENOTYPES FOR YIELD, FRUIT BORER INFESTATION, LITTLE LEAF INCIDENCE AND QUALITY TRAITS

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## INTRODUCTION

Eggplant ( *Solanum melongena* L. ) is a native of India and is extensively grown in all South-East Asian countries. It is one of the most important and popular vegetable crop grown round the year in most of the parts of India. It is highly productive and usually finds its place as poor man's vegetable. Therefore, simultaneous evaluation of large number of genotypes for yield, resistant against major pests and diseases along with quality determining traits is prime important. Such detailed information in the crop is scanty.

## MATERIALS AND METHODS

An experiment comprised of 41 diverse genotypes was conducted at main Vegetable Research Station, Gujarat Agricultural University, Anand during 1995-96 (September to February) using randomised block design with 4 replications. There was natural insects and diseases infestation in the field as no spraying was made to control it.

## RESULTS AND DISCUSSION

The relative performance of different eggplant genotypes for various traits is presented in Table 1. Of these, Banaras Giant, CHBR 1, PLR 1 and Local Alwar found to be high yielder. The data on other traits revealed that ample variability existed in the genotypes under investigation. From the present findings, it can be concluded that positive and desirable traits would be utilized in the eggplant programme for the development of high yielding varieties incorporating disease and insect resistance along with quality parameter.

Table 1 : Mean values of different characters in eggplant.

| Sr. No | Genotypes | Fruit yield/plant (kg) | Fruit borer infestation (%) | Little leaf incidence (%) | Dry matter (mg/100 mg) | Anthocyanin content (mg/100 g fresh peel wt.) | Polyphenol oxidase activity (ρO.D/min) | Total phenols (mg/100 mg dry wt.) | Glyco-alkloid content (O.D.) | Total soluble sugars (mg/ 100 mg dry wt.) | Reducing sugars (mg/100 mg dry wt.) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Pant Rituraj | 3.59 | 18.09 | 27.55 | 6.738 | 11.920 | 0.963 | 2.188 | 0.550 | 32.475 | 6.195 |
| 2. | PLR 1 | 4.45 | 17.12 | 41.15 | 6.808 | 18.165 | 2.287 | 2.250 | 1.205 | 38.132 | 7.280 |
| 3. | DBR 8 | 4.03 | 18.14 | 39.12 | 7.125 | 27.635 | 2.015 | 2.325 | 0.830 | 35.255 | 10.217 |
| 4. | Aruna | 2.03 | 18.66 | 20.21 | 11.212 | 13.350 | 1.190 | 1.445 | 1.075 | 27.510 | 4.313 |
| 5. | KS 224 | 3.42 | 24.12 | 43.07 | 7.130 | 19.452 | 3.010 | 2.410 | 0.845 | 22.300 | 3.313 |
| 6. | JC 2 | 2.33 | 26.62 | 35.70 | 7.307 | 26.615 | 1.665 | 1.145 | 0.435 | 40.225 | 8.710 |
| 7. | DBSR 44 | 2.09 | 23.33 | 26.52 | 7.605 | 40.737 | 2.287 | 1.690 | 1.907 | 36.122 | 6.165 |
| 8. | BB-3-1 | 4.08 | 15.38 | 38.06 | 7.205 | 17.327 | 2.007 | 4.615 | 0.918 | 39.097 | 11.302 |
| 9. | Manjari Gota | 2.06 | 18.80 | 29.91 | 6.725 | 3.170 | 2.648 | 2.153 | 0.837 | 25.372 | 10.858 |
| 10. | CHBR 1 | 4.59 | 23.76 | 30.12 | 7.518 | 34.240 | 2.990 | 1.043 | 0.725 | 37.228 | 11.410 |
| 11. | KS 233 | 3.33 | 20.50 | 40.65 | 6.765 | 6.315 | 3.047 | 2.180 | 1.225 | 48.140 | 14.180 |
| 12. | Junagadh Ravaiya | 2.69 | 18.93 | 31.75 | 7.670 | 19.042 | 2.858 | 2.452 | 0.815 | 42.677 | 5.675 |
| 13. | AB 2 | 3.72 | 18.81 | 30.60 | 7.520 | 24.800 | 2.718 | 2.845 | 0.705 | 38.472 | 7.935 |
| 14. | BB 102 | 3.52 | 13.64 | 20.35 | 7.365 | 0.292 | 0.423 | 1.850 | 1.905 | 14.395 | 6.173 |
| 15. | Sel 1 | 1.84 | 15.86 | 41.27 | 8.230 | 54.212 | 3.257 | 2.320 | 0.428 | 34.573 | 5.415 |
| 16. | JB 64-1-2 | 3.47 | 14.83 | 48.92 | 7.163 | 20.333 | 2.523 | 1.850 | 0.957 | 27.443 | 4.735 |
| 17. | DBSR 91 | 2.43 | 18.19 | 24.10 | 6.905 | 30.535 | 2.025 | 2.318 | 0.815 | 29.702 | 6.370 |
| 18. | ABV 1 | 1.35 | 15.70 | 20.02 | 7.142 | 8.925 | 2.457 | 0.900 | 0.725 | 16.388 | 4.162 |
| 19. | Green round | 3.19 | 15.68 | 22.17 | 7.080 | 0.000 | 0.345 | 1.438 | 0.567 | 12.053 | 3.878 |
| 20. | Local Deesa | 2.38 | 15.66 | 56.07 | 7.355 | 44.525 | 2.017 | 1.092 | 0.405 | 10.282 | 3.933 |
| 21. | Alwar | 3.03 | 17.24 | 35.30 | 7.455 | 0.292 | 3.468 | 0.978 | 0.740 | 12.148 | 4.620 |
| 22. | Local Alwar | 4.17 | 22.89 | 43.82 | 6.747 | 0.655 | 2.910 | 2.706 | 0.405 | 28.167 | 10.070 |
| 23. | Local Anand | 3.61 | 20.05 | 48.52 | 7.053 | 13.815 | 2.035 | 2.457 | 0.750 | 26.140 | 7.035 |
| 24. | Morvi-4-2 | 4.18 | 28.73 | 45.50 | 6.110 | 19.717 | 1.015 | 2.520 | 0.180 | 61.095 | 12.832 |
| 25. | Bombay Gulabi | 3.98 | 32.84 | 34.95 | 6.535 | 27.655 | 2.440 | 1.065 | 0.703 | 56.702 | 10.970 |
| 26. | Bilimora | 3.19 | 30.09 | 52.52 | 6.645 | 112.162 | 1.447 | 1.300 | 0.420 | 56.005 | 4.980 |
| 27. | Surti Ravaiya | 2.43 | 28.11 | 33.45 | 7.455 | 19.138 | 2.245 | 2.240 | 0.132 | 50.325 | 6.830 |
| 28. | Banaras Giant | 4.63 | 25.70 | 19.55 | 7.640 | 0.000 | 0.653 | 1.605 | 0.297 | 38.387 | 5.548 |
| 29. | White Bhada | 2.93 | 18.56 | 20.50 | 7.575 | 0.000 | 0.512 | 2.520 | 0.935 | 21.465 | 7.748 |
| 30. | DBR 31 | 2.87 | 22.31 | 31.85 | 6.905 | 28.620 | 2.530 | 1.840 | 0.835 | 37.315 | 6.535 |
| 31. | White Bhada Bareli | 2.15 | 18.08 | 20.17 | 7.258 | 0.063 | 0.627 | 2.715 | 0.942 | 17.342 | 8.825 |
| 32. | H 8 | 2.94 | 20.46 | 39.42 | 7.838 | 15.408 | 2.250 | 3.550 | 1.215 | 42.360 | 12.715 |
| 33. | AB 1 | 3.37 | 23.46 | 27.95 | 6.912 | 26.725 | 2.273 | 3.845 | 0.765 | 44.007 | 11.400 |
| 34. | CH-157-16- 4-1 | 2.56 | 17.68 | 27.90 | 7.520 | 0.000 | 0.410 | 2.027 | 1.710 | 16.195 | 7.570 |
| 35. | PN 1 | 2.84 | 19.81 | 33.62 | 8.555 | 43.260 | 2.817 | 1.602 | 1.027 | 32.713 | 6.483 |
| 36. | BR-16-3 | 1.94 | 18.16 | 22.12 | 6.738 | 28.637 | 2.847 | 2.545 | 1.415 | 30.715 | 6.785 |
| 37. | CHBR 2 | 2.65 | 20.32 | 30.60 | 8.370 | 36.905 | 2.895 | 0.898 | 0.425 | 27.825 | 5.842 |
| 38. | CHBR 3 | 2.17 | 16.84 | 34.00 | 6.573 | 29.940 | 2.013 | 0.933 | 0.315 | 18.070 | 2.173 |
| 39. | PBR 7 | 3.20 | 20.61 | 39.57 | 7.240 | 13.350 | 2.143 | 1.493 | 0.207 | 30.335 | 6.175 |
| 40. | Jottana local | 1.51 | 19.09 | 48.65 | 8.438 | 0.627 | 0.918 | 1.070 | 0.430 | 24.323 | 3.197 |
| 41. | Dishana local | 3.14 | 21.92 | 41.87 | 6.912 | 41.365 | 2.270 | 2.483 | 0.325 | 31.662 | 6.953 |
| | Mean | 2.81 | 20.36 | 34.14 | 7.340 | 21.460 | 2.035 | 2.026 | 0.781 | 31.980 | 7.260 |
| | S.Em. ± | 0.17 | 0.50 | 1.15 | 0.112 | 1.580 | 0.145 | 0.145 | 0.050 | 1.360 | 0.500 |
| | C.D. at 5% | 0.47 | 1.40 | 4.25 | 0.313 | 4.430 | 0.408 | 0.408 | 0.140 | 3.800 | 1.400 |

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# INTER-SPECIFIC HYBRIDIZATION IN EGGPLANT FOR RESISTANCE TO SHOOT AND FRUIT BORER

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## Introduction

Eggplant (*Solanum melogena* L) commonly known as brinjal is widely grown in tropical and sub tropical parts of the world. It is susceptible to several insect pests including a major insect, shoot and fruit borer (*Leucinodes orbonalis* Guen.). This insect starts attacking eggplant from juvenile phase of the plants to maturity of fruits. Number of related species of *Solanum melongena* have been found resistant to shoot and fruit borer under field condition. It was considered desirable to transfer such a characteristic of agricultural significance through hybridization into different popular varieties of egg plant. Hence, the studies on inter-specific hybridisation in relation to shoot and fruit borer resistance are of great significance in eggplant breeding.

## Materials and Methods

The experimental materials comprised of inbreds of eggplant namely, *Solanum incanum, Solanum indicum, Solanum gilo* and three inbred cultivars of *Solanum melongena*, namely, Annamalai, Aushey and Pusa Kranti. The hybrid seeds of Pusa Kranti x *S. incanum,* Pusa Kranti x *S. indicum,* Pusa Kranti x *S. gilo,* Annamlai x *S. gilo* and Aushey x *S. gilo* were obtained by crossing. These hybrids and their parents were screened against shoot and fruit borer infestation under natural field conditions at three different stages of plant growth namely, seedling stage, adult stage and fruit bearing stage. Each genotype was classified as per the scoring pattern given below (Table 1). The seeds of parental lines and five hybrids were sown during third week of June and transplanted at spacing of 75 x 60 cm after one month of sowing. Each treatment (genotype) consisted of 20 plants of which 16 plants were used for taking observations. All recommended agronomic practices (excluding insecticidal spraying) were followed to grow the crop under irrigated condition. The percentage of seedling, shoot and fruit (both number and weight basis) infestation was recorded and analyzed.

Pollen fertility of inter-specific hybrids was determined by the stainability of the pollen grains from the freshly opened flowers with 1 % acetocarmine solution. The turgid and darkly stained pollen were considered as fertile while the shrunken and colourless ones as sterile. About 1000 pollen grains were observed to estimate the pollen fertility of $F_1$ hybrids. For cytological study, the flower buds were fixed early in the morning in 1:3 acetic-and alcohol mixture for half an hour. They were then transferred to 70 % alcohol and stored at $10^0$ C. The chromosomes were studied from acetocarmine squashes of pollen mother cells (PMCs) under microscope.

## Results and Discussion

*Shoot and fruit borer infestation*

The data on shoot and fruit borer infestation (per cent) in *Solanum* species, cultivars of *S. melongena* and their hybrids are presented in Table 1. The cultivars Pusa Kranti and Aushey showed maximum seedling infestation of 20.74 and 19.25 per cent respectively. The seedling infestation was lowest in *S. indicum* (6.60 %) among the parents and Pusa Kranti x *S. indicum* (7. 46 %) among the hybrids. The percentage of shoot infestation also found higher in Aushey (13.89 %) and Pusa Kranti (12.06 %) whereas lowest infestation occurred in *S. indicum* (2.99 %). The hybrid, Pusa Kranti x *S. indicum* showed less susceptibility to shoot damage. With regard to fruit infestation, *S. indicum* was immune to shoot and fruit borer while the cultivars Aushey and Pusa Kranti were susceptible to this pest showing more than 30 percent fruit infestation on weight basis.

The hybrid, Pusa Kranti x *S. indicum* showed lowest fruit infestation of 9.39 per cent and 10.52 per cent on number and weight basis respectively but the fruits were abnormal in size.

In the present investigation *S. gilo* was also found to have high degree of resistance against shoot and fruit borer so also *S. incanum* and *S. indicum* . Similar reports were also made by Rao and Kumar (1980) in case of *S. indicum*, Kale *et al* (1986) in case of *S. incanum* and Tejavathu *et al* (1991) in *S. gilo*. The inter-specific hybrids generated in the present study can now be utilized for transfer of shoot and fruit borer resistance gene(s) as well as other agronomically desirable traits from the wild relatives to the cultivars of eggplant. The hybrids of cv. Pusa Kranti x *S. incanum* and cv. Pusa Kranti x *S. indicum* had high level of pollen fertility and showed less problem and they will be most useful in this regard.

*Cytological study*

The pollen fertility of inter-specific hybrids and their parents are given in Table 2. Pollen fertility of $F_1$ hybrids cv. Pusa Kranti x *S. incanum*, cv. Pusa Kranti x *S. indicum*, cv. Pusa Kranti x *S. gilo*, cv. Annamalai x *S. gilo* and cv.Aushey x *S. gilo* were 57.2, 62.6, 12.4, 15.4 and 8.7 per cent, respectively while their parents were highly fertile. The variations in the level of low pollen fertility could be due to either the differences in genetic constitution or cryptic structural differences between the parent chromosomes, which might be too small to be detected cytologically or due to combined effects of both the factors (Rao,1981; Anis *et al.,* 1994).

The chromosomal association and the chiasma frequency of $F_1$ hybrids between different cultivars of *S. melongena* and *S. gilo* are presented in Table 3. The frequency of univalents were found appreciably more compared to bivalents and those were 16.73, 22.60, 14.40 in Pusa Kranti x *S. gilo*, Annamalai x *S. gilo* and Aushey x *S. gilo,* respectively. These hybrids also showed high degree of sterility. The two conditions i.e., maximum univalent coupled with high degree of sterility, indicated that the parental species were separated internally by genetic barriers i.e. there may be large extent of dissimilarities of the parental chromosomes. They also observed as many as 24 univalents in some pollen mother cells of the hybrids with loose association of bivalent chromosome having very low frequency of chiasmata. The low chiasma frequency in all $F_1$ hybrid chromosomes contributing to 'loose' association and occurrence of univalents at diakinesis and metaphase I.

In the present findings, the hybrid plants of all three cultivars of *S. melongena* x *S. gilo* produced small parthenocarpic fruits borne in cluster even though there was profuse flowering. The direct evidence of parthenocarpy has not been studied cytologically in the present findings. But it may be assumed to be because of diplontic sterility of the hybrids. This sterility is typically due to the dis-harmonious genetic constitution or combination of genes of the hybrids with different types of action. They may suppose to interfere with the development of the reproductive organs from the earliest differentiation to the final stage of meiosis.

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**Table 1. Relative incidence of shoot and fruit borer infestation in different species and hybrids of eggplant.**

| Genotype | Infestation of *Leucinodes orbonalis* (%) | | | |
|---|---|---|---|---|
| | Seedling | Shoots | Number of fruits | Fruit weight |
| *S. incanum* | 11.21 (R) | 6.95 (R) | 16.10 (MR) | 15.86 (MR) |
| *S. indicum* | 6.60 (R) | 2.99 (R) | 0.00 (I) | 0.00 (I) |
| *S. gilo* | 7.51 (R) | 6.87 (R) | 14.50 (R) | 9.95 (R) |
| *S. melongena* cv. Annamalai | 14.26 (R) | 6.09 (R) | 24.04 (MR) | 26.10 (MR) |
| *S. melongena* cv. Aushey | 19.25 (MR) | 13.89 (R) | 29.20 (S) | 34.38 (S) |
| *S. melongena* cv. Pusa Kranti | 20.74 (MR) | 12.06 (R) | 24.38 (MR) | 31.34 (S) |
| *S. melongena* cv. Pusa Kranti x *S. incanum* | 14.47 (R) | 6.43 (R) | 20.70 (MR) | 15.16 (R) |
| *S. melongena* cv. Pusa Kranti x *S. indicum* | 7.46 (R) | 5.48 (R) | 9.39 (R) | 10.52 (R) |
| *S. melongena* cv.Pusa Kranti x *S. gilo* | 13.43 (R) | 7.03 (R) | -- | -- |
| *S. melongena* cv. Annamalai x *S. gilo* | 14.59 (R) | 5.99 (R) | -- | -- |
| *S. melongena* cv. Aushey x *S. gilo* | 14.41 (R) | 9.53 (R) | -- | -- |

I = Immune (0 %); R = Resistant (1-15 %); MR = Mederately Resistant (16-30 %); S = Susceptible (30-40 %)

**Table 2. Pollen Fertility of different *Solanum* species and their $F_1$ hybrids**

| Female Parent | Male Parent | Pollen Fertility (%) | | |
|---|---|---|---|---|
| | | Female Parent | Male Parent | Hybrid |
| *S. melongena* cv. Pusa Kranti | *S. incanum* | 92.06 | 79.43 | 57.2 |
| *S. melongena* cv. Pusa Kranti | *S. indicum* | 92.06 | 84.26 | 62.6 |
| *S. melongena* cv.Pusa Kranti | *S. gilo* | 92.06 | 78.20 | 12.4 |
| *S. melongena* cv.Annamalai | *S. gilo* | 89.18 | 78.20 | 15.4 |
| *S. melongena* cv.Aushey | *S. gilo* | 86.47 | 78.20 | 8.7 |

**Table 3. Chromosomal association and chaisma frequency at diakinesis in inter-specific hybrids of *Solanum* species**

| Hybrids | No. of cells analysed | Average frequency per cell | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Quadrivalent | Trivalent | Bivalent | | Univalent | Chiasma per | |
| | | | | Rings | Rods | | Cell | Bivalent |
| *S. melongena* cv. Pusa Kranti x *S. gilo* | 30 | -- | 0.20±0.40 | 2.53±1.31 | 0.80±0.75 | 16.73±2.11 | 2.93 | 0.88 |
| *S. melongena* cv. Annamalai x *S. gilo* | 40 | -- | -- | -- | 0.70±0.45 | 22.60±1.89 | 0.25 | 1.07 |
| *S. melongena* cv. Aushey x S. gilo | 30 | 0.70±0.25 | -- | 2.53±1.50 | 2.13±0.72 | 14.40±3.20 | 4.00 | 0.86 |

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# EVALUATION OF BRINJAL GERMPLASM AGAINST PHOMOPSIS DISEASE

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**Abstract**

Eighty four genotypes of brinjal ( *Solanum melongena L.)* were evaluated against Phomopsis leaf blight and fruit rot disease (*Phomopsis vexans)* (Sacc. & Syd.) Harter). Out of 84 genotypes screened against leaf blight, 7, 34, 33 & 10 gave resistant, moderately susceptible (MS), susceptible (S) and highly susceptible (HS) reaction to the pathogen respectively. BPL-1, Ornamental brinjal, Pant Rituraj, Pusa Kranti, Pusa Uttam,T-3 and IC316237 with≤ 5% severity of leaf blight were found resistant to disease. Of these genotypes, twenty seven genotypes possessing fair degree of resistance to bacterial wilt were evaluated against fruit rot stage of the disease. BPL-1, Ornamental Brinjal, and Ragini hybrid with ≤ 5% fruit rot were found resistant to disease and other genotypes were graded moderately susceptible to susceptible to the disease. Ornamental brinjal and BPL –1 were found free from Phomopsis leaf blight and fruit rot.

**Introduction**

Phomopsis disease of eggplant (*Solanum melongena* L) caused by *Diaporthe vexans* Gratz (aneomorph: *Phomopsis vexans* (Sacc. & Syd.) Harter) is one of the most destructive disease and a major bottleneck in enhancing the productivity of brinjal. The pathogen is specific to brinjal and causes poor germinability of seeds, damping-off of seedlings, stem canker, leaf blight and fruit rot in brinjal. Of these, the rot stage is very destructive affecting the marketable fruits. The disease is reported to cause up to 25% losses in fruit yield (Hasija & Chowdhary, 1980) and hampers the seed production programme drastically. In Himachal Pradesh the disease remains throughout the year on seasonal and perennial cultivars of brinjal (Kumar, 1998). The disease starts appearing in the fields during April and increases exponentially uptil October and then begins to decline, causing heavy losses in the main commercial as well as the seed crops.

**Materials & Methods**

Phomopsis leaf blight was scored on 84 genotypes of brinjal, collected from Department of Vegetables crops, HPKV, Palampur; HAU, Hisar; PAU, Ludhiana ; IARI New Delhi and NBPGR, New Delhi. One month old seedlings of brinjal genotypes were transplanted in plots with plant to plant and row to row spacing of 45 & 60 cm, respectively. Ten plants/genotype, replicated thrice, were evaluated against leaf blight and fruit rot under field conditions. One month after transplanting the plants were spray inoculated with spore suspension (125 spores / ml) of *P. vexans.* Data on leaf blight and fruit rot were recorded with the appearance of disease and thereafter at weekly intervals till the completion of experiment and pooled at the end. Data on leaf blight were scored on 0- 8 point scale (0= 0; 1= 0.1-5; 2=5.1-10; 3= 10.1-20; 4=20.1-30; 5=30.1-40; 6=40.1-50; 7=50.1-75 and 8=75.1-100% leaf area infected )

and fruit rot on 1-5 point scale (1=0; 2=0.1-25; 3=25.1 50; 4=50.1-75 & 5=75.1-100% fruit infected ) and percent disease infection ( PDI ) was determined as follows:

$$PDI = \frac{\text{Sum of all ratings}}{\text{Total ratings x Max. grade}} \times 100$$

**Results and Discussion**

Ornamental brinjal and BPL-1 failed to develop disease inspite of repeated inoculation tests whereas JC-4 developed maximum (63.7%) leaf blight. Rest of genotypes developed disease in the range of 0 to 63.7% (Table 1). The genotypes were grouped into resistant (R), moderately susceptible (MS), susceptible (S) and highly susceptible (HS) based on the development of leaf blight in the range of $\leq 5$; 5.1 - 20.0; 20.1 - 50 and >50 per cent, respectively. Accordingly, seven genotypes namely 'BPL-1', 'Ornamental brinjal', 'Pant Rituraj', 'Pusa Kranti', Pusa Uttam', 'T-3' and 'IC 316237' were graded as resistant as these genotypes developed leaf blight disease in the range of 0 - 5 per cent and ten genotypes : 'HLB 25', 'HOE 444', JC-4, 'Sel - 1', 'IC 89903', 'IC 89932', 'IC 90951', 'IC 136184', 'IC 136321', and 'IC 382590' which developed more than 50 per cent leaf blight were categorized as highly susceptible (Table 2). Rest of genotypes which showed disease in the range of 5.1 - 20 and 20.1 - 50 per cent were graded as moderately susceptible and susceptible to Phomopsis leaf blight. Thus, of 84 genotypes screened for their reaction to leaf blight; 7, 34, 33 and 10 were graded as resistant, moderately susceptible, susceptible and highly susceptible to the disease, respectively.

Progress of Phomopsis fruit rot was scored on 27 genotypes of brinjal, which possessed fare degree of resistance to bacterial wilt except PPL. Ornamental brinjal and BPL-1 remained free from fruit rot and these two genotypes of brinjal also possessed good resistance to fruit borer (Table 3). Ornamental brinjal with no fruit rot and negligible borer attack, have fruits with bitter taste which are not eatable. Genotype SM 6-6 followed by Sel-7 developed maximum and Ragini hybrid followed by SM 6-7 the minimum fruit rot. The brinjal genotypes were grouped into resistant (R), moderately susceptible (MS) and susceptible (S) based on the development of fruit rot in the range of < 5; 5.1-20 and > 20.0%, respectively. Accordingly 3, 16 and 8 genotypes were graded as resistant, moderately susceptible and susceptible to fruit rot (Table 4). The genotypes Arka NeelKanth, Arka Nidhi, JC-1, PPC, PPL, Punjab Barsati, Sel-7 and SM 6-6, which developed more than 20% fruit rot, were graded as susceptible. Ornamental brinjal and BPL-1 with no fruit rot and Ragini hybrid with 5% fruit rot were graded as resistant. Remaining 16 genotypes which developed fruit rot in the range of 5.1-20% were graded as moderately susceptible to Phomopsis fruit rot.

Thus, BPL-1 and Ornamental brinjal with no Phomopsis leaf blight and fruit rot can be exploited for resistance breeding programme against the disease.

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Table 1. Phomopsis leaf blight severity (PDI ) on different genotypes of brinjal. 1 = Brinjal genotypes, 2 = Leaf blight severity.

| 1 | 2 | 1 | 2 | 1 | 2 |
|---|---|---|---|---|---|
| Arka Keshav | 15.1 | Ornamental brinjal | 0.0 | IC 111462 | 12.6 |
| Arka Neelkanth | 11.7 | Pant Rituraj | 3.6 | IC112317 | 14.4 |
| Arka Nidhi | 38.0 | PPC | 26.3 | IC126706 | 12.2 |
| BB 3 –1 | 17.5 | PPL | 14.5 | IC126738 | 18.2 |
| BB 7 | 11.8 | Punjab Barsati | 19.2 | IC126829-1 | 18 |
| BB-44 | 12.8 | Pusa Anupam | 24.0 | IC126906 | 22.2 |
| BB 60-C | 27.4 | Pusa Bindu | 14.7 | IC126911 | 22.5 |
| BB 64 | 42.8 | Pusa Kranti | 3.6 | IC127023 | 19.0 |
| BR 112 | 35.3 | Pusa Uttam | 4.0 | IC127153 | 9.9 |
| CH 243 | 16.8 | Ragini Hybrid | 14.2 | IC 136184 | 52.3 |
| CH 249 | 9.4 | Sel –1 | 56.2 | IC136305 | 26.5 |
| CH 309 | 16.3 | Sel-3 | 27.9 | IC136321 | 51.5 |
| Composite- 2 | 8.7 | Sel –4 | 31.6 | IC136370 | 47.4 |
| DPLB 4 | 21.1 | Sel –7 | 40.0 | IC136465 | 44.5 |
| DPLB 5 | 48.1 | Shiva | 49.2 | IC136481 | 26.0 |
| HLB 25 | 53.4 | SM6-6 | 16.0 | IC136551 | 42.3 |
| HLB 300 | 30.8 | SM6-7 | 19.3 | IC137682 | 31.5 |
| Hisar Jamun | 23.7 | SM-141 | 26.9 | IC144021 | 11.9 |
| Hisar Pragti | 22.7 | Soorya | 11.7 | IC144075 | 49.8 |
| Hisar Shyamal | 17.2 | Swetha | 9.8 | IC144126 | 39.0 |
| HOE 444 | 54.6 | T-3 | 1.7 | IC201132 | 21.7 |
| JC- 1 | 44.1 | IC89903 | 52.0 | IC201231 | 12.1 |
| JC-2 | 18.6 | IC89932 | 59.7 | IC305065 | 37.9 |
| JC –4 | 63.7 | IC90071 | 23.2 | IC316237 | 4.0 |
| JC-5 | 17.9 | IC90109 | 22.7 | IC316274 | 13.2 |
| JC-7 | 13.1 | IC90951 | 62.9 | IC382590 | 51.8 |
| Majri Gota | 19.7 | IC99640 | 16.5 | IC383345 | 12.9 |
| NBBL 1 | 31.2 | IC111024 | 20.6 | BPL- 1 | 0.0 |

Table 2. Reaction of various genotypes of brinjal to Phomopsis leaf blight

| Disease Reaction | Leaf blight severity (%) | Genotypes |
|---|---|---|
| Resistant ( R ) | ≤ 5.0 | Ornamental brinjal, Pant Rituraj, Pusa Kranti, Pusa Uttam, T-3, IC 316237, BPL –1 |
| Moderately Susceptible (MS ) | 5.1 – 20.0 | Arka Keshav, Arka Neelkanth, BB3-1, BB 7, BB 44, CH243, CH 249, CH 3 09, Composite -2, Hisar Shymal ,JC-2, JC-5, JC-7, MqJri Gota, PPL, Punjab Barsati,Pusa Bindu, Ragini hybrid, SM 6 - 6, SM 6-7, Soorya, Swetha, IC 99640, IC 111462, IC 112317,IC 126706, IC 126738, IC 126829 -1, IC 127023, IC 127153, IC 144021, IC 201231, IC 316274, IC 383345 |
| Susceptible (S ) | 20.1 – 50.0 | Arka Niddi, BB 60 - C, BB 64, BR 112, DPLB 4, DPLB 5, HLB 300, Hisar Jamun, Hisar Pragti, JC 1,,NBBL 1, PPC, Pusa Anupam, Sel -3, Sel. - 4, Sel-7,,Shiva, SM 141,IC 90071,IC 90109,IC 111024, IC 126906, IC 126911,IC 136305, IC 136370, IC 136465, IC 136481,IC 13655 1, IC 137682, IC 144075, IC 144126, IC 201132, IC 305065 |
| Highly Susceptible (HS ) | > 50.0 | HLB 25, HOE 444, JC -4, Sel -1, IC 89903, IC 89932, IC 90951, IC 136184, IC 136321, IC 382590 |

Table 3. Performance of different genotypes of brinjal against Phomopsis fruit rot.

| Genotypes | Fruit rot (%) |
|---|---|
| Arka Keshav | 14.7(22.54) |
| Arka Neel Kanth | 21.9(27.90) |
| Arka Nidhi | 20.9(27.20) |
| BB-64 | 13.4(21.47) |
| BB-60C | 13.5(21.56) |
| BPC-1 | 15.2(22.95) |
| BPL-1 | 0.0(0.00) |
| BE 706(Hybrid) | 9.5(17.95) |
| CH 243 | 15.0(22.79) |
| CH 249 | 17.5( 24.73) |
| CH 309 | 16.8(24.20) |
| DPLB-4 | 10.5(18.91) |
| Hisar Shyamal | 18.5(25.47) |
| JC-1 | 25.6(30.40) |
| JC-2 | 13.4(21.47) |
| JC-4 | 20.0(26.57) |
| JC-7 | 14.5(22.38) |
| Ornamental brinjal | 0.0(0.00) |
| PPC | 24.7(29.80) |
| PPL | 21.0(27.27) |
| Punjab Barsati | 24.0(29.33) |
| Pusa Anupam | 19.0(25.84) |
| Ragini hybrid | 5.0(12.92) |
| Sel-7 | 26.5(30.98) |
| SM 6-6 | 27.5(31.63) |
| SM 6-7 | 7.5(15.89) |
| SM 141 | 10.2(18.63) |
| CD (P=0.05) | (4.17) |

Angular transformed values in parentheses.

Table 4. Reaction of various genotypes of brinjal to phomopsis fruit rot.

| Disease reaction | Fruit rot(%) | Genotypes |
|---|---|---|
| Resistant (R) | < 5.0 | BPL-1, Ornamental brinjal, Ragini hybrid |
| Moderately susceptible (MS) | 5.1-20.0 | ArkaKeshav, BB-64, BB-60-C, BPC-1, BE-706(hybrid), CH-243, CH-249, CH-309, DPLB-4, Hisar Shyamal, JC-2, JC-4, JC-7, Pusa Anupam, SM 6-7, SM-141 |
| Susceptible(S) | > 20.0 | Arka Neelkanth, Arka Nidhi, JC-1, PPC, PPL, Punjab Barsati, Sel-7, SM 6-6 |

*Fruits bitter in taste, not eatable.