ANNUAL REPORT OF THE

BEAN IMPROVEMENT COOPERATIVE

A VOLUNTARY AND INFORMAL ORGANIZATION
TO EFFECT THE EXCHANGE OF INFORMATION AND MATERIALS

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Report of The

<u>BEAN IMPROVEMENT COOPERATIVE</u>

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<u>Coordinating Committee</u>

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Karen Cichy   Peter Pauls
Jim Kelly (Ex officio)   Thiago Souza
Ken Kmiecik   Jennifer Trapp
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http://www.css.msu.edu/bic
SITE REGULARLY UPDATED

TABLE OF CONTENTS

RESEARCH PAPERS FOR 2017

Cover photo: pinto bean field in Colorado courtesy of Mark Brick

BIC COMMITTEE MEMBERSHIP - 1957 to 2017

Coordinating Committee (approximate year of appointment):

1957   Dean, Enzie, **Frazier* (BIC Coordinator/President)**, McCabe, Zaumeyer
1960   Anderson, Atkin, Dean, Enzie, **Frazier**, McCabe, Zaumeyer
1962   Anderson, Atkin, Dean, **Frazier**, Pierce, Polzak, Zaumeyer
1968   Anderson, **Coyne**, Dean, Jorgensen, Polzak, Zaumeyer
1971   Briggs, **Coyne**, Dean, Jorgensen, Polzak, Zaumeyer
1972   Burke, **Coyne**, Dean, Jorgensen, Kiely, Polzak, Zaumeyer
1974   Ballantyne, Bravo, Burke, **Coyne**, Dickson, Emery, Evans, Kiely, Saettler, Zaumeyer
1977   Ballantyne, Bliss, Coyne, **Dickson**, Emery, Evans, Graham, Meiners, Morris, Saettler, Zaumeyer
1978   Atkin, Ballantyne, Bliss, Coyne, **Dickson**, Graham, Meiners, Morris, Saettler, Sprague
1979   Atkin, Bliss, **Dickson**, Graham, Hagedorn, Meiners, Morris, Sprague, Wallace
1980   Atkin, Bliss, **Dickson**, Hagedorn, Morris, Sprague, Steadman, Temple, Wallace
1982   Atkin, Coyne, **Dickson**, Hagedorn, Sprague, Steadman, Temple, Wallace, Wyatt
1983   Coyne, **Dickson**, Hagedorn, Saettler, Silbernagel, Steadman, Temple, Wallace, Wyatt
1985   Coyne, **Dickson**, Mok, Saettler, Silbernagel, Steadman, Temple, Wallace, Wyatt
1986   Coyne, **Dickson**, Mok, Saettler, Schoonhoven, Schwartz, Silbernagel, Steadman, Wallace
1988   Brick, Dickson, Emery, Magnuson, Roos, **Schwartz**, Singh, Steadman, Uebersax
1992   Dickson, Emery, Grafton, Magnuson, **Schwartz**, Singh, Stavely, Steadman, Uebersax
1994   Antonius, Dickson, Grafton, Magnuson, Park, **Schwartz**, Singh, Stavely, Uebersax
1996   Antonius, Grafton, Park, **Schwartz**, Singh, Stavely, Myers, Kotch, Miklas, Riley
1998   Antonius, Park, Schwartz (ex officio), Singh, Myers, Kotch, Miklas, Riley, Beaver, Vandenberg, **Kelly**
2000   Antonius, Beaver, **Kelly**, Kotch, Miklas, Myers, Park, Riley, Schwartz, Singh, Vandenberg
2001   Antonius, Beaver, **Kelly**, Kotch, Miklas, Myers, Park, Riley, de Ron, Schwartz, Vandenberg
2003   Beaver, **Kelly**, Kmiecik, Kurowski, Miklas, Myers, Park, Riley, de Ron, Schwartz, Vandenberg
2007   Beaver, **Kelly**, Kmiecik, Miklas, Myers, Park, Riley, de Ron, Schwartz , Shellenberger, Vandenberg
2008   Beaver, **Kelly**, Kmiecik, Miklas, Myers, Pauls, Riley, de Ron, Schwartz , Shellenberger, Vandenberg
2010   Beaver, Kelly, Kmiecik, **Miklas**, Myers, Pauls, Riley, de Ron, Schwartz , Shellenberger, Vandenberg
2011   Bett, Kelly, Kmiecik, **Miklas**, Myers, Osorno, Pastor-Corrales, Pauls, Riley, de Ron, Wahlquist
2016   Bett, Cichy, Kelly, Kmiecik, **Miklas**, Myers, Osorno, Pauls, Souza, Trapp, Wahlquist

Awards Committee:

1971	Baggett, Briggs, Burke, Dean, Wallace	1989	**Coyne**, Silbernagel, Wallace
1973	Burke, Dean, Mauth, Zaumeyer	1995	**Coyne**, Dickson, Stavely
1975	Ballantyne, Frazier, Mauth	1997	**Coyne**, Schwartz, Stavely
1977	Ballantyne, Curme, Frazier, Schuster	2001	**Hosfield**, Magnuson, Schwartz
1979	Ballantyne, Schuster, Silbernagel, Temple	2004	Hosfield, **Schwartz**, Singh
1981	Abawi, Bliss, Monis, Silbernagel	2008	Hosfield, **Schwartz**, Singh
1983	Adams, Bliss, Burke, Dean, Morris	2012	Noffsinger, **Schwartz**, Singh
1985	Emery, Hagedorn, Sandsted, Schwartz	2014	**Beaver**, Noffsinger, Urrea
1987	Emery, Hagedorn, Sandsted	2016	**Beaver**, Myers, Urrea

Genetics Committee

2004   **Bassett** (Chair), Beaver, Blair, Gepts, McClean, Miklas, Welsh (ex officio)
2005   **Beaver** (Acting Chair), Blair, Gepts, McClean, Miklas, Porch, Welsh (ex officio)
2007   Beaver, Blair, Gepts, McClean, Miklas, **Porch** (Chair), Welsh (ex officio)
2008   Bett, Blair, Gepts, McClean, Miklas, **Porch** (Chair), Urrea, Welsh (ex officio)
2014   **Bett** (Chair), Ferreira, Gepts, Goncalves-Vidigal, Kalavacharla, Kelly, Kisha (ex officio), McClean, Osorno, Porch, Urrea

THE 60th ANNUAL REPORT OF THE BEAN IMPROVEMENT COOPERATIVE

The Bean Improvement Cooperative (BIC) invites all members and other interested parties to join us at the Twenty-ninth Biennial Meeting from October 30 through November 1, 2017, in East Lansing, Michigan. The local BIC meeting organizers are: Jim Kelly: kellyj@msu.edu, Karen Cichy: cichykar@msu.edu, Marty Chilvers: chiliver@msu.edu, Greg Varner: varnerbean@hotmail.com, Megghan Honke Seidel: honkemeg@msu.edu. In addition, the associated meetings with our colleagues in the North American Pulse Improvement Association will be from November 1 to November 3, 2017 [NAPIA host contact: Weidong Chen weidong.chen@ars.usda.gov]. The Phaseolus Crop Germplasm Committee, BIC Genetics Committee and the Regional W-3150 Committee are scheduled for November 1. Also on November 1, NAPIA will host a Root Rot Workshop. A field trip is also planned. Please refer to the information provided by the local organizing committee in the current report, and look for additional information and updates on the website https://events.anr.msu.edu/bicnapia2017/ for the conference and or check for information and updates on the BIC web site www.css.msu.edu/bic.

Please review the call for nominations for the **BIC Meritorious Service Award**, **BIC Achievement Award**, and new **BIC Technical Merit Award,** and forward your nominations to the Awards Committee Chairperson, James Beaver (j_beaver@hotmail.com) by May 31, 2017. We will continue to recognize our founding members through the **Frazier-Zaumeyer Distinguished Lectureship**. The Lectureship will be awarded at the meeting in East Lansing and nominations should be sent to James Beaver. A current membership list of BIC Committees and those who have received awards throughout the history of the BIC is provided in the current issue and on the BIC website to assist you in nominating colleagues for these awards. We want to make this a memorable meeting, so please share this information with interested colleagues who might like to attend these meetings and/or join the BIC.

The BIC website continues to be maintained at Michigan State University under the direction of Dr. James Kelly. The BIC recognizes the continued IT support that Dave Krauss provides us for maintaining the website. Please consider submitting updates to the Research section of the website. The goal for this Research section is to provide an overview of appropriate techniques for breeding common beans for a particular trait, identify cultivars and breeding lines that can be used as sources of resistance for a particular stress, and to provide references where researchers can obtain more detailed information. Please feel free to contact us with any new ideas, contributions, or updates for the BIC website. A recent suggestion was to develop a specific links page for all the new genomic resources available for Phaseolus.

To reduce mailing costs and expedite communications, the BIC continues to conduct business by email and through postings on the web page. Furthermore, we are transitioning to an online publication only. A site for members to download the report will be provided as will information concerning publication of the book on demand through a third-party publisher. Appreciate your patience as we make this change. Members are asked to ensure that email addresses are current and to periodically review the web page for information on meetings, deadlines and critical dates. We are always open to new ideas to make the BIC a more effective organization and any thoughts can be shared with members of the Coordinating Committee. See you in East Lansing………….

Dr. Phillip Miklas, BIC President

RECIPIENTS of BIC AWARDS for MERITORIOUS SERVICE, ACHIEVEMENT, TECHNICAL MERIT & FRAZIER-ZAUMEYER DISTINGUISHED LECTURESHIP

Year	Recipients
1970	Melvin E. Anderson- Rogers Bros. Seed Co., Plant Pathologist William A. Frazier- Oregon State Univ., Horticulturist (**BIC Founder & Coordinator**, 1957-67) Walter H. Pierce- Asgrow Seed Co., Plant Pathologist William J. Zaumeyer- USDA, Plant Pathologist
1971	Walter H. Burkholder- Cornell Univ., Plant Pathologist James R. Douglass- USDA, Entomologist Howard S. Gentry- USDA, Plant Explorer Charles W. Hungerford- Univ. of Idaho, Plant Pathologist Herbert A. K. Lamprecht- Pl. Breeding Inst. of Sweden, Geneticist John J. Natti- Cornell Univ., Plant Pathologist Melbourne C. Parker- Gallatin Valley Seed Co., Plant Breeder Francis L. Smith- Univ. of California, Agronomist Robert E. Wester- USDA, Plant Breeder
1973	Leslie L. Dean- Univ. of Idaho, Plant Pathologist Nicolaas Hubbeling- Inst. of Phyto. Res.- Netherlands, Plant Pathologist
1975	M. Wayne Adams- Michigan State Univ., Plant Breeder Dermot P. Coyne- Univ. of Nebraska, Plant Breeder (BIC **Coordinator**, 1968-76) Shigemi Honma- Michigan State Univ., Plant Breeder Max. L. Schuster- Univ. of Nebraska, Plant Pathologist
1977	Douglas W. Burke- USDA, Plant Pathologist Roelof Prakken- Utrecht Univ. of the Netherlands, Geneticist Clibas Vieira- Univ. Federal de Vicosa of Brazil, Agronomist
1979	Barbara J. Ballantyne- New South Wales, Plant Pathologist Donald J. Hagedorn- Univ. of Wisconsin, Plant Pathologist Marshall LeBaron- Univ. of Idaho, Agronomist
1982	Eelco Drijfhout- Agr. Inst. of the Netherlands, Plant Breeder Donald H. Wallace- Cornell Univ., Plant Breeder Donald R. Wood- Colorado State Univ., Plant Breeder
1983	Leland W. Hudson- USDA, Horticulturist Roger F. Sandsted- Cornell Univ., Horticulturist
1987	Michael H. Dickson- Cornell Univ., Plant Breeder (BIC **Coordinator**, 1976-87) Aart van Schoonhoven- CIAT, Entomologist Frederick A. Bliss- Univ. of Wisconsin, Plant Breeder Matt J. Silbernagel- USDA, Plant Pathologist
1989	Axel L. Andersen- Michigan State Univ., Plant Breeder/Pathology John D. Aktin- Asgrow Seed Co., Plant Breeder Colin L.A. Leakey- England, Geneticist Alfred W. Saettler- USDA/ARS, Plant Pathologist Arthur P. Sprague- Del Monte, Plant Breeder James R. Steadman- Univ. of Nebraska, Plant Pathologist J. C. "Mike" Tu- Agriculture Canada, Plant Pathologist

1991   Iver L. Jorgensen- Northrup King & Co., Plant Breeder
        John L. Morris- Rogers/NK Seed Co., Plant Breeder
        Rosario Provvidenti- Cornell University, Plant Pathologist
        Shree P. Singh- CIAT, Plant Breeder
        J. Rennie Stavely- ARS/USDA-Beltsville, Plant Pathologist
        Daniel Debouck- IBPGR, Rome, Plant Geneticist [Achievement Award]
        Paul L. Gepts- Univ. of Calif.-Davis, Plant Geneticist [Achievement Award]
        Pat Barnes-McConnell- Bean/Cowpea CRSP, Director [Achievement Award]

1993   Hubert L. Bannerot- INRA, Versailles, Plant Breeder
        Cesar Cardona- CIAT, Entomologist
        Robert B. Colville- Del Monte Foods, Variety Development
        George L. Hosfield- ARS/USDA, East Lansing, Genetics/Nutrition
        Oswaldo V. Voysest- CIAT, Agronomy/Germplasm Evaluation
        James S. Beaver- Univ. of Puerto Rico, Plant Breeder [Achievement Award]

1995   Howard F. Schwartz- Colorado State University, Plant Pathologist (BIC **President**, 1988-97)
        Kenneth F. Grafton- North Dakota State University, Plant Breeder [Achievement Award]

1997   George Emery- Ferry Morse, Plant Breeder
        James D. Kelly- Michigan State University, Plant Breeder (BIC **President**, 1998-2009)
        Steve Magnuson- Harris Moran, Plant Breeder
        David Nuland- University of Nebraska, Bean Extensionist
        Phillip Miklas-USDA-ARS, Prosser, WA, Plant Geneticist [Achievement Award]

1999   James R. Baggett - Oregon State University, Plant Breeder
        James S. Beaver - University of Puerto Rico, Plant Breeder
        Phillip McClean - North Dakota State University, Geneticist [Achievement Award]
        James Myers - Oregon State University, Plant Breeder [Achievement Award]

2001   Dermot P. Coyne - University of Nebraska, Plant Breeder [Frazier-Zaumeyer Distinguished Lectureship]
        Mark J. Bassett – University of Florida, Plant Geneticist
        Soon J. Park – Agriculture and Agri-Food Canada, Plant Breeder
        Mark A. Brick – Colorado State University, Plant Breeder [Achievement Award]
        Ron Riley – Syngenta, Plant Breeder [Achievement Award]
        Juan Carlos Rosas – Escuela Agricola Panamericana, Honduras, Plant Breeder

2003   Fredrick A. Bliss – Seminis Seeds, Plant Breeder [Frazier - Zaumeyer Distinguished Lectureship]
        Steve Beebe – CIAT, Colombia, Plant Geneticist
        Paul Gepts – University of California, Plant Geneticist
        Marcial A. 'Talo' Pastor-Corrales – USDA-ARS, Beltsville, Plant Pathologist

2005   Perry B. Cregan – USDA-ARS, Beltsville, Geneticist, Soybean Genomics [Frazier - Zaumeyer Distinguished Lectureship]
        Jorge A. Acosta Gallegos, INIFAP, Mexico, Plant Breeder
        Phillip N. Miklas, USDA-ARS, Prosser, Plant Geneticist (BIC **President**, 2010-present)
        David M. Webster, Seminis Seeds, Plant Breeder
        A. 'Bert' Vandenberg, University of Saskatchewan, Plant Breeder [Achievement Award]

2007   Molly Jahn – University of Wisconsin, Plant Geneticist and Dean CALS [Frazier - Zaumeyer Distinguished Lectureship]
        Robert L. Gilbertson, University of California-Davis, Plant Pathologist
        Walter Edwin (Ed) Kee Jr. University of Delaware, Vegetable Specialist
        Hans Henning Muendel, Agriculture and Agri-Food Canada, Lethbridge, Plant Breeder
        Matthew W. Blair, CIAT, Colombia, Plant Breeder [Achievement Award]

2009   Maurice Bennink, Michigan State University, Nutritionist [Frazier - Zaumeyer Distinguished Lectureship]
        Henry Thompson, Colorado State University, Nutritionist [Frazier - Zaumeyer Distinguished Lectureship]
        Mark Brick, Colorado State University, Plant Breeder

2011   Phillip McClean, North Dakota State University, Geneticist [Frazier - Zaumeyer Distinguished Lectureship]
        Kenneth F. Grafton, North Dakota State University, Plant Breeder and Dean, Director, & Vice President of
        Agriculture
        Juan Jose Ferreira Fernández , SERIDA Spain, Plant Breeder [Achievement Award]
        Timothy G. Porch, USDA-ARS, Mayaguez, Plant Geneticist [Achievement Award]
        Carlos A. Urrea Florez, University of Nebraska, Plant Breeder [Achievement Award]

2013   James D. Kelly, Michigan State University, Plant Breeder [Frazier - Zaumeyer Distinguished Lectureship]
        James Nienhuis, University of Wisconsin, Plant Breeder
        K. Peter Pauls, University of Guelph, Plant Geneticist
        Kirstin E. Bett, University of Saskatchewan, Plant Geneticist [Achievement Award]
        Thomas Smith, University of Guelph, Research Technician [Technical Merit]

2015   Paul Gepts, University of California-Davis, Plant Geneticist [Frazier - Zaumeyer Distinguished Lectureship]
        Karen A. Cichy, USDA-ARS, East Lansing, Plant Geneticist [Achievement Award]
        Juan M. Osorno, North Dakota State University, Plant Breeder [Achievement Award]

Please consider nominating your colleagues for the 2017 BIC Awards. Details on nominating
colleagues are provided below

2017 BIC AWARDS – NOMINATION REQUEST

The Bean Improvement Cooperative has proudly acknowledged outstanding contributions made by many of its members to bean research and education. The **Meritorious Service Award** has been presented to over 50 of our colleagues during the 58-year history of the BIC. These recipients have devoted many years of their illustrious careers to bean research and education, and have consistently provided outstanding service to our organization.

The **BIC Achievement Award** acknowledges a scientist with fewer than 15 years of post-graduate service who has demonstrated outstanding contributions to bean research and/or education. This special award recognizes BIC members who have devoted less time to their "bean careers" than our Meritorious Service Award recipients.

The BIC Coordinating Committee and Awards Committee proudly announce the seventh **Frazier-Zaumeyer Distinguished Lectureship**. The purpose of this lectureship is to honor an individual who has contributed significantly to bean research over the past 5-10 years or longer. The recipient will provide the keynote address and a short publication (maximum of 6 pages) for the BIC report. The recipient is not excluded from receiving the BIC Achievement Award or the Meritorious Service Award. Further details can be acquired from the BIC Awards Committee Chair.

NEW! The Technical Merit Award recognizes outstanding and long-standing contributions made to bean research, extension and education by bean program support personnel. Criteria used in selection by the BIC Awards Committee, with approval by the BIC Coordinating Committee, are as follows:

- minimum of 10 years service as a Bean Program Technician, Associate, Assistant
- membership for 5 years in the BIC is desirable
- 1-page typewritten summary giving place of birth, date and name of institution granting each degree, career history
- summary of accomplishments, innovations, and impacts made by the nominee to the Bean Program and stakeholders locally, nationally and/or internationally
- evidence of participation in development and release of at least one improved germplasm line and/or cultivar; or contribution to the development and application of a technique or process to further knowledge related to pulse crops, pathogens or pests, nutritional uses
- evidence of participation in publication of at least one refereed research and/or extension article

Nomination for these awards should be sent to the BIC Awards Committee Chair (see below). The awards will be presented at the next BIC Biennial Meeting to deserving candidates nominated by their peers and selected by the BIC Awards Committee. Award recipients will be acknowledged at the twenty-ninth Anniversary of the BIC Biennial Meeting in East Lansing, MI, on the 30th of October 2017.

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BIC AWARD NOMINATION

Return by May 31, 2017 to:

James S. Beaver (Chair)
Dept. of Agronomy and Soils
Mayaguez, PR 00681-9030
j_beaver@hotmail.com

The other Awards Committee members are Drs. James Myers and Carlos Urrea.

<u>Nominee:</u>   Name:_____

              Address: _____

                        _____

                        _____

                        _____

Discipline: _____

Nominated for: _____ Meritorious Service Award

                      _____ Achievement Award

                      _____ Frazier-Zaumeyer Distinguished Lectureship

                      _____ Technical Merit Award Nomination

Submitted by: _____

Date of Submission: _____

[Please include a 1-page typewritten summary statement giving place of birth, date and name of institution granting each degree, career history and accomplishments of the nominee]

**FIRST ANNOUNCEMENT FOR THE BIENNIAL BIC/NAPIA 2017 MEETING
IN EAST LANSING MICHIGAN**
https://events.anr.msu.edu/bicnapia2017/

BIC = OCT 29 – NOV 1, 2017   NAPIA = NOV 1-3, 2017

The meeting will be held at the Kellogg Center on campus of MSU, East Lansing MI. A block of rooms has been set aside, and reservations can be made by contacting the hotel directly.

Hotel Address: Kellogg Hotel and Conference Center https://kelloggcenter.com/
Address: 219 S Harrison Rd, East Lansing, MI 48824
Phone: (517) 432-4000

Registration, Abstract, Lodging and other conference information is available at
https://events.anr.msu.edu/bicnapia2017/

LOCAL HOSTS:

Jim Kelly: kellyj@msu.edu   Karen Cichy: cichykar@msu.edu
Marty Chilvers: chiliver@msu.edu   Greg Varner: varnerbean@hotmail.com
Megghan Honke Seidel: honkemeg@msu.edu

BIC business contact: Phil Miklas: Phil.Miklas@ARS.USDA.GOV
NAPIA business contact: Weidong Chen: Weidong.Chen@ARS.USDA.GOV

TENTATIVE SCHEDULE FOR BIC/NAPIA Meeting - 2017

Day	Date	Activity	Time
Sunday	29 -Oct 2017	Welcome mixer BIC	6-9P
Registration BIC			4-6P
Monday	30-Oct 2017	BIC talks and posters	8A-7P
Dinner on your own		evening	
Tuesday	31 -Oct 2017	BIC talks and poster	8A-6P
BIC banquet – Huntington Club MSU		6:30-8:30P	
Wednesday	1-Nov 2017	W3150/PCGC/ BIC Genetics Committee	8A-12P
Tour: visit Saginaw Valley Research Extension Center & Everbest Organic Bean Grower-Processor in Munger MI			11:00 -5:30P
Wednesday		NAPIA Root Rot Workshop	8A-5P
NAPIA mixer			7-9P
Thursday	2-Nov 2017	NAPIA	8A-5P
		NAPIA Awards Luncheon	12 noon
Friday	3-Nov 2017	NAPIA	8A-5P

In Memory of

Guillermo E. Galvez-Enriquez

Guillermo E. Galvez-Enriquez died June 1, 2016 at the age of 85. Guillermo is survived by his children Ana Patricia, Jacqueline, John, Gustavo and Paulina. He was born in Pasto, Colombia, on July 18th, 1931, and received his Ingeniero Agronomo degree from the National University of Colombia, Medellin campus. He worked as research assistant for the Rockefeller Foundation, and then as assistant plant pathologist for the joint project between the Colombian Ministry of Agriculture and the Rockefeller Foundation at the Agricultural Research. As a Rockefeller Foundation (RF) fellow, he studied at the University of Nebraska, specializing in plant virology under the guidance of Drs. Myron Brakke, a member of the National Academy of Sciences, and William Allington. After receiving his Ph.D. degree, he returned to Colombia to organize the virology section of the Colombian Agricultural Institute (lnstituto Colombiano Agropecuario, ICA). While at ICA, he served as a consultant for the Rockefeller Foundation to help solve several virus disease problems in Bolivia, Peru, and Ecuador.

In April 1969, he was the first staff member to be appointed in the newly established International Center for Tropical Agriculture (Centro Internacional de Agricultura Tropical, CIAT). He headed the Plant Protection Program and was responsible for its organization and operation. In 1978, he and co-editor Dr. Howard F. Schwartz, published the internationally acclaimed CIAT book in English and Spanish entitled "Bean Production Problems: Disease, Insect, Soil and Climatic Constraints of *Phaseolus vulgaris*". Ten years later, he contributed as an author with Dr. Francisco Morales and others to the second edition edited by Drs. Schwartz and Marcial A. Pastor-Corrales. He also was a visiting scientist at IRRI in the Philippines and a consultant on rice viruses in Bangladesh. Dr. Galvez served as the coordinator for the CIAT Bean Program for Central America and the Caribbean, and was also in charge of the *Bean golden yellow mosaic virus* (BGYMV) and Web-Blight research projects.

Dr. Galvez has had a distinguished career as a research scientist, virologist, and plant pathologist, mainly dealing with rice and bean diseases. He was considered the world authority in the *Rice hoja blanca virus*. He helped in the development of resistant varieties to the virus vector. Later he isolated and characterized the *Rice tungro virus*, and helped develop a resistant variety to this virus. This increased the production of this basic grain for Bangladesh. In collaboration with CIAT and ICTA (lnstituto de Ciencias y Tecnologia Agricola, Guatemala) scientists, he developed the bean varieties ICTA Quetzal, ICTA-Jutiapan, ICTA-Tamazulapa and Negro Huasteco 81, all of which are resistant to BGYMV, and *Bean chlorotic mottle virus*. Increased dry bean yields resulted from the release of these virus resistant va rieties in Guatemala, El Salvador, Haiti, Argentina, and Mexico. He was the first to isolate a whitefly-transmitted virus, with a twin particle, the BGMV.

Dr. Galvez trained many students and colleagues from different tropical countries. He was active in the founding of the Latin American Phytopathological Society (ALF) and of the Colombian Phytopathological Society (ASCOLFI). Guillermo Galvez was elected – in August

1984 – Fellow of the American Phytopathological Society (APS). He received the "Alejandro Angel Escobar" scientific prize, the most valuable scientific Colombian award, three times for his contributions to the solution of plant pathological problems in Colombia. He also received the Colombian Rice Federation award and the Central American (PCCMCA) scientific prize for his contribution to the development of virus-resistant rice and bean varieties.

A capable administrator with a knack for action, he was out posted in 1980 to Central America with an office in IICA, in San José, Costa Rica as a leader of CIAT's first regional bean project, PROFIJOL. Heading up a research team of a breeder agronomist, Guillermo established a mode of regional coordination with partners that would later be adapted in other regions. Subsequently Guillermo led a similar regional project in the Andean zone, PROFRIZA, and gave support to a project in Haiti. Guillermo will always be remembered for his dynamism, his forceful character and his loyalty to his family, friends and his team, whether in CIAT as a bean pathologist or in his role as regional coordinator or his devotion as a loving father.

OPTIMIZING SPORULATION OF *Pseudocercospora griseola* IN VITRO

Paula F. de Pádua[1], Rafael Pereira[1], Luanna B. W. Gomes[1] and Elaine A. de Souza[1]*

[1]Universidade Federal de Lavras (UFLA), CEP 37200-000, Lavras, MG - Brazil
e-mail: *easouza@dbi.ufla.br

INTRODUCTION

Angular leaf spot of common bean (ALS), caused by fungus *Pseudocercospora griseola* (Sacc.) Crous & U. Braun, is one of the major diseases that affect the crop (Singh & Schwartz, 2010). Development of resistant cultivars is one of main objectives of common bean breeding programs and success in selection of lines and progenies depends on accurate assessments of ALS severity. High conidia production in vitro is difficult and makes the use of artificial inoculation in common bean plants unfeasible (Sanglard et al., 2009). Development of efficient methodologies in conidia production to artificial inoculation has been reported in literature, however, average time to obtain enough sporulation is around 30 to 45 days (Sanglard et al., 2009; Silveira 1967). In this study we present a new protocol for sporulation of *P. griseola* and compare its efficiency with other methods already described in literature.

MATERIAL AND METHODS

Three different protocols were used to sporulation of *P. griseola*: PDA (potato-dextrose-agar) medium using 200g of potato infusion, 20g dextrose, 20g agar and distilled water to complete 1000 ml; LDA (leaf-dextrose-agar) medium using 200g of common bean leaves, 20g agar, 20g dextrose and distilled water to complete 1000 ml (Silveira 1967); V8 medium using 200 ml of V8 juice, 17g agar, 3g $CaCo_3$ and distilled water to complete 1000ml (Sanglard et al., 2009). Three *P. griseola* isolates were evaluated: Psg-3 (race 63-63), Psg-5 (race 63-23) and Psg-115. For evaluation in PDA medium each isolate was transferred to test tubes containing approximately 8 ml of medium and evaluation was carried out after 12 days. For LDA and V8 media evaluations, firstly each isolate was transferred to test tubes containing PDA. After 15 days mycelium scraping was performed using 5 ml of distilled water. Subsequently mycelium was transferred to Petri dishes containing LDA or V8 media and evaluation was carried out after 6 days. Petri dishes and test tubes were incubated (BOD) at temperature 24 ° C until evaluation in all methodologies. For obtaining conidia suspension, colony surface was scraped using 5 ml of distilled water and a brush to releasing conidia. Suspension was filtered through gauze and conidia concentration was determined in a Neubauer® chamber. Experiment was conducted in a completely randomized design (CRD) with four replications and nine treatments arranged in a 3x3 factorial, three culture medium (PDA, LDA and V8 medium) and three isolates (Psg-3, Psg-5, Psg-115). Data were submitted to analysis of variance and treatments were compared by the Scott Knott test. Genes® software was used to perform statistical analysis.

RESULTS AND DISCUSSION

Source of variation isolate was statistically significant in analysis variance (Table 1). Number of conidia was $3.9x10^5$ (Psg-3), $4.9x10^5$ (Psg-5) and $9.2x10^4$(Psg-115) conidia.mL^{-1} for *P. griseola* isolates evaluated. Psg-3 and Psg-5 isolates formed one group and Psg-115 isolate another one by Scott-Knott's mean test. High rate of sporulation and aggressiveness of Psg-3 isolate (race-63-63) has been reported (Pereira et al., 2015; Pádua et al., 2016). All sporulation protocols evaluated were efficient in production of *P. griseola* conidia. LDA and V8 media have been successfully

used in conidia production of *P. griseola*, however, time to producing inoculum is long and methodology is laborious (Sanglard et al., 2009; Silveira 1967). Protocol proposed in this work uses PDA medium directly in test tubes to sporulation. However, it is not necessary to tranfer mycelium to Petri dishes containing culture medium mentioned above. In this case, time to conidia production from mycelium disc is only 12 days, unlike other protocols that required 30 to 45 days to producing conidia. This is great advantage of this methodology that provides a reduction at least 18 days in production of pathogen inoculum. Furthermore, this method uses only one culture medium, making it faster and more practical. This is the first report of PDA use in test tubes to sporulation of *P. griseola.*

Table 1. Summary of analysis of variance to number of conidia.mL^{-1} in evaluation of three different protocols to sporulation of three isolates of *P. griseola*.

SV	df	MS
Isolate (I)	2	83457.69**
Protocols (P)	2	5154.69
P X I	3	10172.64
Error	16	98596.50
Mean[1]		3.2×10^5
Range[1]		2.6×10^4 to 3.7×10^5

** Significantat 5% probability, by the F test. SV = source of variation; d.f. = degrees of freedom; MS = medium square; [1] Concentration of conidia /mL^{-1}.

REFERENCES
Pádua P. F. et al. (2016) Annual Report of the Bean Improvement Cooperative,59: p.115-116.
Pereira R. et al. (2015) Genetic and Molecular Research, p. 5044-5053, 2015.
Sanglard D. A. et al. (2009) Annual Report of the Bean Improvement Cooperative, 52: p.62-63.
Silveira, G.A. (1967) Master Thesis, Turrialba, Costa Rica, Instituto Interamericano de Ciências Agrícolas da OEA, 60 pp.
Singh, S. P.; Schwartz, H. F. (2010) Crop Science, 50: p. 2200 – 2223.

FINE MAPPING THE BROAD SPECTRUM ANTHRACNOSE RESISTANCE GENE IN AMENDOIM CAVALO

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INTRODUCTION: The Andean common bean landrace Amendoim Cavalo (AC) is resistant to races 2, 7, 9, 19, 23, 39, 55, 65, 73, 89, 1545, 2047 and 3481 of *Colletotrichum lindemuthianum* (Nanami et al, 2014). None of the common bean anthracnose resistance genes known to date, were resistant to all 13 races mentioned above, to which AC was resistant. The resistance in AC is conferred by a single and dominant gene (*Co-AC*) that is independent of the other known genes (Nanami et al., 2014 and Gilio et al, 2016). The AC locus has been located in the lower arm of chromosome Pv01 of common bean (Gilio et al., 2016). The objective of this study was to use fine mapping to locate the position *Co-AC* locus in the common bean genome.

MATERIALS AND METHODS: To map the anthracnose resistance gene in AC, 110 F_2 seedlings from the cross PI 207262 (S) x AC (R) were inoculated with the race 3481 of *C. lindemuthianum*. Bulk segregant analysis (BSA) was performed using one susceptible and one resistant bulk, and the parents. Each bulk consisted of equal amounts of DNA from eight plants. To avoid including heterozygous resistance plants in the resistant bulk, $F_{2:3}$ families were phenotypically evaluated using race 3481 of *C. lindemuthianum* to identify heterozygous resistant plants. DNA samples of the two bulks and the parents were screened with the BARCBEAN6K_3 BeadChip containing 5,398 SNPs following the Infinium HD Assay Ultra Protocol. SNP alleles were called using the GenomeStudio Genotyping Module v1.8.4 software. All allele calls were visually inspected. Positive hits for BSA were recorded when a SNP was polymorphic between PI 207262 and AC, and the susceptible bulk clustered with PI 207262 and the resistant bulk clustered with AC. The candidate region containing the *Co-AC* resistant locus was targeted with SSR and KASP markers for genetic mapping in the F_2 population. Genetic distances were estimated using the software JoinMap 4.0. To fine mapping *Co-AC* locus, $F_{2:3}$ families were selected based on the recombination detected between the flanking KASP markers ss56 and ss92 at F_2 level. The number of plants per $F_{2:3}$ families varied from 1 to 15 plants. $F_{2:3}$ families showing recombination between markers ss56 and ss92 were selected for additional phenotyping with race 3481 of *C. lindemuthianum* and genotyping with newly designed KASP markers to narrow the region of the *Co-AC* locus. Candidate genes were verified in the reference genome G19833 with Phytozome 11.0 (www.phytozome.org).

RESULTS AND DISCUSSION: A total of 21 SNPs resulted positive in the BSA with the SNP BARCBEAN6K_3 BeadChip. The physical location of the positive associated SNPs was on linkage group Pv 01 between 48448199 bp and 50301592 bp, spanning a region of 1.85 Mbps. The results of the mapping analysis of F_2 population from cross Amendoim Cavalo x PI 207262 with six SSRs and four KASP markers showed that the resistance *locus* in AC was located between the markers BARCPVSSR01342 (1.1 cM) and ss92 (1.3 cM). The closest KASP markers in this genetic map was ss55 and ss56 (3.4 cM) upstream and ss92 (1.3 cM) downstream (Figure 1A). The flanking KASP markers ss56 and ss92, were chosen to screen 62 $F_{2:3}$ families

(with genotype heterozygous or recombinants) to explore additional recombinants events. KASP marker ss56 (49,895,862 bp) and the KASP marker ss92 (50,527,176 bp), flanking a physical region of 631 kbp (Figure 1B). A total of 700 $F_{2:3}$ plants were inoculated with race 3481 of *C. lindemuthianum* and genotyped with the flanking KASP markers. From 700 $F_{2:3}$ plants, 86 had genotypic recombinants events, between the flanking markers (ss56 and ss92). These 86 $F_{2:3}$ plants were selected for fine mapping and the region between the flanking markers (631,314 bp) were saturated with seven KASP markers. Based on the recombination events we determined that the AC resistance locus was positioned between KASP markers ss102 (50,377,247 bp) and ss95 (50,442,472 bp) (Figure 1B), spanning a small region of 65.22 Kbp. Nine candidate genes were found in the reference genome (Figure 1C). These results demonstrated that the *Co-AC* anthracnose resistance gene was different from *Co-x* present in Pv01 (physical region 50,332,737 to 50,322,583 bp) (Richard et al., 2014).
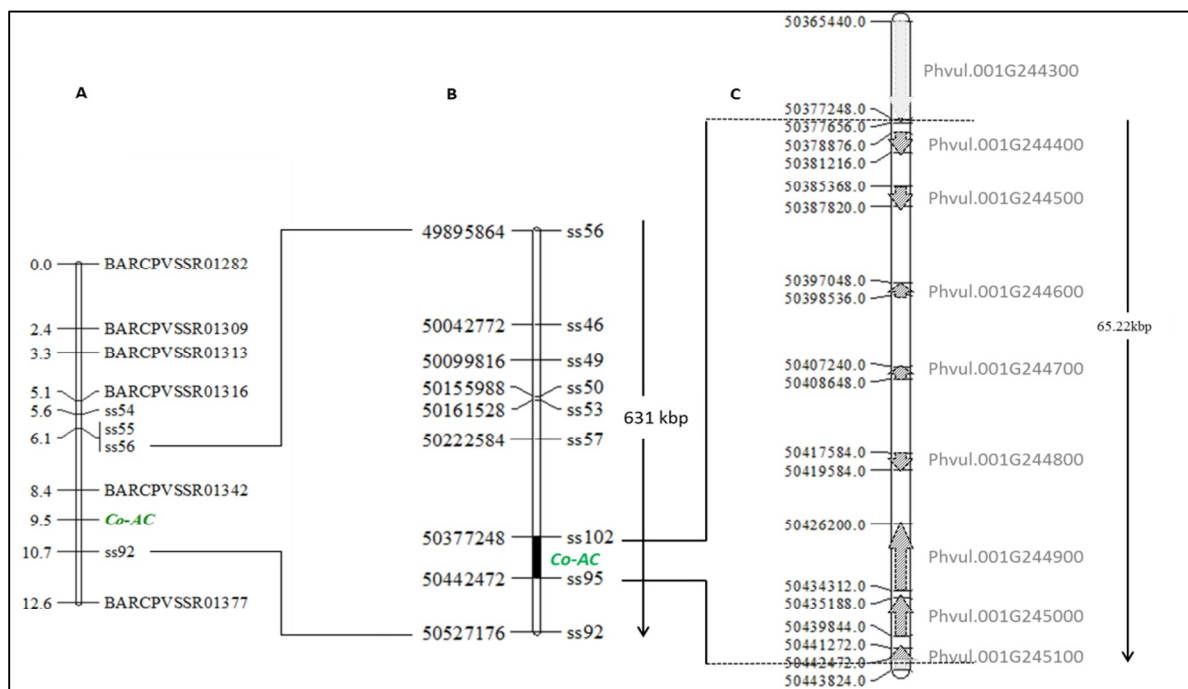
Figure 1. Genetic map of common bean linkage group Pv01 containing the *Co-AC* locus, SSRs and, SNP KASP markers: 1A, flanking KASP markers ss92 and ss56 were used to find recombinants genotypes plants in $F_{2:3}$; 1B, seven KASP were used to fine mapping the *Co-AC* locus to 65.22 Kbp; 1C, nine candidate genes were found between the flanking markers ss102 and ss95 according to the reference genome G 19833.

REFERENCES

Gilio, T.A.S., D.S.Y. Nanami, M.C. Gonçalves-Vidigal, G. Valentini G., Q. Song Q., and M.A. Pastor-Corrales. 2016. Further characterization of the broad-spectrum anthracnose resistance in Andean common bean amendoim cavalo. Annu. Rep. Bean Improv. Coop. 59:127-128.

Nanami, D.S.Y., A.A.T. Frias, S.A.L. Castro, J.C.F. Elias, G,F. Lacanallo, and M.C. Gonçalves-Vidigal. 2014. Inheritance and allelic relationships of anthracnose resistance in common bean Amendoim Cavalo. Annu. Rep. Bean Improv. Coop. 57:165-166.

Richard, M.M., S. Pflieger, M. Sevignac, V. Thareau, S. Blanchet, Y. Li, S.jackson, and V. Geffroy. 2014. Fine mapping of *Co-x*, an anthracnose resistance gene to a highly virulent strain of *Colletotrichum lindemuthianum* in common bean. Theor Appl Genet 127:1653–1666.

CO-SEGREGATION OF RECOMBINANT INBRED LINES OF THE COMMON BEAN TO RACES 65 AND 73 OF *Colletotrichum lindemuthianum*

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INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is one of the most important legumes for direct human consumption (Lin et al. 2008). Anthracnose, caused by *Colletotrichum lindemuthianum* is the most widespread, recurrent and devastating disease of the common bean in Latin America and Africa (Pastor-Corrales and Tu 1989). The genetic mapping is carried out using segregating populations. Obtaining segregating populations for genetic mapping have been standard practice in the common bean research (Gepts et al. 1993; Blair et al. 2006). These populations have led to detailed studies and promising to greater efficiency in the genetic breeding of the crop worldwide, but have as limitation, the small size (Sanglard et al. 2013). Thus, the objective of this research was to phenotype the recombinant inbred lines (RIL's) population from AND 277 × Rudá cross with the 65 and 73 races of *Colletotrichum lindemuthianum*.

MATERIAL AND METHODS

This work was conducted at the Núcleo de Pesquisa Aplicada a Agricultura (Nupagri) of the Universidade Estadual de Maringá (UEM), Paraná state, Brazil. The genetic material consisted of 393 Recombinant Inbred Lines (RIL's), and two parent (AND 277 and Rudá). Seed samples of the RILs population were provided by Dr. Thiago Lívio Pessoa Oliveira de Souza of the CNPAF/Embrapa. These seeds were sown in plastic trays containing peat base substrates. In each tray were sown 10 RIL's to further inoculation with the races 65 and 73 of *C. lindemuthianum*, both obtained from the mycology collection of Nupagri. The inoculum preparation was performed according to the methodology proposed by Cárdenas et al. (1964). The counting of spores in each pathogen race was made with the aid of hemacytometer (Chamber of Neubauer- Preciss) and trinocular biological microscope, Motic® brand - mod BA210. After counting, the spore suspension was adjusted to a concentration of 1.2×10^6 spores mL^{-1}. The visual evaluation of symptoms in each plant was performed 7 to 10 days after inoculation. Severity scale proposed by Pastor-Corrales et al. (1995) was considered, with score ranging from 1 to 9. Plants with scores from 1 to 3 were considered resistant, whereas those having scores from 4 to 9 were considered susceptible.

RESULTS AND DISCUSSION

A total of 393 RIL's from cross Rudá × AND 277 were evaluated and it was observed a segregation of 202 resistant lines and 191 susceptible lines to race 65 ($\chi^2 = 0.307$; p = 0.579) and 203 resistant lines and 190 susceptible lines to the race 73 ($\chi^2 = 0.430$; p = 0.512), which set the monogenic ratio of 1:1. This results shows that the RIL's co-segregated for both inoculated races, indicating that a same gene confer resistance two races 65 and 73 of *C. lindemuthianum* (Table 1).

Table 1. Inheritance test in Recombinants Inbred Lines derived from the cross Rudá × AND 277 inoculated with races 65 and 73 of *C. lindemuthianum*

Races	Score		Observed ratio		Expected ratio		χ^2	p value
	AND 277	Rudá	R[a]	S[b]	R	S		
Race 65	1	5	202	191	1	1	0.307	0.579
Race 73	1	6	203	190	1	1	0.430	0.512

[a] = Resistant; [b] = Susceptible

A segregation fitted a 1:1 (R:S) ratio in 393 RIL's population for races 65 and 73, indicating that there was no distortion of segregation (changing of genetic structure) for this character, which highlights the potential of these lines in genetic mapping. It was noted that the 393 RIL's showed similarity in the phenotypic behavior. Except one, all the lines resistant to the race 65 also were resistance to race 73, and susceptible lines to the race 65 were also susceptible to the race 73. This fact highlights the co-segregation of RIL's in relation to resistance to the both races (65 and 73). Genetic analysis revealed that the recombinant inbred lines co-segregated for 65 and 73 races of *Colletotrichum lindemuthianum*, showing that the *Co-1^4* gene present in AND 277 confers resistance to both races.

The reaction of RIL's to the races 65 and 73 of *Colletotrichum lindemuthianum* presented monogenic inheritance, being governed by a single gene. The recombinant inbred lines derived from the cross AND 277 × Rudá due to the large size, can be used in mapping genes that control quantitative and qualitative traits and molecular markers, hence they present the potential for the development of a consensus map to common bean.

REFERENCES

Blair et al. (2006). Theor Appl Genet 112:1149-1163
Cárdenas et al. (1964). Euphytica 13:178-186
Gepts et al. (1993). Ann Rep Bean Improv Coop 36:24-38
Lin et al. (2008). Food Chemistry 107:399–410
Pastor-Corrales and Tu (1989). CIAT 77-104
Pastor-Corrales et al. (1995). Plant Disease 79:63-67
Sanglard et al. (2013). Ann Rep Bean Improv Coop 56:23-24.

CHARACTERIZATION OF *Colletotrichum* spp. STRAINS FROM COMMON BEAN ANTHRACNOSE AND SCAB LESIONS

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INTRODUCTION: Common bean anthracnose, caused by the fungus *Colletotrichum lindemuthianum* is one of the most important diseases of this crop. However, another disease that has caused damage to common bean crop is the scab that *Colletotrichum truncatum* has been reported as causal agent. Strains isolated from anthracnose and scab lesions have been investigated in recent years and strains of *Glomerella* spp. (teleomorphic form) and *Colletotrichum* spp. (anamorphic form) have been obtained and identified by morphological, cytological, molecular and pathogenic analyses (Barcelos et al., 2014; Mota et al., 2016). Barcelos et al. (2014) classified *Glomerella* sp. strains from anthracnose lesions in two distinct groups, group I and II. Group I strains did not cause symptoms in common bean and amplified for the HMGGlo primer, specific for *G. cingulata* (Barcelos et al., 2011) and did not amplified by the HMGCl primer, specific for *C. lindemuthianum* (Garcia-Serrano et al., 2008). On the other hand, group II presented mild symptoms 10 days after inoculation and did not amplified for both HMG primers. Recently, Mota et al. (2016) studying *Glomerella* sp. Group II and other strains from scab lesions, observed that both caused symptoms in the common bean plants similar to those anthracnose, differing only in pods, which presented typical scab symptoms, indicating that a complex of species may be causing the disease. Due to simultaneous occurrence and similarity of scab and anthracnose symptoms in the leaves and stems of the common bean plants, this study aimed to compare the pathogenicity in common bean of strains obtained from anthracnose and scab lesions. Molecular analysis of these strains also was carried out for possible identification of species.

MATERIAL AND METHODS: 30 strains were evaluated, being one monosporic and three monoascosporic strains from anthracnose lesions and 22 monosporic and four monoascosporic strains from scab lesions. Pathogenicity test was carried out from the inoculation of 30 strains in Pérola common bean cultivar. Strains were grown in Petri dishes containing BDA medium for 15 days at 22°C in the darkness. Spore suspension was obtained according to the methodology described by Pinto et al. (2012). Experiment was conducted in DIC with three replicates, being 31 treatments (30 strains and one control). Each plot consisted of a pot with three plants. Plants in V3 stage were inoculated according to the methodology described by Pinto et al. (2012). Disease severity was evaluated 10 days from inoculation by the descriptive scales proposed by Schoonhoven and Pastor Corrales (1987). Strains were also tested for two species-specific primers of the HMG region, one specific for *G. cingulata*, HMGGlo (Barcelos et al., 2011) and another specific for *C. lindemuthianum*, HMGCl (Garcia-Serrano et al. , 2008), as described by Barcelos et al. (2014).

RESULTS AND DISCUSSION: According to average scores of pathogenicity test, strains were separated in five groups by Scott-Knott test (Table 1). In molecular analysis, all strains from anthracnose lesions not amplified for any of the primers used and monosporic strains presented high averages score for disease severity. Mota et al. (2016) and Barcelos et al. (2014) observed

that strains from scab and anthracnose lesions that not amplified for both HMG primers caused mild anthracnose symptoms in leaves 10 days after inoculation and typical scab symptoms in common bean pods. Strains that amplified for HMGCl primer were the most virulent. Similar results were obtained by Mota et al. (2016). Strains that amplified for HMGGlo primer presented variable average scores. In contrast to the results obtained, in studies carried out by Barcelos et al. (2014) and Mota et al. (2016), all strains that amplified for HMGGlo primer were not pathogenic. Results obtained in this work show that a complex of species are actuating in the common bean and therefore, studies of phylogeny should be carried out, as well as the investigation of diseases caused by them. These information are important for breeding programs that aim to obtain resistant cultivars to anthracnose and scab of common bean.

Table 1 Average scores of disease severity in Pérola cultivar and primers HMG amplified for each *Colletotrichum* and *Glomerella* strain.

Strain	Primer*	Severity**	Strain	Primer*	Severity**
18-1A[MA]	-	4,33 **C**	19-1D[MS]	1	6,33 **B**
38-2A[AA]	-	6,00 **B**	19-2A[MS]	1	8,10 **A**
38-2B[AA]	-	6,83 **B**	19-2B[MS]	1	8,53 **A**
38-3A[AA]	-	6,83 **B**	19-2C[MS]	1	6,66 **B**
2-1A1[MS]	1	6,96 **B**	19-3A[MS]	1	6,33 **B**
12-1D1[MS]	1	7,83 **A**	19-3B[MS]	1	7,66 **A**
14-1[MS]	1	8,20 **A**	19-3C[MS]	1	7,76 **A**
17-3C[MS]	1	7,43 **A**	19-4A[MS]	1	7,76 **A**
17-3D[MS]	1	4,86 **C**	19-4B[MS]	1	8,66 **A**
17-3A[MS]	-	6,83 **B**	40-2A[MS]	2	3,33 **D**
19A[MS]	-	4,83 **C**	9 A[AS]	-	5,00 **C**
19B[MS]	-	6,43 **B**	13-1A1[AS]	2	4,96 **C**
19-1A[MS]	1	7,86 **A**	13-1C[AS]	2	5,10 **C**
19-1B[MS]	1	8,00 **A**	13-2A[AS]	2	2,10 **E**
19-1C[MS]	1	6,66 **B**	51-2A[AS]	2	5,76 **B**

[MA]Monosporic and [AA]monoascosporic strains from anthracnose lesions; [MS]Monosporic and [AS]monoascosporic strains from scab lesions.*1-Strains that amplified to HGMCL primer; 2- Strains that amplified to HGMglo primer.
**Means followed by the same letter belong the same group (P <0.05) according to test of Scott-Knott.
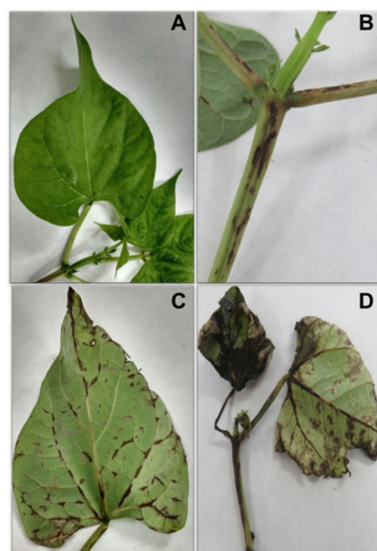
Figure 1 Symptoms in common bean plants 10 days after inoculation. **A)** control, no inoculation. **B, C** and **D)** plants inoculated with strains 19B, 38-2B and 19-2B, respectively.

LITERATURE CITED
    Barcelos, Q. L. et al. 2014. PLoS ONE. 9(3), 90910.
    Barcelos, Q. L. et al. 2011. BIC, 54(1), 224:225.
    Garcia-Serrano, M. et al. 2008. Mycoscience, 49, 312:317.
    Mota, S. F. et al. 2016. GMR, 15(2),1:22.
    Pinto, J. M. A. et al. 2012. Phytopathology. 102(5), 490:497.
    Schoonhoven, A. and Pastor-Corrales, M. A. 1987. CIAT.

GENETIC VARIABILITY OF *Colletotrichum lindemuthianum* BY SEQUENCING ITS REGIONS

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INTRODUCTION

Anthracnose, caused by *Colletotrichum lindemuthianum,* is one of the most important diseases of the common bean (*Phaseolus vulgaris* L.) in Brazil and in other regions of the world (Pastor-Corrales et al. 1994). Anthracnose occurs more severely in places where relative humidity conditions above 91% and temperatures ranging from 18° and 22°C predominate (Kelly et al. 1994). High genetic variability of *C. lindemuthianum* has been described worldwide, and more than 247 different races of the pathogen have been identified, which 35 occur exclusively in Brazil (Nunes et al. 2013). The high number of physiological races and the complexity in the use of genetic resistance of the *C. lindemuthianum* fungus are evidence of wide virulence diversity (Pastor-Corrales et al. 1994). Therefore, the objective of this work was to characterize isolates of *C. lindemuthianum* from Pernambuco state of Brazil through sequencing of ITS regions.

MATERIAL AND METHODS

These researches were conducted at Laboratório de Melhoramento do Feijoeiro Comum e Biologia Molecular do Núcleo de Pesquisa Aplicada a Agricultura (Nupagri), Universidade Estadual de Maringá and at the Centro de Estudos do Genoma Humano, Universidade de São Paulo. Seventeen isolates of *C. lindemuthianum* from Pernambuco state were used for ITS regions analyses. Genomic DNA extraction from mycelia mass was performed according to Cárdenas et al. (2012). PCR were carried out according to (Gardes and Bruns 1993) for the primer ITS 1F (5' CTTGGTCATTTAGAGGAAGTAA 3') and ITS 4 (5'TCCTCCGCTTATTGATATGC 3') (White et al. 1990). The PCR products were analyzed on 1.2% agarose gels stained with SYBR Safe (0.02%). The purification of the PCR products were performed utilizing the Kit PureLink PCR Purification Kit (Invitrogen®) and the sequencing was conducted in the ABI 3730 DNA Analyser with BigDye® Terminator v 3.1 Cycle Sequencing Kit. The analyses sequence were made by the BioEdit (version 7.0) and MEGA 5.2 software.

RESULTS AND DISCUSSION

As illustrated in Table 1, the DNA sequences of 12 out of the 17 *C. lindemuthianum* isolates were compared with the sequences obtained from GenBank. The sequence analysis revealed that the race 2047 from the database showed 100% similarity with theses isolates analyzed in the present study. The greatest genetic divergence was observed among the isolates CLPE 53 with the CLPE 87 and CLPE 55, which magnitude was 0.050. Conversely, the most of isolates were similar with genetic distance value of 0.000. The greater genetic variability presented by the 12 isolates was observed in the ITS 2 region, which are positioned between 391 a 463. Similar results were obtained by Balardin et al. (1999) who identified variability at ITS 2 region of *C. lindemuthianum*. The isolate CLPE 53 was the most divergent among all the isolates, revealing different SNPs at the ITS 2 region. Likewise, at ITS 2 region, the sequences of

CLPE 37, 38, 53, 56, 57 and 63 isolates showed the substitution of **A** by **C,** at position 455. Meanwhile, the isolate CLPE 87 which presented six SNPs, it were observed the following substitutions: **A** by **C,** at position 72, **A** by **G** at position 74, **A** by **G** at position 391, **C** by **A** at position 424, **C** by **A** at position 430 and **A** by **G** at position 434. It is emphasized that the region ITS 1 presented SNP at position 73, taking place **T** by **G** in the sequence of the isolates CLPE 29, 37, 38, 43, 49, 55, 56, 57 and 63 of *C. lindemuthianum*. These same isolates also showed the substitution of **A** for **G** at position 153. Based on sequencing the results presented here, revealed that the isolates from the state of Pernambuco presented reduced genetic variability.

Table 1. Single nucleotide polymorphisms (SNP) on sequences of the *Colletotrichum lindemuthianum* isolates at the ITS 1 and ITS 2 regions

Isolates	Positions																							
	72	73	74	153	391	424	430	431	434	435	436	437	440	441	442	443	444	446	447	448	455	461	462	463
Race 2047	C	G	G	G	G	A	A	C	G	C	A	G	C	A	C	T	C	T	G	C	C	C	A	A
CLPE 29	-	T	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CLPE 37	-	T	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-
CLPE 38		T	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-
CLPE 43	-	T	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CLPE 49	-	T	-	A	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CLPE 53	-	-	-	-	-	-	T	T	C	G	C	A	G	C	A	C	T	C	T	G	A	-	C	-
CLPE 55	-	T	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	T
CLPE 56	-	T	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-
CLPE 57	-	T	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-
CLPE 63	-	T	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-
CLPE 87	A	-	A	-	A	C	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SNP	A/C	T/G	A/G	A/G	A/G	C/A	T/C/A	T/C	C/A/G	G/C	C/A	A/G	G/C	C/A	A/C	C/T	T/C	C/T	T/G	G/C	A/C	A/C	C/A	T/A

REFERENCES
Balardin et al. (1999). Mycological Research 103:841-848
Cárdenas et al. (2012). Plant Disease 96:456012
Gardes and Bruns (1993). Mol. Ecol. 2, p. 113–118
Kelly et al. (1994). Plant Disease, 78(9), 892-894
Nunes et al. (2013). Biennial Meeting of the Bean Improvement Cooperative, Portland, State Oregon/USA
Pastor-Corrales et al. (1995). Plant Disease 78:959-962
White et al. (1990). San Diego, Academic Press, p. 315-322

POTASSIUM FERTILIZATION INFLUENCE IN ANTHRACNOSE CONTROL IN COMMON BEAN

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INTRODUCTION: The common bean (*Phaseolus vulgaris* L.) crop is affected by more than 200 diseases, capable to narrow the production and decrease the product quality physiological, nutritional and commercial, including anthracnose. The bean anthracnose, caused by the fungus *Colletotrichum lindemuthianum* (Sacc. & Magnus), is one of the most serious bean diseases, affecting susceptible cultivars worldwide (Bianchini et al., 1989). Potassium has a big relation with the incidence reduction and diseases severity in plants, acting in the inoculum potential reduction (Huber and Arny, 1985). Thus, the research objective was evaluating the severity and the bean anthracnose control under different levels of potassium.

MATERIALS AND METHODS: The experiment was conducted in greenhouse with 5 liter pots in the Imperatriz city, in the Maranhão state, Brazil. Three common bean genotypes were used, being the Pérola cultivar (susceptible to anthracnose), BRS Estilo cultivar (moderately resistant to anthracnose) and BRS Madrepérola cultivar (resistant to anthracnose), in a completely randomized design with five treatments (0, 80, 120, 140 And 180 kg.ha^{-1} as potassium chloride) and 4 replicates. The inoculation was done with a manual sprayer at the end V4 stage at 35 days after emergence, with a 1.2×10^6 conidia.mL^{-1} inoculum suspension. Severity assessments were made at 9 and 15 days after inoculation (DAI) according to the descriptive diagram scale proposed by Tamayo (1995), which ranges from 1 to 9 and the closer to 1 the lower the severity. For the analysis, was used the statistical program GENES (Cruz, 2013).

RESULTS AND DISCUSSION: There was significant difference (p > 0.05) between the treatments applied on the Peróla cultivar and no significant difference (p ≤ 0.05) between the treatments applied in the BRS Estilo cultivar and BRS Madrepérola cultivar to the severity scores at 9 and 15 days after inoculation. According to the averages in Table 1, a decrease in the disease severity at 9 DAI was observed for the Pérola cultivar as the potassium dose increased, the same was verified at 15 DAI, and for treatment 5 (180 kg.ha^{-1}) this reduction was numerically higher, thus showing that potassium influenced the anthracnose severity, controlling the disease. For the BRS Estilo cultivar (moderately resistant) was observed that at potassium doses of 80 to 180 kg.ha^{-1} at 9 DAI the severity scores were constant, with 2.2 approaching 3.0 (presence of few small size lesions covering approximately 1% of the leaf area), being that of the dose 120 to 180 kg.ha^{-1} at 15 DAI it was observed that the severity scores suffered a reduction. In the BRS Madrepérola cultivar (resistant) at doses 80 to 140 kg.ha^{-1} there was a reduction in anthracnose severity scores at 9 DAI and at 15 DAI and at the dose of 180 kg.ha^{-1} the severity scores were lower than 1 to 15 DAI (without the disease symptoms presence). Gadaga (2009), evaluating the potassium phosphite effect on the common bean anthracnose control in greenhouse, also observed that there was efficiency in the disease severity.

Table 1: Severity averages at 9 DAI (S. 9 DAI) and Severity at 15 DAI (S. 15 DAI), evaluated in 3 cultivars under different K doses, Imperatriz, MA.

KCl (kg.ha^1)	Cv. Pérola[*]		Cv. BRS Estilo[NS]		Cv. BRS Madrepérola[NS]	
	S. 9 DAI	S. 15 DAI	S. 9 DAI	S. 15 DAI	S. 9 DAI	S. 15 DAI
0	5.0	5.0	3.0	3.0	2.2	2.2
80	3.5	4.0	2.2	2.2	1.2	0.7
120	3.0	3.0	2.2	1.7	1.2	1.2
140	1.5	1.5	2.2	1.7	1.5	1.0
180	0.7	0.7	2.2	1.2	1.7	0.7
Averages	2.8	2.9	2.4	2.0	1.6	1.2

[*] Significant at 5 % probability by the F test; [NS] no significant at 5% probability, by the F test

In cowpea, the potassium fertilization use was also efficient in the web blight reduction and severity caused by *Thanatephorus cucumeris*, where the K$_2$O dose of 100 kg.ha^{-1} provided a higher number of pods per plant and a higher harvest (Adebenan, 1998). According to Santos (2008), the deficiency, the excess or the imbalance in the nutritional elements combinations can influence the plants reaction to infection by pathogens so to increase the level of defense or favor the occurrence of diseases.

CONCLUSIONS: Potassium fertilization is efficient in the anthracnose control in the Pérola cultivar, reducing the disease severity at the 180 kg.ha^{-1} potassium dose in KCl form. Mineral fertilization can contribute to the establishment of an integrated management program that allows greater efficiency in the anthracnose control, in addition to the strategy based on escape and protection, in order to reduce the bean anthracnose damage.

REFERENCES:

Adebitan, S.A. 1998. Evaluation of potassium fertilizer and tillage depth for the control of web blight of cowpea. International Journal of Tropical Plant Diseases 16:173-182.

Bianchini, A. Menezes, J. R. Maringoni, A. C. 1989, pp. 189-216. Doenças e seu controle. In: Instituto Agronômico do Paraná. O feijão no Paraná. Londrina, PR.

Cruz, C. D. 2003. Genes – A software package for analysis in experimental statistics and quantitative genetcs. Acta Scientiarum 35: 271-276.

Gadaga, S. J. C. 2009. pp. 93. Fosfitos na proteção do feijoeiro (*Phaseolus vulgaris* L.) contra a antracnose. Lavras, MG.

Marschner, H. 1995, pp. 889. Mineral nutrition of higher plants. Academic. London, UK

Huber, D. M. Arny, D. C. 1985. pp. 467-488. Interactions of potassium with plant disease. In: Munson, R. D. Potassium in agriculture. Madison, IN.

Santos, M. C. 2008. pp.74. Efeito de diferentes doses de silício, nitrogênio e potássio, na incidência de traça-do-tomateiro, pinta-preta e produtividade de tomate industrial. Brasília, DF.

Tamayo, P. J. 1995. pp. 40. Manejo y control de las enfermedades del frijol voluble. Piloto. Rio Negro, PR.

ANTHRACNOSE REACTION OF COMMON BEAN PROGENIES DERIVED FROM RECURRENT SELECTION FOR WHITE MOLD RESISTANCE

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INTRODUCTION

Several diseases have contributed to limit the bean crop yield. Among the diseases white mold (*Sclerotinia sclerotiorum*) and anthracnose (*Colletotrichum lindemuthianum*) stand out. One way to reduce losses caused by both *S. sclerotiorum* and *C. lindemuthianum* is using resistant cultivars. Recurrent selection allows improving quantitative traits such as white mold resistance, as well as improve several characters simultaneously, if the selected parents have alleles for these traits. LEITE et al. (2016) and DIAS (2015) performed recurrent selection for resistance to white mold intercrossing several parents using the circulant diallel procedure and, some of them had anthracnose resistance alleles (*Co-4²* and *Co-5*). Although there was no selection for those alleles, they may have remained in the population after successive selective cycles. The objective of this study was to verify anthracnose resistance among the selected progenies in the cycle VII, VIII and IX from recurrent selection program for white mold resistance, and to identify the presence of resistance alleles *Co-4²* and *Co-5*.

MATERIAL AND METHODS

Twenty-two progenies of cycle VII, 23 of cycle VIII and 21 of cycle IX, all of them of the $S_{0:4}$ generation and derived of recurrent selection program for resistance to white mold, and the controls Pérola (susceptible) and M20 (resistant due to *Co4²* allele) were evaluated. The experiments were set up using a completely randomized design, with two replicates and plots with 16 plants in greenhouse. The races 65 and 1609 were inoculated in all experiments according to PIO-RIBEIRO & CHAVES (1975) and, the anthracnose reaction was evaluated visually using a descriptive scale with scores from 1 to 9 (SCHOONHOVEN & PASTOR-CORRALES 1987).

In order to verify the presence of the *Co-4²* resistance allele, DNA of the progenies and the controls were extracted through a procedure similar to the used by PARRELLA et al. (2008), and the PCR was carried out using the primer SAS13, which is linked to *Co-4²* allele at the distance of 0,34cM.

RESULTS AND DISCUSSION

The experiments were set up with high experimental precision with accuracy value higher than 90%. There were selected 54% of resistant progenies to anthracnose, with mean lower than the control M20. Some progenies and the controls are present in the Table 1. The cycle VII had a higher number of resistant progenies than cycles VIII and IX, probably because in cycle VII five new white mold sources were intercrossed and, three of them were also anthracnose resistant. In cycles VIII and IX the frequency of the resistant alleles for anthracnose should have become more diluted in the population, as long as the selection was based only on white mold resistance.

Table 1. Reaction of progenies from cycles VII, VIII and IX of recurrent selection to the *C. lindemuthianum* races 65 and 1609.

Cycle VII	Race 65			Race 1609		
Progeny	Group	Mean	% of R*	Group	Mean	% of R*
81/15	a	1.00	91	a	1.15	74
81/13	a	1.00		a	1.07	
81/17	a	1.18	(M<3)	a	1.00	(M<3)
M20	b	2.10		b	3.85	
Pérola	d	5,22		d	5,76	
Cycle VIII						
8\04	a	1.00	65	a	3.25	0 (M<3)
5\23	a	1.04		a	4.41	35
14\17	a	1.32	(M<3)	a	4.43	
M20	b	2.44		a	4.57	(M<M20)
Pérola	d	6,13		b	6,19	
Cycle IX						
34\3	a	1.10	64	a	1.74	3 (M<3)
28\11	a	1.47		a	1.71	47
24\14	a	2.40	(M<3)	b	3.30	
M20	a	2.57		b	4.27	(M<M20)
Pérola	c	5,55		c	5,44	

* % of resistant progenies with mean less than 3 (M<3) and mean less than resistant control (M<M20) according to *Scott Knott* grouping (p<0.05).

Although large number of the selected progenies had less infection than M20 control, the *Co4²* allele could not be identified by molecular marker SAS13 like in M20. Therefore, the resistant progenies should have the *Co5* allele, known to be present in some original parents. However, it is matched by the race 1605 and responsible for resistance only to 65 race. So, those progenies resistant to both races should also have other alleles that are present in the parents. We can realize that recurrent selection was efficient in contributing to generate variability, and consequently, to make feasible the selection of resistant progenies, both to the white mold and anthracnose.

REFERENCES
DIAS, J.A. Master Dissertation, Universidade Federal de Lavras, MG, Brazil, 2015.
LEITE, M.E. et al. Scientia Agricola, v.73, n.1, p. 71-78, 2016
PARRELLA, N.S.D. et al. Ciência e Agrotecnologia, v.32, p. 1503-1509, 2008.
PIO- RIBEIRO. G.; CHAVES G. M.; Experientiae, v.19, n. 4, p. 59-71, fev.1975.
SCHOONHOVEN, A.; PASTOR-CORRALES, M. A. Standard system the evaluation of bean germoplasm. Cali: CIAT, p. 54, 1987.

GENETIC ANALYSIS OF ANTHRACNOSE RESISTANCE IN OURO NEGRO, ESPLENDOR AND MAJESTOSO COMMON BEAN CULTIVARS

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INTRODUCTION

Anthracnose, caused by the hemibiotrophic fungus *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara, is one of the most devastating diseases of common bean. Genetic resistance is the most effective and economical method of defence against this pathogen (Singh and Schwartz, 2010). Thus, characterization of resistant cultivars to prevalent *C. lindemuthian*um strains in growing region is important for choosing appropriate strategies in breeding programs to anthracnose resistance. Ouro Negro, Majestoso and Esplendor are common bean cultivars widely used by farmers in Brazil, having high resistance to different races of *C. lindemuthianum* predominant in the country. Therefore, this study aimed to characterize the genetic resistance of these cultivars to three different strains of *C. lindemuthiam*, race 65.

MATERIAL AND METHODS

The study of resistance inheritance were performed in F_2 populations derived from crosses between the resistant cultivars, Ouro Negro, Majestoso and Esplendor with the susceptible cultivar, União. Allelism tests also were performed in F_2 populations derived from crosses between resistant cultivars. The strains of *C. lindemuthianum*, race 65 used in all these tests were: Cl1740, Cl1614 and Cl1532. The strains were inoculated in bean pod culture medium and incubated at 22°C for 10-15 days in the darkness to obtain high sporulation. Then, a suspension of 1.2 x10^6 conidia.ml^{-1} was prepared and sprayed in seedlings of parents, F_1 and F_2 populations derived from each cross. The inoculated plants remained in greenhouse with humidity (95%) and temperature (22°C) controlled. 10 days after inoculation, plants were visually evaluated using a scale from 1 to 9 proposed by Schoonhoven and Pastor Corrales, (1987). Plants scoring below 3 were considered resistant, whereas plants scoring more than 3 were considered susceptible. Pérola cultivar was used as susceptible control in all inoculations. The genetics analysis of F_2 populations were performed through Chi-Square Test (χ^2) using Genes Software (Cruz, 2013).

RESULTS AND DISCUSSION

The results of inheritance and allelism tests are presented in Table 1. Segregation observed in F_2 populations derived from crosses between the resistant cultivars Ouro Negro, Esplendor and Majestoso with the susceptible cultivar, União fitted a 3R:1S ratio for Cl1614 and Cl1532 *C. lindemuthianum* strains (race 65) indicating that the resistant cultivars differs only in one gene from União and the dominant allele is responsible to promote the resistance. However, F_2 populations derived from cross between the resistant cultivar, Esplendor and the susceptible cultivar, União segregated in ratio of 15R:1S for Cl1740 strain. This result indicates that the resistance to this strain in Esplendor is conferred by duplicated genes, which the dominant allele is responsible to the resistance. Allelism test to Cl1614 strain in F_2 population derived from cross between the resistant cultivars Majestoso and Esplendor, no segregation was observed.

This results suggest that the resistance of cultivars Majestoso and Esplendor to Cl1614 strain is provided by the same allele or by different alleles from the same gene. On the other hand, for this same strain, it was observed segregation which fitted a 15R:1S ratio in F_2 populations from the crosses: Ouro Negro x Majestoso and Ouro Negro x Esplendor; indicating that Ouro Negro has one different resistance gene to Cl1532 strain, from those present in the Esplendor and Majestoso cultivars. Segregation ratio of 15R:1S was also observed in the allelism test to strain Cl1532 involving F_2 populations from the crosses: Ouro Negro x Majestoso and Esplendor x Majestoso, showing that Majestoso has one different resistance gene to Cl1532 strain from those present in the cultivars Esplendor and Ouro Negro. Therefore, these cultivars have different resistance genes to *C. lindemuthianum* strains, having great importance in breeding programs that aimed anthracnose resistance. Further research may be carried out in order to establish if the alleles identified in these cultivars are different from those described in other papers, as it was done for other common bean lines (Ferreira et al. 2013).

Table 1 - Reaction of parents and expected ratio of resistant (R) and susceptible plants (S) in F_2 populations in each cross inoculated with Cl1740, Cl1614 e Cl1532 strains of *C. lindemuthianum,* race 65.

Crosses	Strain	Reaction	Observed Ratio		Expected Ratio	χ^2	p-value
			R	S	R:S		
Ouro Negro x União	Cl1740	R x S	37	17	3:1	1.21	0.27
Esplendor x União	Cl1740	R x S	45	2	15:1	0.32	0.57
Majestoso x União	Cl1740	R x S	43	7	3:1	3.23	0.07
Ouro Negro x Esplendor	Cl1740	R x R	47	0	1:0	-	-
Ouro Negro x Majestoso	Cl1740	R x R	48	0	1:0	-	-
Esplendor x Majestoso	Cl1740	R x R	48	0	1:0	-	-
Ouro Negro x União	Cl1614	R x S	37	17	3:1	1.21	0.27
Esplendor x União	Cl1614	R x S	44	10	3:1	1.21	0.27
Majestoso x União	Cl1614	R x S	40	10	3:1	0.67	0.41
Ouro Negro x Esplendor	Cl1614	R x R	47	5	15:1	1.00	0.32
Ouro Negro x Majestoso	Cl1614	R x R	47	6	15:1	2.33	0.13
Esplendor x Majestoso	Cl1614	R x R	54	0	1:0	-	-
Ouro Negro x União	Cl1532	R x S	35	15	3:1	0.67	0.41
Esplendor x União	Cl1532	R x S	71	27	3:1	0.34	0.56
Majestoso x União	Cl1532	R x S	42	12	3:1	0.22	0.64
Ouro Negro x Esplendor	Cl1532	R x R	54	0	1:0	-	-
Ouro Negro x Majestoso	Cl1532	R x R	81	9	15:1	2.16	0.14
Esplendor x Majestoso	Cl1532	R x R	52	2	15:1	0.60	0.44

REFERENCES
Cruz, C. D. Acta Scientiarum. Agronomy, v. 35, n. 3, p. 271–276, 2013.
Ferreira, J. J. et al. In: Varshney, R. K., Tuberosa, R. Crop Breeding. p. 151–176, 2013.
Schoonhoven, A., Pastor-Corrales, M. A. Cali: CIAT, 1987. 54 p
Singh, S. P., Schwartz, H. F. Crop Science, v. 50, n. 6, p. 2199–2223, 2010.

THE PUTATIVE COMMON BEAN KINASE *COK-4* PLAYS A ROLE IN PLANT DEVELOPMENT

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INTRODUCTION

Several anthracnose resistance loci have been identified in common bean (*Phaseolus vulgaris* L.), including the broad-based resistance locus *Co-4* (Melotto et al., 2004). *Co-4* contains several paralogs of the *COK-4* gene that is predicted to code for a protein kinase. The predicted COK-4 protein is highly similar to FERONIA (FER), a membrane-localized receptor-like kinase of Arabidopsis that has been implicated in the regulation of plant growth. We observed that the *fer-5* kinase null mutant has some developmental defects as it accumulates lower levels of fresh and dry weight than the wild type plants. Interestingly, genetic complementation of *fer-5* with *COK-4* partially rescued the wild type phenotype. Altogether, these data provide evidence that COK-4 is a structural and functional ortholog of FER kinase domain and these proteins may be involved in the control of growth-defense balance in plants.

MATERIAL AND METHODS

Genetically complemented *fer-5* mutant with the *COK-4* gene isolated from the resistant bean genotype SEL1308 was obtained using the floral-dip method (Clough and Bent, 1998). Transgenic plants were selected by spraying a solution of BASTA (0.0114% of glufosinate ammonium) supplemented with 0.005% of Silwet. Col-0 and *fer-5* plants containing the empty vector (35S-*GFP*), and the *fer-5* expressing *COK-4* (35S-*GFP*::*COK-4*) lines, were grown at 22-24°C, 60±5% relative humidity, and 12 h photoperiod. Three sets of 10 four- to five-week old plants were collected to evaluated fresh and dry weights. The rosette of each genotype was kept at 70°C during 72 hours to measure dry weight. The fresh weight was obtained immediately after removing the rosette from the soil. In both cases, the plant weight was obtained using analytical balance (OHAUS AV114 Adventurer Pro Analytical Balance) according with Abe et al. (2003).

RESULTS AND DISCUSSION

The *fer-5* mutant has lower fresh and dry weights than the Col-0 wild type plants (Fig. 1a and b). Phenotypic analysis showed that *fer-5* complemented with *COK-4* plants have significant increase in both fresh and dry weight when compared to the mutant lacking the kinase domain of FER (Fig. 1c and d). Nevertheless, the complemented lines still presented lower measurements than the Col-0 expressing empty vector (Col-0/GFP). These results suggest that COK-4 may not only be involved in common bean resistance (Oblessuc et al., 2015), but also have an additional function in the regulation of plant growth similar to FER. In addition, we demonstrate that the domain kinase of FER is required for protein function, reinforcing the importance of COK-4 as its functional ortholog. FER participates in several processes in the plant, such as growth, development and reproduction (Lindner et al, 2012; Wolf and Hofte, 2014). Additionally, FER has important roles in the signal transduction pathways of several hormones, including auxin and brassinosteroid that control plant growth (Duan et al., 2010; Deslauriers and Larsen, 2010). Further investigation of the possible role of COK-4 in these plant responses would add valuable information on common bean biological processes.
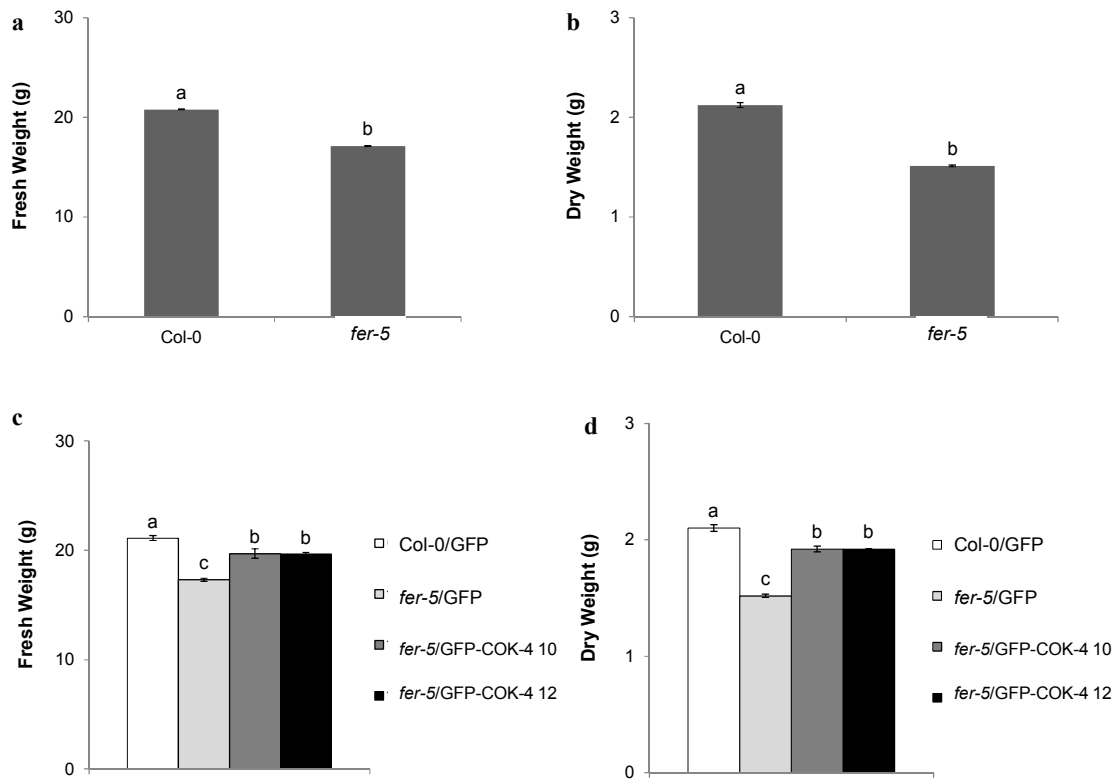
Fig. 1 (a) *fer-5* mutant has lower fresh and (b) dry weight than the Col-0 wild type plants. (c) *fer-5* expressing *COK-4* shows increased fresh weight as compared to *fer-5*. (d) *fer-5* complemented with *COK-4* lines has higher dry weight when compared with *fer-5* mutant. All data points are shown as average (n=30) ± standard deviation (SD) and statistical significance between the means was calculated with Tukey´s test at 5% of probability

REFERENCES
Abe H, Urao T, Ito T, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2003). Plant Cell 15: 63-78
Clough SJ, Bent AF (1998). Plant J 16: 735-743
Deslauriers SD, Larsen PB (2010). Mol Plant 3: 626-640
Duan Q, Kita D, Li C, Cheung AY, Wu HM (2010). Proc Natl Acad Sci USA 107: 17821–17826
Lindner H, Muller LM, Boisson-Dernier A, Grossniklaus U (2012). Curr Opin Plan Biol 15: 659–669
Melotto M, Coelho MF, Pedrosa-Harand A, Kelly JD, Camargo LEA (2004). Theor Appl Genet 109: 690–699
Oblessuc PR, Francisco C, Melotto M (2015). Theor Appl Genet 128: 1193-1208
Wolf S, Hofte H (2014). Plant Cell 26: 1848-1856

PHYLOGENETIC ANALYSIS OF *Macrophomina phaseolina* (Tassi) Goid. FROM COMMON BEANS AND OTHER HOSTS

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The fungus *Macrophomina phaseolina* (Tassi) Goid. (MP) is the causal agent of charcoal rot disease that affects more than 100 plant families worldwide and causing significant yield losses (Hernández-Delgado *et al*., 2011). Despite the broad host range, only a single species is currently recognized: *M. phaseolina* (NCBI, 2017; http://ncbi.nlm.nih,gov). Charcoal rot disease is favored by drought and high temperatures stresses. MP symptoms are characterized by dark lesions at seedling stems that after cover the complete plant destroying vascular vessels. Finally, systemic chlorosis, wilting, defoliation and microsclerotia and pycnidia growing on stem epidermis are distinguished (Kaur *et al*., 2012). Recently, Sarr *et al*. (2014) reported two species within the genus *Macrophomina: M. phaseolina* and *M. pseudophaseolina* supported by slight differences on conidia morphology and phylogenetic analysis based on five loci on MP isolates from Senegal and other countries. Our results support that even *M. phaseolina* shows a broad genetic variability due host and geographical origin variations, no species or subspecies are evident (Reyes-Franco *et al*., 2006). The aim of this work was to analyze MP isolates from different host and geographical origins based on four genetic markers and to determine their phylogenetic relationships.

The sequences of four genomic regions: Internal Transcribed Spacer (ITS), Cytochrome Oxidase I (COXI), Elongation Factor 1α (EF-1α), and RNA Polymerase II (RPII) of 90 *M. phaseolina* isolates from México (26 isolates), Brazil (18), USA (17), Japan (10), Italy (10), Australia (5), Colombia (2) and Argentina (2) were amplified by PCR. Plant hosts of isolates were variable: beans, maize, soybean, sunflower, chickpea, cotton, sorghum, etc. Two markers (ITS and COXI) were derived from rDNA while the other two (EF-1 α and RPBII) were obtained from nuclear DNA. ITS oligonucleotides were described by Babu *et al*. (2007); the other three were designed in this work. Amplified fragments were sequenced, edited and aligned. Sequences were subjected to bioinformatic analyses, firstly creating a multiple-aligned matrix by the merged sequences and after data matrix was subjected to cluster analyses by maximum parsimony, maximum likelihood and Neighbor-Joining methods. Finally, same statistical analyses were individually conducted by each marker. At all cases sequences of *Aspergillus flavus* was used as outgroup.

Topology of phylogenetic trees by combined markers showed variable grouping regardless host or geographical origins although three major clusters were found: one included the outgroup *A. flavus*; the second showed three MP isolates from Brazil, USA and Colombia; the third included 64 MP isolates (Table 1). Similar results were reported by Chakraborty *et al*. (2011) analyzing MP isolates from mandarin. When grouping MP isolates by each single marker, the clearest grouping was found using COXI due only one group including 85 MP isolates was

produced and it was clearly separated from the outgroup. MP isolates were subdivided into two subgroups: one including 74 isolates sorted by geographical origin and the other included 11 MP isolates from different host and geographical origins (Brazil, Japan, USA, México, Italy and Colombia). COXI was more efficient that ITS (Chakraborty *et al.*, 2011) for grouping MP isolates. Results indicated that *M. phaseolina* can be separated by geographical origins (Reyes-Franco *et al.*, 2006; Sharma *et al.*, 2012) but cannot be separated by species or subspecies yet. Therefore, we ratified that the fungus must be considered as a genus with only one species: *M. phaseolina*.

Table 1. Clustering of 67 *M. phaseolina* isolated based on four genomic regions and three clustering methods.

Origins	Maximum parsimony			Maximum likelihood			Neighbor-Joining		
	Cluster I	Cluster II	Cluster III	Cluster I	Cluster II	Cluster III	Cluster I	Cluster II	Cluster III
Brazil	0	1	14	0	1	14	0	1	14
Japan	0	0	7	0	0	7	0	0	7
Australia	0	0	5	0	0	5	0	0	5
Italy	0	0	7	0	0	7	0	0	7
Colombia	0	1	0	0	1	0	0	1	0
Argentina	0	0	1	0	0	1	0	0	1
USA	0	1	16	0	1	16	0	1	16
México	0	0	14	0	0	14	0	0	14
A. flavus	1	0	0	1	0	0	1	0	0
	1	3	64	1	3	64	1	3	64

REFERENCES
- Babu B.K. *et al.* (2007) Mycology 99:733-739.
- Chakraborty B. *et al.* (2011) Global J. Mol. Sci. 6:26-34.
- Hernández-Delgado S. *et al.* (2011). Phytopathology in the Omics era. Research Ringspot. Kerala, India. pp. 231-250.
- Kaur S. *et al.* (2012) Crit. Rev. Microbiol. 38:136-151.
- Reyes-Franco M.C. *et al.* (2006). J. Phytopathol. 154:447-453.
- Sarr M.P. *et al.* (2014) Phytopathol. Mediterr. 53:250-268.
- Sharma M. *et al.* (2012). Afr. J. Biotecnol. 11:8948-8959.

Phaseolus vulgaris GENES OBTAINED BY SUBTRACTIVE HYBRIDIZATION UNDER *Macrophomina phaseolina* INFECTION

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INTRODUCTION

The fungus *Macrophomina phaseolina* (Tassi) Goid. causes charcoal rot in bean (*Phaseolus vulgaris* L.). The fungus causes dark lesions on epicotyls and hypocotyls of seedlings and they die due to obstruction of the xylem vessels and vascular wilting. In adult plants, the pathogen causes yellowing of roots, the stems show dark longitudinal lesions and the plant was defoliated and withered. The charcoal rot is promoted by high temperatures and drought conditions, which are frequent in beans growing areas in Mexico. *M. phaseolina* is responsible for the 65% on loss of bean yield (Abawi and Pastor-Corrales, 1990; Mayek-Perez *et al.,* 1995;, Mayek-Perez *et al.,* 1997). The study of genes differentially expressed in plants under *M. phaseolina* infection, will allow to gain a better understanding about plant defense mechanism. The objective of this study is the identification of *P. vulgaris* genes related to plant defense after fungal infection by subtractive hybridization (SSH) technique.

MATERIALS AND METHODS

The HMP05 strain of *M. phaseolina* and Pinto UI-114 *P. vulgaris* plants (susceptible variety to *M. phaseolina*) were grown in MS medium and incubated in a climate chamber at 25º C with 16-h light and 8-h darkness period, during 5 and 15 days for the fungus and plantlets, respectively. For interaction assays, the plants were place on the mature mycelium and incubated in a climate chamber at same conditions of growth. After 48 h three replicates of the interaction samples, and the controls (only fungus and only plants without interaction) were collected. For expressed assays, RNA from interactions and control samples was extracted by TRIzol® (Invitrogen®) method. The SSH was performed with the PCR-Select™ cDNA Subtraction Kit as describe the manufacturer (637401, Clontech®). SSH library products were sent to Eurofins MWG Operon for sequencing. The expressed sequences tag (EST) obtained from the SSH were analyzed using Blastn and Blastx algorithms against the complete transcriptome of *P. vulgaris* (NCBI repository). Thereafter the ESTs corresponding with *P. vulgaris* genes that coding to hypothetic or unknown protein were analyzed using Blastp algorithm.

RESULTS AND DISCUSSION

A total of 68 ESTs from the *P. vulgaris* were obtained during the interaction with *M. phaseolina* and displayed a significant match against deposited sequences in the no redundant protein database. The sequences identified were related to senescence-related proteins like glyoxysomal malate dehydrogenase, several proteases like aleurain, asparagine synthetase, auxin response factor, chloroplastic pyruvate phosphate dikinase 1, glyoxysomal malate synthase, beta-galactosidase, catalase, ATP sulphurylase, isocitrate lyase and osmotin. A fraction of the ESTs obtained (26.4 %) matching with several metabolic process proteins (e.g. ubiquitination, glycolysis or fatty acid oxidation) like ubiquitin activating enzyme 1, ATP synthase, P450 cytochrome protein and carnitine acyl carrier protein. Additionally, 3 ESTs corresponding to proteins involved in the cell wall biosynthesis like a hydroxyproline-rich glycoprotein, synthase

1 and proline-rich glycoprotein. Finally, we identified 5 ESTs with similarity to plant defense genes previously reported in other plants (such as *Arabidopsis thaliana*) during pathogen interaction. The feature of the identified genes are shown in table 1.

Table 1. Some *Phaseolus vulgaris* genes obtained during the interaction with *Macrophomina phaseolina* by SSH.

Gene name	Protein coding	Function
Pvped1	3-ketoacyl-CoA 2-peroxisomal thiolase	Mitochondrial protein involved in fatty acids beta-oxidation and is required for induce the jasmonic acid local and systemic biosynthesis on pathogen (e.g. *Botytris cinerea*) and mechanic stresses.
Pvext3	Extensin 3	A glycoproteins constituent of the plant cell walls that differentially express during mechanic and pathogen induced stresses.
Pvlfs2	2-hydroxy Isoflavone synthase 1	An enzyme involved in the flavonoids, isoflavonoids and phytoalexins biosynthesis (antimicrobial secondary metabolites). It is express on hydric, mechanic, thermic and pathogen induced stresses.
Pvebgla	Endo 1,3-Beta-D-glucanase	Hydrolyzing essential components of the fungal and bacteria cell walls. And is co-express with chitinases by pathogen elicitors induction.
Pvuvpr	Protein induced by UV-B	Cytoplasmic protein involved in the possible NADH-depending ROS regulation and also participate in the jasmonic acid and ethylene signaling.

These results are reported by first time in *P. vulgaris* under the interaction with the phytopathogen fungus *M. phaseolina*. The information obtained shows a panorama about particular genes that are involved in *P. vulgaris* defense under fungus infection. This work represent a new insight for new studies about the differential expression of plant defense genes, and could elucidate the mechanisms of *P. vulgaris* defense against this important pathogen.

REFERENCES

Abawi, G. S., & Corrales, P. (1990). Root rots of beans in Latin America and Africa: diagnosis, research methodologies, and management strategies.

Mayek-Pérez, N., Flores, P. J., García, V. L., & Lozano, V. C. (1995). Factores genéticos y ambientales relacionados con la dinámica temporal y efecto de las enfermedades en fríjol (*Phaseolus vulgaris* L.) en Marín, Nuevo León, México. *Revista Mexicana de Fitopatología*, *13*(1), 1-9.

Mayek-Pérez, N., López-Castañeda, C., & Acosta-Gallegos, J. A. (1997). Variación en características culturales in vitro de aislamientos de Macrophomina phaseolina y su virulencia en fríjol. *Agrociencia*, *31*(2), 187-195.

IDENTIFICATION AND MAPPING OF QTLS ASSOCIATED WITH RESISTANCE TO *Macrophomina phaseolina* AND DROUGHT STRESS IN COMMON BEANS

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Common beans (*Phaseolus vulgaris* L.) are native from Mexico. The crop has a great economic and social importance and it is a major source of protein and essential nutrients. Drought is the main stress factor on bean production in Mexico and frequently is combined with high incidences of diseases caused by fungi, bacteria, viruses or nematodes. One emerging pathogen in beans and other crops is the fungus *Macrophomina phaseolina* (Tassi) Goid., causal agent of charcoal rot which incidences are favored by water deficits (Hernández-Delgado *et al.*, 2011; García-Olivares *et al.,* 2012). This work was developed to apply DNA molecular markers to develop a genetic map for identification of molecular markers associated to genes that confer resistance to combined charcoal rot disease/drought stress.

A population of 94 RILs $F_{2:9}$ from crosses between BAT 477 (resistant to both charcoal rot and water stresses) and cv. Pinto UI-114 (susceptible) was generated. Evaluations of reactions to *M. phaseolina* and drought stress were conducted under both field and controlled conditions. Controlled evaluations were conducted in Reynosa, México; field experiments were carried out in Rio Bravo, Cotaxtla and Isla, México and were described by García-Olivares et al. (2012). A genetic linkage map was built with genotypic data obtained with 30 +3/+3 AFLP marker combinations which generated 476 polymorphic markers, 190 of them segregating in a 1:1 ratio. Finally, QTLs associated with resistance to both stresses were identified using R ver. 2.10.1 (R Development Core Team, 2012)

A genetic linkage map was obtained with 68 AFLP markers distributed in 10 linkage groups (LG) with coverage of 718.1 cM. This map showed nine QTLs associated with resistance to *M. phaseolina* and three with resistance to drought stress, while Hernández-Delgado *et al.* (2009) only detected one QTL associated to charcoal rot resistance in BAT 477 using a F_2 population. Markers BPC40M127 and BPC54M150 (associated with charcoal rot resistance) and BPC63M217 (near to the genomic site C5.LOC20, and associated to drought resistance) are proposed as candidates to be transformed as SCAR markers and then used in Marker-Assisted Selection programs in order to identify and develop bean germplasm with resistance to both adverse factors (Méndez-Aguilar *et al.*, 2013).

REFERENCES

- García-Olivares J.G. *et al.* (2012) J. Phytopathol. 160:98-105.
- Hernández-Delgado S. *et al.* (2009) J. Plant Pathol. 91:163-170.
- Hernández-Delgado S. *et al.* (2011). Phytopathology in the Omics era. Research Ringspot. Kerala, India. pp. 231-250.
- Méndez-Aguilar R. *et al.* (2013) Phyton, Intl. J. Exp. Bot. 82:215-226.
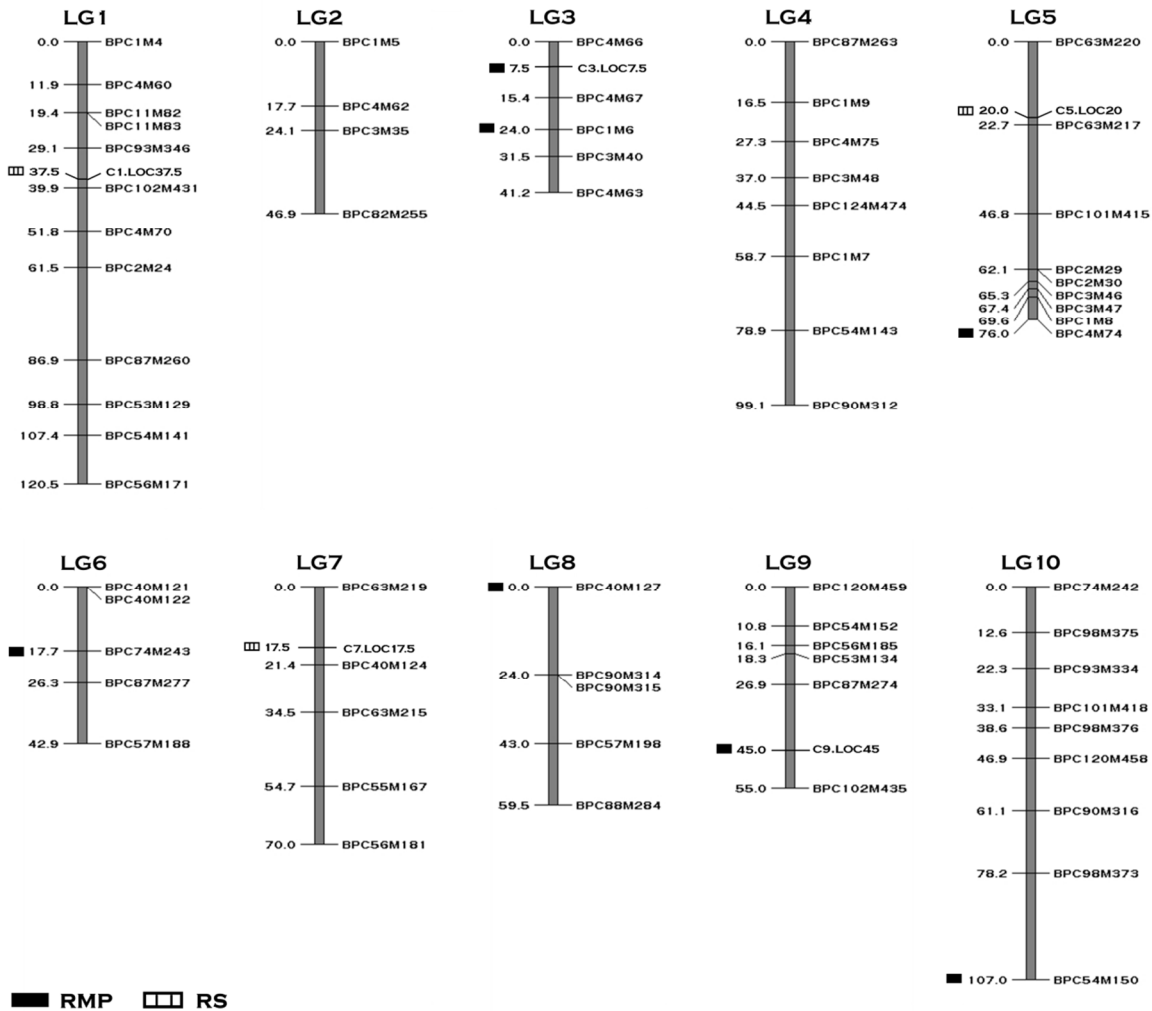
Fig. 1. Linkage map on $F_{2:9}$ RILs derived from BAT 477 x Pinto UI-114. Linkage Groups (LGs) included 68 AFLP markers (right) and distances between markers (left) are centiMorgans (cM). RMP = QTL of *M. phaseolina* resistance; RS = QTL of drought resistance.

SCREENING COMMON AND TEPARY BEAN GENOTYPES AGAINST TWO ISOLATES OF *MACROPHOMINA PHASEOLINA* BY THE CUT-STEM METHOD

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INTRODUCTION: Ashy stem blight (ASB) caused by *Macrophomina phaseolina* (Tassi) Goidanich (*Mp*) is an important tropical disease that affects common bean in all growth stages. Resistance has been identified in common (*Phaseolus vulgaris* L.) and tepary (*P. acutifolius* A. Gray) bean gene pools (Miklas et al. 1998). Most of the ASB screening methods in the greenhouse have been conducted in seeds, seedlings, and plants at vegetative stages (Estévez et al., 2014; Pastor-Corrales and Abawi, 1988). However, a reliable screening technique that covers the entire cropping season needs to be assessed. Our objective was to evaluate the response of *Phaseolus* spp. genotypes to two *M. phaseolina* isolates throughout the crop season by the cut-stem method.

MATERIALS AND METHODS: *Phaseolus* spp. genotypes were planted in a randomized complete block design with three replications at Isabela Research Substation. Plants were inoculated one and three times with the PRI16 and PRJD16 isolates in the 4th internode of the main stem and lateral branches according to the methodology described by Viteri et al. (2015). Inoculated plants were kept with mean day temperature of 36°C and night temperature of 28°C with 12 h light and humidity < 60% in the greenhouse, with watering as needed for plant growth. Ashy stem blight disease severity and the area under disease progress curve (AUDPC) were recorded on a single-plant basis from 7 to 42 days post inoculation using a 1 to 9 scale, where 1= no sign of infection in the inoculated internode and 9= *Mp* invasion beyond the third node with or without plant death. Plants with scores of 1 to 3 were considered resistant; 4 to 6 intermediate, and 7 to 9 susceptible (Singh et al., 2014). Also, resistant plants (scores ≤ 3) were verified at harvest.

RESULTS AND DISCUSSION: Significant differences ($P ≤ 0.05$) were noted among replications, isolates, and genotypes (Table 1). PRI16 isolate was more aggressive (mean scores of 7.3) than PRJD16 (scores of 6.4). Similar results were reported by Miklas et al. (1998). ICA Bunsi, NY6020-4, 'Othello', VAX 6, and 'Verano' were susceptible (scores ≥ 7.0) to both isolates. None of the genotypes tested had resistant mean scores (≤ 3) to the less or more aggressive isolates (Table 2). Tepary beans PI 321637 and PI 440806 were intermediate to both isolates and BAT 477 was susceptible to PRI16 (Table 2). These results are different from those reported by Miklas et al. (1998) and Pastor-Corrales and Abawi (1988) where these three genotypes were resistant to different *Mp* isolates. Differences might be related to (1) inoculation and evaluation at different plant stages, V_4 to harvest vs seed or earlier vegetative stages; (2) the inoculation method used, cut-stem method vs soil infected with sclerotia; (3) different levels of aggressiveness of *Mp* isolates; (4) the number of inoculations per plant; and/or (5) the days to evaluation. To the best of our knowledge, three inoculations of *Mp* per plant and delayed evaluations (even at harvest) have not been reported for ASB screening. Therefore, this is the most severe test reported for selecting *Phaseolus* spp. with resistance to ASB in vegetative and reproductive stages in the greenhouse. Although A 195, 'Badillo', PC 50 and the tepary beans

had intermediate scores and no significant AUDPC values to both isolates (Table 2), A 195 had higher percentage of resistant plants at harvest (56%), followed by tepary PI 321637 (33%) to PRJD16. In contrast, Badillo, PC 50, and tepary PI 440806 had 22% of resistant plants to the same isolate at harvest.

REFERENCES

Estévez, C., Vargas, A., Porch, T.G., and Beaver, J. 2014. *Ann. Report of the BIC* 57: 227-228.
Miklas, P., Schwartz, H., Salgado, M.O., Nina, R., and Beaver, J. 1998. *HortSci.* 33: 136-139.
Pastor-Corrales, M.A., and Abawi, G.S. 1988. *Plant Disease* 72: 39-41.
Singh, S.P., Schwartz, H.F., and Steadman, J. 2014. *Ann. Report of the BIC* 57: 231-232.
Viteri, D., Otto, K., Terán, H., Schwartz, H.F., and Singh, S.P. 2015. *Euphytica* 204: 457-472.

Table 1. Analysis of variance of *Phaseolus* spp. genotypes evaluated against two isolates of *Macrophomina phaseolina* at 42 days after inoculation in the greenhouse in 2016.

Source	df	Mean square
Replication	2	38.5*
Isolate (I)	1	57.5*
Genotype (G)	25	28.6*
I x G	25	3.2
Error	414	2.6

*Significant at $P \leq 0.05$.

Table 2. Disease range, mean disease scores, and area under disease progress curve (AUDPC) of *Phaseolus* spp. genotypes evaluated against PRI16 and PRJD16 *Macrophomina phaseolina* isolates at 42 days after inoculation in the greenhouse in 2016.

	PRI16 (Isabela)			PRJD16 (Juana Díaz)		
Genotype	Disease range	Mean	AUDPC[a]	Disease range	Mean	AUDPC
P. acutifolius						
PI 321637	4-9	5.9[b]	138.8	3-7	5.1	144.7
PI 440806	3-9	6.3	143.1	3-8	5.3	153.2
P. vulgaris						
A 195	4-8	5.4	115.5	3-8	4.1	143.9
Badillo	3-7	5.1	131.1	3-8	5.4	144.7
BAT 477	5-9	8.0	163.3	3-9	6.1	243.8
ICA Bunsi	6-9	8.3	172.3	4-9	7.0	247.7
NY6020-4	7-9	8.7	209.2	4-9	7.3	255.9
Othello	9	9.0	302.2	9	9.0	301.4
PC 50	4-9	6.2	142.7	3-7	4.9	173.4
VAX 6	7-9	8.6	194.0	5-9	7.7	219.7
Verano	9	9.0	286.6	9	9.0	292.1
Mean	…	7.3	181.7	…	6.4	211.0
LSD (*P* < 0.05)	…	1.3	45.0	…	1.6	44.8

[a] AUDPC, area under disease progress curve; [b] Ashy stem blight scored on a 1 to 9 scale, where 1= no sign of infection in the inoculated internode, and 9= invasion beyond the third node with or without plant death. Genotypes with scores 1 to 3 were considered resistant, 4 to 6 intermediate, and 7 to 9 susceptible.

RESISTANT REACTION OF ANDEAN COMMON BEAN LANDRACE G19833, REFERENCE GENOME, TO 13 RACES OF *UROMYCES APPENDICULATUS* SUGGESTS BROAD SPECTRUM RUST RESISTANCE

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INTRODUCTION

The Andean common bean landrace G19833 (Chaucha Chuga) was recently used to obtain the first reference genome of *Phaseolus vulgaris* (Schmutz et al, 2014). A large quantity of sequence information is available for this landrace which includes BAC libraries, cDNA libraries, SNP databases, gene expression profiles from various tissues (RNAseq), etc. Additionally, G19833 has been used to generate RIL populations for mapping traits such as phosphorous acquisition, agronomic performance, and disease resistance. G19833 has also been reported as resistant to the anthracnose, angular leaf spot, and Ascochyta blight pathogens and susceptible to the bean golden mosaic and bean common mosaic, viruses (Reviewed by Broughton et al, 2003). However, little is known about the reaction of G19833 to the bean rust pathogen. We report here the reaction of G19833 to a set of 13 races of the rust pathogen, 10 Mesoamerican and three Andean. Together, these races overcome all known rust resistance genes in common bean. Rust resistance in the under-utilized Andean beans lags behind that of Mesoamerican beans. The objective of this study was to determine the spectrum of resistance of Andean G19833 to 13 different virulent races of the bean rust pathogen.

MATERIALS AND METHODS

All disease evaluations were conducted under greenhouse conditions using published methodologies (Stavely, 1983). Landrace G19833 was inoculated with races 15-1 (41), 6-10 (44), 15-3 (47), 22-6 (49), 31-1 (53), 31-7 (58), 31-22 (67), 21-0 (72), 73 (6-15), 84 (37-1), 85 (6-31), 102 (29-0), and 108 (22-52). Rust resistance controls included Pinto 114, Aurora (*Ur-3*), Mexico 235 (*Ur-3+*), Early Gallatin (*Ur-4*), Mexico 309 (*Ur-5*), Golden Gate Wax (*Ur-6*), Great Northern 1140 (*Ur-7*), Pompadour Checa 50 (*Ur-9/Ur-12*), PI181996 (*Ur-11*), Redlands Pioneer (*Ur-13*), Ouro Negro (*Ur-14*), PI260418 (*Ur-Unnamed*), and PI310762 (*Ur-Unnamed*), and Compuesto Negro de Chimaltenango, CNC (*Ur-Unnamed*). Rust phenotypic evaluations were conducted twelve days after inoculations.

RESULTS AND DISCUSSION

The Andean landrace G19833 was resistant to all thirteen diverse races of the rust pathogen. The spectrum of resistance of G19833 was broader than all known rust resistance genes in common bean, including PI181996 (*Ur-11*), Ouro Negro (*Ur-14*), PI260418 (*Ur-Unnamed*), and PI310762 (*Ur-Unnamed*), known to be resistant to all but one race of more than 80 races of the rust pathogen maintained at ARS-Beltsville (Table 1). Most of these broad spectrum resistance genes are of Mesoamerican origin. G19833 was resistant to Mesoamerican races 22-52 that overcomes *Ur-11* and *Ur-14*, race 6-31 that overcomes PI310762, races 31-7 and 31-22 that overcome *Ur-3*, *Ur-4*, *Ur-5*, *Ur-6*, *Ur-7*, *Ur-9* and *Ur-12*, and *Ur-13*, and Andean race 37-1, the only race that overcomes PI260418 (Table 1). It is worth noting that the reaction of G19833 to all races used in this study was characterized by tiny pustules. No hypersensitive response (HR) was observed on G19833 when challenged with the thirteen races reported here (Table 1).

To our knowledge, this is the first report comparing the reaction of G19833 to that of all known rust resistance genes in common bean to 13 Mesoamerican and Andean races of the rust pathogen. G19833 was the only bean resistant to all 13 races, suggesting the broad spectrum of resistance of this Andean landrace. Elucidation of the genetic basis of bean rust resistance in this landrace will be greatly accelerated when the results reported here are combined with the study of segregating populations resulting from the inheritance of resistance study G19833, and with the abundant genomic resources developed for G19833.

Table 1. Reactions of common bean genotype G19833 and of cultivars with known rust resistance genes to 13 races of *Uromyces appendiculatus*

Bean Genotype	Ur gene	Races of *Uromyces appendiculatus*[1]												
		15-1	6-10	15-3	22-6	31-1	31-7	31-22	21-0[*]	6-15	37-1[*]	6-31	29-0[*]	22-52
G19833		3,f2	3	3,f2	3	3	3	3,f2	3	3	3	3	3,f2	3,f2
Pinto 114	-	4, 5	4, 5	4, 5	4, 5	4, 5	4, 5	4, 5	4, 5	4, 5	4, 5	4, 5	4, 5	4, 5
Aurora	*Ur-3*	2++	4, 5	4, 5	4, 5	2+	4, 5	5, 4	2	4, 5	2	4, 5	2	2, 2+
Mx 235	*Ur-3+*	3,f2	4, 5	3,f2	3,f2	3,f2	3,f2	3,f2	3,f2	4, 5	3,f2	4, 5	3,f2	3,f2
E.Gallatin	*Ur-4*	4, 5	2	4, 5	2+	4, 5	4	4, 5	4,5	2	4, 5	2, 2+	4, 5	2+
Mx 309	*Ur-5*	3,f2	3,f2	3,f2	4, 5	3,f2	4, 5	4, 5	3,f2	4, 5	3,f2	4, 5	3,f2	4, 5
G.G. Wax	*Ur-6*	4, 5	2	2+	4, 5	4, 5	4, 5	4, 5	4, 5	2	2	2	4, 5	4, 5
G.N. 1140	*Ur-7*	4, 5	3,f2	4, 5	3,f2	4, 5	4, 5	3,f2	3,f2	4, 5	4, 5	4, 5	3,f2	3,f2
P.C. 50	*Ur-9/12*	4, 5	2	4, 5	2	4, 5	4, 5	4, 5	2	2	2	2	4, 5	2
PI181996	*Ur-11*	f2	f2, 3	f2	f2	f2	f2, 3	f2	f2	f2	f2	3,f2	f2	4, 5
R.Pioneer	*Ur-13*	4, 5	4, 5	4, 5	4, 5	4, 5	4, 5	4, 5	2	4, 5	2	4, 5	2	4, 5
O. Negro	*Ur-14*	3,f2	3,f2	3,f2	3,f2	3,f2	3,f2	3,f2	3,f2	3,f2	3,f2	3,f2	3,f2	4,5
PI260418	*UNK*	3	3	3,f2	3,f2	3,f2	3	3	3,f2	3,f2	4, 5	3	3	3
PI310762	*UNK*	3,f2	f2	3,f2	3,f2	3,f2	3,f2	f2, 3	f2	3,f2	3,f2	4,5	f2	3,f2
CNC	*UNK*	3,f2	3,f2	3,f2	3,f2	3,f2	3,f2	4, 5	3,f2	3,f2	3,f2	4, 5	3,f2	4, 5

[1]Name of races of the rust pathogen according to the system adopted in South Africa and their original names in parenthesis.*Andean race. UNK = Unnamed *Ur* gene. Mx = Mexico. G.G.Wax = Golden Gate Wax. P.C. 50 = Pompadour Checa 50; G.N. 1140 = Great Northern 1140; R. Pioneer = Redlands Pioneer. CNC = Compuesto Negro Chimaltenango. The observed reaction (phenotype) of each common bean cultivar to each of the 13 races of the rust pathogen, was annotated using an Standard Bean Rust Grading Scale: HR = Hypersensitive resistant reaction (HR) visualized as necrotic spots and annotated as 2; R = Resistant reaction visualized as tiny pustules less than 0.3 mm in diameter and annotated as f2,3and 3,f2; S = Susceptible reaction visualized as large pustules from 0.5 to 0.8 mm in diameter and annotated as 4, 5, 6 (shown in gray).

REFERENCES

Schmutz et al., 2014. A reference genome for common bean and genome-wide analysis of dual domestications. Nature Gen. 46: 707-713.

Broughton W. J., Hernandez G., Blair M., Beebe S., Gepts P., and Vanderleyden J. 2003. Bean (*Phaseolus ssp.*)-model food legumes. Plant and Soil 252: 55-128.

Stavely, J.R. 1983. A rapid technique for inoculation of *Phaseolus vulgaris* with multiple pathotypes of *Uromyces phaseoli*. Phytopathology. 73: 676-679.

SIMPLE SEQUENCE REPEAT DNA MARKERS LINKED WITH GENES FOR RESISTANCE TO MAJOR DISEASES OF COMMON BEAN

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INTRODUCTION: Rust, anthracnose (ANT), and angular leaf spot (ALS) are widespread and devastating diseases of the common bean in Latin America and Africa. Using cultivars with disease resistance is the most cost-effective strategy to manage these diseases. Single and dominant genes condition the resistance to rust, ANT, and ALS in common bean. Combining different genes into single cultivars to manage one or more of these diseases is one of the main objectives of many common bean breeding programs. Bean cultivars with broad resistance to some of these diseases have been developed by combining several resistance genes using traditional, laborious, and time-consuming methods to pyramid these genes. To accelerate and facilitate gene pyramiding, effective, inexpensive, and easy to use DNA markers are needed. Simple sequence repeat (SSR) DNA markers are effective, co-dominant, and easy to visualize in most laboratories in developing or developed nations. The objective of this study was to discover SRR markers closely linked to genes for resistance to the rust, ANT, and ALS diseases.

MATERIAL AND METHODS: F_2 populations for the rust resistance genes reported here were developed using the following crosses: Pinto 114 × Aurora (*Ur-3*), Mexico 309 (*Ur-5*) × Early Gallatin (*Ur-4*), Pinto 114 × PI 181996 (*Ur-11*), Pinto 114 × PI 310762 (*Ur-PI₃₁₀₇₆₂*). The parents and F_2 segregating populations were evaluated for their reaction to specific races of bean rust pathogen. The *Ur-14* rust resistance gene and the gene cluster *Co-3⁴/Phg-3* that confer resistance to anthracnose (*Co-3⁴*) and ALS (*Phg-3*) present in Ouro Negro cultivar were studied using 105 $F_{2:3}$ families from the Rudá × Ouro Negro cross. Co-segregation analysis between *Ur-14* gene and the *Co-3⁴/Phg-3* loci was performed using these $F_{2:3}$ families, which were evaluated for their reaction to the rust and anthracnose pathogens independently. Total genomic DNA was isolated from the parents and the segregating population. To perform the molecular analysis, susceptible F_2 plants were used for bulk segregant analysis (BSA) for *Ur-3, Ur-4, Ur-5, Ur-11* and *Ur- PI₃₁₀₇₆₂*. Genotyping was also performed on the 105 $F_{2:3}$ families from the Rudá × Ouro cross. The DNA from the parents, the susceptible bulks and the 105 $F_{2:3}$ families were screened using the BARCBean6K_3 Illumina BeadChip with 5,399 single nucleotide polymorphism (SNP) DNA markers following the Infinium assay. SNP alleles were called using the Genome Studio and all allele data were manually checked. Positive hits for BSA were recorded when a SNP was polymorphic between the susceptible and the resistant parents and susceptible bulks clustered with the susceptible parent. Similarly, positive markers for *Ur-14* and *Co-3⁴/Phg-3* were recorded when a SNP was polymorphic between Rudá and Ouro Negro. Susceptible $F_{2:3}$ families clustered tightly with Rudá (S), resistant $F_{2:3}$ families clustered tightly with Ouro Negro (R), and the heterozygous families were in a separate group. The sequence scaffolds containing polymorphic SNPs were evaluated for the presence of SSR using the Perl script "MISA". Flanking markers to the SSR regions were designed using Primer3. Each primer pair was used to amplify genomic DNA of the parents. Polymorphic SSR markers were validated in the segregating populations. Genomic DNA of each plant was amplified using PCR and the products were then analyzed using agarose gel electrophoresis.

RESULTS: We have used existing new technologies for SNP genotyping to identify SSR DNA markers closely linked with genes that confer resistance to the rust, ANT, and ALS diseases of common bean. BSA and the BARCBean6K_3 chip were used to identify many SSR DNA markers linked to several important genes that confer resistance to the pathogens that cause rust (*Ur-3, Ur-4, Ur-5, Ur-11,* and *Ur-PI_{310762}*) in common bean. We have identified three flanking SSR markers linked to *Ur-4* that were very effective in identifying Andean and Mesoamerican beans with the *Ur-4* gene alone or in combination with other rust resistance genes. This is a significant improvement over a previously published molecular marker linked to *Ur-4* (OA14_{1100}) that was present in all Andean bean lines with or without the *Ur-4* gene. Furthermore, we have also identified the SSR markers BARCPVSSR14078 and BARCPVSSR04582, linked to *Ur-5* at 0.0 cM. These markers were effective in differentiating cultivars with *Ur-5* from those without this gene. We also identified two SSR markers linked to *Ur-14* at 0.0 cM. The close physical linkage between the *Ur-14* and the *Co-3^4/Phg-3* cluster ensures that these genes are inherited together. Thus, the SSR markers discovered during this study are most useful to detect the presence of the three genes in this cluster with resistance to rust, ANT, and ALS. Moreover, some of the SSR markers discovered is this study are flanking and closely linked to genes conditioning resistance to these three diseases of common bean. These markers would be very effective when used in marker-assisted selection. We still searching for new, specific, thus more effective, SSR markers linked to *Ur-3* and *Ur-11*.
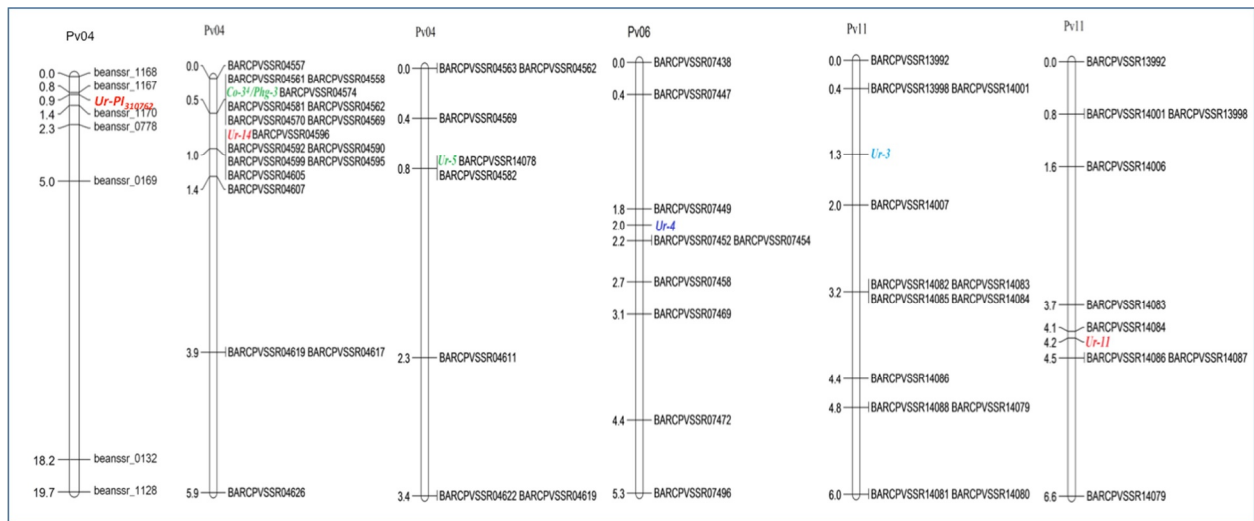
Figure 1. Genetic linkage maps of the major resistance genes: *Ur-14, Co-3^4/Phg-3* (F_{2:3} Rudá × Ouro Negro); *Ur-PI_{310762}* (F_2 Pinto 114 × PI 310762); *Ur-5, Ur-4* (F_2 Mexico 309 × Early Gallatin); *Ur-3* (F_2 Pinto 114 × Aurora); *Ur-11* (F_2 Pinto 114 × PI 181996).

MUTI SITE SCREENING IDENTIFIES AND VERIFIES LEVELS OF RESISTANCE TO WHITE MOLD IN COMMON BEAN IN 2016

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The development of common bean cultivars with partial resistance and/ or avoidance to white mold (WM) caused by *Sclerotinia sclerotiorum* would benefit producers by reducing yield loss and reducing input costs for fungicides. Our main objective in this study is to identify bean germplasm supplied by bean breeders from across the USA and Belgium with levels of partial resistance to WM.

Breeders sent seed of 9 bean lines for field testing and 25 bean lines for greenhouse testing with putative sources of resistance to our laboratory. The seeds were divided in equal amounts for field (400g/line) and/or greenhouse (25 seeds/ line) tests and then sent to eight locations to be evaluated by standardized greenhouse and/or field screening methods. Three bean lines were included in both tests as controls: a good source of partial resistance G122, Bunsi with mostly field avoidance and susceptible GN Beryl.

The use of multisite screening in the field was justified when only three sites had data. Weather was the primary factor in locations with field plots that did not report any data. There were eight lines with resistance higher than Beryl and five with resistance similar to the resistant G122 (Table 1).

Table 1. The mean infection rating using the CIAT scale* and t Grouping** in field plots from three white mold resistance screening locations.

Line	Mean score by location*				t Grouping**					
	BEL	OR	WI	Mean						
Beryl	7	9.0	8.3	8.1	A					
P14815	8	7.3	6.7	7.3	A	B				
WM91212-4-3	7	6.0	6.0	6.3		B	C			
N15341	6	5.3	5.7	5.7			C	D		
Ex Rico-Bunsi	5	5.0	6.3	5.4			C	D		
B15430	7	4.0	4.3	5.1			C	D	E	
USPT-WM-12	4	4.3	5.7	4.7				D	E	F
PS08-039-A5	4	4.7	5.0	4.6				D	E	F
R13752	5	4.3	4.3	4.5				D	E	F
G122	4	5.0	3.0	4.0					E	F
R12844	3	4.0	4.0	3.7						F
ASS 1865	3	3.3	4.0	3.4						F

*CIAT Scale: 1 = no disease, 9 = plants dead **Alpha = 0.05; LSD = 1.38

Greenhouse screening identified seven lines similar to Beryl and Bunsi, and two lines similar to G122. North Dakota submitted 15 lines representing different classes of bean but no lines statistically were similar to Bunsi, known to be more escape than resistant (Table 2).

Table 2. The mean straw test rating* and t Grouping** in greenhouse screening from six locations.

Line	Mean score by location						Overall Mean	t Grouping									
	CO	WI	WA	OR	NE	MI											
N15341	8.4	9.0	7.9	8.4	8.8	8.7	8.5	A									
P14815	8.2	9.0	6.6	6.9	7.9	8.0	7.8	A	B								
NDF140436	7.4	9.0	6.5	6.9	8.7	7.9	7.7	A	B	C							
B15430	5.2	9.0	7.5	7.8	8.9	7.3	7.6	A	B	C	D						
NDF140443	6.2	9.0	5.6	7.0	8.8	8.6	7.5	A	B	C	D	E					
NDF140446	6.6	8.6	6.3	6.4	8.6	8.4	7.5	A	B	C	D	E					
NDF140422	5.7	8.6	6.8	6.3	8.4	8.7	7.4	A	B	C	D	E					
NDF140433	5.7	9.0	6.3	6.3	8.8	7.5	7.3	A	B	C	D	E	F				
WM91212-4-3	7.4	8.3	6.5	5.9	7.3	6.2	6.9		B	C	D	E	F	G			
NDF140461	4.2	6.6	6.9	6.6	8.3	8.0	6.8		B	C	D	E	F	G	H		
ASS 1865	6.1	7.8	5.2	4.8	7.1	8.9	6.7		B	C	D	E	F	G	H		
NDF140423	4.8	6.8	6.7	6.0	8.3	7.2	6.6		B	C	D	E	F	G	H		
NDF140405	5.2	7.6	6.6	6.1	7.3	6.7	6.6		B	C	D	E	F	G	H		
NDF141308	7.2	8.0	5.0	4.2	7.6	6.7	6.5		B	C	D	E	F	G	H		
R13752	6.2	8.3	6.3	4.4	6.9	6.3	6.4			C	D	E	F	G	H		
NDF140427	4.8	8.3	5.7	5.4	7.1	7.0	6.4				D	E	F	G	H		
Beryl	5.8	5.8	5.8	5.1	7.7	8.0	6.4				D	E	F	G	H		
NDF140409	4.9	8.2	6.2	6.1	7.5	5.0	6.3				D	E	F	G	H		
NDF140415	5.4	7.3	5.4	6.1	7.5	6.0	6.3				D	E	F	G	H		
NDF140408	5.2	7.4	6.0	6.0	6.8	6.1	6.3					E	F	G	H	I	
NDF140406	5.1	4.9	6.3	5.0	8.2	7.9	6.2					E	F	G	H	I	
NDF140460	3.5	7.3	4.7	6.0	7.4	7.5	6.1						F	G	H	I	
PS08-039A-5	5.1	7.2	5.3	5.0	5.9	5.9	5.7							G	H	I	
R12844	4.6	5.8	5.7	5.2	5.5	6.8	5.6							G	H	I	
Bunsi	4.3	5.6	5.8	4.9	7.8	5.1	5.6								H	I	
G122	4.4	4.7	4.1	4.2	5.2	6.9	4.9									I	J
USPT-WM-12	3.3	4.3	3.8	3.7	4.2	5.0	4.1										J
031-A-11	3.3	3.0	4.4	3.7	4.2	5.2	4.0										J

*Straw test rating scale based on modified Petzoldt and Dickson scale (Teran *et al.,* 2006)
(1-3 = resistant, 4-6 = intermediate, 7-9 = susceptible) **Alpha = 0.05, LSD = 1.3

REFERENCES:

1. CIAT (Centro Internacional de Agricultura Tropical). 1987. Standard system for the evaluation of bean germplasm. Van Schoonhoven, A. and M.A. Pastor-Corrales (compilers). Cali, Columbia. 54 p.

2. Teran, H. M. Lema, H. Schwartz, R. Duncan, R. Gilbertson, and S. Singh. 2006. Modified Petzoldt and Dickson scale for white mold rating of common bean. Ann. Rep. Bean Improv. Coop. 49: 115-116.

PHYSIOLOGICAL RESISTANCE OF COMMON BEAN LINES TO
Sclerotinia sclerotiorum

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INTRODUCTION. White mold (WM), caused by the fungus *Sclerotinia sclerotiorum*, is a serious constraint of common bean during the fall-winter season in Brazil. The most commonly used control measure is fungicide application. However, the high cost and the potentially deleterious effects on human health and environment have motivated the search for new options of WM management. Genetic resistance is a key component of the WM management, because it is easier for farmers to adopt and is environmentally safe. Since 2008, we have screened common bean lines/cultivars for WM resistance from the field trials named Value for Cultivation and Use (VCU) conducted under WM pressure. The lines screened in these trials have been developed by Federal University of Viçosa, Federal University of Lavras, EPAMIG and Embrapa Rice and Beans. Beginning in 2015, we have evaluated the genotypes screened in the VCU trials in comparison to three WM-resistant checks (A195, G122, and Cornell 605) in advanced field trials, straw and/or detached leaflet tests. Here, we present results from the straw and the detached leaflet tests to assess physiological resistance of lines/cultivars originally screened for resistance to foliar diseases and high yield.

MATERIAL AND METHODS. Seventeen genotypes screened in the VCU field trials [12 with putative resistance, two with intermediate resistance (Pérola and BRS Estilo), and three with susceptibility to WM (Ouro Negro, Ouro Vermelho and BRSMG Majestoso)] along with the WM-resistant checks were evaluated in a straw test (greenhouse) and in a detached leaf test (laboratory), both in Viçosa, Minas Gerais. In both tests, an isolate of *S. sclerotiorum* with high aggressiveness collected in Itararé, State of São Paulo, was used. In the straw test, three plants of each genotype were grown in 3.0 L-pots as described by Lehner et al. (2015). WM severity was evaluated 7 days after inoculation, using the 1-9 scale of Terán et al. (2006), in which 1 = no infection and 9 = invasion of the third internode > 2 cm or plant death. In the detached leaf test, leaflets of the youngest fully expanded trifoliate leaves of 5-week-old plants were placed on filter paper moistened with 5 mL of sterilized distilled water inside plastic boxes. Two-day-old mycelial discs from the first subculture were placed between the main vein and the leaflet edges; one disc on each side of the main vein. Boxes containing inoculated leaves were kept at 23°C in the dark. The lesion diameter was assessed 24 h after inoculation. In both trials, treatments were replicated four times in a completely randomized design.

RESULTS AND DISCUSSION. The common bean genotypes were separated into three groups based on WM severity and lesion length in the straw test and into four groups based on lesion diameter in the detached leaflet test (Table). The resistant checks (A195, G122, Cornell 605) confirmed their high physiological resistance to WM, especially A195. The lines CNFC 10720, CNFP 10798, VC17 and the cultivar Ouro Branco did not differ significantly from the WM-resistant checks G122 and Cornell 605. Among the most susceptible genotypes to WM were

CNFC 11946, CNFC 10432 and the cultivar BRS Estilo. The susceptible cultivars to WM in the field, Ouro Negro and Ouro Vermelho, exhibited some level of physiological resistance to WM.

Table. Lesion diameter evaluated 24 hours after inoculation on the detached leaf test and white mold (WM) score and lesion length in the straw test.

Genotype	Detached leaflet test	Straw test	
	Lesion diameter (cm)	WM score	Lesion length (cm)
CNFC 10720	12.6 C[1]	5.50 C	4.52 C
CNFC 10722	12.1 C	5.75 B	5.28 C
CNFP 10798	12.2 C	5.13 C	5.71 C
G122	11.8 C	5.08 C	3.46 C
Ouro Branco	12.4 C	5.00 C	3.23 C
VC17	12.6 C	5.25 C	3.90 C
VC26	13.4 B	5.00 C	3.00 C
BRS Vereda	11.5 C	5.78 B	5.63 B
Ouro Negro	14.6 B	5.00 C	2.96 C
A195	9.1 D	5.00 C	2.69 C
Ouro Vermelho	13.3 B	5.08 C	3.36 C
BRSMG Majestoso	16.6 A	6.00 B	6.14 B
CNFC 11946	13.6 B	6.67 A	8.88 A
Pérola	10.7 D	5.75 B	5.38 B
CNFC MG11-06	14.6 B	5.17 C	3.78 C
Cornell 605	11.6 C	5.00 C	2.69 C
CNFC 10432	15.7 A	6.67 A	7.88 B
CNFP 11990	14.1 B	5.25 C	3.88 C
BRS Estilo	15.3 A	5.92 B	6.12 A
VC27	13.4 B	5.38 C	4.07 B

[1] Means followed by the same letters belong to the same group (Scott-Knott test, $p = 0.05$).

REFERENCES

Lehner, M.S.; Teixeira, H.; Paula Júnior, T.J.; Vieira, R.F.; Lima, R.C.; Carneiro, J.E.S. Adaptation and resistance to diseases in Brazil of putative sources of common bean resistance to white mold. Plant Disease 99: 1098-1103, 2015.

Terán, H.; Lema, M.; Schwartz, H.F.; Duncan, R.; Gilbertson, R.; Singh, S.P. Modified Petzoldt and Dickson scale for white mold rating of common bean. Annual Report of the Bean Improvement Cooperative 49:115-116, 2006.

SSR MARKERS LINKED TO META-QTLS FOR WHITE MOLD RESISTANCE IN COMMON BEAN

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INTRODUCTION

Several studies have been conducted to identify QTLs for resistance to white mold in *Phaseolus vulgaris*. SSR markers have the advantages of being codominant and distributed throughout the genome, being considered one of the best genetic markers for mapping purposes and efficient to identify QTLs (Soule et al. 2011). In the meta-QTL analysis, SSR markers can be used to identify stable genomic regions in populations, and therefore, be more promising in marker-assisted selection programs. The objective of the study was to identify SSR markers nearby and/or within the meta-QTL intervals described by Vasconcellos et al. (2017), in order to evaluate the possibility of using these markers for resistance to *Sclerotinia sclerotiorum* in common bean.

MATERIAL AND METHODS

Two progenies derived of a recurrent selection program for resistance to white mold were used one from Cycle X (CX 53/3) and one from Cycle XII (CX 11/185) and two checks: Cornell 605 (resistant) and Beryl (susceptible). The Cornell 605 line is derived from the cross between the Redkote (Cornell University, NY, USA) and the Cornell line 6603 (Griffiths et al., 2012). The Beryl line is highly susceptible to white mold and is used as a negative control in several studies involving this pathosystem (Griffiths et al. 2012; Lehner et al., 2015). The plants had its DNA extracted and were evaluated for the reaction to *S. sclerotiorum* by the Straw test method, described by (Petzoldt and Dickson, 1996) and modified by (Terán et al., 2006). SSR primers located within or very close to the meta-QTLs (Vasconcellos et al. 2017) and, that had polymorphism between check lines were selected thus, marking it possible to identify more stable QTLs of resistance and/or susceptibility (Table 1).

RESULTS AND DISCUSSION

From the 19 SSR markers, 8 presented polymorphism between the checks and only one (IAC27) presented a band in common with the resistant check and the two progenies (Cornell 605, CX 11/185 and CX 53/3). This band is linked to the white mold stable QTL, that is, the marker IAC27 is inside the WM2.2 meta-QTL that has been reported to occur in at least six populations, and has been detected in both greenhouse and field trials (Vasconcellos et al., 2017). Therefore, it is certainly a useful marker for helping the selection of resistance common beans in our conditions (Table 2). Three more markers (IAC74, PVBR79 and GATS91) were also present in one or other progeny derived from recurrent selection, indicating the presence of another meta-QTL that probably helps to explain their high level of resistance. However, we can see that most of the meta-QTLs presented in Cornell 605 are not present in the progenies, as was expected, because this source of resistance was not used as parent in the recurrent selection program, that aims carioca grain type as well. The results indicate that other white mold resistant alleles are expressing in the progenies and need to be identified for helping the selection. Those meta-QTLs present in Cornell 605 and absent in the progenies are promising to be included in the recurrent selection aiming an even higher level of resistance to white mold.

Table 1. SSR primers located within or very close to the meta-QTLs and polymorphic in the checks and progenies.

Meta-QTL	SSR Marker	Reference
WM3.1	IAC 07	(BENCHIMOL et al., 2007)
WM2.2	IAC 27	(BENCHIMOL et al., 2007)
WM1.1	IAC 37	(BENCHIMOL et al., 2007)
WM1.1	IAC 45	(BENCHIMOL et al., 2007)
WM2.2	IAC 51	(BENCHIMOL et al., 2007)
WM7.4	IAC 63	(BENCHIMOL et al., 2007)
WM8.3	IAC 71	(BENCHIMOL et al., 2007)
WM8.3	IAC 74	(BENCHIMOL et al., 2007)
WM3.1	IAC 77	(BENCHIMOL et al., 2007)
WM3.1	IAC 98	(BENCHIMOL et al., 2007)
WM3.1	IAC 117	(BENCHIMOL et al., 2007)
WM3.1	PVBR 21	(BUSO et al., 2006)
WM3.1	PVBR 23	(BUSO et al., 2006)
WM2.2	PVBR 78	(GRISI et al., 2007)
WM1.1	PVag003	(YU et al., 2000)
WM8.3	BMC 222	(BLAIR et al., 2009)
WM2.2	GATS 91	(GAITÁN-SOLÍS et al., 2002)
WM5.4	BM 142	(GAITÁN-SOLÍS et al., 2002)
WM3.1	BM 197	(GAITÁN-SOLÍS et al., 2002)

Table 2. SSR markers polymorphic in the checks and in some progenies.

Lines	Markers								Mean[1]
	IAC27	IAC51	IAC71	IAC74	PVBR21	PVBR79	GATS91	BM197	
Cornell	2	2	2	2	2	2	2	2	3.55
Beryl	1	1	1	1	1	1	1	1	6.82
CX 11/185	2	1	1	2	1	1	1	1	3.09
CX 53/3	2	1	1	1	1	2	2	1	2.74

[1] Reaction do white mold from 1 (susceptible) to 9 (dead plant) (Singh et al. 2014).

REFERENCES

BENCHIMOL, L. L. et al. **Genetic Resources and Crop Evolution**, v. 54, n. 8, p. 1747–1762, 22 nov. 2007.

BLAIR, M. W. et al. **BMC Plant Biology**, v. 9, n. 1, p. 100, 2009.

BUSO, G. S. C. et al. **Molecular Ecology Notes**, v. 6, n. 1, p. 252–254, mar. 2006.

GAITÁN-SOLÍS, E. et al. **Crop Science**, v. 42, n. 6, p. 2128, 2002.

GRIFFITHS, et al. **HortScience**, v. 47, n. 7, p. 952–954, 2012.

GRISI, M. C. M. et al. **Genetics and Molecular Research Genet. Mol. Res**, v. 6, n. 63, p. 691–706, 2007.

LEHNER, M. S. et al. **Plant Disease**, v. 99, n. 8, 2015.

PETZOLDT, R.; DICKSON, M. H. **Annual Report of the Bean Improvement Cooperative**, v. 39, p. 142–143, 1996.

SINGH, S.P. et al. **Annual Report of the Bean Improvement Cooperative**, v. 57, p. 231–232, 2014.

SOULE et al. **Crop Science**, v. 51, p. 123-139, 2011.

TERÁN, H. et al. **Annual Report of the Bean Improvement Cooperative**, v. 49, p. 115–116, 2006.

VASCONCELLOS, R. et al., **PLoS One**, 12(2), 22p, 15 fev. 2017

YU, K. et al. **Journal of Heredity**, v. 91, n. 6, p. 429–434, 1 nov. 2000.

PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF RELEVANT *Sclerotinia sclerotiorum* ISOLATES

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Knowledge of pathogen population structure is useful to identify isolates for screening cultivars and lines for resistance. For *S. sclerotiorum*, causal agent of white mold in more than 400 plant species, including common bean and soybean, breeding for resistance is particularly challenging. The objective of this study was to characterize phenotypic and genotypic variation of *S. sclerotiorum* isolates from soybean production areas of the U.S.A. (15), Brazil (75), and Argentina (5) to compare them with 366 isolates from dry bean characterized previously (Everhart *et al*., 2016).

Genotyping – DNA of 95 isolates was extracted and genotyped at 11 SSR loci (Sirjusingh, and Kohn, 2001). Identified were 92 multilocus genotypes, with only four represented by more than one isolate. Our results showed these isolates had greater genotypic richness and diversity compared with the 366 isolates genotyped previously (Everhart *et al.,* 2016). Pairwise genetic distances between isolates was estimated using Bruvo's distance, which utilizes a stepwise model of mutation. The matrix of pairwise distances was used to construct a minimum spanning network (MSN); using the R package *poppr* (Fig. 1). Within the MSN was a core set of 12 MLG that were closely related (thick lines in MSN) and from six states in Brazil. These results are consistent with that expected for a soilborne organism, such as *S. sclerotiorum*.

Table 1. Number of isolates (N), number of multilocus genotypes (MLG) and genotypic diversity (*h*) within populations of *S. sclerotiorum*

Populations	N	MLG	*h*
Nebraska	14	14	0.929
Argentina	5	5	0.800
Bahia	14	13	0.918
Rio Grande do Sul	16	16	0.938
Paraná	16	16	0.938
Mato Grosso do Sul	6	6	0.833
Goiás	17	15	0.927
Minas Gerais	7	7	0.857

Figure 1. MSN generated for 52 isolates using distance-based analysis methods.

Mycelial compatibility group – A sub-set of 69 isolates were paired and grown on DS medium and evaluated for compatibility after 10-days growth. Identified were 25 MCGs, with 44% of these groups represented by only one isolate (Fig. 2). The most abundant MCG was represented by 16 isolates from Brazil that were geographically partitioned into 10 regions from 5 states. One MCG was identified in the U.S.A. (one isolate; Bellwood, NE) and Brazil (two isolates; Chapadão do Sul, Mato Grosso do Sul). Within the same field in Mead, NE and Auburn, NE were two different MCGs, each represented in both fields.

Figure 2. Frequency distribution of the 25 MCG identified (A-W, Y, Z) among the 69 isolates characterized in this study.

Phenotyping – Aggressiveness of 70 isolates was determined using a detached leaf bioassay (DLB), wherein leaves of partially resistant soybean cultivar "Dassel" were inoculated with an agar plug of mycelium. The three youngest and fully expanded leaves were collected at 21, 28, and 35 days after emergence and lesion areas observed 48 hours after inoculation. Preliminary analysis (Fig. 3) showed leaf age influenced results of aggressiveness assays, with larger necrotic lesion areas observed on leaves of younger plants (21 days). Necrotic area varied among 70 isolates.

Importance for breeding programs – our current work will enable selection of isolates that are representative of the *S. sclerotiorum* populations throughout deployment regions. Differences in isolate aggressiveness and genetic variation have identified *S. sclerotiorum* that can be used for cultivar evaluation studies.

Conclusions – Preliminary results showed more variation in isolates from South American soybean than those genotyped previously in dry bean (Everhart *et al*., 2016); necrotic lesions on leaves decreased with increasing plant age. The overall approach we used in our soybean/dry bean research is applicable to facilitate identification of white mold resistance in other susceptible crops including canola, pulses, and sunflower.

Future Direction – MCGs and MLGs of isolates in the present study will be used for comparison with 366 isolates genotyped previously; validation of fungicide sensitivity methods is currently underway by assessing closeness of estimate to true 50% inhibitory dose. Further analysis of isolate aggressiveness is also underway.

Figure 3. Necrotic lesion area (cm^2) formed on soybean leaves collected at 21, 28, and 35 days after emergence and lesion areas observed 48 hours after inoculation. Each plot represents 10 repetitions of 70 isolates (700 total).

REFERENCES

Everhart, S.E., B.S. Amaradasa, R. Jhala, R. Higgins, and J.R. Steadman. 2016. Population structure and fungicide sensitivity of 366 *Sclerotinia sclerotiorum* isolates from dry common bean. *Bean Improv. Coop.* 59:131-132.

Sirjusingh, C. and L.M. Kohn. 2001. Characterization of microsatellites in the fungal plant pathogen, *Sclerotinia sclerotiorum. Molecular Ecology* 1:267-269.

SEEDLING STRAW TEST: A RAPID AND RESOURCE-EFFICIENT METHOD FOR EVALUATING WHITE MOLD RESISTANCE

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The Seedling Straw Test is a modified version of the Petzoldt and Dickson (1996) straw test. Most steps are similar, including using normal clear or colored straw segments and production of the pathogen culture. The main difference between the two tests is in the stage of inoculation and the scoring system. Timing of mycelia production is more critical in this test compared to the standard straw test as there is a very narrow window for inoculation. The test can be can be completed within 25 days whereas the original straw test requires at least 35 days. Fewer resources (potting media, bench space) and less labor are required for the seedling straw test.

The Seedling Straw Test procedure is as follows:

- Plant seeds in eight to 12 cm pots with one seedling per pot (plant 2-3 seeds and thin to one at emergence) or multiple seedlings in larger pots may be grown. Because plants remain small by the time the test is completed, there is no need to use large pots unless plants will be retained for seed.
- Immediately after all seeds are germinated and emerged, prepare the inoculum. Growing the pathogen in petri dishes on PDA is exactly same as with conventional method but seedling growth must be judged carefully so that the pathogen culture is ready at the right stage of plant growth.
- For inoculum preparation, a two stage system is used. First, sterile sclerotia are plated and grown on 3.9 % PDA in a 100 x 20 mm petri dish for three days at 23ºC. At this stage, mycelia should cover ¾ or so of the petri plate. Secondly, to ensure mycelia growth uniformity, a set of sub-cultures are produced by transferring a plug of agar with mycelia from the original plate to the center of a new petri dish containing PDA and incubating at 23ºC for three days. At this point the culture is ready to use.
- The seedling will be ready for inoculation about seven to ten days after germination. The critical stage is when the apical meristem has grown at least 2 cm above the primary leaves. While large differences in internode length may be observed among cultivars at later stages of development, the selected stage generally shows a little variation in internode length. It is important to make sure that all the seedlings have enough internode length to be inoculated.
- Cut the stem about 2-3 cm above the primary leaves but below the apical meristem. A straw 1-2 cm length that has been stapled on one end is used to cut two plugs of agar containing fungal mycelia. The plugs should be taken from the outer zone of mycelia where the fungus is actively growing. The straw with the mycelial plugs is placed on the decapitated stem with the plugs in contact with the stem.
- Disease severity is scored at 4 days after inoculation using a 1-9 scale (Table 1, Figs. 1 & 2).

Table 1. Rating scale for the seedling straw test.

Score	Description
1	No symptoms beyond cut stem.
2	Lesion to a point midway between the cut stem and the primary leaf node.
3	The lesion reaches the primary leaf node.
4	The lesion passes the primary leaf node (within the first quarter of the distance between the primary leaf node and the cotyledonary node).
5	The lesion reaches the half-way point between the primary leaf node and the cotyledonary node.
6	The lesion passes the half-way point between the primary leaf node and the cotyledonary node.
7	The lesion reaches the cotyledonary node.
8	The lesion passes the cotyledonary node, the first half between the cotyledonary node and the soil surface.
9	The seedling completely collapses and dies.
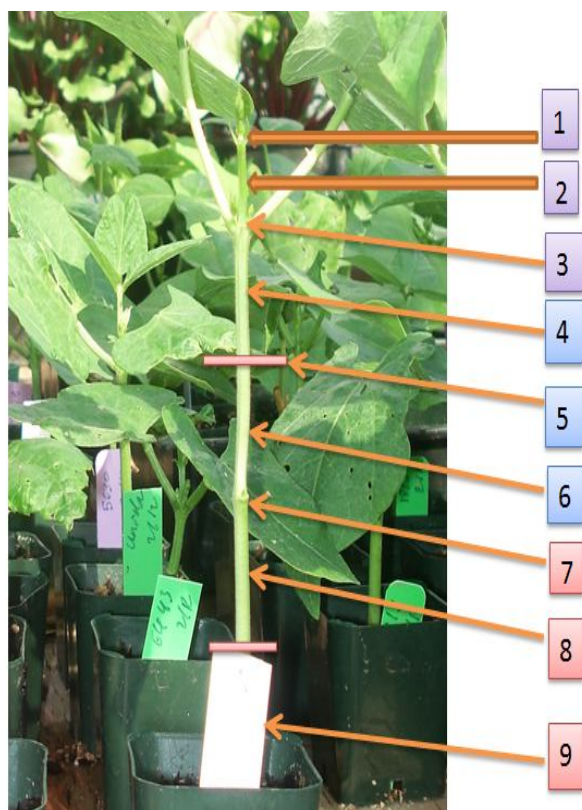
Figure 1. Optimal stage of growth of bean plants for inoculation with white mold for the seedling straw test.

Figure 2. Scoring of bean seedlings inoculated with white mold in the seedling straw test at four days post inoculation.

REFERENCES

Petzoldt, R., and M.H. Dickson. 1996. Straw test for resistance to white mold in beans. Annu. Rpt. Bean Improv. Coop. 39:142-143.

COMPARISON OF THE CONVENTIONAL AND SEEDLING STRAW TESTS FOR QUANTIFYING WHITE MOLD RESISTANCE

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INTRODUCTION: White mold, caused by *Sclerotinia sclerotiorum* (Lib.), is one of the most important pathogens of common bean (*Phaseolus vulgaris* L.), causing complete crop failure under certain conditions. Several methods have been developed to evaluate physiological resistance to white mold in the laboratory and greenhouse, such as spraying plants at bloom with a suspension of ascospores, limited-term inoculation, and the excised stem test (Abawi & Grogan, 1975; Hunter et al., 1981; Miklas et al., 1992). The straw test developed by Petzoldt & Dickson (1996), is widely used to evaluate and screen for physiological resistance. This method was subsequently modified by Terán et al. (2006). The original and modified straw test use plants that are three to five weeks old at the time of inoculation, with another week of growth prior to scoring, which requires larger pots, more bench space, and more hand labor maintaining the plants. We modified the conventional straw test so that fewer resources and less time and effort are required. A companion paper (Arkwazee & Myers, 2017) provides details of the procedure, and the seedling straw test was able to successfully detect major QTLs associated with white mold resistance in common bean (Vasconcellos et al., 2017).

MATERIALS AND METHODS: Two experiments were conducted to compare the conventional (original) and seedling straw test with two different sets of accessions being used, the first being six common bean lines with known levels of resistance or susceptibility to white mold and second 28 accessions from 2017 National White Mold Nursery. The six common bean lines included G122 and NY6020-5 (resistant), Ex Rico (moderate resistance), and OSU 5630, OR 91G, and Beryl (susceptible) (Table 1.) and the 28 national white mold trial accessions are shown in Table 2. Four seeds from each line (thinned to 3 seedlings) were planted in a 2L pot with four replications for each accession.

Plant stems were cut about 5 cm after the third node and 2-3 cm straws with one end closed containing a plug of agar with fungal mycelia put on top of the cut stem for the conventional method. For the seedling straw test, stems were cut 1-2 cm above the primary leaves and inoculated by 1-2 cm straw with 2 plugs of fungal mycelia. Plants were scored 7 days after inoculation for the conventional method and 4 days for the seedling method. A 1-9 scale was used to score the development of the disease severity for both methods but using the Terán et al. (2006) scale for the conventional test and the scale reported in the companion paper for the seedling straw test. (Arkwazee & Myers, 2017).

Table 1. Mean comparison of white mold resistant and susceptible common bean accessions using conventional straw test (left) and the seedling method (right).

Accession	Conventional	Seedling
	Score (1-9)	
OSU5630	7.25a	9.00a
OR91G	7.08a	9.00a
Ex Rico	7.83a	8.92a
Beryl	6.89a	8.67a
G122	5.08b	2.92b
NY 6020-5	5.08b	3.00b

RESULTS: Tukey's pairwise comparison test showed similar overall result when the data of both methods was analyzed separately. NY6020-5 and G122 were significantly more resistant than other lines (Tables 1). Differences between resistant and susceptible lines was much greater in the seedling compared to the conventional straw test. Regression of conventional onto the seedling straw test revealed a highly significant association between methods ($R^2 = 0.55$; Fig. 1). While there was change in rank in the center of the table, both methods were in agreement in identifying the resistant and susceptible ends of the spectrum (Fig. 1, Table 2). In both tests, Ex Rico/Bunsi appeared to be more susceptible in the seedling than in the conventional straw tests. Ex Rico/Bunsi generally shows moderate resistance in the field but often appears moderately susceptible in the straw test. The seedling straw test appears to exaggerate this effect. Visually, differences between susceptible and resistant accessions are more apparent in the seedling than in the conventional straw test.

Table 2. Mean comparison of white mold resistance using conventional straw test and the seedling straw test on the 2016 National White Mold Nursery conducted in Oregon.

Entry	Seedling	Conventional
	Score (1-9)	
USPT-WM12	2.6	3.7
031A-11	2.5	3.7
G122	3.0	4.2
NDF141308	3.4	4.2
R13752	3.7	4.4
ASR 1865	5.8	4.8
Bunsi	5.9	4.9
PS08-039A-5	3.2	5.0
NDF140406	5.3	5.0
Beryl	6.1	5.1
R12844	4.5	5.2
NDF140427	5.7	5.4
WM91212-4-3	3.7	5.9
NDF140423	5.6	6.0
NDF140408	5.7	6.0
NDF140460	6.0	6.0
NDF140415	5.1	6.1
NDF140405	5.2	6.1
NDF140409	5.6	6.1
NDF140433	6.1	6.3
NDF140422	6.3	6.3
NDF140446	6.9	6.4
NDF140461	6.3	6.6
P14815	4.8	6.9
NDF140436	5.4	6.9
NDF140443	7.0	7.0
B15430	5.7	7.8
N15341	7.3	8.4
LSD 0.05	1.6	1.0

Figure 1. Regression of conventional straw test on the seedling straw test for the National White Mold Nursery data from Oregon in 2016.
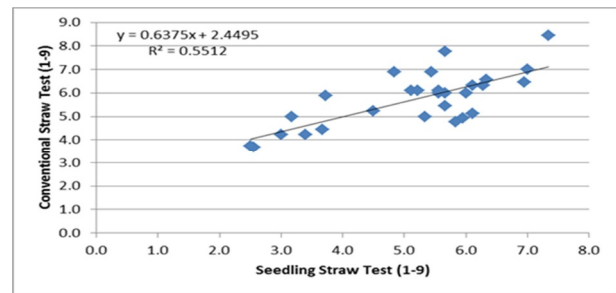
REFERENCES

Abawi, G.S., and R.G. Grogan. 1975. Source of primary inoculum and effects of temperature and moisture on infection of beans by *Sclerotinia sclerotiorum*. Phytopathology 65:300–309.

Arkwazee, H. and J.R. Myers. 2017. Seedling straw test: A rapid and resource-efficient method for evaluating white mold resistance. Ann. Rep. Bean Improv. Coop. 60:xx-xx.

Hunter, J.E., M.H. Dickson and J.A. Cigna. 1981. Limited-term inoculation: a method to screen bean plants for partial resistance to white mold. Plant Dis. 65:414-417.

Miklas, P.N., K.F. Grafton, and B.D. Nelson. 1992. Screening for partial physiological resistance to white mold in dry beans using excised stems. J. Am. Hortic. Sci. 117:321-327.

Petzoldt, R., and M.H. Dickson. 1996. Straw test for resistance to white mold in beans. Annu. Rpt. Bean Improv. Coop. 39:142-143.

Terán, H., M. Lema, H.F. Schwartz, R. Duncan, R. Gilbertson and S.P. Singh. 2006. Annu. Rpt. Bean Improv. Coop. 49:115-116.

Vasconcellos, R.C.C., O.B. Oraguzie, A. Soler, H. Arkwazee, J.R. Myers, J.J. Ferreira, Q. Song, P. McClean and P.N. Miklas. 2017. Meta-QTL for resistance to white mold in common bean. PLoS ONE 12(2): e0171685. doi:10.1371/ journal.pone.0171685.

PRELIMINARY SURVEY OF RNA GENOME VIRUSES IN LIMA BEAN

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INTRODUCTION: In Brazil, at least nine species of virus have been reported infecting common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata* (L.) Walp.). Among these, several stand out because of their predominance in the field: *Cowpea severe mosaic virus* (CPSMV - Secoviridae, *Comovirus*), *Cowpea aphid-borne mosaic virus* (CABMV - Potyviridae, *Potyvirus*) and *Cucumber mosaic virus* (CMV - Bromoviridae, *Cucumovirus*). The viruses that infect cowpea and common bean should be considered potential pathogens for lima bean (*Phaseolus lunatus* L.), mainly because they belong to the same botanical family and are cultivated simultaneously in the same areas. Considering the importance of lima bean and the acknowledged scarcity of information about viruses in this crop, a survey was carried out of RNA genome viruses, which cause mosaic diseases, in lima bean-producing regions in Piauí state, one of the main producers of this leguminous crop in Brazil. The data obtained reveal a predominance of CABMV and CMV, and an absence of CPSMV in the samples analyzed. The collection and analysis of more samples is ongoing, and will supply an overview of the distribution of these pathogens. It will also offer the possibility of detecting other species of virus, because symptomatic samples presented a negative result for the three species evaluated.

MATERIALS AND METHODS: Twelve leaf samples from lima bean plants, exhibiting symptoms of mosaic and leaf deformity (Figure 1) were collected in lima bean fields in the municipalities of Teresina and Várzea Grande, Piauí state, Brazil. Each sample consisted of a plant stem presenting typical symptoms of viral infection. All the samples collected were dried, identified and stored at 20 ºC. The samples were diagnosed in the Instituto Biológico de São Paulo. Twelve samples were analyzed using the plate-trapped antigen - enzyme linked immunosorbent assay (PTA-ELISA) with specific anti-CABMV and anti-CMV polyclonal antiserum, and the RT-PCR protocol (with specific primers for CPSMV) according to Barros et al. (2013). Absorbance at 405 nm was read in an ELISA reader (Microplate Reader 3550- UV, Bio-Rad) in triplicates, after the application of p-nitrophenyl phosphate as substrate. Results obtained were expressed as the ratio of mean absorbance of infected samples to mean absorbance of healthy samples. Samples were considered positive when mean absorbance readings were at least three times as high as negative control absorbance values (Barros et al., 2013). Total RNA was extracted from 0.1 g lima bean leaf tissue in Trizol® medium (Thermo Fisher Scientific) according to the manufacturer's instructions. We conducted RT-PCR using approximately 1 g total RNA and specific primers designed to amplify the protein coat gene of CPSMV (antisense 5'-CTCAAACCCCTGTTGGGACCACA-3'; sense 5'-GGATGAATTTTTGATGGCATGG-3') (Barros et al., 2013). Samples were then placed in a thermocycler and, after initial heating at 94 °C for 5 min, the amplification was conducted as follows: 30 cycles at 94 °C for 1 min, followed by 47 °C for 2 min and 72 °C for 3 min, and a final extension at 72 °C for 7 min. The size of the PCR product expected was 592 bp. Amplified DNA fragments were visualized on agarose gels 1.2% in the presence of ethidium bromide, under ultraviolet light (Sambrook et al., 1989).

RESULTS: Four samples (33.3%) were infected by CABMV, two (16.6%) were infected by CMV (Table 1) and none was infected by CPSMV (data not shown). Mixed infections were not detected. Six samples (50%) with symptoms of mosaic and leaf deformity were not infected by any of the three viruses, which evidences the presence of at least one species naturally present in plants in the field, but which has not yet been described in lima bean. Studies are underway to identify the virus. In the 1980s, in Piauí state, Brazil, Santos et al. (1984) characterized two potyviruses by sorology, morphology and host-range

tests. The authors concluded that the two viruses were distinct from the other bean potyviruses known in the country.

CONCLUSIONS: Based on the diagnosis by ELISA or RT-PCR with anti-serum and specific primers, respectively, the species CABMV and CMV are etiological agents of lima bean mosaic virus in Brazil. Furthermore, there is at least one unidentified species naturally infecting lima bean.
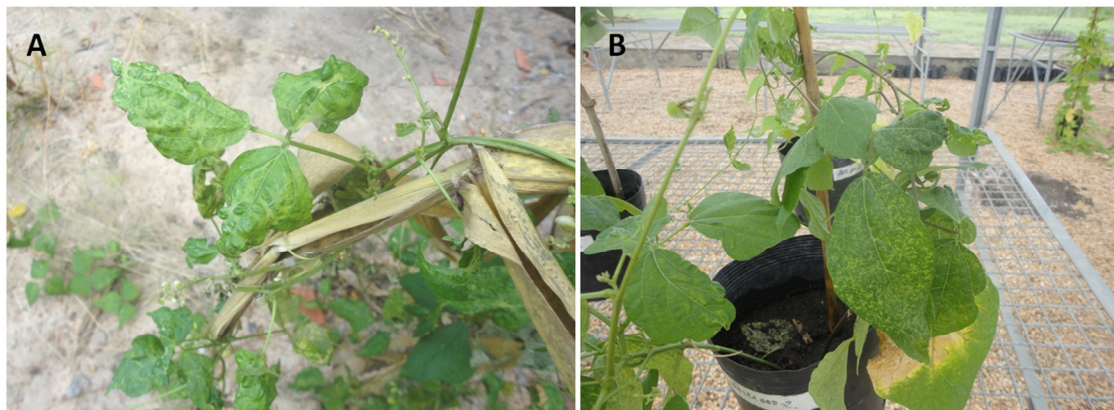
Figure 1. Lima bean (*Phaseolus lunatus* L.) plants infected by CABMV (A) and CMV (B), exhibiting symptoms of mosaic and deformity.

Table 1. Index of lima bean (*Phaseolus lunatus* L.) samples for CABMV (A) and CMV (B) by PTA-ELISA with specific anti-serums. The values within brackets refer to absorbance readings at 405 nm.

A	Sample	Absorbance
	217	+ (A_{405nm} = 1.900)
	228	+ (A_{405nm} = 1.000)
	239	+ (A_{405nm} = 0.900)
	620	- (A_{405nm} = 0.300)
	666	+ (A_{405nm} = 0.900)
	Positive control	+ (A_{405nm} = 0.900)
	Negative control	+ (A_{405nm} = 0.400)
B	Sample	Absorbance
	SC1	- (A_{405nm} = 0.200)
	SC2	- (A_{405nm} = 0.150)
	VG1	- (A_{405nm} = 0.100)
	VG2	- (A_{405nm} = 0.040)
	G28	+ (A_{405nm} = 1.900)
	220	- (A_{405nm} = 0.090)
	250	+ (A_{405nm} = 1.900)
	Positive control	+ (A_{405nm} = 0.600)
	Negative control	+ (A_{405nm} = 0.150)

REFERENCES:

Barros, G.B., Nogueira, M.S.R., Oliveira, C.R.R., Freire Filho, F.R., Ribeiro, V.Q., Veiga, C.F.M., Brioso, P.S.T., Eiras, M. 2013. Obtenção de plantas de feijão-caupi resistentes ao *Cowpea severe mosaic virus* e ao *Cowpea aphid-borne mosaic virus.* Summa Phytopathologica 39: 130-136.

Sambrook, J., Fritsch, E.F., Maniatis, T. 1989. Molecular Cloning, 2 ed. Cold Spring Harbor Press, New York.

Santos, A.A., Lin, M.T., Kitajima, E.W. 1984. Caracterização de dois potyvirus isolados de caupi (*Vigna unguiculata*) no Estado do Piauí. Fitopatologia Brasileira 9: 567-581.

ASSESSMENT OF THE RESISTANCE CONFERRED BY THE *bc-1* ALLELES TO *Bean common mosaic necrosis virus*

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INTRODUCTION: *Bean common mosaic necrosis virus* (BCMNV) is a potyvirus comprising several strains classified into two pathogroups according to the interactions with six recessive resistance alleles in common bean (1). These pathogroups (PGs), numbered III and VI, are defined by the ability (PG-VI) or inability (PG-III) of a BCMNV isolate to replicate in bean differential lines carrying *bc-1* or *bc-1²* resistance alleles. The biological and molecular basis for this differential response of BCMNV isolates to the presence of *bc-1* alleles is not known. Conversely, the genetic determinants involved in interactions of BCMNV strains with *bc-1* resistance alleles have not yet been identified either. We performed a complete biological and molecular study of three isolates of BCMNV belonging to PG-III and VI, collected in California and in Oregon. Particular attention was paid to BCMNV isolates' performance in common bean lines from host groups 2, 3, and 9, harboring *bc-1* and *bc-1²* alleles. The data obtained suggest that the *bc-1* alleles restricted systemic movement of PG-III isolates of BCMNV, while cell-to-cell movement of the virus in inoculated leaves did not seem to be affected.

MATERIALS AND METHODS: The reference BCMNV isolate TN1 used in this work was described previously (2). BCMNV isolate NL8-CA was collected in 2015 near Davis, CA, as a field sample from an heirloom common bean cultivar 'Zuni Gold' displaying mosaic and stunting. The TN1a isolate of BCMNV was found in a sample of common bean exhibiting mosaic found in Corvallis, OR, in 2015. BCMNV isolate 1755b was initially identified in a field sample 91-1755 collected in Willamette Valley, OR, in 2013 (3); this original sample contained mixed infection comprising a 1755a isolate of BCMV and a 1755b isolate of BCMNV. The 1755b isolate of BCMNV was biologically separated from BCMV by passaging it through common bean cultivar Redlands Greenleaf B, non-permissive for the PG-VIII isolate 1755a of BCMV (3). All virus isolates were propagated in the bean cultivar 'Dubbele Witte' using mechanical inoculation and then maintained under greenhouse conditions. The whole-genome cloning strategy, sequencing, and sequence analysis for BCMNV isolates 1755b, TN1a, and NL8-CA were conducted as described previously (2).

RESULTS: Of the three BCMNV isolates subjected to the biological typing on bean indicators, one (NL8-CA) was unable to systemically infect cultivars 'Redlands Greenleaf B' (HG-2) and 'Redlands Greenleaf C' (HG-3), and hence was typed as belonging to pathogroup III, while the other two (TN1a and 1755b) infected cultivars 'Redlands Greenleaf B' and 'Redlands Greenleaf C' systemically, and were typed as belonging to pathogroup VI. The two latter isolates, TN1a and 1755b had the pathogenicity profile identical to the control BCMNV isolate TN1 belonging to PG-VI. Isolate NL8-CA (PG-III) induced only local necrosis on inoculated leaves in cultivars 'Top Crop' and 'Jubila' harboring *I* gene protected with the *bc-1* allele, while isolates TN1, TN1a, and 1755b (all PG-VI) induced rapid whole plant necrosis (WPN) in 'Top Crop' 7-14 days

45

post-inoculation, and severe systemic necrosis, but not WPN, in 'Jubila' 3-5 weeks post-inoculation. In cultivars 'Redland Greenleaf C' expressing *bc-1* and 'Redland Greenleaf B' expressing *bc-1²* alleles, isolate NL8-CA was able to systemically infect only a small proportion of upper, uninoculated leaves, below 13% and below 3%, respectively. The whole genomes of BCMNV isolates NL8-CA, TN1a, and 1755b were cloned and sequenced, using the approach described previously (2). Upon sequence assembly, NL8-CA was found to be 9,627-nt long, excluding the poly (A). Based on conceptual translation, the BCMNV NL8-CA genome encoded a single polyprotein of 3,071 aa. Both TN1a and 1755b genomes were found to be 9,628-nt long, excluding the poly (A), and both genomes encoded a single polyprotein of the same size as NL8-CA (3,071 aa).

Fig. 1. Recombination analysis of the three studied BCMNV isolates, TN1a, 1755b, and NL8-CA, in comparison to the BCMNV isolates from the GenBank, NL8, NL3, and TN1. Manual distance plot based on the aligned full-length nucleotide sequences of BCMNV isolates NL8, NL3, TN1, TN1a, 1755b, and NL8-CA; NL3 (GenBank accession AY282577) was used as the reference strain. X axis represents nucleotide position in the alignment, Y axis represents relative distance from the reference sequence which is calculated using Kimura model (Kimura, 1980).

CONCLUSIONS: The whole genomes of isolates 1755b, TN1a, and NL8-CA were sequenced and sequence analysis revealed that despite the overall high nucleotide sequence identity between PG-III and PG-VI isolates (ca. 96%), two areas of the BCMNV genome in the P1/HC-Pro and HC-Pro/P3 cistrons appeared to be more divergent between these two pathotypes of BCMNV (Fig. 1). The data obtained suggest that the phenotypic differences among PG-III and PG-VI isolates of BCMNV in common bean cultivars from host resistance groups 2, 3, and 9, carrying *bc-1* alleles, were related to the impaired systemic movement of the PG-III isolates to the upper, uninoculated leaves, and also suggest a role of the recessive *bc-1* gene in interfering with systemic spread of BCMNV.

REFERENCES:
1. Drijfhout, E. and Morales, F. 2005. Bean Common Mosaic. In Compendium of bean diseases. Second edition (Scwartz, H.F., Steadman, J.R., Hall, R., and Forster, R.L., editors). The American Phytopathological Society: St. Paul, MN; pp. 60-62.
2. Feng, X., Poplawsky, A.R., Nikolaeva, O.V., Myers, J.R., and Karasev, A.V. 2014. Recombinants of *Bean common mosaic virus* (BCMV) and genetic determinants of BCMV involved in overcoming resistance in common beans. *Phytopathology* 104: 786-93.
3. Feng, X., Myers, J.R., and Karasev, A.V. 2015. *Bean common mosaic virus* isolate exhibits a novel pathogenicity profile in common bean, overcoming the *bc-3* resistance allele coding for the mutated eIF4E translation initiation factor. *Phytopathology* 105: 1487-1495.

IDENTIFICATION OF TROPICAL BLACK BEAN BREEDING LINES RESISTANT TO BGYMV USING MOLECULAR MARKERS

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INTRODUCTION: The incidence of diseases significantly reduces seed yield in the tropical environments of common bean production areas in southern Mexico, which includes the states of Veracruz and Chiapas. Yield losses up to 100% have been reported when plant infection by BGYMV occurs at the seedling stage (López et al., 2002a). Moreover, it has been known since the early 90´s that BGYMV reduces dry bean yield in tropical Mexico, and the state of Chiapas is not the exception since yield loses have been reported to reach 18.2 to 40.5% for commercial cultivars Negro Tacaná and Negro Huasteco-81, respectively (López et al., 1993); while in the northern areas of the state of Veracruz the reduction could get up to 87.6% for cultivar Negro Jamapa (Rodríguez and Yoshii, 1990). The objective of this study was to identify Mesoamerican black common bean germplasm with resistance to BGYMV by artificial inoculation and verification by the presence of molecular markers associated with virus resistance.

MATERIALS AND METHODS: 70 common bean genotypes (54 recombinant inbred lines and 16 improved cultivars) were used for the scrutiny using BGYMV clones in agrobacterium, pBGMXAbin (DNAA) and pBGMXBbin (DNA-B) previously sequenced for both components collected in Chiapas, Mexico (Garrido et al., 2000). Ten plants of each of all genotypes were planted in 300 mL Styrofoam cups filled with sterilized soil substrate and kept in the greenhouse. 16 days after planting, at fully extended primary leaf stage, plants were inoculated with a 1:1 mixture of cell suspensions from the BGYMV clones in both agrobacterium previously cultivated in LB+Kanamycin liquid medium, which was shaken for 48 hours and verified by PCR for the stability of both components. The inoculated plants were kept in the greenhouse and the BGYMV symptoms were evaluated 15 days after inoculation. The resistance or susceptibility of the inoculated genotypes was determined based on the percentage of infection by genotype and type of symptoms shown. Samples of the third trifoliate were taken from each plant and the DNA was extracted by the Dellaporta method (Gilbertson et al., 1991), for PCR analysis of the presence of BGYMV and resistance genes. To verify the presence of the *bgm*-1 resistance gene in the inoculated genotypes, the SR2 marker was used by PCR following the protocol proposed by Miklas et al. (2005), which were 34 cycles of 10 seconds at 94 °C, 40 seconds at 60 °C and 120 seconds at 72 °C, followed by a 5-minute cycle at 72 °C. To determine the nature of the marker (codominant or dominant), the temperature mating was varied from 60 °C to codominant and 65 °C to dominant (Miklas et al., 2005). PCR products were visualized on 1% agarose gels, with the GelRed dye. In addition, markers were selected for the *bgm*-3 and *Bgp*-2 genes (Osorno et al., 2007).

RESULTS AND DISCUSSION: In relation to the *bgm*-1 gene, the results indicated that there was a broad variability among the evaluated genotypes. Table 1 presents the results of molecular detection of the *bgm*-1 gene with a molecular marker type SCAR SR2 and symptoms observed in bean plants of the evaluated germplasm. A group of 11 breeding lines plus DOR 448, Negro Papaloapan, Verdín and T-39 had a $R2_{570/530}$ (-/+) phenotype indicating that these are resistant

homozygous (*bgm*-1/*bgm*-1) plants, which did not show any BGYMV infection symptoms and virus amplification was not achieved by PCR. Another group the five breeding lines and cultivars Negro 8025 Negro Veracruz, Negro Huasteco-81 and Negro Comapa, which despite having a phenotype R$2_{570/530}$ (-/+) which indicates a homozygous (*bgm*-1/*bgm*-1) and expected to perform as resistant, symptoms of BGYMV infection were observed in inoculated plants (data not shown).

Table 1. Reaction of common black bean genotypes to inoculation with BGYMV.

Breeding line or cultivar	Seed Germination (%)	R2 570/530		Symptoms[b]					Death plants [c]
		-/+	+/-	PS	MD	AE	DV	Ab	
Papaloapan/SEN 46-1-8	80	1	0	0/2	-	-	-	-	8
Papaloapan/SEN 46 -6-2	90	3	0	0/3	-	-	-	-	6
Papaloapan/SEN 46-6-4	90	3	0	0/4	-	-	-	-	5
Papaloapan/SEN 46-7-8	50	4	0	0/4	-	-	-	-	1
Papaloapan/SEN 46-7-11	60	4	0	0/6	-	-	-	-	0
Papaloapan/SEN 46 -7-12	80	4	0	0/6	-	-	-	-	2
Papaloapan/SEN 46-7-13	60	4	0	0/4	-	-	-	-	2
Negro Citlali/RAV-187-3-1-5	60	4	0	1/4	-	-	-	-	2
Negro Citlali/RAV-187-3-1-6	30	3	0	0/3	-	-	-	-	3
Negro Citlali/RAV-187-3-2-2	50	4	0	0/4	-	-	-	-	1
Negro Citlali/RAV-187-3-16-7	80	2	0	0/8	-	-	-	-	0
Negro Papaloapan	30	3	0	0/3	-	-	-	-	0
Verdín	80	4	0	0/6	-	-	-	-	2
T-39	50	4	0	0/4	-	-	-	-	1

A SCAR SR2 molecular marker linked to the *bgm*-1 resistance gene. + and - presence and absence of the 570 bp or 530 bp fragment amplified with this marker - / + homozygous resistant (*bgm*-1/*bgm*-1), +/- susceptible homozygous (*Bgm*-1/*Bgm*-1), [b] Symptoms produced by the inoculation of BGYMV by agrobacterium. [C] Plants that died from causes not attributable to the inoculated virus. PS = plants with symptoms/total #plants; MD = Golden mosaic, AE = Internode shortening, DV =Pod distortion, Ab = Abortion of flowers and pods.

CONCLUSIONS: The agroinoculation technique was effective on infecting common bean seedlings with BGYMV, and facilitated the identification of resistant bean germplasm using the SCAR SR2 molecular marker linked to the *bgm*-1 resistance gene. Eleven recombinant inbred common bean lines, the elite line DOR 448 and cultivars Negro Papaloapan, Verdín and T-39 were identified as BGYMV resistant.

REFERENCES

López, S. E.; *et al*. 1993. Agric. Téc. Méx. 19(2):99-109.
López, S. E., *et al*., 2002a. Rev. Mex. Fitopatol. 20(2):193-199.
Rodríguez, R. R. y Yoshii, O. K. 1990. Agric. Téc. Méx. 16:19-32.
Garrido-Ramírez, E. R., *et al*., 2000. Phytopathology 90:1224-1232.
Gilbertson, R. L., *et al*., 1991. Plant Dis. 75:336-342.
Miklas, P.N. 2005. [Reviewed in February 2016].
http://www.ars.usda.gov/sp2UserFiles/Place/53540000/Miklas/SCARtable.pdf.
Osorno, J. M. *et. al*,, 2007. J. Am. Soc. Hortic. Sci. 132:530-533.

SINGLE NUCLEOTIDE POLYMORPHISM (SNP) MARKER DISCOVERY AND GENETIC MAPPING ASSOCIATED WITH RESISTANCE TO BEAN GOLDEN YELLOW MOSAIC VIRUS

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INTRODUCTION: Bean Golden Yellow Mosaic Virus (BGYMV) is a severe disease of common bean (*Phaseolus vulgaris* L.) that causes important yield losses. BGYMV is a geminivirus (family Geminiviridae) transmitted by Whitefly in tropical and sub-tropical countries of Latin America and the Caribbean. Breeding for resistance to the virus has been the most effective strategy for controlling the disease. One resistance source, A429, derived from the Durango landrace Garrapato (G2402), and subsequent DOR lines developed at CIAT, were shown to contain a single recessive gene, *bgm-1*, which reduces mosaic and yellowing symptoms. The marker SR2 has been used in marker-assisted selection programs because it is linked to the *bgm-1* gene for resistance to BGYMV. In this study, SNP markers were developed from Genotyping by Sequencing (GBS) data on the region associated with the bgm-1 gene. These SNPs markers could constitute an important tool for marker-assisted selection programs for improvement of common bean cultivars with resistance to BGYMV.

MATERIALS AND METHODS: Two common bean populations, each 100 recombinant inbred lines (RILs), generated at the International Center for Tropical Agriculture from the cross SEL 1309 x DOR476 and SMC33 x SCR16 were used for this study. The Mesoamerican genotypes DOR 476 and SCR 16 were resistant to BGYMV. The DNA, extracted using the urea protocol from a set of genotypes resistant to BGYMV, was sequenced and genotyped according to Elshire et al. (2014) protocol. Total genomic DNA for each RIL in both populations was isolated from single seed using the extraction method described by Xin et al., 2003 as modified by the Bean Molecular Genetics Laboratory-CIAT. The two populations of 100 RILs were tested in field in El Salvador. Experimental design was a randomized complete block with two replications. Plants were evaluated for chlorosis using a 1–9 scale where 1 was equivalent to very resistant and 9 to very susceptible for each of the symptoms.

Bowtie2 v2.2.3 was used to align reads against the current reference genome of *P. vulgaris*, downloaded from the Phytozome website. NGSEP pipeline was used to discover and genotype SNPs and to perform functional annotation of variants, filtering and conversion from VCF to other formats for further downstream analysis. Primers were designed according to Wang et al. 2005 using the software Primer 3. The markers were amplified by PCR on a fluorescence-detecting thermocycler (CFX384 Real-Time System, Bio-Rad) with EvaGreen© fluorescent dye. Melting point analysis for allele determination of the template DNA was performed using the same equipment.

A linkage map of the target segment around *bgm-1* gene was generated using Mapdisto® with a LOD value 3.0 as the threshold to confirm the order of markers, and the recombination values were converted into Kosambi genetic distances (cM). Additional markers (SSR's, SCAR's, SNP's) from previous studies were incorporated into this map. QTL were identified with QTL IciMapping® v.4.0.6. Map associations were identified with Inclusive composite interval mapping (ICIM) analysis.

RESULTS AND DISCUSSION: In both populations analyzed were found one QTL on chromosome 3 linked to bgm-1 gene. SEL 1309 x DOR476 was a maximum PVE of 62.28% and SMC 33 x SCR 16 of 89.23%. CB_00352 was the SNP marker with higher correlation with the bgm-1 gene, between 79 and 84% in these populations. These results suggest that the CB_00352 SNP is better than SR2, which has been used in marker-assisted selection programs for resistance to BGYMV.
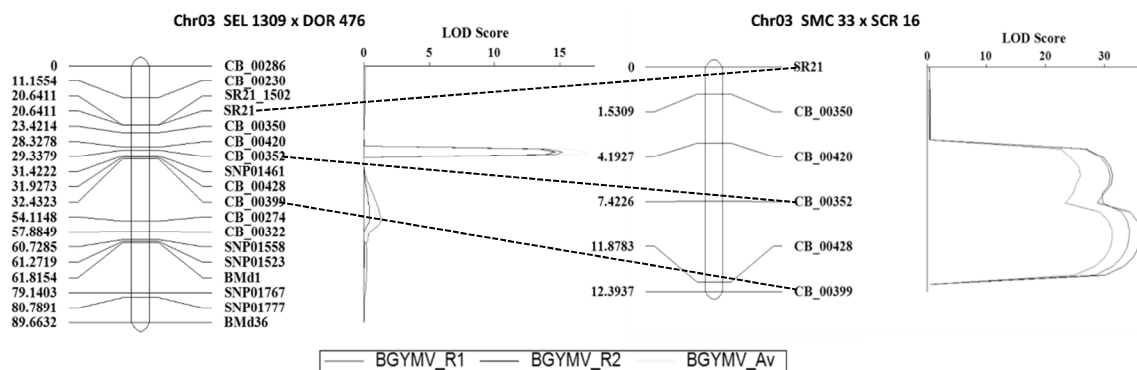
Figure 1. The SEL 1309 x DOR476 and SMC 33 x SCR 16 linkage chrm03. One QTL identified on chromosome 3 for resistance to BGYMV. R1: Replication 1, R2: Replication 2 and AV: Average between replications.

Table 1. Quantitative trait loci (QTL) for resistance to BGYMV for the recombinant inbred line (RIL) population from the cross SEL1309 x DOR 476 and SMC 33 x SCR 16. R1: Replication 1, R2: Replication 2 and AV: Average between replications.

Trait	RIL Population	Chrm	Left Marker	Physical Position(bp)	Right Marker	Physical Position(bp)	LOD	PVE(%)	Add
BGYMV_R1	SEL 1309 x DOR 476	3	CB_00352	2,488,398	SNP01461	2,620,505	15.21	58.10	1.63
BGYMV_R2	SEL 1309 x DOR 476	3	CB_00352	2,488,398	SNP01461	2,620,505	14.64	57.47	1.54
BGYMV_Av	SEL 1309 x DOR 476	3	CB_00352	2,488,398	SNP01461	2,620,505	17.06	62.28	1.59
BGYMV_R1	SMC 33x SCR16	3	CB_00420	2,111,735	CB_00352	2,488,398	30.95	86.55	2.01
BGYMV_R1	SMC 33x SCR16	3	CB_00352	2,488,398	CB_00428	2,747,044	34.23	87.06	2.02
BGYMV_R2	SMC 33x SCR16	3	CB_00420	2,111,735	CB_00352	2,488,398	25.97	85.07	1.96
BGYMV_R2	SMC 33x SCR16	3	CB_00352	2,488,398	CB_00428	2,747,044	31.32	87.66	2.00
BGYMV_Av	SMC 33x SCR16	3	CB_00420	2,111,735	CB_00352	2,488,398	31.39	87.64	1.97
BGYMV_Av	SMC 33x SCR16	3	CB_00352	2,488,398	CB_00428	2,747,044	36.13	89.23	2.00

REFERENCES

Blair, M. W., Rodriguez, L. M., Pedraza, F., Morales, F., & Beebe, S. (2007). Genetic mapping of the bean golden yellow mosaic geminivirus resistance gene bgm-1 and linkage with potyvirus resistance in common bean (*Phaseolus vulgaris* L.).

Elshire R. J., Glaubitz J. C., Sun Q., Poland J. A., Kawamoto K., et al. (2011) A Robust, Simple Genotyping-by-Sequencing (GBS) Approach for High Diversity Species.

Wang, J., Chuang, K., Ahluwalia, M., Patel, S., Umblas, N., Mirel, D., Germer, S. (2005). High-throughput SNP genotyping by single-tube PCR with Tm-shift primers.

Xin, Z., Velten, J. P., Oliver, M. J., & Burke, J. J. (2003). High-throughput DNA extraction method suitable for PCR.

TERMINAL WATER STRESS IMPOSED TO IDENTIFY DROUGHT TOLERANT MESOAMERICAN BLACK COMMON BEAN BREEDING LINES IN SOUTHERN MEXICO

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INTRODUCTION: Productivity of the dry bean crop is low in the humid tropical environments of southeastern Mexico, due in large part to the effect of different biotic and abiotic factors which reduce grain yield. The most important abiotic factor is the presence of terminal drought that occurs frequently after flowering, during pod filling stages and physiological maturity (Acosta-Gallegos *et al*., 1998), particularly in the crop season with residual moisture, which restrict yield and cause partial and severe losses in commercial crops. The development of improved varieties with resistance to this environmental factor is a viable alternative to increase yields of beans under conditions of water stress (Frahm *et al*., 2003). The objective of this study was to field test recombinant inbred Mesoamerican black dry bean lines for their resistance and/or adaptation to terminal drought and high plant relative yield efficiency grown under non-stress and water stress conditions in tropical southeastern Mexico.

MATERIALS AND METHODS: A dry black dry bean nursery was set up and included 50 recombinant inbred lines derived from three crosses: Jamapa Plus/RAV, Negro Citlali/RAV and Negro Papaloapan/SEN 46 and cultivars Negro Comapa, Negro Tacana and Negro Grijalva were used as regional checks. Field experiments were planted under water stress a non-stress conditions the 12th of February, 2016 during the Winter-Spring (January-May) dry season. Two identical bean nurseries were used, each one included a single row of each of 50 breeding lines sown in plots 5.0 m long and 0.80 m rows; the three check cultivars were planted every 10 breeding lines giving a total of 68 rows arranged in two blocks. Water control was provided by furrow irrigation applied at pre-sowing, 23, 30, 43, 56 and 65 days after planting (DAP) for the non-stress condition, while the water stress condition (terminal drought) included just the pre-sowing and two more irrigations, 23 and 30 (DAP). Samples of soil cores were taken at different soil depths (0-40 cm) every week to reveal soil water content during the growing cycle in both conditions. The testing of the breeding nursery was undertaken using the reduction of seed yield, the drought susceptibility index (Fischer and Maurer,1978) and the plant relative yield efficiency (Graham, 1984) index for each line.

RESULTS AND DISCUSSION: In this study the Drought Intensity Index obtained was high (DII = 0.73), as a result the average yields under drought stress conditions were generally low (316 kg ha^{-1}) in comparison to non-stress (1156 kg ha^{-1}). Results for this field test indicated that among the three breeding populations, Jamapa Plus/RAV had higher seed yield and less yield reduction (48%) in comparison to the other two populations and all three check cultivars (Table 1); its DSI=1.18 was also higher than 1.0 which indicates low drought tolerance; however, it had a higher relative efficiency index (REI=2.67) than the rest of the genotypes but similar to Negro Grijalva (REI=2.61) check cultivar (Table 2). DSI among the 50 breeding lines ranged from DSI = 0.22 (Negro Citlali/RAV-187-3-7-2) to DSI = 1.36 (Negro Citlali/RAV-187-3-2-2) with up to DSI = 1.11 for all cultivar checks. Plant relative efficiency index among recombinant inbred

lines fluctuated from REI = 0.03 to REI = 4.18 and Negro Grijalva check cultivar presented a REI = 2.61. A group of nine recombinant inbred lines was identified as to have less yield reduction than check cultivars, a DSI < 1.0 and high relative efficiency index, this included three from the Jamapa Plus/RAV cross, four from Negro Citlali/RAV and two more from Papaloapan/SEN 46 cross (Table 2). Soil water content in both conditions, non-stress and water stress, was similar during the first 30 (DAP). After this period, the moisture content in the bean nursery with water stress was significantly reduced so that in less than 10 days it had exceeded the maximum permissible abatement limit (p = 0.45 - 0.50) for the bean crop (Allen *et al*., 2006). The continuous depletion of soil moisture had a linear tendency during the following 8 to 11 days (data not shown) until reaching the permanent wilting point, this reveals that bean plants were under severe water stress from flowering to maturity in the water stress condition.

Table 1. Seed yield and drought stress indexes of three bean breeding populations grown under non-stress and terminal water stress field conditions in Veracruz, Mexico. Winter-Spring, 2016 season.

Population	Non-stress (kg ha^{-1})	Water stress (kg ha^{-1})	Reduction (%)	DSI[&]	REI*
Papaloapan/SEN 46	1082	231	78	1.08	0.73
Negro Citlali/RAV	1149	414	64	0.89	1.47
Jamapa Plus/RAV	1364	696	48	0.66	2.67
Average	1156	316	73	1.00	1.12

[&]Drought susceptibility index. * Relative efficiency index.

Table 2. Agronomic characteristics, seed yield and drought tolerance indices of recombinant inbred bean lines identified for their response to terminal drought conditions in Veracruz, Mexico. Winter-Spring, 2016 season.

Breeding line/cultivar	DF[§] (d)	PM[£] (d)	Non-stress (kg ha^{-1})	Water stress (kg ha^{-1})	Reduction (%)	DSI[&]	REI*
Jamapa Plus/RAV-3-1-8	36	66	1861	822	55.8	0.77	4.18
NegroCitlali/RAV-187-3-14-7	36	68	1561	917	41.3	0.57	3.91
Negro Citlali/RAV-187-3-1-6	39	70	1417	857	39.5	0.54	3.32
Jamapa Plus/RAV-3-1-2	34	66	1306	861	34.0	0.47	3.08
NegroCitlali/RAV-187-3-16-7	36	68	1722	583	66.1	0.91	2.75
Jamapa Plus/RAV-3-4-4	36	70	1122	728	35.1	0.48	2.23
Papaloapan/SEN 46-6-6	40	77	1317	583	55.7	0.77	2.10
NegroCitlali/RAV-187-3-14-6	36	73	1128	672	40.4	0.56	2.07
Papaloapan/SEN 46-3-7	36	70	1289	500	61.2	0.84	1.76
Negro Comapa**	39	74	1832	408	77.7	1.58	2.04
Negro Grijalva**	40	74	1949	489	74.9	1.69	2.61
Negro Tacaná**	41	71	1775	344	80.6	1.54	1.67
Average	38	74	1156	316	72.6	1.00	1.10
Standard deviation	1.9	2.4	494	334	19.1	0.26	1.04

[§]Days to flower, [£]Physiological maturity, [&]Drought susceptibility index. *Relative efficiency index. **Cultivar checks.

CONCLUSIONS: Breeding lines Jamapa Plus/RAV-3-1-2, Jamapa Plus/RAV-3-4-4, Negro Citlali/ RAV-187-3-14-6, Negro Citlali/RAV-187-3-1-6 and Negro Citlali/RAV-187-3-14-7 were the most tolerant to terminal drought based on low yield reduction and a DSI < 1.0; the last two lines together with Jamapa Plus/RAV-3-1-8 also had the highest relative productive efficiency index (REI > 4.18).

REFERENCES.
   Acosta-Gallegos, J. A.*et al*. 1998. Ann. Rep. Bean Improv. Coop. 41:151-152.
   Allen, G. R., et al., 2006. Riego y Drenaje No. 56. FAO. Roma, Italia. 323 p.
   Frahm, M., *et al*. 2003. Agron. Mesoam. 14:143-150.
   Fischer, R.A. and Maurer, R. 1978. Australian Journal of Agricultural Research, 29:897-912.
   Graham, R. D. 1984. Advances in Plant Nutrition, 1:57-102.

WATER REGIME EFFECTS ON PHENOLOGY AND SEED YIELD OF TWO COMMON BEAN CULTIVARS GROWN IN DURANGO, MÉXICO

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INTRODUCTION: In the State of Durango, México, common bean (*Phaseolus vulgaris*) is the most important food product used for direct human consumption, after grain cooking. Common bean is cultivated mainly under rainfed conditions in despite of its decisive role in local economy, human nutrition and nutraceutical contribution for human health. Under rainfed conditions water shortage (intermittent drought) and yield losses in common beans are the main factors causing negative impacts on the farmer´s economy. Some attempts have been made to use common beans under irrigated conditions in order to improve water productivity and to stabilize seed yield. Early maturity and low water requirements were introduced in modern 'pinto' common bean cultivars in order to improve seed yield. Characterization of modern cultivars is required in order to implement the use of technological tools for precise irrigation scheduling; as well as for phenology and yield prediction. The growing degree days (°D) concept, which is based on actual temperatures, is considered as a simple and accurate method to predict when a certain plant stage will occur (Miller *et al*., 2001). The objective of this study was to evaluate effects of three water regimes on phenology and seed yield of common bean at three experimental sites in the State of Durango, México.

MATERIALS AND METHODS: In 2015 and 2016, an irrigation experiment was conducted at INIFAP´s experiment stations located in Durango and Canatlán, México. Two common bean cultivars (Pinto Saltillo and Pinto Centauro) were included in the study. The cultivars were sown in July 10th (Durango 2015), July 13th (Canatlán, 2015) and July 7th (Durango, 2016), using randomized block design with split plot arrangement and four (2015) to eight (2016) replications. The experimental plot consisted of 32 rows 10 m in length (Durango, 2015), 16 rows in 8 m length (Canatlán, 2015) and 12 rows with 10 m in length (Durango, 2016) and 0.81 m apart in all the experimental sites. Fertilizer was applied during the first mechanical weeding at the rate of 35-50-00 (N-P_2O_5-K_2O). Data was collected for days to flowering (DF) and physiological maturity (DPM), minimum and maximum temperatures, amount of water (rainfall + irrigation), seed yield and water productivity. Degree days (°D) were estimated according to the following conditions (Ojeda *et al*., 2004): $°D = T_a - T_{c\text{-}min}$, $T_a < T_{c\text{-}max}$; $°D = T_{c\text{-}max} - T_{c\text{-}min}$, $T_a \geq T_{c\text{-}max}$; $°D = 0$, $T_a \leq T_{c\text{-}min}$. Where T_a is daily mean temperature, $T_{c\text{-}max}$ and $T_{c\text{-}min}$ represent the air maximum and minimum temperatures related to the range required for plant growth in common beans (10 and 28 °C, respectively). Three irrigation treatments were applied (100, 80 and 60 % of soil available water) in order to avoid severe water stress in plants and to determine precise amounts of water required to optimize yield in common bean. At maturity, three plant samples were taken for yield determination, and then the average value was obtained by replication. Plant samples consisted of two rows with 5 m in length by 0.81 m in width (8.1 m^2). Water productivity was calculate using the equation WP= yield/applied water. The analysis of variance was obtained under a randomized complete block design with split plot arrangement and four to eight replications. Mean comparisons were performed using Tukey´s honestly significant difference test ($P \leq 0.05$).

RESULTS AND DISCUSSION: The total amount of water applied across water regimes showed fluctuations between 377 mm (60 %) to 765 mm (100 %) (Table 1). Pinto Centauro cultivar showed precocious flowering (40-41 DAP) and physiological maturity (92-98 DAP) across sites and water regimes, resulting in lower accumulation for °D at the flowering (406-430 °D) and physiological maturity (893-958 °D). Fluctuation observed on phenological stages and accumulated °D showed low influence

over seed yield across water regimes. Higher and stable seed yield values were observed during 2016, across water regimes in both common bean cultivars, showing values among 3,200 kg/ha to 3,687 kg/ha. Across years, both cultivars showed higher values for seed yield under the 80 % and 100 % treatments of soil available water. In 2015, differences were observed between sites and higher yields were observed in the 80 % treatment (Durango) and in the 100 % treatment (Canatlán). Yield reductions were observed under 100 % treatment due to excessive amount of water (765 mm) which surpassed theoretical crop water requirements (300-362 mm), thus causing seed yield reductions. Results suggest that common beans grown in Durango require a water supply ranging from 425 to 631 mm in order to achieve increments in seed yield, registering values for water productivity from 3.9 to 8.0 kg/ha/mm (Figure 1).

Table 1. Phenology and seed yield observed in three sites and three water regimes applied to two common bean cultivars.

Cultivar	Durango 2015			Canatlán 2015			Durango 2016		
	[1]DF	DPM	Yd	DF	DPM	Yd	DF	DPM	Yd
	*100 % (765 mm)			*100 % (631 mm)			*100 % (566 mm)		
P. Saltillo	$43_{(448)}$	$103_{(1,015)}$	3,129	$45_{(459)}$	$101_{(950)}$	$3,344^a$	$43_{(448)}$	$95_{(917)}$	3,576
P. Centauro	$41_{(427)}$	$97_{(967)}$	2,546	$40_{(406)}$	$99_{(937)}$	$3,157^a$	$41_{(430)}$	$92_{(893)}$	3,548
	80 % (425 mm)			80 % (497 mm)			80 % (538 mm)		
P. Saltillo	$42_{(438)}$	$102_{(1,007)}$	3,380	$45_{(459)}$	$99_{(937)}$	$1,946^b$	$42_{(440)}$	$94_{(909)}$	3,466
P. Centauro	$41_{(427)}$	$96_{(958)}$	2,890	$41_{(416)}$	$98_{(930)}$	$2,190^b$	$41_{(430)}$	$93_{(900)}$	3,687
	60 % (377 mm)			60 % (401 mm)			60 % (440 mm)		
P. Saltillo	$43_{(448)}$	$103_{(1,015)}$	2,665	$44_{(447)}$	$100_{(943)}$	$1,645^c$	$43_{(448)}$	$93_{(900)}$	3,292
P. Centauro	$41_{(427)}$	$97_{(967)}$	2,686	$41_{(416)}$	$97_{(923)}$	$1,705^c$	$41_{(430)}$	$92_{(893)}$	3,200
Mean	$\mathbf{42_{(438)}}$	$\mathbf{99_{(986)}}$	**2,883**	$\mathbf{43_{(436)}}$	$\mathbf{99_{(937)}}$	**2,331**	$\mathbf{42_{(440)}}$	$\mathbf{93_{(900)}}$	**3,461**

[1]DF= days to flowering, DPM= days to physiological maturity, subscript represents accumulated ºD; Yd= seed yield; *Relative value based on soil available water. Letters in each column indicate significant differences according to Tukey´s test ($P \leq 0.05$) between cultivars [a-c].
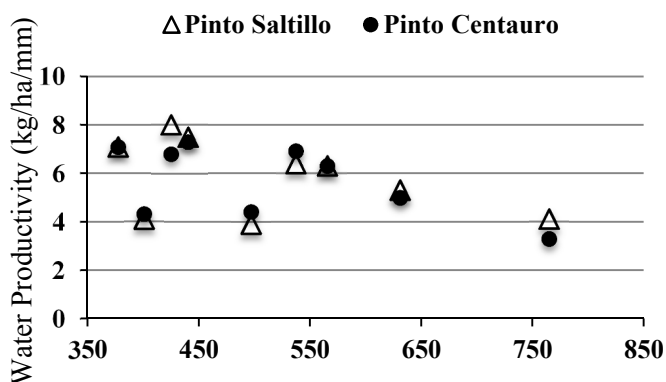
REFERENCES

Miller, P.; W. Lanier; S. Brandt. 2001. Using growing degree days to predict plant stages. Montana State University. Bozeman, MT. 7 p.

Ojeda, W.; E. Sifuentes; D. C. Slack; M. Carrillo. 2004. Generalization of irrigation scheduling parameters using the growing degree days concept: application to a potato crop. Irrigation and Drainage. 53: 251-261

CHANGES IN THE PHENOLICS METABOLISM OF COMMON BEAN PLANTS GROWN UNDER DEFICIT IRRIGATION

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INTRODUCTION: The common bean is a traditional crop in México which is produced in many agricultural regions throughout the country. Nonetheless, its quality depends of environmental factors that have place during the plant development; in this sense, the metabolic response to these factors can be modify by abiotic factors such as a limited water supply, and this can be observed in different plant tissues. The objective was to obtain a secondary metabolites differentiation regarding the phenylpropanoid pathway of two Flor de Junio (FJ) bean cultivars.

MATERIAL AND METHODS: Cultivars FJ Dalia and Victoria were sown in July 2016 at the INIFAP-Zacatecas Research Center. Plants were subjected to different water supply regimens: T1, 100/50 % of water availability in vegetative/reproductive stage; T2, 75/12.5; T3, 50,100 and T4, 12.5,75. Sampling was conducted when plants reached the reproductive stage; foliar tissue was collected and immediately drown in liquid nitrogen, freeze dry and processed in a domestic grinder, samples were kept at room temperature until analysis. Extraction of polyphenols was performed using 1 g of sample and 10 mL of acidic acetone [acetone/water/acetic acid (70:29.5:0.5, v/v/v)] as described by Xu et al. (2007). Content of total phenols (mg GAE/g), total flavonoids (mg CAE/g), condensed tannins (mg CAE/g) and anthocyanins (mg C3GE/g) was determined.

RESULTS AND DISCUSSION: The phenolic compounds content among treatment and common bean cultivars is shown in Table 1. Results clearly show that an interaction effect was obtained since the concentration of polyphenols depends on the relation between the water supply regime and the cultivar's genotype. Karaqbourniotis et al. (2014) reported that annual plants exposed to a deficit irrigation tend to water saving instead of carbon gain. Therefore, priority is given to protection, including the stimulation of antioxidant enzymes activity and the synthesis of phenolic compounds in order to counteract the accelerated production of ROS in different cellular organelles. Significant differences ($p < 0.5$) were observed for all variables evaluated, the trend in the synthesis of condensed tannins was very similar in both bean cultivars with very slight changes in their content, however, comparing the water supply treatments, total flavonoids were synthetized in greater amount when plants were grown under a non-well-watered treatment (2 and 4 for Dalia and Victoria, respectively); in the case of total phenols, a 10% increase in the foliar tissue sampled from FJ Dalia was observed in comparison to the FJ Victoria cultivar, while the maximum concentration of anthocyanins was found in the samples collected from FJ Victoria with a small increase of 4%, in both cases from treatment 3. In this context, André et al. (2009) reported that drought stress induces changes in the expression of phenylalanine ammonia-lyase, which is the key regulator of the phenylpropanoid pathway, and thus, synthesis of polyphenols.

Table 1. Phenolic compounds content in foliar tissue of common bean plants subjected to different water supply regimes.

Compound	Treatment	Cultivar	
		Victoria	Dalia
Total phenols[1]	T1	14.4 ± 0.0 c	13.5 ± 0.1 d
	T2	14.6 ± 0.1 c	17.2 ± 0.2 a
	T3	15.5 ± 0.1 b	17.1 ± 0.2 a
	T4	14.8 ± 0.6 c	12.6 ± 0.1 e
Total flavonoids[2]	T1	7.0 ± 0.1 c	5.1 ± 0.3 e
	T2	5.7 ± 0.2 e	12.5 ± 0.0 a
	T3	5.7 ± 0.5 e	7.6 ± 0.0 b
	T4	12.9 ± 0.2 a	6.8 ± 0.1 d
Condensed tannins[2]	T1	46.3 ± 0.5 a	39.8 ± 0.9 e
	T2	36.8 ± 0.9 f	39.9 ± 0.2 e
	T3	46.9 ± 0.3 a	42.2 ± 0.0 c
	T4	44.3 ± 0.3 b	41.2 ± 0.4 d
Total anthocyanins[3]	T1	240.4 ± 3.4 g	319.7 ± 1.2 b
	T2	296.9 ± 2.7 e	258.5 ± 3.7 f
	T3	336.0 ± 3.1 a	320.8 ± 0.2 c
	T4	333.2 ± 0.7 a	312.6 ± 0.5 d

Values are presented as mean ± SD. Means in the same section with a common letter are not significantly different ($p<0.05$, Tukey). Data are expressed as: [1]mg equivalent of gallic acid/g of dry sample. [2]mg equivalent of (+) catechin/g of dry sample. [3]mg equivalent of cianidin-3-glucoside /kg of dry sample.

REFERENCES:

André, C.M., Schafleitner, R., Legay, E., Lefévre, I., Alvarado, A.A., Norberto, G., Hoffman, L., Hausman, J-F., Larondelle, Y. & Evers, D. 2009. Gene expression changes related to the production of phenolic compounds in potato tubers grown under drought stress. Phytochemistry 70:1107-1116.

Karaqbourniotis, G., Liakopoulos, G., Nokolopoulos, D., Bresta, P., Stavroulaki, & Sumbele S. 2014. "Carbon gain vs. water saving, growth vs. defense": two dilemmas with soluble phenolics as a joker. Plant Science 227: 21-27.

Xu BJ, Chang SKC. 2007. A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. Journal of Food Science. 2: 159-166.

DEFICIT IRRIGATION IMPROVES SAPONINS CONTENT IN DIFFERENT COMMON BEAN PLANT TISSUES

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INTRODUCTION: Common bean plants are often subjected to water deficiency due to the large extent of territory destined to production under rainfed conditions, which may lead to a critical abiotic stress that affects plant physiology and development. Plants that are exposed to drought stress generally produce higher levels of secondary metabolites, including triterpenoids such as saponins (Nasrollahi et al., 2014). The objective was to quantify the total saponins content of different plant tissue of two Flor de Junio (FJ) common bean cultivars.

MATERIAL AND METHODS: Cultivars FJ Dalia and Victoria were sown in July 2016 at the INIFAP-Zacatecas Research Center, under a rainout shelter to protect against rainfall; plants were subjected to different water supply regimens: 1, 50/50 % of water availability in vegetative/reproductive stage; 2, 100/100; 3, 50,100 and 4, 100,50. Root and foliar tissue were collected when plants reached the reproductive stage, samples were immediately freeze dry and processed in a domestic grinder. In brief, total saponins were extracted adding 4 mL of hexane to 0.5 g of dry sample, after shaking for 6 h, samples were centrifuged and acetonitrile was added to the hexanoic extracts (Hiai et al., 1976). Total saponins quantification was conducted on the basis of a reaction with vanillin and sulfuric acid. Results were expressed as µg equivalent of oleanolic acid/gram of dry sample (µg OAE/g).

RESULTS AND DISCUSSION: It has been reported that saponins are phytochemicals significantly influenced by factors that compromise an environmental stimulus such as water supply in crops, and the increase of these compounds can be achieved by applying special moisture regimes during the crop cycle (Szakiel et al., 2011). Figure 1 shows significant differences in the total saponins content of root tissue among treatments and cultivars evaluated. FJ Dalia exhibited a greater capacity of synthesis in comparison to FJ Victoria, which indicates that, regardless of the water supply treatment, genotype is an important trait. Although saponins concentration of this cultivar was higher due to the effect of the well-watered treatment, the foliar tissue had the highest amount of this compounds due to a water deficiency during the reproductive stage (Figure 2). Our results are in accordance with Nasrollahi et al. (2014) who mentions that modifying the soil moisture availability, it may be possible to improve the production of saponins in agricultural crops, however, this depends on the plant tissue and the cultivar's genotype. In this context, FJ Victoria had a different tendency, since the maximum concentration of saponins in the root tissue was found in plants subjected to water deficiency during the whole cycle, while foliar tissue displayed a higher synthesis in the well-watered plants.
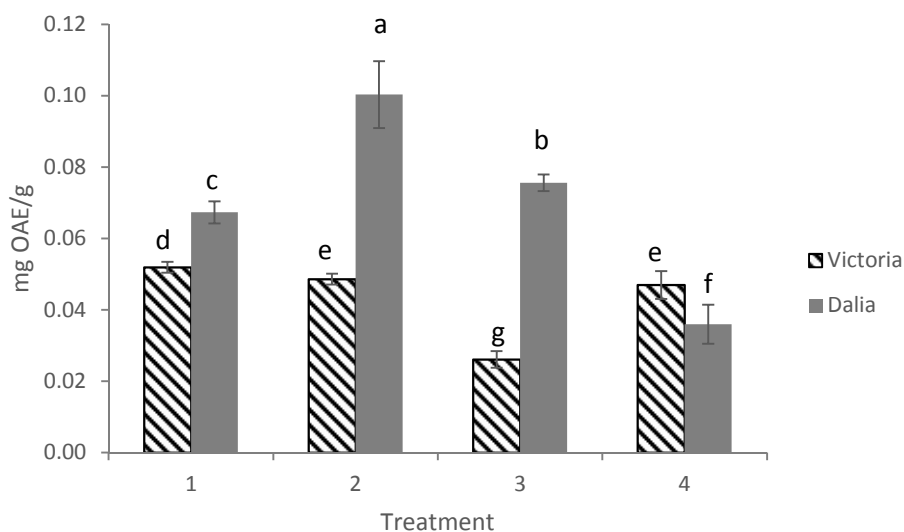
Figure 1. Total saponins content in plants root tissue of two common bean cultivars.
Values are presented as mean ± SD. Means in the same bar with a common letter are not significantly different ($p < 0.05$, Tukey).
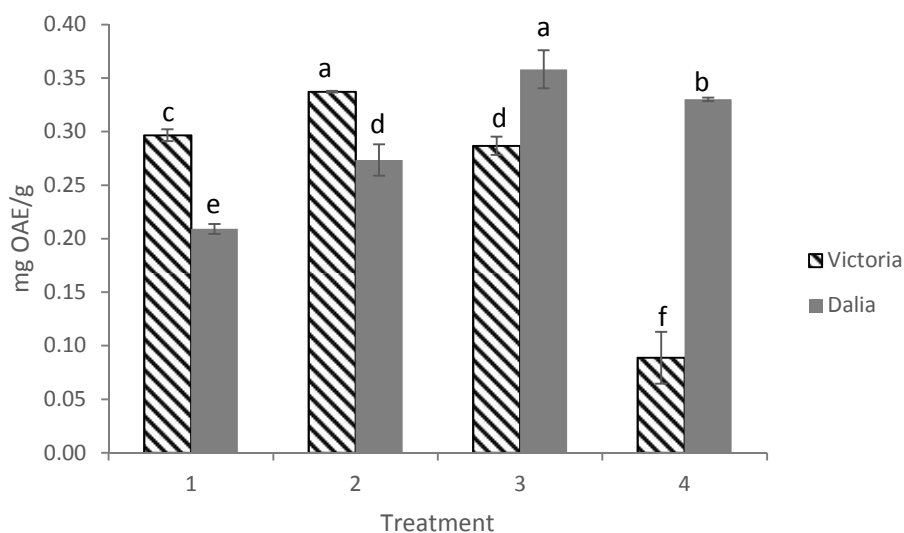
Figure 2. Total saponins content in plants foliar tissue of two common bean cultivars.
Values are presented as mean ± SD. Means in the same bar with a common letter are not significantly different ($p < 0.05$, Tukey).

REFERENCES:

Hiai, S., Oura, H., y Nakajima, T. (1976). Color reaction of some sapogenins and saponins with vanillin and sulfur1c acid. Planta medica. 29: 116-122.

Nasrollahi, V., Mirzaie-asl, A., Piri, K., Nazeri, S. & Mehrabi, R. The effect of drought stress on the expression of key genes involved in the biosynthesis of triterpenoid saponins in liquorice (Glycyrrhiza glabra). Phytochemistry 103: 32-37.

Szakiel, A., Paczkowski, C. & Henry, M. 2011. Influence of environmental abiotic factors on the content of saponins in plants. Phytochemistry Reviews 10: 471-491.

DROUGHT TOLERANCE OF COMMON BEAN GENOTYPES CHARACTERIZED BY PHYSIOLOGICAL AND BIOCHEMICAL MARKERS

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is considered the main legume for human consumption, being an important source of proteins, carbohydrates and minerals. However, most of its farming occurs under drought conditions, with water deficit being one of the main limiting factors for production, which can reduce it by up to 80% (ROSALES et al., 2012). The effects of drought on the common bean depend on the frequency, duration and intensity of the stress and the phenological stage of the crop, which can affect the photosynthetic efficiency, stomatal conductance, transpiration rate and solute accumulation. Quantifying these components related to drought tolerance is extremely important to define strategies for their use in breeding programs. Therefore, the present study aimed to evaluate two common bean genotypes for tolerance to drought through biochemical and physiological characteristics.

MATERIAL AND METHODS

The experiment was conducted in greenhouse, at Agronomic Institute of Paraná State, the experimental design was randomized blocks with six replications and two tolerant cultivars were evaluated, IAPAR 81 and BAT 477. The plants were grown in pots with substrate under 80% of the pot capacity until the phenological stage R5, when the drought began in plots subjected to stress, in which was adopted the water treatment of 30% of the pot capacity for 19 days. Liquid photosynthesis, stomatal conductance, transpiration and carboxylation efficiency of the plant were measured in the morning on sunny days, on the last day of stress, using the portable system Photosynthesis LI-6400XT (LI-COR Biosciences, Lincoln, NE, EUA). For the biochemical analyzes (GPX, APX, protein and proline) it was collected, also in the last day of stress, a leaflet of each plant, arranged in bulk and diluted in proportion 1:5 in four different buffer solutions according to the analyzes. Data were submitted to analysis of variance and Tukey test at 5% of probability by the computer program R (http://www.r-project.org) using the ExpDes and MVar.pt packages.

RESULTS AND DISCUSSION

The results indicated physiological and biochemical changes when the cultivars were submitted to water deficit in the reproductive period. The values of liquid photosynthesis, stomatal conductance and transpiration decreased in both genotypes when submitted to water deficit, but it was not observed statistical difference between the genotypes under stress conditions (Table 1). The first reaction of plants to water deficit is the stomata closing for less water loss by transpiration, reducing the availability of CO_2 inside the leaf, which causes a decrease in the rate of photosynthesis (ANDROCIOLI et al., 2016). The carboxylation efficiency decreased only in BAT 477 under drought conditions and IAPAR 81 presented statistically higher values for this variable, besides having the highest rate of liquid photosynthesis, demonstrating physiological efficiency when submitted to drought. In the biochemical analyzes, an accumulation of GPX and

proline was observed only in BAT 477 under water stress and it was verified statistical difference between the cultivars only for GPX, with BAT 477 presenting the highest value for this characteristic (Table 1). One of the most common responses to stresses in plants is the overproduction of different types of organic solutes (ASHRAF; FOOLAD, 2007). The accumulation of proline in plants under water deficit has been correlated with stress tolerance, and their concentration has generally been shown to be higher in tolerant plants than in plants sensitive to stress. It was observed reduction of APX and protein for both genotypes. Still both cultivars being drought tolerant, IAPAR 81 presented physiological adaptions to drought while BAT 477 showed higher values on biochemical characteristics for the same conditions.

Table 1 - Averages comparison by Tukey method (P <0.05) for physiological and biochemical traits of common bean genotypes evaluated in the presence and absence of water deficit.

Genotypes	A		g_s		T		CE	
	Control	WD[2/]	Control	WD	Control	WD	Control	WD
BAT 477	16.31Aab	3.35Bab	0.422Aa	0.034Ba	3.70Ab	0.59Ba	0.053Aab	0.014Bb
IAPAR 81	14.50Ab	6.34Ba	0.286Ab	0.049Ba	4.74Aa	1.03Ba	0.050Aab	0.035Aa
Genotypes	GPX		APX		Protein		Proline	
	Control	WD	Control	WD	Control	WD	Control	WD
BAT 477	0.345Ba	0.608Aa	1.289Aa	0.784Bab	18.853Aa	4.379Ba	0.643Bc	0.957Ab
IAPAR 81	0.233Ab	0.248Ab	1.004Aab	0.623Bb	19.538Aa	3.626Ba	0.887Ab	0.843Ab

[1/]A: liquid photosynthesis (mol CO_2 m^{-2} s^{-1}), g_s: stomatal conductance (mol m^{-2} s^{-1}), T: transpiration (mol H_2O m^{-2} s^{-1}), CE: carboxylation efficiency (mol CO_2 m^{-2} s^{-1} Pa^{-1}), GPX: glutathione peroxidase (activity per minute), APX: ascorbate peroxidase (activity per minute); [2/]WD: water deficit, Means followed by the same lowercase letter in the column and upper case in the row do not differ from each other by the Tukey test at 5% of probability.

REFERENCES

ANDROCIOLI, L. G.; TOMAZ, J. P.; MODA-CIRINO, V. Physiological responses of two drought tolerant common bean cultivars. Annual report of the bean improvement cooperative, v. 59, p. 35-36, 2016.

ASHRAF, M.; FOOLAD, M. R. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany. v. 59. p. 206-216. 2007.

ROSALES, M. A. et al. Physiological analysis of common beans (*Phaseolus vulgaris* L.) cultivars uncovers characteristics related to terminal drought resistance. Plant Physiology and Biochemistry, v. 56, p. 24-34, 2012.

HIGHER CONCENTRATION OF Fe LEADS TO HISTONE MODIFICATION IN COMMON BEAN TISSUE

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INTRODUCTION

Over more than 100 years agricultural scientists all over the world have developed high yielding crop varieties to meet the energy demand of increasing population. However the nutritional qualities have not been given priorities as a result world is facing with serious malnutrition problem. Two of the most prominent deficiencies affecting the world are of iron (Fe) and zinc (Zn). Among others Fe is vital in building proteins of red blood cells, whereas Zn is essential in cellular growth and development. Epigenetic mechanisms such as DNA methylation, histone modification, and small interfering RNA (sRNA) regulate the transcription of DNA in living organisms (He et al. 2011). It has been reported that the abiotic and biotic stresses such as changes in temperature, pests, drought, disease, and the concentration of minerals and metals in the surrounding soil are involved with the changes in epigenetic and transcriptomic components (Hu et al. 2012) in plant. The long-terms goals of our work are to identify the epigenetic and transcriptomic components involved in the acquisition and translocation of micronutrients in common bean.

MATERIALS AND METHODS

In our work, we applied higher concentrations of Fe to a common bean genotype G122 which was previously identified as a genotype that is highly responsive to elevated Fe and other health-related minerals (Bauduin et al. 2014). The treated and control plants were planted in 8.5"x11" pots filled with Sunshine Mix. The sunshine mix was kept soaked with water until germination. After germination we kept the clear saucers beneath the treated pot filled with a solution of Fe (200 mg-^{1L}) until the leaves reached 50% senescence while the control plant continued to receive water. At 50% leaf senescence, the stems of the plants were harvested and chromatin and total histone were isolated using the Chromaflash Plant Chromatin Extraction Kit (P-2022) and Epiquik Total Extraction Kit (OP-0006) respectively (www.epigenetek.com). One hundred nanograms of total histone of treated and control samples were added into the wells of 20 H3 modification sites in duplication for identifying each of the 20 histone modification patterns. Following the procedure described in the EpiQuik Histone H3 Modification Multiplex Assay Kit (P-3100), the intensity of absorbance of the H3 modification sites was measured at 450 nm wavelength by a BioTek Microplate reader (Elx808). Using the formula (given below) provided by the EpiQuik Histone H3 Modification Multiplex Assay Kit, histone modifications for 20 of the 21 H3 modification sites were calculated in ng/µg of histone 3 protein and compared between treated and control stems and presented as fold change (Table. 1).

$$H3\ Modification\ or\ total\ H3\ (ng/\mu g\ protein) = \frac{(Sample\ OD - Blank\ OD) \div S}{(Assay\ Control\ OD - Blank\ OD) \div P} \times 1000$$

Where, blank OD was 0.063, assay control OD was 0.530 and **S** was the amount of input sample protein in ng (100 ng) and **P** is the amount of input assay control in ng (25 ng).

Table 1. H3 modification and fold of modification in Fe treated common bean Stem compared with control.

	3	4	5	6	7	8	9	10	11	12
A	0.184	0.186	0.182	0.278	1.98	0.241	0.248	0.258	0.309	0.553
B	0.20	0.168	0.199	0.287	1.99	0.266	0.225	0.227	0.283	0.435
H3 Modification (ng/μg) protein	68.984	60.963	68.182	117.38	1027.81	101.8717	92.7807	95.9893	124.5989	230.4813
C	0.116	0.087	0.18	0.135	0.248	0.194	0.159	0.235	0.243	0.389
D	0.156	0.102	0.099	0.158	0.214	0.199	0.177	0.237	0.278	0.392
H3 Modification (ng/μg) protein	39.037	16.845	40.909	44.6524	89.8396	71.39037	56.1497	92.51337	105.615	175.1337
Fold Change	1.7671	3.619	1.6667	2.62874	11.4405	1.426966	1.65238	1.037572	1.179747	1.316031
E	0.203	0.116	0.157	0.192	0.241	0.669	0.263	1.114	0.335	0.239
F	0.194	0.103	0.163	0.15	0.202	0.612	0.251	1.281	0.397	0.206
H3 Modification (ng/μg) protein	72.46	24.866	51.872	57.754	84.7594	308.8235	103.743	606.6845	162.0321	85.29412
G	0.144	0.146	0.132	0.135	0.22	0.292	0.265	0.668	0.269	0.368
H	0.154	0.068	0.155	0.142	0.205	0.277	0.245	0.659	0.281	0.301
H3 Modification (ng/μg) protein	45.9893	23.5294	43.0481	40.374332	79.946524	118.4492	102.6738	321.12299	113.36898	145.18717
Fold Change	1.57558	1.05682	1.20497	1.4304636	1.0602007	2.6072235	1.0104167	1.889259	1.4292453	0.587477

RESULTS AND CONCLUSIONS

We observed 2-11 fold increase in four H3Kme1, H3K27me2, H3K36me1, and H3K79me1 of the 20 H3 sites in common bean stems due to higher concentrations of Fe. Using different tissues (such as root, stem, pod, and seed) of selected varieties of treated and control samples, significance of the 21 sites of H3 modification can be further analyzed and antibody specific chromatin fragments can be identified with CHiP-Seq analysis. Analysis of the other histones sites, such as Histone 4, may also provide a better understanding in how higher concentrations of minerals and metals affect epigenetic changes.

REFERENCES

Bauduin, M., Shimpa, L., Johnson, MA., and K. Hossain. 2014.k. Influence of Increased Concentration of Fe and Zn on Different Health Related Mineral Concentrations among Common Bean Genotypes. North Dakota INBRE Annual Symposium for Undergraduate Research. Oct. 23. Alerus Center, Grand Forks, ND.

He, X., Chen, T. and Zhu 2011. Regulation and function of DNA methylation in plants and animals. Cell Research 21:442–465.Hu, Y., Zhang, L., He, S., Huang, M., Tan, J., Zhao, L., Yan, S., Li, H., Zhou, K., Liang, Y., Li, L. 2012. Cold stress selectively unsilences tandem repeats in heterochromatin associated with accumulation of H3K9ac. Plant Cell Environ 35:2130-2142

ACKOWLEDGEMENT

HIGHER CONTENT OF FE CAUSES DIFFERENTIAL GENE EXPRESSION IN COMMON BEAN

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INTRODUCTION

The insight into the complex processes of biological systems encoded by the plant and animal genome can be focused by studying the network of gene products (Pandy and Mann, 200) which are resulted from gene expression determined by the complex interactions among transcription factors, chromatin proteins, and epigenetic modifications. The long-term goals of our research are to understand the transcriptomic and epigenetic components involved in the translocation of health related micronutrients in common bean. Previously, we identified a common bean genotype highly responsive to higher concentration of Fe (Bauduin et al. 2014). In this work, we applied higher concentrations of Fe to a responsive common bean genotype and analyzed isolated proteins to identify differences in protein expression between treatments using SDS-PAGE and mass spectrometry.

MATERIALS AND METHODS

At Mayville State University, we grew three replications of the bean genotype (G122) with controls. We planted the seeds in 8.5''x11'' pots filled with "Sunshine Mix". The sunshine mix was soaked with water until germination. After germination, we kept filling the plastic saucer beneath the treated plants' pot with solutions of 150mg-^{1L} and (200mg-^{1L}) Fe until 50% leaf senescence, while the controls continued receiving water. After harvesting, seed samples were sent to a Proteomics laboratory at University Maryland College Park for SDS-PAGE and Mass Spectrometry analysis.

For mass spectrometry, the protein (100 µg) was reduced and digested with Porozyme immobilized trypsin (Thermo Fisher Scientific, Waltham, MA). The digested peptides were purified and analyzed by 2D nano-LC (Shimadzu) equipped with LTQ Orbitrap XL mass spectrometer (ThermoFinnigan). All MS/MS samples were analyzed using Mascot (Matrix Science, London, UK; version 1.4.0.288) and Sequest (Thermo Fisher Scientific, San Jose, CA, USA; version 1.4.0.288). Mascot was set up to search Pvulgaris_218_v1.0.protein.fasta (unknown version, 29605 entries) assuming the digestion enzyme trypsin. Scaffold (version Scaffold_4.4.1.1, Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications. Protein identifications were accepted if they could be established at greater than 78.0% probability to achieve an FDR less than 5.0% and contained at least 2 identified peptides The proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony. Spectral abundance was used for differential protein expression.

RESULTS AND DISCUSSION

In the SDS-PAGE analysis, we observed an additional protein band of about 125 kDa in bean seed when treated with 200mg-^{1L} (figure 1). Mass spectrometry revealed significant reduction of storage protein associated enzymes, starch phosphorylase, and granule bound starch

synthase due to Fe treatment. In addition, several other membranes bound and metabolic proteins were differentially expressed due to Fe treatment.

Increasing the concentration of Fe in the common bean, makes significant changes to the expression of proteins.
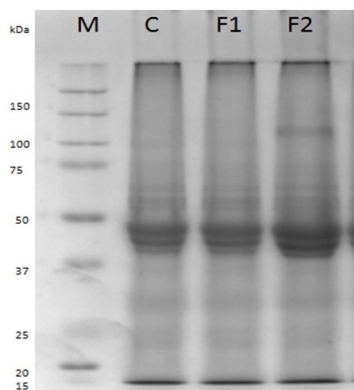
Figure 1. Protein patterns of Control (C) and Iron treated (F1-150mg^{-1L}, F2-200mg^{-1L}) common bean seeds. Soluble proteins were extracted from the bean seed using 6M urea in 100mM tris. Protein concentration was determined by bicinchoninic assay according to manufacturer directions (Pierce, Rockford, IL, USA). Protein (10 µg) was separated in 10% acrylamide and stained with Coomassie blue (G-250, BioRad, Hercules, CA, USA) following electrophoresis.

REFERENCES

Bauduin, M., Shimpa, L., Johnson, MA., and K. Hossain, k. Influence of Increased Concentration of Fe and Zn on Different Health Related Mineral Concentrations among Common Bean Genotypes. 2014 North Dakota INBRE Annual Symposium for Undergraduate Research. Oct. 23. Alerus Center, Grand Forks, ND.

Pandy, A., Mann, M., 2000. Proteomics to study genes and genomes, *Nature*, 2000: 837–846.

ACKOWLEDGEMENT

DIFFERENTIAL RESPONSE OF FIFTEEN PINTO BEAN CULTIVARS TO TWO NITROGEN RATES

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INTRODUCTION

Dry bean (*Phaseolus vulgaris* L.) is the main source of protein (20 to 25%) for most people in the world; protein from soybean is higher but is primarily use for livestock. Dry bean yield is often lower than 1000 kg ha^{-1} in most dry bean producing regions except the US. Besides drought, low soil fertility and ineffective nitrogen (N) management strategies are the most important yield-limiting factors for dry bean production worldwide (Fageria et al., 2013). Use of N-efficient dry bean genotypes, optimal timing of N application(s), and identifying a cost-effective N rate are good strategies to optimize dry bean profitability. Therefore, the aim of this study was to evaluate fifteen pinto bean cultivars grown in the greenhouse with two rates of nitrogen fertilizer for physiological/growth traits and their tolerance to low N.

MATERIAL AND METHODS

Seed of (Bill Z, Centennial, CO46348, COSD-25, COSD-35,Croissant, El Dorado, ISB1231-1, La Paz, Lariat, Long's Peak, ND307, Othello, Poncho, and UIP-40) were sown in 11.3 L pots (8 kg of soil) in the greenhouse (four pots per cultivars) on 20 September 2016 in Laramie WY (2200 m elevation). Seed were inoculated with a commercial inoculant at planting. The soil mix was 33% sand, 33% soil amendment, and 33% native soil. Seedlings were thinned to three per pot at two weeks. Aqueous fertilizer treatments (NH_4NO_3) were applied at (25, 32, 39, and 46 days after planting, dap) in two rates (0 and 67 kg N ha^{-1} seasonal equivalent). A randomized complete block design was used with two replicates. Leaf chlorophyll (CHYL) was measured on the third uppermost fully-expanded leaf by using a chlorophyll meter (SPAD-502) at (26, 33, 40, 47, and 54 dap). The height, root mass, and stalk mass was determined at maturity. Seed yield, pod harvest index (PHI), and nitrogen susceptibility index (NSI) were also determined at maturity. Pod harvest index equaled seed weight divided by the sum of pod wall plus seed weight; NSI was calculated as the cultivar's percentage reduction in yield due to zero N divided by the average yield reduction due to zero N.

RESULT AND DISCUSSION

Adding 67 kg N ha^{-1} increased most traits except for PHI which was reduced (Table 1). Adding N increased CHYL at 33 dap and at 40 dap (data not shown). The seed yield increase due to N was explained by increased number of pods per pot (12.9 vs. 10.3) whereas seed size and seed per pods did not explain any of the difference (data not shown). The only nitrogen-by-cultivar interaction we detected was on CHYL at 33 dap. This was due to several cultivars responding more intensely to N as opposed to others responding modestly (Table 2). Pod harvest index was greatest in Othello, CO-46348, and COSD-25 than the other twelve cultivars. The seed yield was not significantly different among cultivars due to unexplained variability within three cultivars. Nevertheless, we observed numerical differences in yield response to N and their associated NSI. High-N CHYL at 33 dap was positively correlated with seed yield at High-N (r = 0.61). We are repeating this experiment to validate or refute these observations.

Table 1. Influence of two nitrogen levels on leaf chlorophyll, growth, leaf traits, and yield of dry bean averaged across fifteen cultivars. The non-reproductive biomass and shoot:root ratio included root and shoot tissue only (no leaves were included because they had already senesced).

Treatment kg N ha^{-1}	Chlorophyll 33 dap	Plant Height cm	Total Non Repro Biomass	Shoot: Root Ratio	Specific Leaf Wt. mg cm^{-2}	Seed Yield g/pot	Pod Harvest Index
0	32	40.7	3.00	1.92	4.23	14.9	0.820
67	36	52.9	3.75	2.33	4.71	17.5	0.802
P-value	0.0001	0.0036	0.0080	0.0195	0.0090	0.0244	0.0001

Table 2. Leaf chlorophyll, root mass, pod harvest index, seed yield, and nitrogen susceptibility index (NSI) of fifteen dry bean cultivars grown at two N levels in the greenhouse.

Treatment	Zero-N Chlorophyll 33 dap	High-N Chlorophyll 33 dap	Root Mass g/pot	Pod Harvest Index	Zero-N Seed Yield g/pot	High-N Seed Yield g/pot	NSI
Bill Z	34	37	0.62	0.80	11.7	16.5	1.96
Centennial	37	40	0.92	0.78	12.1	13.8	0.81
CO-46348	27	33	0.66	0.84	13.6	25.3	3.11
COSD-25	36	38	0.92	0.83	12.5	16.7	1.69
COSD-35	34	37	1.02	0.82	16.7	18.7	0.68
Croissant	35	40	0.94	0.81	16.0	18.2	0.81
El Dorado	33	36	1.69	0.79	14.4	17.2	1.28
ISB1231-1	31	33	1.12	0.77	15.5	15.2	- 0.14
La Paz	33	36	0.98	0.81	16.2	15.3	- 0.41
Lariat	28	35	2.00	0.79	15.8	16.9	0.44
Long's Peak	28	36	1.05	0.82	12.6	16.3	1.55
ND307	30	35	1.69	0.79	21.8	16.8	- 2.03
Othello	32	38	0.85	0.86	13.2	21.5	2.64
Poncho	35	37	0.79	0.82	21.6	17.7	- 1.49
UIP-40	26	35	1.90	0.81	10.5	15.8	2.23
Mean	32	36	1.14	0.81	14.9	17.5	n/a
LSD (0.05)	3	3	0.64	0.02	N.S.	N.S	n/a

REFERENCES: Fageria, N. K., Melo, L. C., and Oliveria, J. D. 2013. Nitrogen use efficiency in dry bean genotypes. Journal of plant nutrition 36: 2179-2190.

IDENTIFICATION OF BLACK COMMON BEAN BREEDING LINES ADAPTED TO ACID SOILS OF SOUTHERN VERACRUZ, MEXICO

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INTRODUCTION

In the area of Isla Plains and Juan Rodríguez Clara, located south of Veracruz, Mexico, beans are sown annually in low fertility acid soils, mainly Cambisols and Acrisols, of sandy texture, with pH lower than 5.0 and organic matter content less than 1.5%, which confer a low cation exchange capacity (Zetina et al., 2002). In this type of soil, low dry black bean seed yields are obtained, which may be lower than 300 kg ha^{-1}, particularly when there is a combined effect of soil acidity and drought (Tosquy et al., 2008). The objective of this research was to identify black bean genotypes with adaptation to low fertility acid soils of southern Veracruz with high yield efficiency when grown with and without application of dolomitic lime.

MATERIALS AND METHODS

In the Fall-Winter crop season of 2015-16, two identical common bean breeding nurseries were established in an acid soil with a pH of 4.48 in the municipality of Juan Rodríguez Clara, Ver., Mexico; one nursery was conducted under natural conditions of acid soil stress while the other, 44 days before sowing, 2.9 t ha^{-1} of dolomitic lime were applied to soil to reach a pH of 6.0 (Zetina et al., 2002). Fifty F_{10} black bean recombinant inbred lines and three commercial varieties used as cultivar checks (Negro Comapa, Negro Tacaná and Negro Grijalva), developed and released by INIFAP for southeastern Mexico, were evaluated. The genotypes were planted without experimental design, in plots of a 5 m long rows with a plant density of 250,000 plants ha^{-1}, and check cultivars planted systematically every 10 entries. Rainfall was recorded from sowing to harvest. Seed yield in kilograms per hectare was quantified and the effect of soil acidity was estimated using the plant Relative Efficiency Index (REI) proposed by Graham (1984), which indicates the average response of each genotype, with and without application of lime, and allows to identify those genotypes with greater plant productive efficiency.

RESULTS Y DISCUSSION

During the growing cycle of the bean breeding nursery, a total 177.1 mm of rainfall was recorded of which only 27.4 mm occurred during the reproductive phase of the crop, as a result low average seed yields were obtained 846 and 732 kg ha^{-1} with and without dolomitic lime application, respectively. Papaloapan/SEN46-4-10, Negro Citlali/RAV-187-3-1-8, Jamapa Plus/RAV-3-1-2, Papaloapan/SEN46-3-2, Negro Citlali/RAV-187-3-1-5 and Negro Citlali/RAV-187-3-14-7 were the most productive breeding lines in the soil that received dolomitic lime. The first four breeding lines also showed the best adaptation to natural acid soil and drought stress conditions, with higher seed yields similar to Negro Grijalva (best check cultivar), low percentage seed yield reduction and higher indicating high productive efficiency with and without dolomitic lime application (Table 1). Furthermore, the check cultivar Negro Tacaná along with 23 other recombinant breeding lines showed poor adaptation to these environmental conditions, since the RE Index values were lower than the unit (data not shown).

Table 1. Seed yield (kg ha^{-1}), seed reduction (%) and plant productive efficiency of common improved bean breeding lines and regional check cultivars with and without lime application in Juan Rodríguez Clara, Veracruz, Mexico during Fall-Winter 2015-16 season.

Genotype	With lime	Without lime	Reduction	REI[&]
Papaloapan/SEN 46-4-10	1257	1220	2.9	2.48
Negro Citlali/RAV-187-3-1-8	1226	1131	7.7	2.24
Papaloapan/SEN 46-3-2	1111	1091	1.8	1.96
Jamapa Plus/RAV-3-1-2 3	1134	1000	11.8	1.83
Negro Citlali/RAV-187-3-1-5	1177	811	31.1	1.54
Papaloapan/SEN 46-3-7	1051	857	18.5	1.45
Papaloapan/SEN 46-4-5	937	909	3.0	1.38
Papaloapan/SEN 46 -7-10	946	886	6.3	1.35
Papaloapan/SEN 46-4-8	946	880	7.0	1.34
Papaloapan/SEN 46-2-6	903	886	1.9	1.29
Negro Citlali/RAV-187-3-14-7	1111	714	35.7	1.28
Papaloapan/SEN 46-1-10	957	811	15.3	1.25
Papaloapan/SEN 46-6-6	883	857	2.9	1.22
Papaloapan/SEN 46-7-8	971	774	20.3	1.21
Negro Grijalva*	946	774	18.2	1.18
Negro Comapa*	934	735	21.3	1.11
Negro Tacaná*	709	621	12.4	0.71
Average	846	732	13.5	1.0

* Cultivar checks. [&]REI = Plant productive efficiency index.

CONCLUSIONS

Papaloapan/SEN 46-4-10, Negro Citlali/RAV-187-3-1-8, Papaloapan/SEN46-3-2 and Jamapa Plus/RAV-3-1-2, showed the best adaptation to acid soil and drought field conditions of southern Veracruz, Mexico, these breeding lines responded well to application of dolomitic lime and showed the highest plant productive efficiency with and without application of lime.

REFERENCES

Graham, R. D. 1984. Breeding for nutritional characteristics in cereals. Adv. Plant Nutr. 1:57-102.

Tosquy, V. O. H., López, S. E., Zetina, L. R., Ugalde, A. F. J., Villar, S. B. y Cumpián, G. J. 2008. Selección de genotipos de frijol con adaptación a suelos ácidos. TERRA Latinoamericana 26(3):227-233.

Zetina, L. R., Pastrana, A. L., Romero, M. J. y Jiménez, Ch. J. A. 2002. Manejo de suelos ácidos para la región tropical húmeda de México. Libro Técnico Núm. 10. INIFAP. CIRGOC. Campos Experimentales Papaloapan y Huimanguillo. Veracruz, México. 170 p.

TOLERANCE TO ALUMINUM TOXICITY IN COMMON BEAN GENOTYPES

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INTRODUCTION

Approximately one third of areas cultivated with common bean in Brazil, more specifically in the states of Paraná, Minas Gerais, São Paulo, Goiás and Bahia, have high aluminum (Al) concentration and low fertility in soils (Giannakoula, 2008). In conditions of acidity and low fertility, the presence of soluble Al in the soil solution is one of the most limiting abiotic factors of crop development and yield. The Al exchangeable interferes in the absorption and movement of phosphorus, calcium and magnesium in the plant, contributing also to the adsorption of phosphorus in the soil (Echart and Cavalli-Molina, 2001). Considering the nature of Al stress, the hydroponic medium offers advantages in interaction studies of this element with plants, such as easy access to the root system, the possibility of monitoring and control pH, Al concentrations and other ions important to sensibility or tolerance reactions expression (Rossielo and Netto, 2006). Thus, the objective of this study was to evaluate common bean genotypes of the carioca and black commercial groups to reaction for the toxicity by aluminum.

MATERIAL AND METHODS

The experiment was carried out in the greenhouse at Agronomic Institute of Paraná (IAPAR), Londrina - Parana – Brazil, in December 2013 to February 2014. Two independent experiments were performed to evaluate the toxicity caused by aluminum, one for the carioca and another for the black commercial group, with ten genotypes each one. The experimental design was a completely randomized with three replicates, arranged in a factorial design with ten genotypes and two concentrations of Al, 0 ppm and 10 ppm, added as $AlCl_3$. The seedlings with uniform shoot and roots were transplanted into polyethylene vases with 3.35 L capacity containing nutrient solution Hoagland and Arnon (1950), modified by Pavan and Bingham (1982). The redox potential (pH) and electrical conductivity (EC) of the nutritive solution were monitored daily, the pH was maintained at 4.0 ± 0.1 by addition of HCl or NaOH 0.1M and the initial electrical conductivity (EC) was 0.35 ± 0.02 dS cm^{-2}. The solution was aerated continuously. After 25 days of growth in the nutrient solution, at the V4 development stage, the following characteristics were evaluated: Maximum root length (RL), plant height (PH), shoot dry matter (SDM) and root dry matter (RDM). It was also calculated the Reduction Index (RI) for each characteristic, using the expression RI (%) = $\{[(CSA - CCA) / CSA] \times 100\}$ (Molina et al, 2001), in which: CSA: characteristic at the concentration of 0 ppm of Al; CCA: characteristic evaluated at the concentration of 10 ppm of Al. Analysis of variance was performed using the Genes computational program (Cruz, 2006).

RESULTS AND DISCUSSION

For all characteristics evaluated in both commercial groups the genotype effect did not present statistical difference. There was a significant effect for aluminum factor in all evaluated characteristics (RL, PH, SDM and RDM) for both commercial groups studied. It was also observed a significant effect of interaction to genotype x [Al] for PH and SDM in the carioca group and for RL, SDM and RDM in the black group. The RI was positive for all the characteristics, proving that the presence of the toxic element Al in the concentration of 10 ppm

caused physiological changes in the plants, like reduction in RL, PH, SDM and RDM. The main effect of the aluminum toxicity observed was the inhibition of the root system of the plants, which can be visualized in the first days of cultivation in nutrient solution. The roots of the plants grown in solution containing Al did not develop normally, becoming short, thick and without the fine branches. Based on the root length and reduction index (Figure 1), in the commercial group carioca, the cultivar BRS Estilo and the breeding line LP08-187 can be classified as aluminum toxicity moderately tolerant, presenting a below-average reduction index and root development moderate to above of the average. In the black commercial group the breeding line LP08-71 was distinguished from the others genotypes being classified as aluminum toxicity moderately tolerant, with a root reduction index below the average and moderate root development.
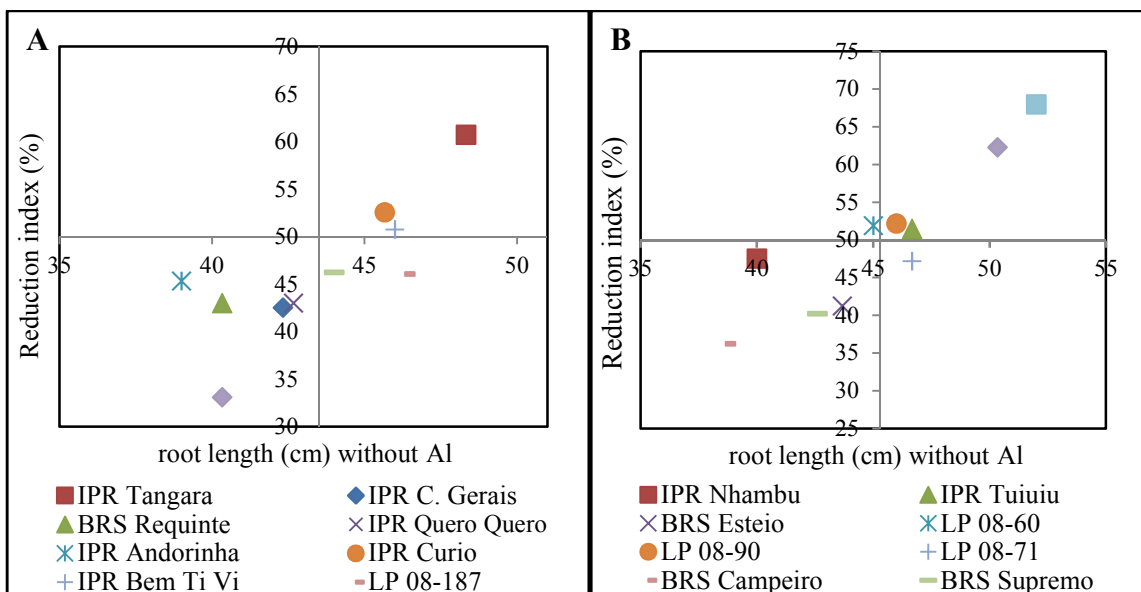
Figure 1 - Relationship between root length (cm) in condition without aluminum (0 ppm) and root length reduction index (%) when submitted to stress by Al (10 ppm) of ten common bean genotypes of the Carioca (A) and Black (B) commercial groups.

REFERENCES

CRUZ, C. D. Programa Genes: Biometria. Editora UFV. Viçosa (MG). 382p. 2006.

ECHART, C. L.; CAVALLI-MOLINA, S. Fitotoxicidade do alumínio: efeitos, mecanismo de tolerância e seu controle genético. Ciência Rural, Santa Maria, v. 31, n. 3, p. 531-541, 2001.

GIANNAKOULA, A. et al. Aluminum tolerance in maize is correlated with increased levels of mineral nutrients, carbohydrates and proline, and decreased levels of lipid peroxidation and Al accumulation. Journal of Plant Physiology, v.165, p.385-396, 2008.

HOAGLAND, D. R.; ARNON, D. I. The water culture method for growing plants without soil. California Agricultural Experimental Station, 1950. (Circular, 374).

PAVAN M. A.; BINGHAM F. T. Toxicity of aluminum to coffee seedlings grown in nutrient solution. Journal of Soil Science Society of America 46. 1982. 993-997.

MOLINA, J. C.; et al. Response of common bean cultivars and lines to water stress. Crop Breeding and Apllied Biotechnology, v.1, n.4, p.363-372, 2001.

ROSSIELLO ROP e JACOB NETTO J (2006) Toxidez por alumínio em plantas: novos enfoques para um velho problema. In: Fernandes MS (ed.) Nutrição mineral de plantas. Sociedade Brasileira de Ciências do Solo, Viçosa, p.376-418.

GROWTH OF COMMON BEAN AFFECTED BY SOIL CONTAMINATED WITH CHROMIUM VI

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INTRODUCTION: High levels of trace elements are characteristic of Brazilian soils. However, the presence of these trace elements may increase with incorrect application of agricultural inputs, industrial waste, agricultural chemicals, and mining waste. Many trace elements have known biological functions. Neglecting the permissible limits of these trace elements in the soil can lead to plant ecological and nutritional imbalances. Chromium (Cr VI) causes oxidative and mutagenic changes to plant cells, therefore, can be a highly toxic threat to both plants and the animals feeding on these plants. The harmful effects of Cr VI are greater than other forms of this element due to its mobility, capacity to penetrate plant tissue, oxidative and mutagenic capacity, and ability to inhibit soil biochemical processes. Even at low concentrations Cr VI can be toxic and cause inhibition of germination, limit root and shoot growth, and cause foliar chlorosis. When absorbed by plants, the accumulation of Cr VI is concentrated predominantly in the roots, with proportional translocation to the shoots. Due to its detrimental behavior in soil and ability to be absorbed by plants in the form of chromate, the investigative study of Cr VI is imperative. The effects of Cr VI on plants can be observed and investigated in the common bean.

This study aims to evaluate the emergence and initial development of *Phaseolus vulgaris* cv. BRSMG Madrepérola grown on soil contaminated with Cr VI.

MATERIALS AND METHODS: An experiment was carried out in a greenhouse of the Department of Soil Science of the Federal University of Lavras, Lavras, Minas Gerais, Brazil. A randomized experimental design was used with four replicates of six treatments of Cr VI (0, 75, 150, 300, 450 and 600 mg kg^{-1}). The recommended fertilizer was applied to each test and corrections were performed following the analysis of results.

Each test was composed of a 500 cm³ pot filled with soil samples taken from the 0-20 cm topsoil horizon of a Red-Yellow Latosol (Oxisol). The contaminate used was potassium dichromate ($K_2Cr_2O_7$). Ten seeds were sown into each pot following contaminate application. Potassium dichromate was applied to all treatments equally. It was applied as potassium chloride to ensure no nutritional differences.

The test was concluded 21 days after the emergence of 50% of the control plants. The variables evaluated were: emergence, number of leaves, shoot length, shoot dry weight, stem diameter, root length and root dry weight. The normality and homogeneity of the data were tested. The significance in the differences of the endpoints was tested by a one-way ANOVA. In the case of significant differences ($p \leq 0.05$), a Dunnett's test was performed to detect the difference between the treatments and the control (no observed effect concentration -NOEC). The EC_{50} (concentrations that reduce endpoints by 50% when compared with the control) were estimated through non-linear models. All analyses were conducted using STATISTICA version 7.

RESULTS AND DISCUSSION: All variables evaluated were affected by Cr VI (Figure 1). The emergence of plants (EC_{50}: 421 ± 38.6 mg kg^{-1}) and stem diameter (EC_{50}: 498 ± 37.6 mg kg^{-1}) were the least sensitive variables. Effects on emergence and stem diameter were observed at doses greater than 150 mg kg^{-1}, for all other variables the effects were observed at doses greater than 75

mg kg^{-1}. The most sensitive variable was biomass dry weight in which a reduction of 50% was observed in comparison to the control treatment. The EC$_{50}$ (114 ± 16.5 mg kg^{-1}) derived for shoot dry weight was about 3.7 times lower than the EC$_{50}$ for the emergence of plants. Effects on leaf number, root and shoot length were intermediate and had EC$_{50}$ ranging from 173 to 237 mg kg^{-1}. The presence of Cr VI damaged plant photosystems, nutrient uptake, chloroplasts and, therefore, effected plant photosynthesis and growth. There was inhibition of root formation from 150 mg Cr kg^{-1}, together with the other points discussed, this also contributed to the deleterious effect on plant growth. The results for LVAd cannot be applied to other soil classes as the toxicity of the treatment is related to availability of the element in soil solution. Thus, for the same treatment, soils with properties that provide greater available Cr VI tend to have more pronounced deleterious effects. Moreover, factors that affect Cr speciation also affect phytotoxicity. More studies are needed to verify the effect of Cr VI contamination on the development, production, and transfer to grains and to verify the location of this element in plants.

Figure 1. No observed effect concentrations (NOEC), and 50% (EC$_{50}$) effect concentration (with 95% confidence intervals in parentheses) for the toxicity of the chromium to *Phaseolus vulgaris* L. cv. BRSMG Madrepérola.
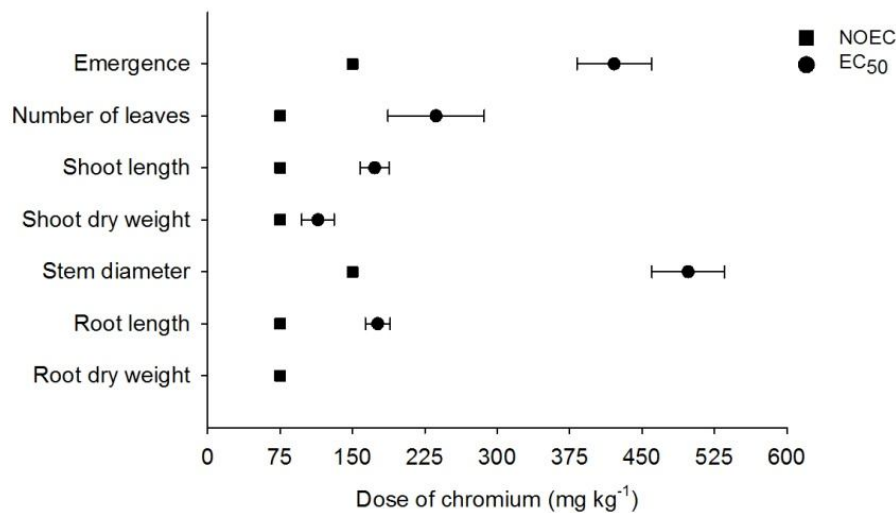
CONCLUSIONS: Harmful effects on the production of common bean biomass occur at lower levels than the observed effects for plant emergence. Among the evaluated variables, shoot dry weight was the most sensitive. Chromium levels greater than 75 mg kg^{-1} are harmful to bean crops. Contents greater than 114 mg kg^{-1} should be considered highly phytotoxic, because the biomass production is reduced by 50%.

MERCURY PHYTOTOXICITY IN COMMON BEANS IN SOILS OF MINAS GERAIS, BRAZIL

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INTRODUCTION: Problems caused by contaminated areas have been increasingly found around the world. Mercury (Hg) is a trace element potentially toxic to humans and the environment. Thus, monitoring Hg levels in the environment has great importance. The transfer of trace elements from soils to plants is a complex process, that depends on soil attributes such as pH, cation exchange capacity, texture, and mineralogy and also plant attributes, such as root system lenght and others. In Brazil, agricultural areas with levels of Hg higher than 12 mg kg^{-1} are considered contaminated, since such levels of Hg are likely to come from non-natural sources.

The State of Minas Gerais is the third largest producer of common beans in Brazil (2015/2016 farming year). The area planted with common beans is estimated in 339,000 hectares. The soil most used for such crop in the State of Minas Gerais is classified as Latosol (Oxisol).

The plants of *Phaseolus vulgaris* L. cv. BRSMG Madrepérola have indeterminate growth habit, low tolerance to lodging, and can be classified as an early-season variety. This variety has high yield potential and resistance to diseases, such as the common mosaic virose and anthracnose. *P. vulgaris* is one of the preferred plant species for using in ecotoxicological tests to evaluate the potential effect of toxic substances. The objective of this work was to evaluate the emergence and early development of common bean variety BRSMG Madrepérola under doses of mercury chloride (HgCl$_2$), in two Latosols (Oxisols).

MATERIALS AND METHODS: The experiment was conducted in a greenhouse of the Department of Soil Science at the Federal University of Lavras, Lavras, Minas Gerais, Brazil. The soils used were classified as dystroferric Red Latosol (LVdf) and dystrophic Red Yellow Latosol (LVAd), which were collected in the 0-20 cm soil layer. In order to create good conditions to plant growth, base saturation was increased to 60% and pH stabilized at 6. The common bean variety BRSMG Madrepérola was used. Pots were filled with 500 cm^3 of dry soil contaminated with HgCl$_2$ in the concentrations of 0; 2.5; 5.0; 10.0; 20.0; 40.0 and 80.0 mg kg^{-1}. Treatments were arranged in a completely randomized design with seven treatments and four replications. In each pot, 10 seeds were sown 24 hours after the HgCl$_2$ application. After the plant emergence, thinning reduced the number of plants per pot to 6. The experiment was conducted for 21 days, starting after 50% of emergence of the control pot.

At the end of the experimental period, shoot dry weight, emergence rate, emergence speed index (ESI), shoot length, leaf number, stem diameter, and SPAD index were evaluated. The normality and homogeneity of the data were tested. The significances of the differences in the endpoints were tested via a one-way ANOVA. In the case of significant differences (p≤0.05), a Dunnett's test was performed to detect the difference between the treatments and the control (no observed effect concentration - NOEC). The EC10 (concentrations that reduce endpoints by 10% when compared with the control) were estimated through non-linear models. All analyses were performed using STATISTICA, version 7.

RESULTS AND DISCUSSION: The doses of HgCl$_2$ did not caused differences in plant emergence, stem diameter, and SPAD index in both soils, when compared with the control plot (Table 1). For

ESI, significant differences were observed in relation to the control treatment from the dose of 20 mg kg^{-1} in both soils. For this endpoint, the EC10 found in the LVAd was 32.9 mg kg^{-1}. The Hg caused an emergence delay in *P. vulgaris* plants. Hg also inhibited *P. vulgaris* growth, with a NOEC of 40 mg kg^{-1} for both soils. However, the EC10 observed for plant height was 57.1 mg kg^{-1} for LVdf and greater than 80 mg kg^{-1} in the LVAd. The presence of trace elements in germination can affect water absorption and inhibit the solution absorption. This may affect the seedling development. The number of leaves was significant different only in the LVAd, with NOEC of 40.0 mg kg^{-1} and EC10 of 54.6 mg kg^{-1}. This variation in relation to soil type can be inferred to the different soil attributes, such as clay content and organic material. These different attributes cause variation in availability of Hg for the studied crop. The shoot dry weight was influenced by the concentration of Hg in the soil. For shoot dry weight, a reduction of 48% in the LVdf and 58% in the LVAd were observed for the highest concentration of Hg, when compared with the control. The reduction in growth can be attributed to the effect of Hg on the photosynthetic process and the damage caused by oxidative stress.

Table 1. No observed effect concentrations (NOEC), and 10% (EC$_{10}$) effect concentration (with 95% confidence intervals in parentheses) for the toxicity of Hg to *Phaseolus vulgaris* L. cv. BRSMG Madreperola growing in different soils.

Endpoint	LVdf		LVAd	
	NOEC	EC$_{10}$	NOEC	EC$_{10}$
	-- mg kg^{-1} --			
Emergence	> 80	> 80	> 80	> 80
Speed of emergence-index	20	Not validated	20	32.9 (20.6 – 45.1)
Shoot length	40	57.1 (43.3 - 71.0)	40	> 80
Stem diameter	> 80	Not validated	> 80	>80
Number of leaves	> 80	Not validated	40	54.6 (46.9 -62.3)
SPAD index	> 80	Not validated	> 80	> 80
Shoot dry weight	20	21.6 (11.0 - 32.1)	20	25.8 (13.6 - 38.0)

Mercury caused phytotoxic effects on *P. vulgaris* cv. BRSMG Madreperola leading plants to death and abnormal growth in concentrations above 40.0 mg kg^{-1} in the soil, especially in the LVAd. Chlorosis spots in leaves and necrosis of the plants were observed (Figure 1).

Figure 1. Phytotoxical effect of Hg (80 mg kg^{-1}) in plants of *Phaseolus vulgaris L.* cv. BRSMG Madreperola in two different Latosols (oxysols) dRL (A) and dRYL (B).
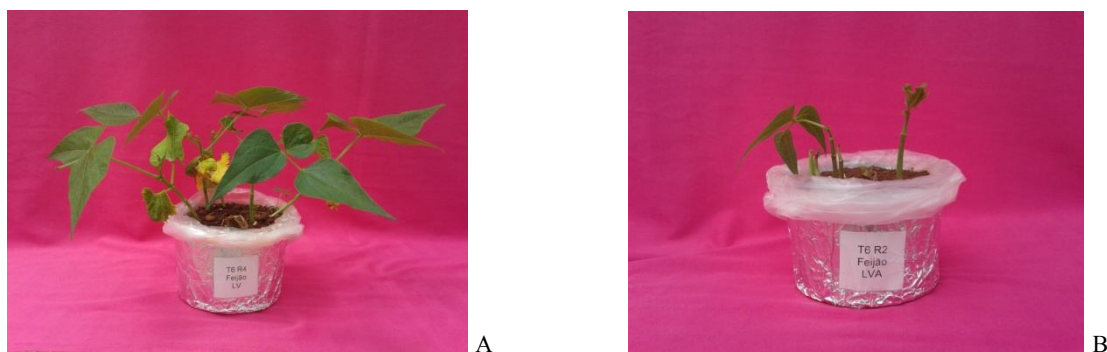
CONCLUSIONS: The presence of Hg in the soil caused reductions in ESI, plant height, number of leaves, and shoot dry mass. The biggest reductions in these variables were observed in the LVAd, which had more Hg availability for absorption by *Phaseolus vulgaris* L..

NICKEL APPLICATION RATES ON DRY BEAN CULTIVARS IAC FORMOSO AND BRS NOTÁVEL

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INTRODUCTION: Nickel (Ni) is required by crops since it is a structural component of the enzymes urease and hydrogenase, which act in assimilation of nitrogen (N) by plants. In spite of its importance, there is a lack of information in regard to use of Ni in fertilization programs for dry bean. Thus, the aim of this study was to define application rates of Ni through the soil adequate for dry bean growth.

MATERIALS AND METHODS: The experiment was carried out in a greenhouse of the Soil Science Department of the Universidade Federal de Lavras, Brazil, from December 2015 to March 2016. A randomized block statistical design was used, with three replications and a 5×2 factorial arrangement, involving five application rates of Ni (0.0, 0.25, 0.75, 1.5, and 3.0 mg dm^{-3}) and two cultivars of dry bean (IAC Formoso and BRS Notável). The pots contained 3 dm^3 of *Latossolo Vermelho* soil.

The source of Ni was hexahydrate nickel sulfate ($NiSO4.6H2O$), applied together with base fertilization according to Malavolta (1980). An inoculant with Rhizobium tropici at a rate of 10 g.kg of seed^{-1} was used as a source of N. The inoculant contained approximately 10^9 viable cells per gram. The cv. IAC Formoso was registered in 2010 and released on the Brazilian market in 2011. It has tolerance to lodging and resistance to bacterial blight and to bacterial wilt (Curtobacterium). The cv. BRS Notável was also released in Brazil in 2010 and is recommended for cultivation in twenty Brazilian states. Both have a semi-early cycle, type II upright growth habit, high yield potential, and resistance to anthracnose (CARBONELL et al., 2010; PEREIRA et al., 2012).

At full flowering, the following determinations were made: relative chlorophyll index (RCI), photosynthetic rate (PhR), transpiration rate (TransR), stomatal conductance (Cond), internal CO_2 concentration (ICO_2C) and internal and external CO_2 concentration ($IECO_2C$), and the activity of the enzyme urease, as well as plant height and shoot dry matter (SDM). All the data were subjected to analysis of variance. In the cases of significant effect of cultivars, comparison of means was performed by the F test ($P \leq 0.05$). The effects of Ni application rates were evaluated through regression analysis, selecting the equations through significance of the models.

RESULTS AND DISCUSSION: The highest RCI, PhR, Cond, and plant height were observed in cv. BRS Notável (Table 1), certainly resulting from variation in genetic material. Values similar to those of cv. IAC Formoso, however, were observed in the other variables analyzed. In relation to the effects of Ni, an increase in the application rate did not affect RCI, PhR, ICO_2C, $IECO_2C$, SDM, and plant height, the mean values of which were in the order of 32, 15 µmmol m^{-2} s^{-1}, 291 mol m^{-2} s^{-1}, 0.8 mol m^{-2} s^{-1}, 2.7 g plant^{-1}, and 76.0 cm, respectively. In regard to the physiological parameters TransR and Cond, as well as in regard to urease activity, an increase in the Ni application rate led to increases in the variables; rates greater than 2.0 (TransR – Fig. 1A and Cond – Fig. 1B) and 2.30 mg Ni dm^{-3} (urease activity – Fig. 1C), however, were harmful. The effect of greater application rates indicates reduction in stomatal opening, which reduced transpiration, but not the supply of CO_2 for photosynthesis. Even so, the initial rates appear to have met plant demands, not compromising the plants during the vegetative stage.

As Ni is the enzymatic activator of urease, its presence in the soil at low concentrations increased the activity of this enzyme. In contrast, high concentrations of this nutrient have a negative effect in degradation of urea into carbon dioxide and ammonia, as also reported by Sreekanth et al. (2013).

Table 1. Relative chlorophyll index (RCI), photosynthetic rate (PhR), transpiration rate (TransR), stomatal conductance (Cond), internal CO_2 concentration (ICO$_2$C) and internal + external CO_2 concentration (IECO$_2$C), urease enzyme activity, plant height, and shoot dry matter (SDM) of dry bean in the full flowering stage.

Cultivar	RCI	PhR	TransR	Cond	ICO$_2$C	IECO$_2$C	Urease Activity	Height	SDM
	-	(μmmol m^{-2} s^{-1})	(mmol m^{-2} s^{-1})	- (mol m^{-2} s^{-1}) -		(μmmol m^{-2} s^{-1})	(μmol N-NH$_4^+$ g massa fresca^{-1} h^{-1})	(cm)	(g planta^{-1})
IAC Formoso	30.7 B	14.4 B	5.0 A	0.5 B	290.8 A	0.82 A	14.5 A	68.8 B	2.83 A
BRS Notável	33.7 A	16.9 A	5.6 A	0.6 A	291.3 A	0.83 A	15.3 A	83.3 A	2.56 A
Mean	32.2	15.6	6.3	0.6	291.1	0.82	14.9	76.0	2.70

Mean values followed by the same uppercase letter in the column do not differ by the F test at the level of 5% probability.
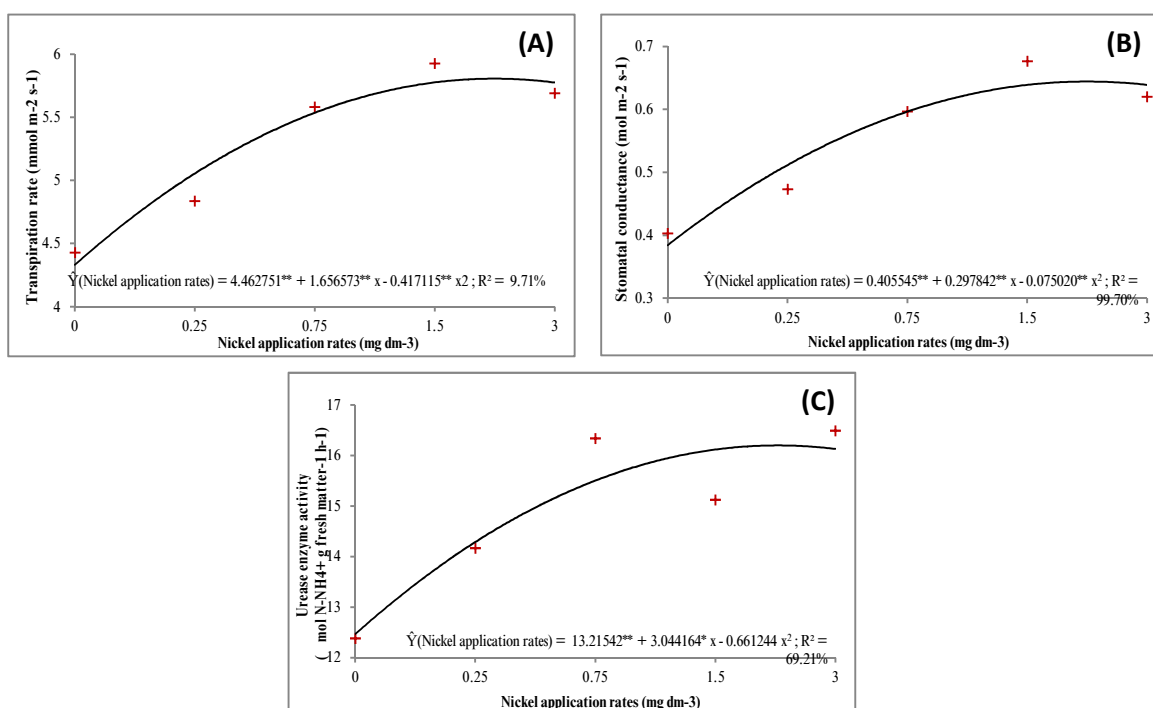
Figure 1. Transpiration rate (A), stomatal conductance (B), and urease enzyme activity (C) in dry bean (means of two cultivars, IAC Formoso and BRS Notável, two plants per cultivar) at full flowering (R6), as a function on nickel application rates on soil.

Considering these preliminary evaluations, new investigations should be carried out so as to establish the Ni application rates recommended for dry bean.

REFERENCES

CARBONELL, S. A. M et al. IAC Formoso: new carioca common bean cultivar. *Crop Breeding and Applied Biotechnology*, v. 10, p. 374-376, 2010.

MALAVOLTA, E. *Elementos de nutrição mineral de plantas.* São Paulo: Agronômica Ceres, 1980. 251p.

PEREIRA, H. S. et al . BRS Notável: a medium-early-maturing, disease-resistant Carioca common bean cultivar with high yield potential. *Crop Breeding Applied Biotechnology* v. 12, p. 220-223, 2012.

SREEKANTH, T. V. M. et al. Occurrence, physiological responses and toxicity of nickel in plants. *International Journal of Environmental Science and Technology*, v. 10, p. 1129-1140, 2013.

BIOMASS AND YIELD OF COMMON BEAN (*Phaseolus vulgaris* L.) AS A FUNCTION OF THE NITROGEN SOURCE

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INTRODUCTION

Beans in grain (*Phaseolus vulgaris* L.) are important for food due to its nutritional and medicinal properties. Thus, research that generates strategies to increase the yield is justified. Nitrogen fertilization has been determinant in increasing the yield of this legume (Escalante *et al*., 2015a). The farmers usually fertilizes with the nitrogen source available on the market, whether ammonium sulfate, ammonium nitrate, urea, etc. However, the results may be different depending on the source of nitrogen applied as reported Binoti *et al*. (2009). The aim of this study was to determine the effect of the nitrogen source on the biomass, yield and its components in beans.

MATERIALS AND METHODS

The planting of the dry bean cultivar "Michoacán 12-A-3" of black grain and indeterminate habit, was on 10 june 2014 in Montecillo, Texcoco, State of Mexico, México (19°29'N and 98°53'O and 2250 meters of altitude) of temperate climate (Cw, García 2004), under field conditions and rainfall in a clay loam, with 24 NO_3 me L^{-1} and pH of 7.6. The treatments consisted in the application of 100 kg de N ha^{-1}, as source: ammonium sulphate (AS, 20.5 % de N y 24% de S); ammonium nitrate (AN, 35% N) and ammonium sulphate   (50%) + ammonium nitrate (50%),(AS+AN). The sowing row distance was 0.80 m and between plants 0.30 m. The population density was 4.16 plants m $^{-2}$. The total biomass (TB, dry matter), grain yield (YG, grain weight), number of pods with grain (PN) and the number of racemes (RN) and the harvest index (HI) it was recorded. An analysis of variance and the Tukey multiple comparison test, were applied to the variables in study. Also the occurrence   of days to phenological phases (with the criteria presented in Escalante and Kohashi, 2015b), the seasonal mean of the maximum and minimum temperature, the rainfall and the evaporation of the tank type A.

RESULTS AND DISCUSSION

During the development of the crop, the mean maximum and minimum temperature was 24 and 8° C, respectively; rainfall of 380 mm and evaporation of 467 mm. No differences were observed as a result of treatments in the days to phenological phases. The emergence was at 8 days of sowing (das), beginning of flowering at 40 days and physiological maturity at 120 days. Table 1 shows that N sources caused significant changes in TB, GY, PN and RN, but not in HI. The highest production of TB, PN, RN and consequently in GY was achieved with ammonium sulfate and the combination AS + AN and was higher in 18%, 15%, 11% and 21% on average to fertilization with AN. This response may be related to the higher acidity of the AS, which could favor a greater availability of nutrients for the crop because it is initially an alkaline soil.

Table 1. Total biomass (TB, gm^{-2}), harvest index (HI, %), grain yield (GY, gm^{-2}), pod number (PN, m^{-2}) and racemes number (RN, m^{-2}) in dry beans cutivar Michoacán 12-A-3 in relation to nitrogen source. Montecillo, Texcoco Mexico. Mexico. 2014.

N SOURCE	TB gm^{-2}	HI (%)	GY gm^{-2}	PN m^{-2}	RN m^{-2}
AS+AN	222 a	64	141 a	153 a	126 a
AS	216 a	63	136 a	150 a	126 a
AN	186 b	62	114 b	132 b	113 b
Mean	208	63	131	145	122
Tukey 0.05	20	17	21	14	10

Columns with similar letter values are statistically the same (Tukey, 0.05).

CONCLUSION

Under crop development conditions, the source of nitrogen fertilizer affects biomass production, grain yield and its components, but not the harvest index. The ammonium sulfate application results in higher biomass production, grain yield and its components in the bush bean Michoacan 12-A-3 of indeterminate growth habit.

REFERENCES

Binotti, Flávio Ferreira da Silva, Arf, Orivaldo, Sá, Marco Eustáquio de, Buzetti, Salatier, Alvarez, Angela Cristina Camarim, & Kamimura, Karina Marie. 2009. Fontes, doses e modo de aplicação de nitrogênio em feijoeiro no sistema plantio direto. *Bragantia*, *68*(2), 473-481. https://dx.doi.org/10.1590/S0006-87052009000200022.

Escalante-Estrada, José A.; Rodríguez-González, María T.; Escalante-Estrada, Yolanda I. 2015a. Nitrógeno, distancia entre surcos, rendimiento y productividad del agua en dos cultivares de frijol. Bioagro 27 (2):75-82. Disponible en:<http://www.redalyc.org/articulo.oa?id=85741585003> ISSN 1316-3361.

Escalante E. J. Alberto y J. Kohashi Shibata.2015b. El Rendimiento y Crecimiento del Frijol. Manual para toma de datos. Centro de Botánica. Colegio de Postgraduados. Montecillo, Edo. De México. 84 páginas.

García, E. 2004. Modificación al sistema de clasificación climática de Köppen para adaptarlo a las condiciones de la República Mexicana. Cuarta edición. UNAM. México, D.F. 217 p.

PHOSPHORUS CONTENT IN SEEDS OF COMMON BEAN GROWN IN DIFFERENT PHOSPHORUS LEVELS

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INTRODUCTION

Seed phosphorus (P) is a fundamental energy source that acts on germination and seedling development. In addition to the inorganic P (Pi), the seed also accumulates as phytate salts and other forms of phosphate compounds denominated organic P (Po). Although necessary for the seed development, an excess of Po may interfere with the accumulation of calcium, iron and zinc nutrients, which are easily complexed with P and become unavailable for human diet and animal feed (RABOY, 2009). The availability of P in the soil or nutrient solution may influence the storage forms of this mineral in the seed. Therefore, the study aimed to evaluate the partition of the absorbed P in common bean genotypes grown in different levels of P in hydroponic solution.

MATERIAL AND METHODS

The experiment was carried out in the greenhouse at Agronomic Institute of Paraná (IAPAR), Londrina, Paraná, Brazil, in September to December 2015. The experimental design was a randomized block with six replicates and treatments arranged in a factorial design with two genotypes (BRS Estilo and IPR Tangará) and five P levels (2, 4, 6, 8 e 10 mg L^{-1}). The seedlings with uniform shoot and roots were transplanted into polyethylene pots with capacity 3.35 L containing nutrient solution Hoagland and Arnon (1950), modified by Pavan and Bingham (1982). The redox potential (pH) and electrical conductivity (EC) of the nutritive solution were monitored daily. The solution was aerated continuously and when the electric conductivity reached 0.14 dS cm^{-2} the nutrient solution was exchanged. To determine total P content the seeds were ground in Perten 3100 mill and submitted to nitroperchloric digestion according to the methodology of Miyazawa et al. (1999). Pi content was extracted according to the method proposed by Raboy and Dickson (1984) and it was quantified by the method described by Chen et al. (1956). Po content was obtained by subtracting the Pi from the total P. The phytate content was determined according to the methodology of Oomah et al. (2008). The data have been subjected to analysis of variance and test Tukey ($p \leq 0.05$) for mean comparison. The statistical analyzes were performed using the Sisvar version 5.3 software (FERREIRA, 2010).

RESULTS AND DISCUSSION

Among the genotypes, no significant differences were observed in the total P, Pi, Po and phytate contents at the different P levels. In the seeds of BRS Estilo and IPR Tangará, the contents of total P, Pi, Po increased as there was an increase in the P concentrations of the nutrient solution (Table 1). Po values present in genotypes accounted for about 95% of total P seeds and these values ranged from 4.29 to 7.27 g Kg^{-1} in BRS Estilo and 4.56 to 7.04 g Kg^{-1} in IPR Tangará (Table 1). The BRS Estilo and IPR Tangará genotypes showed low Pi content in relation to the Po content. The mean values of Pi for BRS Estilo ranged from 0.28 to 0.38 g Kg^{-1} and from 0.25 to 0.35 g Kg^{-1} for IPR Tangará (Table 1). In the present study, the phytate content in the genotypes showed significant differences in the different P levels. Phytate contents in BRS Estilo and IPR Tangará ranged from 11.85 to 15.11 mg g^{-1} and from 12.53 to 15.27 mg g^{-1}. These

values were higher than observed by Vasić et al. (2012) in different common bean genotypes whose values ranged from 5.34 to 10.47 mg g^{-1}. The increase of P concentration in the nutrient solution caused a significant linear increase in the content of Po in the seed of BRS Estilo (R^2 = 0.76) and IPR Tangará (R^2 = 0.67). However, variations in phytate content did not increased linearly with P levels (R^2 = 0.23 and R^2 = 0.35 for BRS Estilo and IPR Tangará cultivars). The increase in the level of P in the nutrient solution increased the total P and Po content in the seed without, however, changing the phytate values in the seed, maintaining the availability of other nutrients for the human diet and animal feed.

Table 1. Total P, Pi, Po and phytate content in common bean grown in hydroponic condition with different phosphorus concentrations. Londrina - PR, Brazil, 2015.

P* (mg L^{-1})	Total P (g Kg^{-1})		Pi (g Kg^{-1})		Po (g Kg^{-1})		Phytate (mg g^{-1})	
	BRS Estilo	IPR Tangará	BRS Estilo	IPR Tangará	BRS Estilo	IPR Tangará	BRS Estilo	IPR Tangará
2	4.57aA	4.81aA	0.28aA	0.25aA	4.29aA	4.56aA	11.85aA	12.53aA
4	5.68aA	6.52bA	0.35abA	0.28abA	5.33aA	6.24bA	14.72bA	14.67abA
6	7.62bA	7.30bA	0.35abA	0.31abA	7.27bA	6.98bA	15.11bA	15.27bA
8	7.45bA	6.85bA	0.38bA	0.33abA	7.07bA	6.52bA	13.39abA	14.54abA
10	7.42bA	7.39bA	0.38bA	0.35bA	7.04bA	7.04bA	14.53bA	14.54abA
CV(%)	13.00		18.02		13.77		9.19	

* Phosphorus level (mg L^{-1}) in the nutrient solution.
Means followed by the same letter, lower case letter in the column and capital letter in the line, are not significantly different by Tukey at 5% probability.

REFERENCES

CHEN, P. S. et al.,1956. Microdetermination of phosphorus. Anal. Chem, 28:1756-1758.

FERREIRA. D.F. SISVAR - Sistema de análise de variância. Versão 5.3. Lavras G: UFLA. 2010.

HOAGLAND, D. R.; ARNON, D. I. 1950. The water culture method for growing plants without soil. California Agricultural Experimental Station, (Circular, 374).

MIYAZAWA, M., et al., 1999. Análise química de tecido vegetal. In: SILVA, F. C. (Org). Manual de análises químicas de solos, plantas e fertilizantes. Brasília: Embrapa, p. 171-223.

OOMAH, D.Bet al.,2008. Phytic acid, phytase, minerals, and antioxidant activity in Canadian dry bean (*Phaseolus vulgaris* L.) cultivars. J. Agric. Food Chem. 56:11312-11319.

PAVAN M. A.; BINGHAM F. T. 1982. Toxicity of aluminum to coffee seedlings grown in nutrient solution. Journal of Soil Science Society of America 46. 993-997.

RABOY, V. 2009. Approaches and challenges to engineering seed phytate and total phosphorus. Plant Scie. 177:28-296.

RABOY, V., DICKINSON, D.B. 1984. Effect of phosphorus and zinc nutrition on soybean seed phytic acid and zinc. Plant Physiol. 75:1094-1098.

VASIC, M.A., TEPIC, A.N., MIHAILOVIC, V.M., MIKIC, A.M., GVOZDANOVIC-VARGA, J.M., SUMIC, Z.M., TODOROVIC, V.J. 2012. Phytic acid content in different dry bean and faba bean landraces and cultivars. Seeds. 29:79-85.

NON-PREFERENCE FOR FEEDING OF *Heliothis virescens* BY BEAN GENOTYPES

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INTRODUCTION

The species *Heliothis virescens* (Fabricius, 1781) (Lepidoptera: Noctuidae), is considered an important pest of various crops, including the common bean, *Phaseolus vulgaris* Linnaeus, in which it can feed from all plants structures (Moreira, 2009). For its management the most used tactic is chemical control, however, if used improperly can cause risks to the environment and human health.

A safer alternative is the utilization of resistant cultivars, which can reduce populations of pest insects below the levels of economic damage, production costs and risks of imbalances in the agro-ecosystem, beyond the possibility of harmonious use with other control tactics (Boiça Júnior et al., 2013). Therewith, the aim of this work it was evaluate the non-preference for feeding of first instar caterpillars of *H. virescens* by different bean genotypes.

MATERIAL AND MÉTHODS

We conducted the experiment in UNESP/FCAV, in the Laboratório de Resistência de Plantas a Insetos, Jaboticabal, SP, Brazil, under controlled conditions of temperature ($25 \pm 1\ ^{o}C$), relative humidity ($60 \pm 10\%$) and phtofase (12 hours). We used the completely randomized design, with 10 treatments composed by genotypes Pérola, Raz 49, BRS Supremo, IAC Carioca Tybatã, IAC Galante, IAC Diplomata, IAC Harmonia, IAPAR 81, IAC Una, IAC Carioca Eté, with ten replicate in free-choice and no-choice test.

In the first, were utilized arena constituted of glass circular trays (23 cm of diameter), where were distributed equidistant from the center, leaf discs of the respective genotypes. In the no-choice test the leaf discs were individualized in Petri dish (nine centimeters of diameter). In both tests were released, five caterpillars of instar first by genotypes and arenas and Petri dishes utilized, were papered with filter paper moistened with deionized water. The leaf disc were obtained with aid of a punch of $4.91\ cm^{2}$.

We evaluated the average number of caterpillars on leaf discs to 1, 3, 5, 10, 15 and 30 minutes and 1, 2, 6, 12, 24, 36 and 48 hours. At the end of both experiments, the leaf discs were submitted a visual evaluation of injury, in which three evaluators attributed note of 0 a 100. For data analysis, we calculated the average of present caterpillars number in the genotypes in the evaluations of 1 to 30 minutes, 1 to 6 hours, 12 to 24 hours and 36 to 48 hours and gave a note (0-100%) for the injuries caused by the caterpillars. We submitted the data to Levene ($\alpha=0.05$) and Cramer-von Mises ($\alpha=0.05$) test, when homogeneous and normal the data were submitted to ANOVA test and the averages compared by Tukey test ($\alpha=0.05$). If not, the data were analyzed by nonparametric test of Kruskal-Wallis and the averages compared by Dunns test ($\alpha=0.05$).

RESULTS AND DISCUSSION

The caterpillars number of *H. virescens* present in the leaf discs of genotypes, in the free-choice test, differed significantly in the evaluations means of 36 a 48 hours, being lower in the genotype IAC Harmonia compared with IAC Carioca Tybatã. The injuries caused by caterpillars were significantly lower in the IAC Harmonia in comparison with other genotypes (Table 1).

In no-choice test the genotype IAC Carioca Tybatã was significantly less injured that the BRS Supremo, IAC Diplomata e IAC Carioca Eté (Table 1). We emphasize that the genotypes IAC Harmonia e IAC Carioca Tybatã, can own one or more allelochemicals which confer this kind resistance. We conclude that these genotypes were less preferred for feeding, being still necessary more studies to investigate the possible causes.

Table 1. Average (± standard error) of caterpillars number of *Heliothis virescens* presents in the leaf discs of bean genotypes in the assessments at different time and the injury notes (%), in free-choice and no-choice tests.

Genotypes	Minutes	Hours			Injury scores (%)
	10 to 30	1 to 6	12 to 24	36 to 48	
Free-choice					
Pérola	1.4 ± 0.25	0.6 ± 0.19	2.4 ± 0.65	1.0 ± 0.22 ab	11.8 ± 6.28 a
RAZ 49	1.4 ± 0.25	0.7 ± 0.15	2.1 ± 0.47	1.2 ± 0.28 ab	9.5 ± 2.16 a
BRS Supremo	1.4 ± 0.34	0.5 ± 0.14	2.2 ± 0.46	0.4 ± 0.13 ab	9.6 ± 2.41 a
IAC Carioca Tybatã	0.6 ± 0.19	0.4 ± 0.16	2.6 ± 0.68	1.5 ± 0.45 a	21.9 ± 8.30 a
IAC Galante	1.1 ± 0.25	0.4 ± 0.14	1.4 ± 0.36	1.1 ± 0.28 ab	11.0 ± 3.48 a
IAC Diplomata	1.4 ± 0.49	0.7 ± 0.24	2.5 ± 0.68	0.8 ± 0.08 ab	10.3 ± 2.84 a
IAC Harmonia	1.5 ± 0.28	0.6 ± 0.14	0.6 ± 0.17	0.1 ± 0.06 b	1.0 ± 0.40 b
IAPAR 81	1.4 ± 0.21	0.8 ± 0.22	2.3 ± 0.44	0.8 ± 0.32 ab	10.3 ± 2.96 a
IAC Una	1.5 ± 0.30	0.8 ± 0.17	1.5 ± 0.25	0.8 ± 0.17 ab	6.8 ± 1.34 a
IAC Carioca Eté	2.0 ± 0.24	0.9 ± 0.21	2.1 ± 0.27	1.2 ± 0.31 ab	9.9 ± 2.65 a
F (Genotypes)	-	0.85^{ns}	-	-	3.16**
H (Genotypes)	12.67^{NS}	-	16.42^{NS}	22.06**	-
No-choice					
Pérola	1.6 ± 0.25	2.0 ± 0.18	3.4 ± 0.40	2.4 ± 0.35	9.1 ± 2.56 ab
RAZ 49	2.4 ± 0.44	2.9 ± 0.47	3.7 ± 0.28	3.7 ± 0.28	13.5 ± 2.70 ab
BRS Supremo	1.7 ± 0.26	2.0 ± 0.28	3.8 ± 0.31	3.0 ± 0.20	27.3 ± 6.17 a
IAC Carioca Tybatã	2.0 ± 0.38	2.5 ± 0.28	3.5 ± 0.37	3.2 ± 0.33	3.5 ± 1.38 b
IAC Galante	2.2 ± 0.24	2.3 ± 0.32	3.7 ± 0.27	3.3 ± 0.19	16.8 ± 6.40 ab
IAC Diplomata	2.1 ± 0.42	2.7 ± 0.25	4.0 ± 0.17	3.3 ± 0.17	28.2 ± 7.97 a
IAC Harmonia	1.9 ± 0.24	2.3 ± 0.26	3.8 ± 0.17	3.4 ± 0.27	7.6 ± 1.71 ab
IAPAR 81	2.1 ± 0.36	2.9 ± 0.20	4.5 ± 0.17	3.2 ± 0.29	28.9 ± 4.70 a
IAC Una	1.7 ± 0.30	2.7 ± 0.21	4.2 ± 0.20	3.6 ± 0.16	10.3 ± 0.90 ab
IAC Carioca Eté	1.2 ± 0.47	2.5 ± 0.37	3.7 ± 0.33	3.5 ± 0.37	28.5 ± 8.22 a
F (Genotypes)	1.09^{ns}	2.12^{ns}	1.38^{ns}	1.76^{ns}	4.56**

Means followed by different letters in the column, differ significantly by Tukey test at 5% of probability. [NS]Not significant by Kruskal-Wallis test at 5% probability. [ns]Not significant by ANOVA test at 5% probability. **Significant by ANOVA test at 1% probability.

REFERENCES

Boiça Júnior, A.L.; Souza, B.H.S.; Lopes, G.S.; Costa, E.N. Moraes, R.F.O.; Eduardo, W.I. 2013. Atualidades em Resistência de Plantas a insetos In: Busoli, A.C.; Alencar de, J.R.C.C.; Fraga, D.F.; Souza, L.A.; Souza, B.H.S.; Grigolli, J.F.J. Tópicos em Entomologia Agrícola – VI. Jaboticabal: Gráfica e editora Multipress, p. 207-224.

Moreira, M.D. 2009. Biologia de *Heliothis virescens* (Lepidoptera: Noctuidae) em dietas artificial e natural. *Revista Brasileira de Agroecologia*, 4: 1716-1719.

RESISTANCE OF DIFFERENT BEAN GENOTYPES (*Phaseolus vulgaris* L.) TO *Aphis craccivora* Koch, 1854

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INTRODUCTION

The black aphid (*Aphis craccivora* Koch, 1854, Hemiptera: Aphididae) is an important pest to the species of beans *Phaseolus vulgaris L*. and *Vigna unguiculata* (L.) Walp., due to its ability to cause damage by sucking of sap and the virus transmission as Cowpea aphid borne mosaic virus (CABMV), Blackeye cowpea mosaic virus (BICMV) and Bean common mosaic virus (BCMV) which lead to losses in production. The main control method has been chemical insecticides spraying, which often cause imbalance to the agroecosystem and can cause resistance problems. Thus it is desirable to look for more sustainable controlling methods, following the example of the use of cultivars that show some type of resistance. The study aimed to evaluate possible resistance of different genotypes of common beans *P. vulgaris* to the *A. craccivora*.

MATERIAL AND METHODS

The experiment was carried out in the period from May to July 2016 in a greenhouse at the Agronomic Institute of Paraná (IAPAR), Londrina, Paraná, Brazil. The air temperature and relative humidity were 25 ± 4ºC and $62 \pm 10\%$, respectively. The genotypes of *P. vulgaris* evaluated were: Raz 49, IAPAR 81, IAC Alvorada, Negão, IPR Celeiro, IPR Tangará, MD 1133, IPR Curió and IPR Maracanã. The experimental design used was randomized blocks, with nine treatments (genotypes) and eight repetitions. Each plot consisted of a pot with two plants. The genotypes were sown in pots of 5L with a mixture of clay soil, sand and manure in the ratio of 2:1:1, respectively, plus 20 g fertilizer 4-30-10 (N, P_2O_5, K_2O) per pot. The aphids (*A. craccivora*), used in the study were obtained from IAPAR collection. When the genotypes were between flowering and early fruiting, the infestation was performed. On the leaf's abaxial surface of each plant four adult females were placed with the aid of a small cage. After seven days of the infestation, the leaf were detached from the plant and taken to the laboratory for the accounting of females and nymphs, verifying the aphids establishment success and the colonies development. The variable evaluated were: adult females number alive after seven days of infestation; viability (%), which corresponds to the percentage of females alive; total abundance, which corresponds to the sum of females alive plus the nymphs; adult females fecundity, obtained by dividing the number of nymphs by adult females. The data complied with the assumptions for parametric analysis and were submitted to analysis of variance and Scott-Knott test at 5% significance level.

RESULTS AND DISCUSSION

It was not possible to verify differences among the genotypes in terms of the number of females alive after seven days of infestation (Table 1). The genotypes Raz 49, IAPAR 81, IAC Alvorada, IPR Celeiro and IPR Maracanã had lower number of aphids by leaflets differing from other genotypes. The smallest amount of aphids in the genotypes aforementioned occurred due to lower females fertility, with the exception of genotype Raz 49, which is related to a lower number of females which survived during the period after inoculation, and resulted in a lower

number of nymphs (Table 1). Studies indicate different levels of resistance to *A. craccivora* for genotypes of *Vicia faba* L.and *V. unguiculata* (Laamari et al., 2008; Moraes and bleicher, 2007), interfering in the aphids colonies development. This interference can be related to the trichomes presence (Johnson, 1953) and plant height (Soffan and Aldawood, 2014). Moreover, the presence of tannins in groundnuts leaves reduces the fecundity of *A. craccivora* (Grayer et al., 1992), being the resistance occurrence by antibiosis checked for the cultivars of *V. unguiculata* (Obopile and Ositile, 2010). Future studies evaluating the anatomical and biochemical characteristics of the cultivars evaluated in this study may elucidate the resistance mechanism involved.

Table 1. Average number (N) of adult females, viability, total abundance and fecundity after seven days of infestation with *Aphis craccivora* in nine genotypes of common beans (*Phaseolus vulgaris L.*). Londrina, Paraná, Brazil, 2016.

Genotypes	Adult females		Total abundance (nymphs + females)	Fecundity (nymphs/ females)
	N	Viability (%)		
Raz 49	2.1*	53.1	51.9 b[**]	23.4 a
IAPAR 81	2.6	64.1	39.9 b	15.2 b
IAC Alvorada	1.9	46.9	27.8 b	15.9 b
Negão	2.8	68.8	71.3 a	24.7 a
IPR Celeiro	2.4	60.9	45.4 b	18.4 b
IPR Tangará	2.5	62.2	60.0 a	26.7 a
MD 1133	2.6	65.6	68.6 a	28.6 a
IPR Curió	2.6	65.6	60.9 a	22.6 a
IPR Maracanã	2.6	65.6	49.6 b	17.7 b
p-value	0.20	-	< 0.01	0.03
CV(%)	26.9	-	39.9	41.7

CV: Coefficient of Variation
* average values of 16 repetitions.
** Means followed by the same letter do not differ among themselves according to Scott-Knott test at 5% probability.

REFERENCES

Grayer RJ et al. (1992). Condensed tannin levels and resistance of groundnuts (*Arachis hypogeae*) against *Aphis craccivora*. Phytochemistry, 31 (11): 3795-3800.

Johnson B (1953). The injurious effects of the hooked epidermal hairs of french beans (*Phaseolus vulgaris*) on *Aphis craccivora*. Bulletin of Entomological Research, 44: 779-788.

Laamari M et al. (2008). Resistance source to cowpea aphid (*Aphis craccivora*) in broad bean (*Vicia faba*) Algerian landrace collection. African Journal of Biotechnology, 7 (14): 2486-2490.

Moraes JGL & Bleicher E (2007). Preferência do pulgão-preto, *Aphis craccivora*, a diferentes genótipos de feijão-de-corda, *Vigna unguiculata*. Ciência Rural, 37 (6): 1554-1557.

Obopile M & Ositile B (2010). Life table and population parameters of cowpea aphid, *Aphis craccivora* (Homoptera: Aphididae) on five cowpea *Vigna unguiculata* varieties. Journal of Pest Science. 83(1): 9-14.

Soffan A & Aldawood AS (2014). Biology and demographic growth parameters of cowpea aphid (*Aphis craccivora*) on faba bean (*Vicia faba*) cultivars. Journal of Insect Science, 14(120): 1-10.

RESISTANCE OF DIFFERENT COMMON BEAN GENOTYPES (*Phaseolus vulgaris* L.) TO WHITEFLY (*Bemisia tabaci* GENNADIUS, 1889) B BIOTYPE (HEMIPTERA: ALEYRODIDAE)

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INTRODUCTION

The whitefly (*Bemisia tabaci* Gennadius, 1889) B biotype is one of the most harmful pests that attack common bean crops, mainly for extracting large quantities of sap, excreting honeydew that causes sooty mould, and transmitting the bean golden mosaic vírus (BGMV) (Yuki et al., 1998). The disease is the largest constraint to bean production in Latin America and causes significant yield losses (40 to 100%) in South and Central America (Morales, 2006). In the hottest regions of Paraná State (Brazil), in the dry season (sowing from January to April), the BGMV incidence has reached 80 to 100% of plants, with production losses of 30% to 100% (Bianchini et al., 1989). The keeping insect populations below economic threshold levels can be reached with use of resistant cultivars, reducing the need of insecticides (Kavitha and Reddy, 2012). This is an important component of Integrated Pest Management and it is considered as non-monetary input at common bean farmers. This work aimed evaluate the resistance of common bean genotypes to *B. tabaci* B biotype.

MATERIAL AND METHODS

The experiment was carried out in the period from July to August 2016 in a climatized room (temperature of 25 ± 2ºC, relative air humidity of 70 ± 10%, 14h of photophase) at the Agronomic Institute of Paraná (IAPAR), Londrina, Paraná, Brazil. Tubes of 275 ml capacity were filled with commercial substrate (Plantmax®), plus fertilization with the formulation 4-30-10 (N, P_2O5, K_2O) and osmocot (commercial fertilizer). The dose used for both fertilizers was 0.35 g/tube. After that were sow the following common bean genotypes: Arc 1, Raz 49, IAPAR 81, IAC Alvorada, MD Negão, IPR Celeiro, IPR Tangará and IPR Curió. The experimental design was a randomized block with eight treatments (genotypes) and five replicates. Each plot consisted of a tube with a plant. The insects (*B. tabaci*, B biotype) used in the study were obtained from IAPAR collection. Eighteen days after sowing (the genotypes presented phenological stage V3), 40 adults were released on the first trifolium of each plant. These insects were confined within a small cage attached to the leaf surface for a period of 24 hours. This procedure was performed for oviposition on foliar suface. Ten eggs were marked for each infested trifolium, which were monitored daily until the emergence of adults. Mortality and development period (from egg to adult) of insects was recorded. Data were submitted to variance analysis and Scott-Knott test at 5% probability. When the data did not meet the assumptions for parametric analysis, a Friedman analysis (α = 5%) was performed.

RESULTS AND DISCUSSION

The highest percentages of mortality of whitefly nymphs were observed in Arc 1 (44%) and IAC Alvorada (46%), differing from the other genotypes. The highest percentages of mortalities occur in the first and second instars for the genotype Arc 1, and in the third instar for the IAC Alvorada genotype (Table 1). There were no significant differences in the insect

development period among the different genotypes. In this study, this period ranged from 21.5 to 22.2 days (Table 1).

Table 1 - Mortality percentage during nymphal phase and development period from egg to adult in *Bemisia tabaci* B biotype avaluated in differents common bean genotypes under laboratory conditions. Temperature of 25 ± 2ºC, relative air humidity of 70 ± 10%, 14h of photophase. Londrina, Paraná, Brazil, 2016.

Genotypes	Percentage of mortality					Time (days)
	1º instar	2º instar	3º instar	4º instar	Total	Egg-adult
Arc 1	18*	18	4	4	44 a**	22,2 a***
Raz 49	0	0	9	3	12 b	21,6 a
IAPAR 81	0	8	10	4	22 b	22,1 a
IAC Alvorada	10	10	16	10	46 a	22,2 a
MD Negão	2	4	20	2	28 b	21,6 a
IPR Celeiro	2	6	8	0	16 b	21,6 a
IPR Tangará	6	2	2	6	16 b	21,9 a
IPR Curió	4	6	0	0	10 b	21,5 a
CV (%)	-	-	-	-	57,2	2,8
p-value	-	-	-	-	0,03	0,22

CV: Coefficient of variation;
 * Mean values: n = 5 for percentage of mortality and n between 22 to 45 for elapsed time from egg to adult.
** Averages followed by the same letter in the column do not differ differ among themselves. Scott-Knott test at 5% probability.
*** Averages followed by the same letter in the column do not differ among themselves. Friedman's test (α = 5%).

Common bean genotypes evaluated showed different levels of resistance to whitefly. These results suggest that the genotypes Arc 1 and IAC Alvorada present some type of resistance (mechanical and /or antibiosis). This resistance is not related to the duration of the egg to adult period, but to the mortality of the whitefly nymphal stage. Torres et al., (2012) also did not verify difference in the whitefly duration life cycle among the IAC Alvorada genotype and the other evaluated genotypes. In relation to the mortality of nymphs, other studies verified resistance of IAC Alvorada and Arc 1 genotypes (Torres et al., 2012; Oriani et al., 2008) with nymphal mortality reaching 96.7% in the cultivar IAC Alvorada and 78% In the Arc 1 genotype. The authors attributed to antibiosis the mortality presented in these genotypes. Future studies evaluating the anatomical and biochemical characteristics of these cultivars can elucidate the mechanism of resistance involved.

REFERENCES
Yuki, V.A. et al. Transmissão experimental do vírus do mosaico dourado do feijoeiro por *Bemisia argentifolia* Bellows & Perring. Anais da Sociedade Entomológica do Brasil, Londrina, v.27, n.4, p.675-678, 1998.
Bianchini, A. et al. Doenças do Feijoeiro. In: O Feijão no Paraná. (Circular nº 63) Londrina: IAPAR, p.199-216, 1989.
Kavitha, K.; Reddy, K. D. Screening Techniques for Different Insect Pests in Crop Plants. International Journal of Bio-resource and Stress Management, v.3, n.2, p.188-195, 2012
Morales, F. J. History and current distribution of begomoviruses in Latin America. Adv. Virus Res. v.67, p.127-162, 2006.
Torres, L. C. et al. Resistência de genótipos de feijoeiro a *Bemisia tabaci* biótipo B. Bragantia, Campinas, v.71, n.3, p.346-354, 2012.
Oriani, M. A. G. et al. Aspectos Biológicos de *Bemisia tabaci* (Genn.) Biótipo B (Hemiptera: Aleyrodidae) em Seis Genótipos de Feijoeiro. Neotropical Entomology, v.37, n.2, p.191-195, 2008.

STRATEGIES FOR WHITEFLY MANAGEMENT ON COMMON BEANS USING NEEM OIL AND INSETICIDE, UNDER GREENHOUSE CONDITIONS

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INTRODUCTION: Infestations of whitefly *Bemisia tabaci* (Gennadius) biotype B on common beans are managed mainly by the use of insecticides. This is owing to the fact that insecticides are highly available on the market, are easy to be applied, and cause rapid insecticidal effect (Boiça Júnior et al., 2006). However, conventional management practices relying on insecticides are sometimes not effective for this pests usually prefer the abaxial leaf surface of plants, in addition to rapid insect resistance development to the active ingredients (Horowitz and Ishaaya, 1995).

The use of natural products alone or integrated with insecticides can be an alternative to reduce the negative effects of chemical control, without losing efficiency in managing the pest. Products based on neem, *Azadirachta indica* A. Juss, are reported to cause repellency, reductions in feeding and oviposition, interruptions of development and ecdysis, lengthening of biological development, reduction in fertility, and other changes in insect behavior (Martinez, 2011). We evaluated strategies based on applications of neem oil, insecticide, and neem oil-insecticide combinations for managing *B. tabaci* biotype B on common beans, under greenhouse conditions.

MATERIAL AND METHODS: Experiment was carried out in a greenhouse (10 x 5 x 3 m) coated with anti-aphid screen mesh, in the School of Agriculture and Veterinarian Sciences, UNESP, in Jaboticabal, SP, Brazil. Seeds of cultivar Pérola were sown in 40 pots (5 L) filled with soil, sand, and manure at 2:1:1 ratio. The pots were kept in the greenhouse arranged in five rows spaced 1 m apart. For whitefly artificial infestation, 16 potted kale plants (cv. Manteiga) infested with whitefly populations were introduced in the greenhouse and evenly distributed among the common bean potted plants. Introduction of infested kale plants in the greenhouse occurred 15 days before the first treatment application.

We used a completely randomized design, with treatments consisting of eight strategies of whitefly management. The strategies were based on two sprayings performed at 30 and 45 days after emergence of plants: (1) control (no treatment); (2) two sprayings of neem oil at 150 ml ha^{-1}; (3) two sprayings of neem oil at 200 ml ha^{-1}; (4) two sprayings of neem oil at 250 ml ha^{-1}; (5) one spraying of pyriproxyfen at 300 ml ha^{-1} + one spraying of neem oil at 150 ml ha^{-1}; (6) one spraying of pyriproxyfen at 300 ml ha^{-1} + one spraying of neem oil at 200 ml ha^{-1}; (7) one spraying of pyriproxyfen at 300 ml ha^{-1} + one spraying of neem oil at 250 ml ha^{-1}; and (8) two sprayings of pyriproxyfen at 300 ml ha^{-1}. Neem oil used in the experiment was Azamax® and the insecticide pyriproxyfen was Tiger®.

Three samplings of whitefly infestation on bean plants were performed. The first sampling took place the day before the first spraying was performed, and two additional samplings were performed three days after each of the two sprayings. At each sampling, the number of adult whiteflies were quantified in the abaxial surface of 10 leaflets, and additional 10 leaflets were randomly collected, kept in paper bags, and taken to the laboratory, each leaflet was considered a repeat. Next, the number of eggs and nymphs were recorded with the aid of a stereoscopic microscope. Data were subjected to Levene (α=0.05) and Cramer-von Mises (α=0.05) tests.

Homogeneous and normally distributed data were analyzed by ANOVA, and treatment means were compared by Tukey test (α=0.05). Whenever ANOVA assumptions were not met, data were analyzed by Kruskal-Wallis non-parametric test, and treatment means compared by Dunns test (α=0.05).

RESULTS AND DISCUSSION: The number of whitefly eggs after the second application was significantly higher in the control as compared to the other management strategies (Table 1). Numbers of nymphs did not differ among strategies in any whitefly sampling (data not shown). In the sampling after the first spraying (FS), strategies 2 and 3 had significantly less numbers of whitefly adults than strategies 6 and 7, not differing from control (without spraying) (Table 1).

After the second application (SS), number of adults in the control was significantly higher than numbers observed in strategies 2, 4, and 5 (Table 1). It is important to note that strategies 2, 4, and 5 succeeded in reducing the number of eggs and adults after the second application, and in all of them neem oil was used in the second spraying. We conclude that neem oil reduces infestations of whitefly eggs and adults in the greenhouse when used in the second application. More studies in the field are needed to confirm the efficiency and residual effect of neem oil.

Table 1. Numbers (± SE) of whitefly eggs and adults per leaflet of common beans treated with different strategies. Sampling before sprayings (BS), sampling after first spraying (FS), and sampling after second spraying (SS).

Management strategies	Eggs per leaflets			Adults per leaflets		
	BS	FS	SS[1]	BS	FS[1]	SS[2]
1. Control (without spraying)	7.6±2,91	27.6±6.48	26.3±5.11c	3.0±0.71	1.2±0.39a	3.3±0.78c
2. Neem 150 mL.ha^{-1} + neem 150 mL.ha^{-1}	5.2±1,22	13.0±6.76	1.6±1.39a	2.8±0.51	1.5±0.50ab	0.3±0.21ab
3. Neem 200 mL.ha^{-1} + neem 200 mL.ha^{-1}	6.1±1,69	12.5±3.73	4.1±1.70ab	3.2±0.89	1.0±0.42a	0.9±0.37abc
4. Neem 250 mL.ha^{-1} + neem 250 mL.ha^{-1}	9.3±3,55	6.8±2.02	3.3±1.37ab	3.8±0.59	1.0±0.39a	0.4±0.16ab
5. Pyriproxyfen + neem 150 mL.ha^{-1}	9.8±3,07	24.0±3.52	3.5±1.22ab	6.4±1.39	2.5±0.56ab	0.1±0.10a
6. Pyriproxyfen + neem 200 mL.ha^{-1}	9.4±1,74	38.3±15.07	8.5±3.13b	6.7±1.38	4.1±0.78b	1.2±0.33abc
7. Pyriproxyfen + neem 250 mL.ha^{-1}	3.3±1,35	14.8±4.78	9.1±3.66b	6.0±1.06	5.0±1.61b	1.7±0.40bc
8. Pyriproxyfen + pyriproxyfen	2.2±0,77	28.3±7.73	6.9±1.65b	2.6±0.60	1.8±0.44ab	0.9±0.37abc
F (SM)	1.66^{ns}	-	8.52**	4.67**		-
H (SM)	-	19.12*	-	15.53*	-	29.29**

Means followed by different letters in columns differ significantly by [1]Tukey and [2]Dunns tests at 5% of probability. [ns]Not significant by ANOVA at 5% probability. *Significant by Kruskal-Wallis test at 5% probability. **Significant by ANOVA at 5% probability ** Significant by Kruskal-Wallis test at 1% probability.

REFERENCES

Boiça Júnior, A. L.; Angelini, M. R.; Costa, G. M. 2006. Efeito do uso de óleos vegetais, associados ou não a inseticida, na eficácia de controle de *Bemisia tabaci* (Gennadius, 1889) e *Thrips tabaci* (Lind., 1888), em feijoeiro comum na época "de inverno". Bioscience Journal 22:23-31.

Horowitz, A. R.; Ishaaya, I. 1995. Chemical control of *Bemisia* - management and application. In: Gerling, D.; Richard, T. Mayer (Eds.). *Bemisia*: taxonomy, biology, damage, control and management. Andover, Hants: Intercept, p.537-556.

Martinez, S. S. 2002. Ação do nim sobre os insetos. In: _____. (Ed.). O nim *Azadirachta indica* – natureza, usos múltiplos, produção. Londrina: IAPAR, p.31-57.

DEVELOPMENT AND FITNESS OF *Spodoptera cosmioides* (WALKER) (LEPIDOPTERA: NOCTUIDAE) ON *Phaseolus vulgaris* L. GENOTYPES

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INTRODUCTION

The black armyworm *Spodoptera cosmioides* (Walker, 1858) (Lepidoptera: Noctuidae), is widely distributed in South America, except for southern Argentina, Chile, and regions located to the west of the Andes in Peru (SILVAIN, LALANNE -CASSOU, 1997). In Brazil, this specie has occurred in several crops, including soybean, cotton and bean, causing injuries on leafs and pods that result in substantial losses in the plant yield (SPECHT et al., 2004).

Thus, Host-Plant Resistance becomes an alternative method as a strategy in the control of this pest, since resistance features expressed by some plants can cause changes in the behavior, and / or biology of phytophagous insects, or provide greater support capacity to their attack (BOIÇA JÚNIOR et al., 2013). Therefore, the aim of this study was to evaluate the development and fitness of *S. cosmioides* larvae fed on bean genotypes.

MATERIAL E METHODS

Bean genotypes used were BRS Pérola, IAC Harmonia, BRS Supremo, BRS Talisman and IPR Campos Gerais. The leaves of the genotypes (treatments) were individualized into 9-cm-diameter Petri dishes lined with deionized water moistened filter paper. In each plate, a newly hatched larvae of *S. cosmioides* was released. Experiment was carried out under a completely randomized design with 30 replications. Data on larval stage duration, larval survival and larval weight of *S. cosmioides* were recorded. In addition, the larval performance of *S. cosmioides* was observed by calculating the Fitness Index (FI), adapted from Boregas et al. (2013), using the formula: FI = larval survival (%) × pupal biomass (mg) / larval development period (days) / 10.

Larval development data were analyzed for residuals normality and variance homogeneity, and when necessary, were transformed to meet the assumptions of analysis of variance (ANOVA). Then, data were submitted to ANOVA (unidirectional ANOVA), and when significant, the means of treatments were compared by Tukey's test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

There were significant differences on survival and larval weight at 10 days, and fitness index of *S. cosmioides* among bean genotypes evaluated. The highest survival was observed in larvae fed on the genotypes BRS Supremo, BRS Pérola and IPR Campos Gerais, in relation to the genotype BRS Talismã. There was total mortality of the larvae that fed on IAC Harmonia genotypes (Figure 1). Similar results obtained by Morando (2015) and Santos (2015) showed that the IAC Harmonia genotype presented resistance features to *Chrysodeixis inculudens* and *Spodoptera frugiperda* (Lepidoptera: Noctuidae), respectively.
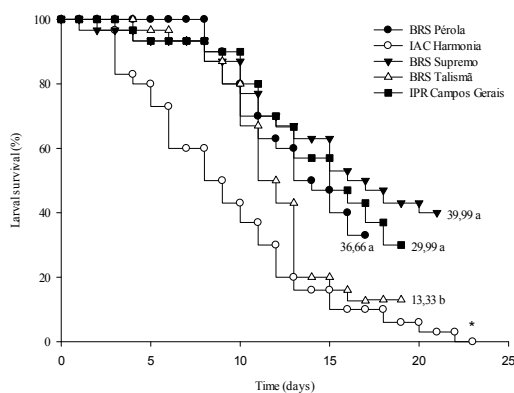
Figure 1. Survival curve (±SE) of *Spodoptera cosmioides* larvae fed with bean genotypes. Different lowercase letters indicate significant difference by the Tukey test ($P < 0.0001$; $F = 18,32$; SE = 9,16). *Data not analyzed due to total insect mortality.
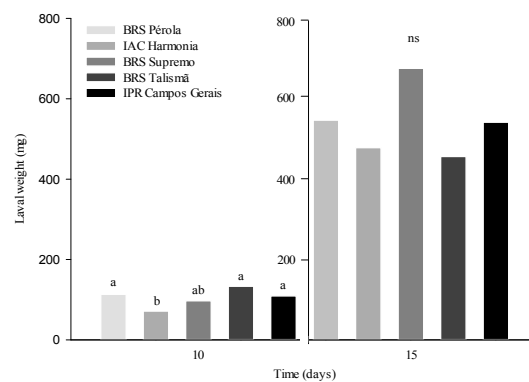
Figure 2. Average larval weight (±SE) of *Spodoptera cosmioides* fed with bean genotypes at 10 and 15 days. Bars topped with different lowercase letters indicate significant difference by the Tukey test ($P = 0.0006$; $F = 5,58$; SE = 5,28). [ns] No significant difference by the Tukey test ($P = 0.8210$; $F = 0,38$; SE = 54,23).
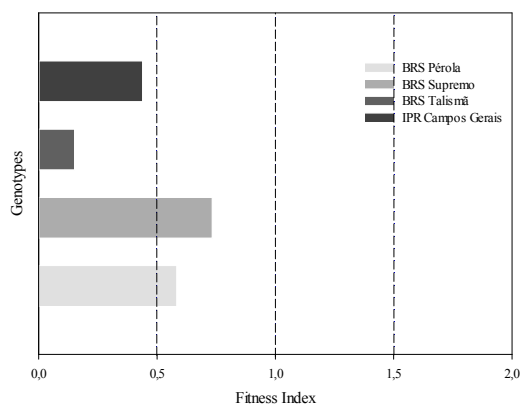
Figure 3. Fitness index of *Spodoptera cosmioides* larvae fed with bean genotypes.

Regarding larval weight (Figure 2), there were significant differences only at 10 days after larvae hatching. Genotypes BRS Supremo, BRS Pérola and IPR Campos Gerais, provided greater *S. cosmioides* weight in relation to genotypes IAC Harmonia and BRS Talisman. The fitness index that demonstrates the host adequacy level for the evaluated population, was considered low (<1.0) for *S. cosmioides* in all bean genotypes used in this experiment (Figure 3). The results found herein demonstrate that bean genotypes evaluated show resistance features in the antibiosis category and provided low fitness level to *S. cosmioides*.

REFERENCES

BOIÇA JÚNIOR, A. L.; SOUZA, B. H. S.; LOPES, G. S.; COSTA, E. N.; MORAES, R. F. O.; EDUARDO, W. I. Atualidades em resistência de plantas a insetos. In: BUSOLI, A. C.; ALENCAR, J. R. D. C. C.; FRAGA, D. F.; SOUZA, L. A.; SOUZA, B. H. S.; GRIGOLLI, J. F. J. (Eds.). Tópicos em entomologia agrícola – VI. Jaboticabal: Gráfica Multipress, 2013b. p. 207-224.

BOREGAS, K.G.B.; MENDES, S.M.; WAQUIL, J.M.; FERNANDES, G.A.W. Estádio de adaptação de *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) em hospedeiros alternativos. Bragantia, v.72, p.61-70, 2013.

MORANDO, R. Resistência de genótipos de feijoeiro a *Chrysodeixis Includens* (Walker) (Lepidoptera: Noctuidae). 2015. 81 f. Dissertação (Mestrado em Agronomia – Área de Concentração: Proteção de Plantas), Faculdade de Ciências Agronômicas, Universidade Estadual Paulista, Botucatu, 2015.

SANTOS, J. D. V. Não preferência para alimentação e antibiose em genótipos de feijoeiro a Spodoptera frugiperda (J. E. Smith, 1797) (Lepidoptera: Noctuidae). 2015. 84 f. Dissertação (Mestrado em Agronomia – Área de Concentração: Entomologia Agrícola) – Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal, 2015.

SILVAIN, J. F.; LALANNE-CASSOU, B. Distinction entre *Spodoptera latifascia* (Walk.) et *Spodoptera cosmioides* (Walk.), bona species (Lepidoptera: Noctuidae). Revue Française d'Entomologie, v. 19, p. 95-97, 1997.

SPECHT, A.; SILVA, E.; LINK, D. Noctuídeos (Lepidoptera, Noctuidae) do Museu Entomológico Ceslau Biezanko, Departamento de Fitossanidade, Faculdade de Agronomia "Eliseu Maciel", Universidade Federal de Pelotas, RS. Revista Brasileira de Agrociências, v. 4, p. 389-409, 2004.

FEEDING PREFERENCE OF *Spodoptera frugiperda* (SMITH) (LEPIDOPTERA: NOCTUIDAE) ON *Phaseolus vulgaris* L. GENOTYPES

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INTRODUCTION

The species *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae) is a widely distributed pest in the American continent and with a polyphagous habit, causing damage to several crops of economic importance, such as bean bean (CASMUZ et al., 2010). The most control measure to manage *S. frugiperda* is based on sprayings of chemical insecticides. However, alternative methods less damaging to the environment have been proposed. The host plant resistance is one of them, through plants that present in their constitution genes that are capable of producing substances that interfere in the behavior and/or development of the insect (BOIÇA JÚNIOR et al., 2015). The ability of these plants to tolerate damage caused by insect pests has been aided in breeding programs to obtain genotypes with resistance characteristics. Thus, the aim of this study was to evaluate *S. frugiperda* larval feeding preference in common bean genotypes.

MATERIAL AND METHODS

The following genotypes were evaluated for resistance to *S. frugiperda* larvae: BRS Pérola, IAC Harmonia, BRS Supremo, BRS Talismã and IPR Campos Gerais. Double and multiple choice tests were performed under a randomized complete block desing, with 10 replications each. In the multiple-choice test, arenas composed of 14-cm-diamenter Petri dishes were used. In the double choice test, 8-cm-diamenter Petri dishes were used, confronting the genotypes two by two. Petri dishes were coated to the bottom with moistened filter paper, and 3-cm-diameter leaf disc of the respective genotype was distributed equidistantly. In both tests, third-instar larvae of *S. frugiperda* was released per genotype.

The evaluation consisted in counting the average number of larvae present in the leaf discs, after, one, six and 12 hours from the beginning of the experiment. In the double-choice test, the Preference Index (PI) was calculated according to Kogan (1972), using the formula: $PI = 2A / (A + T)$, where A = number of *S. frugiperda* present in the first bean genotype confronted, and T = of the second genotype. The value of the Standard Error of the mean (SE) was added / subtracted to the values of PIs. Values of PI ± SE >1 indicate preference for genotype "A", PI ± SE <1 preference for "T" genotype and PI ± SE = 1 neutrality. The PI, in the multiple-choice test was calculated taking into account the BRS Pérola genotype, as a susceptibility standard (SOUZA et al., 2012). At the end of the experiment, two evaluators determined the injury percentage caused by the larvae of *S. frugiperda* in the leaf discs, ranging from zero for uninjured pods to 100% for completely injured.

Percentage of injury data were submitted to homoscedasticity and normality analyzes. Subsequently, the data for the double-chance tests were submitted to the t-test ($p < 0.05$) while the results of the multiple-chance test were submitted to analysis of variance and then to the Tukey test ($p < 0.05$).

RESULTS AND DISCUSSION

The genotypes BRS Talismã and IPR Campos Gerais were classified as stimulants, while IAC Harmonia and BRS Supremo were considered as deterrents in the multiple-choice test (Fig. 1A). Although stimulating, the injury percentage in the genotype IPR Campos Gerais did not differ significantly from the genotypes IAC Harmonia and BRS Supremo, which presented lower injury percentages (Fig. 1B). On the other hand, BRS Talismã showed higher injury percentage.
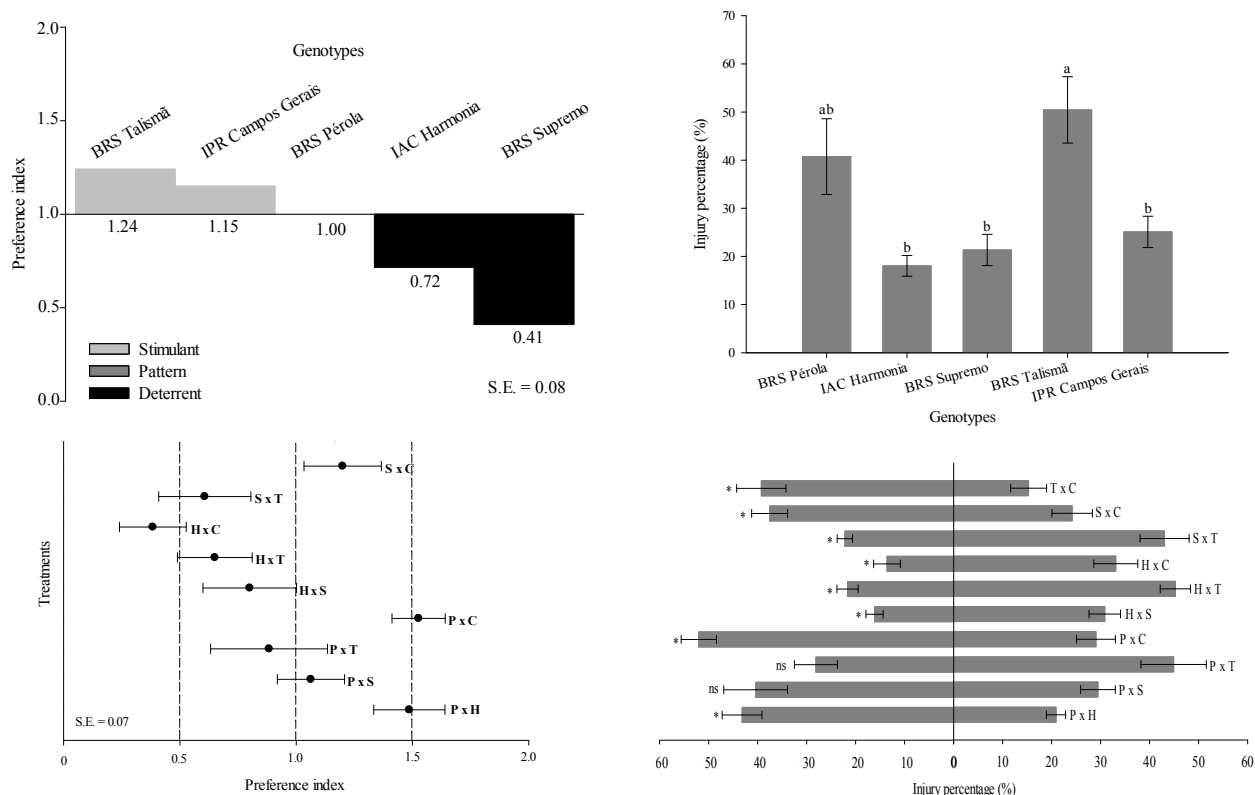
Figure 1. Preference index for feeding in multiple choice (A) and double chance (B) tests and injury percentage in tests with multiple chance (C) and double chance (D) on bean genotypes to *Spodoptera frugiperda* caterpillar. Means followed by the same lowercase letter not differ significantly by the Tukey test (P <0.05); * Significant and ns not significant by t-test (p <0.05). P = BRS Pérola; H = IAC Harmonia; S = Supremo BRS; T = BRS Talismã; C = IPR Campos Gerais. Jaboticabal, UNESP, 2017.

In the double-choice test, IAC Harmonia was classified as deterrent when compared to all genotypes (Fig. 1C), also reflecting lower injury percentages (Fig. 1D). BRS Talismã presented higher injury percentages when compared to the other genotypes. Overall, we conclude that the IAC Harmonia genotype presents resistance in the feeding preference category to *S. frugiperda* larvae, while BRS Talismã showed susceptibility.

REFERENCES

BOIÇA JÚNIOR, A. L. et al. A defesa das plantas ao ataque dos insetos. In: BUSOLI, A. C. et al. (Eds.). *Tópicos em entomologia agrícola – VIII*. Jaboticabal: Maria de Lourdes Brandel-ME. 2015. p. 161-179.

CASMUZ, A. et al. Revisión de los hospederos del gusano cogollero del maíz, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Revista de la Sociedad Entomológica Argentina, Mendoza, v. 69, n. 3-4, p. 209-231. 2010.

KOGAN, M. Feeding and nutrition of insects associated with soybeans. 2. Soybean resistance and host preferences of Mexican bean beetle, *Epilachna varivestis*. *Annals of Entomological Society of America*, College Park, v. 65, p. 675-683, 1972.

SOUZA, B. H. S. et al. Non-preference for feeding of *Spodoptera frugiperda* (Smith) (Lepidoptera) in bean genotypes. *Bean Improvement Cooperative*, v. 55, p. 211-212, 2012.

ATRACTIVENESS AND NON-PREFERENCE FOR OVIPOSITION OF *Tetranychus ogmophallos* (ACARI: TETRANYCHIDAE) ON GENOTYPES OF *Phaseolus vulgaris* L.

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INTRODUCTION

The use cultivars resistant to arthropod pests is a significant tool used in integrated pest management. Indirectly cultivars on which arthropod show prolonged growth and development may expose the organism to the potential natural enemies resulting in natural control of arthopod population (Sarfraz et al., 2007). In Brazil, little information is available on the host plant resistance to *Tetranychus ogmophallos* mites, which presents high potential to infest common bean, peanut and soybean plant.

Bonato et al. (2000) verified that *T. ogmophallos* able to develop on bean and displayed high rates of increase when reared on this plant. In addition, the ability of develop on a wide range host plants, constitutes a factor that raises the status this pest (Bonato et al., 2000). Thus, it is important to analyze the attractiveness and oviposition response of *T. ogmophallos* on bean genotypes in order to determine the genotypes that are less preferred by mite. In this study we evaluated *T. ogmophallos* attractiveness and preference oviposition of common bean genotypes.

MATERIALS AND METHODS

The experiment was conducted under controlled conditions of 25 ± 2 ºC temperature, $70 \pm 10\%$ relative humidity, and 12 h photophase. The following bean genotypes were tested: BRS-Supremo, BRS Pérola, BRS Talismã, IAC-Harmonia and IPR Campos Gerais. Seeds of the common bean genotypes were sown in 5-L-pots and were kept in a greenhouse until use.

Plants were used at the vegetative stage V3-V4. The attractiveness and preference oviposition by *T. ogmopahllos* in free-choice assay was designed in randomized blocks and, no-choice test completely randomized, with 10 replications each.

In the free-choice each replication consisted of a Petri dish arena (15 cm diameter x 1 cm height) containing the leaf discs (2.5 cm diameter) of each genotype distributed equidistantly. To prevent escape of mites and maintenance of moisture, the margins of Petri dishes were surrounded with water-soaked cotton and then covered with plastic film. Thereafter, 25 newly emerged *T. ogmophallos* female were released in the ratio of five females to each genotype.

For the no-choice test, the arena (10 replication) consisted of a petri dish (6 cm diameter x 1 cm height) with an insect pin fixed in the center, where a supernatant leaf disc (2.5 cm diameter) was placed in water attached through the insect pin in order to avoid leaf movement and mite scape. Then, five newly emerged *T. ogmophallos* female were released in each Petri dish arena.

For both assays, after 48 h of oviposition period, leaf disc attractiveness to *T. ogmophallos* female was recorded and then the adults were removed and the eggs laid per plant was counted through stereomicroscope. From the number of eggs laid by *T.ogmophallos* per leaf disc, the oviposition preference index (OPI) was calculated according to Fenemore, 1980.

Data of attractiveness and number of eggs were analyzed for residuals normality and variance homogeneity, and when necessary, were transformed to meet the assumptions of analysis of variance (ANOVA). Next, data were subjected to analysis of ANOVA, and means were compared by Tukey's test ($P<0.05$).

RESULTS AND DISCUSSION

Regarding OPI results observed in no-choice test, genotypes behaved as stimulants for oviposition (Figure 1A). In the free-choice test, the genotypes BRS Supremo and IPR Campos Gerais were classified as deterrents for oviposition (Fig. 1B) Genotypes IAC Harmonia and BRS Talisman showed higher numbers of eggs than the genotype defined as the standard susceptible and therefore were classified as stimulants for oviposition.
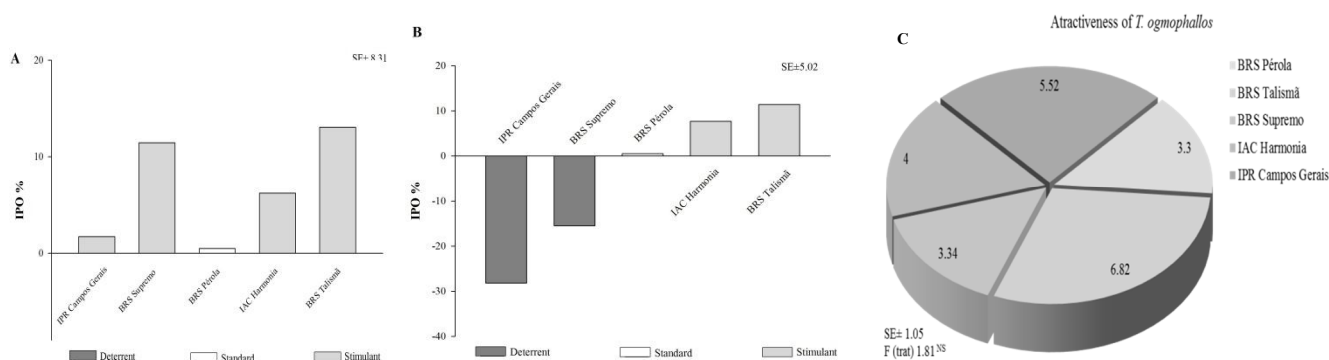
Figure 1. Index preference for oviposition (IPO) and atractiveness of common bean genotypes to *Tetranychus ogmophallos*. IPO in no-choice test (A), free-choice test (B) and number *T. ogmophallos* attractiveness (C). [NS] sectors with same letter are not significantly different by Tukey test at 5% probability.

In addition, in the fre-choice test, the attractiveness of *T. ogmophallos* did not differ between bean genotypes. However, it is worth highlighting that the genotype BRS Supremo was deterrent for oviposition and behaved as less preferred by *T. ogmophallos*.

REFERENCES

BONATO, O.; SANTAROSA, P. L.; LUCCHINI, F. 2000. Suitability of three legumes for development of *Tetranychus ogmophallos* (Acari: Tetranychidae). *Florida Entomologist*. 83:203-205.

SARFRAZ, M.; DOSDALL, L. M.; KEDDIE, B. A. 2007. Resistance of some cultivated Brassicaceae to infestations by *Plutella xylostella* (Lepidoptera: Plutellidae). *Journal of Economic Entomology*. 100:215-24.

FENEMORE, P. G. 1980. Oviposition of potato tuber moth, *Phthorimaea operculella* Zell. (Lepidoptera: Gelechiidae); identification of host-plant factors influencing oviposition response. *New Zealand Journal of Zoology*, 7:5–439.

COMPARATIVE ANALYSIS OF GENOMIC DNA EXTRACTION METHODS IN LIMA BEAN (*Phaseolus lunatus* L.)

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INTRODUCTION: The lima bean (*Phaseolus lunatus* L.) is the second most important legume of the genus *Phaseolus* (Maquet et al. 1999). In Brazil, this species is mainly cultivated in the Northeast region and presenting great socioeconomic importance for family communities. In that region there are water scarcity and predominate, long periods of drought, what favors the selection of drought tolerant genotypes and adaptation to different environments? The development of research on lima bean Genetics, Genetic Resources and Plant Breeding is extremely important considering its socioeconomic potential and the lack of information for its exploitation. Thus, we aimed to compare five genomic DNA extraction methods and to determine the most efficient protocol for *Phaseolus lunatus*, with a view to genotyping.

MATERIAL AND METHODS: Five protocols of DNA extraction in lima bean were evaluated: Dellaporta et al. (1983); Doyle and Doyle (1987), modified; Khanuja et al. (1999); Ferreira and Grattapaglia (1996), Romano and Brazilian (1999). Quality and integrity of DNA samples of each protocol were observed through the comparative analysis of the intensity and the standard of the bands obtained by agarose (0.8%) gel electrophoresis. The spectrophotometer NanoDrop 2000™ also quantified the samples, which provides the absorbance ratios 260/280 nm in addition to the quantification of the nucleic acids. The gels were stained with GelRedTM and visualized with a photo-documenter. The accessions used to compare the protocols were UFPI 1007 and UFPI 804, from the Germoplasma Bank of the Federal University of Piauí.

RESULTS AND DISCUSSION: The analysis of agarose gel electrophoresis showed that all protocols, except of Khanuja et al. (1999), were efficient in obtaining good amount of high molecular weight genomic DNA of the two lima bean accessions (Figure 1). It also observed little degradation of extracted DNA in all protocols. According to the data obtained in NanoDrop 2000TM (Table 1), the protocol that showed superior with respect to the concentration of DNA was Doyle and Doyle (1987), modified (above 2000 ng / l of DNA), for the two lima bean accessions. The protocols Dellaporta et al. (1983), Doyle and Doyle (1987), Romano and Brasileiro (1999) presented an absorbance ratio 260/280 nm equal to or greater than 1.8. This indicates that the DNA extracted by these protocols is free from contaminants such as phenols and proteins. The DNA of the lima bean accessions, UFPI 1007 and UFPI 804, extracted by the protocols Dellapporta et al. (1983), Doyle and Doyle (1987), Romano and Brasileiro (1999) presented high integrity and quality, suggesting the feasibility of using these protocols.
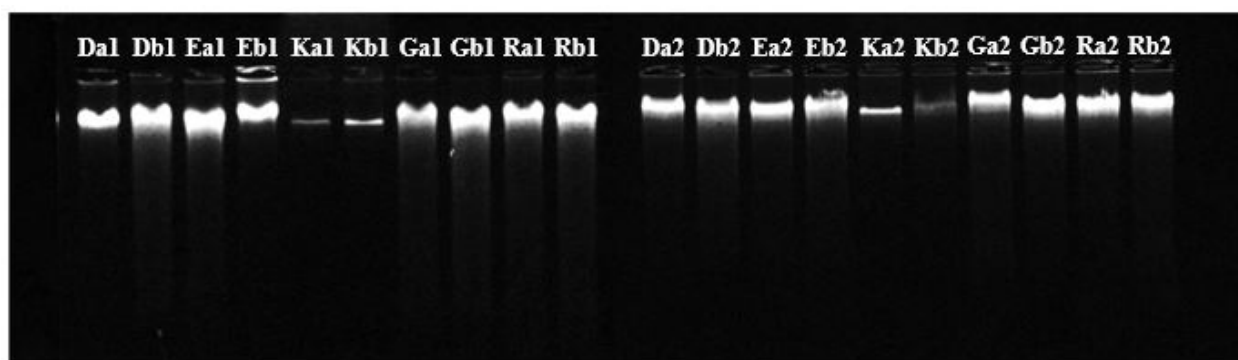
Figure 1. Electrophoretic profile of DNA extracted from young leaves of *Phaseolus lunatus* L. by the methods: D - Dellaporta et al. (1983); E –Doyle and Doyle (1987); K - Khanuja et al. (1999); G – Ferreira and Grattapaglia (1998) and R – Roman and Brasileiro (1998). a - UFPI 1007 and b - UFPI 804. 1 - plant 1 and 2 - plant 2.

Table 1. DNA quantification of two lima bean accessions with spectrophotometer NanoDrop^{TM} 2000.

Amostra*	Concentração ng/µl	260/280	Amostra	Concentração ng/µl	260/280
Da1	1281,3	1,89	Da2	1390,8	1,84
Db1	1084,4	1,72	Db2	1286,3	1,85
Ea1	2712,4	1,89	Ea2	3071,3	1,86
Eb1	2474,5	1,84	Eb2	2009,9	1,93
Ka1	211,6	1,3	Ka2	543,3	1,60
Kb1	344,2	1,52	Kb2	569,9	1,66
Ga1	1231,7	1,82	Ga2	1423,2	1,78
Gb1	1035,7	1,76	Gb2	917,6	1,74
Ra1	1325,2	1,8	Ra2	1624,9	1,85
Rb1	1335,2	1,84	Rb2	1414,2	1,83

*D–Dellaporta et al. (1983); E–Doyle and Doyle (1987); K-Khanuja et al. (1999); G–Ferreira and Grattapaglia (1998) and R-Romano and Brasileiro (1998). a - UFPI 1007 and b - UFPI 804. 1 - plant 1 and 2 - plant 2.

REFERENCES
Dellaporta S.L. et al. (1983). A plant minipreparation: version II. Plant Mol. Biol. Rep. 1: 19-20.
Doyle J.J. & Doyle, J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19: 11-15.
Ferreira M.E. & Grattapaglia D. (1998). Introdução ao uso de marcadores moleculares em análise genética. 3rd edn. Embrapa, Cenargen, Brasília.
Khanuja S.P.S. et al. (1999). Rapid isolation of DNA from dry and fresh samples of plants producing large amounts of secondary metabolites and essential oils. Plant Mol. Biol. Rep. 17: 74-74.
Maquet A. et al. (1999). Phylogenetic study on wild allies of Lima bean, *Phaseolus lunatus* (Fabaceae), and implications on its origin. *Plant Systematics and Evolution* 218: 43-54.
Romano E. & Brasileiro A.C.M. (1999). Extração de DNA de plantas. Biotecnologia, Ciência e Desenvolvimento 2: 40-43.

MORPHO-AGRONOMIC CHARACTERIZATION OF LIMA BEAN GENOTYPES FROM SUBTROPICAL BRAZIL

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INTRODUCTION: The Lima bean (*Phaseolus lunatus)* is distributed worldwide, and characterized by greater tolerance to drought, excess moisture, as well as high temperatures (VIEIRA, 1992). In Brazil, it presents a limited region of cultivation, being mainly emphasized in the northeast region of Brazil, being directly connected to the feeding of the familiar farmers. The objective of this work was to evaluate the genotypes of Lima bean from the subtropical region of Brazil.

MATERIAL AND METHODS: In the agricultural year of 2015-2016 genotypes of lima bean, which were characterized according to: DEF, day number to emergence up to 50% of plants in flowering plot, number of harvests; plant habit: semi-erect (SE) and prostrate (P); kind plant: 3-semi-erect and branched and 4-prostrate and branched; growth habit: semi-determined (SD) and Undetermined (U), postage and flower color: white (W) and yellow (Y). The experiment was conducted at the Experimental field of the Terras Baixas Station, Embrapa Clima Temperado, in the municipality of Capão do Leão, RS, at 31º48'15"S latitude and 52º24'42"W longitude. The climate classified as Cfa according to Köppen and Geiger, presenting a temperature of 18.0ºC and a rainfall of 1.378 mm. Sowing was carried out on 01/13/2016, occurring the emergence in 50% of the seedlings on 01/21/2016 The experiment consisted of two lines of each genotype, spaced 0.65 m between rows and 0.20 m between plants, with a density of 5 plants per linear meter.The experiment consisted of two lines of each genotype, spaced 0.65 m between rows and 0.20 m between plants, with a density of 5 plants per linear meter.

RESULTS AND DISCUSSION: Table 1, it can be verified that the genotypes varied in the behavior of the DEF, ranging from 34 to 67 DEF. The initial genotypes were those that obtained flowering with 34 DEF, in this case the genotypes G 344 and Sel 344. The late genotypes were those that showed flowering greater than 60 DEF, which were genotypes G 198 and G 497. Sel 198 showed flowering Precocious when compared to G198. As for the morphological characters, all genotypes showed climbing, indeterminate growth habit and type 4, only the genotype Sel G344 that presented semi-indeterminate growth and plant type 3. The color of the flowers varied between white and yellow. The flowering data are in accordance with the work of Gomes et al., 2010 and Almeida et al., 2014, who found values for DSF between 48-50; 41-82 and 34-90 days respectively, which show a wide genetic variability in genotypes of southern Brazil. The average number of days for the first harvest was 50 DFC, ranging from 31 to 64 days in relation to the date of flowering and the first harvest. The initial genotype for the harvest was G 198, which presented 31 DFC, and this genotype presented a larger harvest window, presenting 6 harvests occurred between April 24 and June 3, at 72 days after flowering. A period beginning before winter in the southern hemisphere, however, selection of this genotype stands out, showing a higher precocity to flowering than the source material, 6 days, and only one harvest in 52 DFC (Table 2). The posterior genotype for the first harvest was G 344, whose first harvest was carried

out in 64 DFC. It is necessary to emphasize that the genotype G 344 was the one that presented the early flowering and the late harvest, fact related to the behavior of the genotype during the filling and maturation of the grains.

Table 1: Period from emergence at plant flowering (DEF), plant habit, plant type, growth habit and flower color of genotypes of Lima bean (*Phaseolus lunatus*).

Genotype	DEF (dias)	Plant habit	Plant type	Growth Habit	Color Flower
G198	61b	P[1]	4	U	W
Sel 344	34 f	SE	3	SD	Y
Sel 198	55 c	P	4	U	W
G344	34 f	P	4	U	Y
G497	68a	P	4	U	W
G194	50 d	P	4	U	W
G196	50 d	P	4	U	W
G120	55 c	P	4	U	Y
G155	56 c	P	4	U	W
G197A	47 e	P	4	U	W

[1]P: prostrate; SE: semierect; U: undeterminated; SD: semideterminated, W: white, Y: yellow.
*Values followed by the same letter in the column do not differ by Duncan's test at the 5% level of significance

Table 2: Number of days to flowering until harvests of Lima Bean (*Phaseolus lunatus*).

Genotype	DFC1*	DFC2	DFC3	DFC4	DFC5	DFC6
G198	31**	38**	45**	52	57	72
Sel.344	57	64	78	83	x	x
Sel.198	58	x	x	x	x	x
G344	64**	71**	78	x	x	x
G497	50	65	x	x	x	x
G194	49	56	68	83	x	x
G196	49	63	83**	x	x	x
G120	44	51	58	63	x	x
G155	43	50	57	x	x	x
G197A	52	71**	x	x	x	x
Average (AVE)	50	59	67	70		
St.Dev.(SD)	9.19	10.95	13.92	15.39		
Ave + 1SD	58.89	69.73	80.64	85.64		
Ave -1SD	40.51	47.83	52.79	54.86		

* DFC = Number of days of 50% of the plants of the flowering plot until the first harvest.
** Ave ± 1SD = Sum of average and St.Deviation

REFERENCES

CANTERI, M.G. et al. SASM-Agri: Sistema para análise e separação de médias em experimentos agrícolas pelos métodos Scoft-Knott, Tukey e Duncan. Revista Brasileira de Agrocomputação, v.1, n.2, p.18-24, 2001.

FRAZÃO, J.E.M. et al. Morfologia e fenologia de dez variedades de fava nas fases vegetativa e de inflorescência. Agropecuária Técnica, Areia, v. 7, n. 1, p.18-24, 2010.

GOMES, S.O. et al. Avaliação de componentes de produtividade de grãos em sub-amostras de feijão-fava de crescimento determinado. Anais da Academia Pernambucana de Ciência Agronômica, Recife, v. 7, p.312-317, 2010.

EFFECT OF CLIMATE CHANGE ON THE POTENTIAL DISTRIBUTION OF WILD LIMA BEAN (*Phaseolus lunatus L.*, Fabacea)

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INTRODUCTION

The distribution of wild bean *Phaseolus lunatus* in Mexico is broad and adapted to several climatic conditions. Recent studies reveal a low conservation status of wild beans in most populations due anthropogenic disturbance of their habitats. Thus the need to implement strategies of conservation *in situ* arises, based on the knowledge of populations on risk by ecological or climate change disruptions, or by means of *ex situ* conservation in germplasm banks of individuals in threatened areas, which will allow the maintenance of genetic diversity. The aim of this study was to evaluate the impact of climate change in the years 2020 and 2080 on the potential distribution model (DM) of wild *P. lunatus* in Mexico.

MATERIALS AND METHODS

Accessions with geographical information (126) were obtained from several herbarium databases and germplasm banks. Bioclimatic variables (19) were calculated for Mexico based on climate date of the periods 1961-2009 (reference climatology) and compared with 2020s (2015-2039) and 2080s (2075-2099) representing the early and late 21st century, respectively, under two representative concentration pathways of greenhouse gases (RCP 4.5 y 8.5). Distributions were modeled by using Maxent (Philips *et al*., 2006) and fitted using cross-validation (10 iterations) each one randomly dropping 25% input points, and average test AUC ≥ 0.90. Models were validated by check accuracy of 10% random reservoir dataset. Potential distribution surface results were classified on three categories as percentages of environmental fitness: 0-0.3 probability LEA (Low Environmental Aptitude); 0.3-0.7 probability MEA (Medium Environmental aptitude) and 0.7-1.0 probability HEA (High Environmental Aptitude).

RESULTS AND DISCUSSION

Wild lima bean was distributed across 17 states of Mexico, with Chiapas and Campeche being represented by 15.9 and 13.5%, respectively. All models had an average test AUC > 0.93, suggesting a good aptitude of the models to discriminate the specie´s fundamental climatic niche. The validation dataset indicated ranges of probability from 0.11 to 0.83, suggesting a good discrimination between presence and absence of the species. Temperature seasonality and mean temperature of the coldest quarter were the variables with the highest contribution percent to DM.

The current distribution potential of surface of HEA was 416,748.3 km^2, whereas surface of MEA was 43,421.3 km^2 (table 1 and fig. 1a). Table 1 shows the changes of patterns of distribution of MEA and HEA surface under future climate scenarios with respect to the current scenario. The projected changes in the distribution of *P. lunatus* are concentrated in Yucatan Peninsula areas for the scenario RCP 4.5 in both periods, as well as in the Trans-Mexican Volcanic Belt present in the Jalisco and Michoacan states (Fig. 1).

Table 1. Variation on the surface of distribution of *P. lunatus* according to two categories of environmental fitness, two climate change models and three periods: at present, 2020s and 2080s.

Medium Environmental Aptitude					High Environmental Aptitude				
1961-2009	2020s		2080s		1961-2009	2020s		2080s	
Surface km^2	Δ% RCP 4.5	Δ% RCP 8.5	Δ% RCP 4.5	Δ% RCP 8.5	Surface km^2	Δ% RCP 4.5	Δ% RCP 8.5	Δ% RCP 4.5	Δ% RCP 8.5
416,748.3	-26.3	-7.3	-7.4	-21.8	43,421.3	-18.7	-28.8	-41.3	-18.8
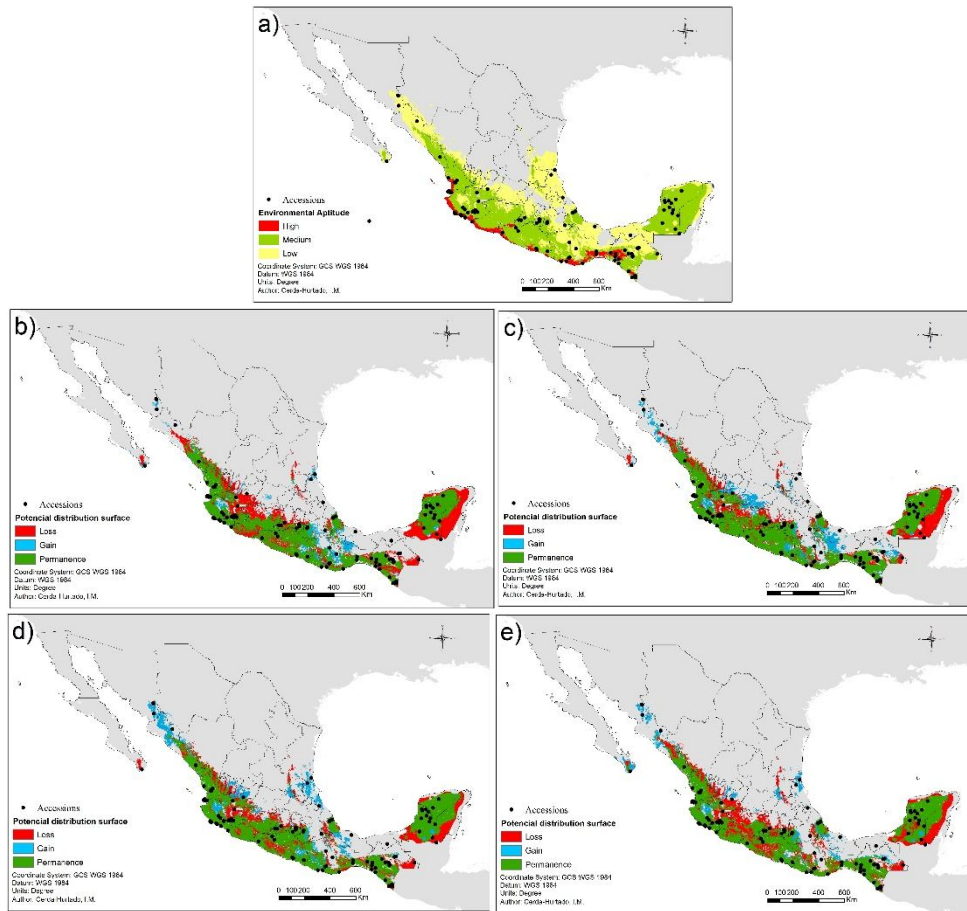
Figure 1. Spatial patterns changes of MEA and HEA of *P. lunatus* surface in Mexico. a) Model current distribution (1961-2009); b) Changes for scenario RCP 4.5 at 2020s; c) Changes for scenario RCP 4.5 at 2080s; d) Changes for scenario RCP 8.5 at 2020s; e) Changes for scenario RCP 8.5 at 2080s.

CONCLUSIONS

Climate change will decrease the surface with medium and high environmental fitness for distribution of *P. lunatus*. It is necessary to design conservation strategies in the near future, to protect and prevent the loss of these genetic resources.

REFERENCES

Andueza-Noh, R. H., et al., 2016. Polibotánica 41:101-113.

Cuervo-Robayo, A. P., et al., 2014. Int. J. Climatol. 34:2427-2437.

Fernández-Eguiarte, A., et al., 2014. *Actualización de los escenarios de cambio climático para estudios de impacto, vulnerabilidad y adaptación en México y Centroamérica*.

Phillips, S. J., et al., 2006. Ecological Modelling 190: 231-259.

PHYSIOLOGICAL EFFECTS OF HEAT STRESS ON LIMA BEAN (Phaseolus lunatus) AND DEVELOPMENT OF HEAT TOLERANCE SCREENING TECHNIQUES

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INTRODUCTION

Heat stress reduces yields of May and early June-planted lima beans (*Phaseolus lunatus*) on the Delmarva Peninsula. High temperatures during flowering reduce or delay pod set and can result in later harvest, lower yield and split sets. We are working to develop heat tolerant baby and Fordhook type lima bean cultivars that are adapted to the Mid-Atlantic Region. Both field and greenhouse screening methods have been used to test inbred lines for heat stress response, but greenhouse screening has been particularly useful in determining the physiological effects of heat stress.

METHODS

Eight lima bean inbred lines were grown in two climate controlled chambers inside of the greenhouse under hot and cool night temperature regimes. Experiments were arranged in a randomized complete block design with 5 replications. Target night temperatures were 27 °C in the hot chamber and 18 °C in the cool chamber. For experiment 1, target daytime temperatures were 32-35 °C in the hot chamber and 27-30 °C in the cool chamber. For experiment 2 target daytime temperatures for both chambers were 32-35 °C. Newly opened flowers were collected from plants grown under hot and cool night conditions. The style, with pollen adhering to it, was removed from the flower, stained with acetocarmine, then viewed and photographed under 40x magnification. We later counted stained pollen grains visible in the photograph. We harvested pods from the plants at maturity and noted the number pods, number of seeds per pod, total number of seeds and total weight of seeds for each plant.

RESULTS

Heat Effects on Flower Production - In experiment 1, hot night temperatures reduced time to flowering in all genotypes. On average, plants in the hot chamber began flowering 11 days earlier than those in the cool chamber. Plants in the hot chamber produced many flowers and re-flowered repeatedly on racemes that failed to set pods. Heat stress does not appear to cause yield loss by inhibiting or delaying flowering.
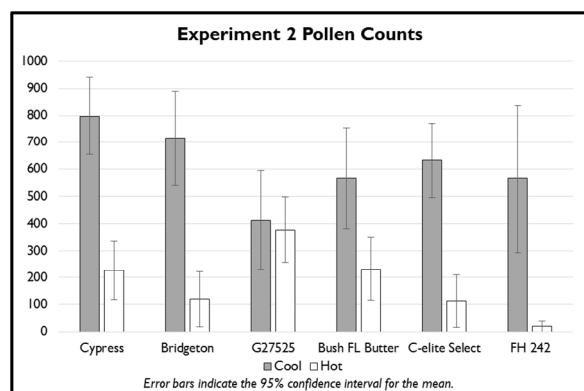
Figure 1. Experiment 2 pollen counts
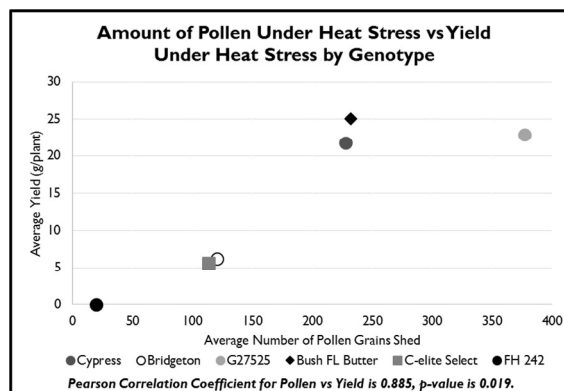
Figure 2. Amount of pollen shed under heat stress vs. yield under heat stress by genotype

Heat Effects on Pollen Production and Release - In both experiments, flowers from plants grown in the hot chamber had less pollen shed onto the stigma and style (Figure 1). In some cases no shed pollen was observed, but in most cases some pollen was present. The maximum number of pollen grains observed was over 1,400. In experiment 2 there was a positive correlation between the amount of pollen shed under heat stress and yield under heat stress (Figure 2).

Heat Effects on Fertilization - The acetocarmine stain used in this assay is reported to be useful as a test for viability. Unstained pollen grains were not counted but were also very rarely observed. Germinated pollen grains were only occasionally seen, but this is not unexpected as flowers were collected only a few hours after opening. We observed that in some flowers the style and stigma were not completely enclosed in the keel. This could interfere with self-pollination. Stigma location was noted for the flowers collected on four different dates in experiment 2. The stigma was located outside of the keel in 47% of the flowers from the hot chamber and 11% of the flowers from the cool chamber (means differ significantly by Student's T-Test p-value=0.0041). Additional experiments are needed to determine if high temperatures affect other aspects of fertilization such as pollen germination, pollen tube growth, stigma receptivity, and egg viability.

Heat Effects on Seed Fill and Maturation - In experiments 1 and 2, yield loss was due to fewer seeds produced per plant. There was not a significant difference in per seed weight. In some genotypes there was not a significant difference in the number of pods set. In all genotypes there were significantly fewer seeds per pod in plants grown in the hot chamber. Fewer seeds per pod indicate heat effects on pollination or fertilization.

Whole Plant vs Localized Effects of Heat Stress - In spring 2015 a vining, heat susceptible genotype (Dr. Martin) was grown inside the hot chamber and trained out into the greenhouse (cool night temperatures) and vice versa. Yield and pollen production were affected by the location of the flower -- either in the hot chamber or in the cooler greenhouse.

Genotype Differences in Heat Tolerance - Under high nighttime temperature conditions, genotypes that were heat tolerant shed more pollen onto the style and set more seed than genotypes that were heat susceptible. A large-seeded genotype, 'Fordhook 242', was the most heat susceptible genotype that was tested. Among the small-seeded genotypes, 'Bridgeton' and 'C-elite Select' are heat susceptible and 'Bush Florida Butter', 'Cypress' and G27525 are more heat tolerant.

CONCLUSIONS

Heat stress related yield loss in lima bean is due, in part, to reduction in the amount of pollen shed onto the stigma and style. The heat effects on pollen production and release are determined by conditions experienced by the flower, not the plant as a whole. Other factors, such as pollen viability, pollen tube growth and location of the stigma inside or outside of the keel may also play a role in heat stress response. By growing in a greenhouse chamber under sustained high nighttime temperature conditions, we were able to identify several heat tolerant genotypes which are now being used as parents in the University of Delaware lima bean breeding program.

OCCURRENCE OF SOIL DISEASES IN INTERCROPPING OF LIMA BEAN LANDRACES WITH MAIZE

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INTRODUCTION: The lima bean culture, if technically exploited, may cause great changes in the socio economic reality of the producing regions of Northeast Brazil. The commitment to the development of technologies for lima bean culture will result in a significant increase in yield, improvement in quality, which directly affecting in the quality of life's farmer (Neto Barreiro et al. 2015). In this work, we aimed to perform the agronomic evaluation of eight lima bean landraces to identify those with potential for cultivation.

MATERIAL AND METHODS: The experiment was carried out in area of the Department of Agriculture of the Federal University of Piauí, in Teresina, Piauí, Brazil. We evaluated twelve lima bean landraces from six Northeastern Brazilian states (Table 1). The landraces present an indeterminate growth habit and the cultivation was intercropped with corn, variety AL Piratininga, which offered support to the legume. A completely randomized experimental design was used, with four replications. Each plot was composed four rows of 5.0 m, spaced 0.80 m x 0.70 m. In corn, fertilization at planting consisted of 30 kg ha^{-1} of N from ammonium sulfate, 270 kg ha^{-1} of P_2O_5 from single superphosphate and 85 kg ha^{-1} of K_2O from potassium chloride, based on soil analysis. Three topdressing fertilizations were made with 180 kg ha^{-1} of N and 90 kg ha^{-1} of K_2O at the rate of 40% with 4 to 6 leaves; 40% with 8 to 10 leaves, and 20% with 12 leaves. The sowing of the bean was done in pits, soon after the emergence of the corn. The fertilization consisted of one kg of bovine manure, 20 kg ha^{-1} of N, 40 kg ha^{-1} of P_2O_5 and 30 kg ha^{-1} of K_2O, at the planting time. Weed control was performed with manual weeding during the crop cycle.

Table 1 – Lima beans landraces with their respective origin and seed color, evaluated in intercropping with corn in Teresina, Piauí State, Brazil, 2016.

Landrace	Origin	Seed color
Fava branca	Pedra Branca - CE	Branca
Fava branca	Riachão - MA	Branca
Fava branca	Riachão - MA	Branca
Fava branca	Nova Colina - MA	Branca
Boca de moça	Palmeirais - PI	Rajada (branco e marrom)
Fava branca	Picuí – PB	Branca
Cara larga	Remígio – PB	Rajada (branco e vermelho)
Rosinha	Remígio – PB	Rosa
Fava rosa	Areios – PE	Rosa
Fava branca	Surumbi – PE	Branca
Fava branca	Surumbi – PE	Rajada (branco e preto)
Fava branca	Maceió – AL	Branca

RESULTS AND DISCUSSION: The experiment was conducted in an area previously cultivated with lima bean. Sequence cultivation of this legume caused a favorable environment for the multiplication of soil fungus, Macrophomina phaseolina n Rhizoctonia solani, which caused death in the plants. The diseased plants had necrotic lesions on the roots, stems, branches and stems, reaching the pods. M. phaseolina is an important phytopathogenic fungus that infecting more than 500 plant species, including crops of economic importance such as soybeans, beans, corn, cotton, sunflower, peanut and castor bean (Gupta et al., 2012). In Brazil, this pathogen has caused damage to several cultivated species (Almeida et al., 2001). Seeds become the main source of dissemination in the most affected crops (Ndiaya, 2007) Cultural control can be done with early planting to close the canopy earlier, contributing to the reduction of soil temperature. In addition, dense populations, adequate levels of P and K, and soil water stress should be avoided. Chemical control is done by fungicides. R. solani fungi are classified as imperfect fungi that live on the soil saprophytically or exert parasitism on several annual or perennial crops, animals and other fungi present in this environment. The action of these fungi is related to the symptoms of seedling felling and root rot of plants. According to Goulart (2008), the control of R. solani is done through the combination of fungicides. In this experiment, even with the severity of these fungi, pods were harvested in the "Fava branca" of Riachão - MA, "Cara larga" of Remígio - PB, "Fava branca" and "Fava rajada" of Surubim - PE. Seed production in these landraces may indicate sources of resistance to M. phaseolina and R. solani.

REFERENCES

Almeida A.M.R. et al. 2001. *Macrophomina phaseolina* em soja: sistema de semeadura, sobrevivência em restos cultura e diversidade genética. Londrina: Embrapa Soja. 47 p.

Goulart A.C.P. 2008. Controle de tombamento de plântulas de algodoeiro causado por Rhzoctonia solani pelo tratamento de sementes com fungicidas. Dourados: Embrapa Agropecuária Oeste 31 p. Boletim de Pesquisa e Desenvolvimento.

Gupta G. K., Sharma S. K., RAMTEKE R. 2012. Biology, epidemiology and management of the pathogenic fungus *Macrophomina phaseolina* (Tassi) Goid with special reference to charcoal rot of soybean (Glycine max (L.) Merrill). Journal of Phytopathology 160: 167-180.

Neto Barreiro M. et al. 2015. Características morfológicas e produtivas em acessos de feijão fava consorciado. Tecnologia & Ciência Agropecuária 9:23 – 27.

Ndiaye M. 2007. Ecology and management of charcoal rot (*Macrophomina phaseolina*) on cowpea in the Sahel. PhD Thesis, Wageningen University, the Netherlands. 114pp.

HOST REACTION OF COMMON BEAN GENOTYPES TO ROOT-KNOT NEMATODES

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Common bean (*Phaseolus vulgaris*) have great economic and social importance worldwide and is the widely distributed crop in the world and the main food legume in Americas, especially in Brazil, Mexico and the USA (Sikora et al., 2005). Brazil is the second largest producer and is responsible to 15% from the world production with an annual mean consumption of 3.25 million ton (MAPA, 2013). However, the productivity is compromised due to pests, diseases and weeds that occur in the crop, with emphasis to phytonematodes.

In bean growing areas, yield losses caused by nematodes from genera *Meloidogyne*, especially *M. incognita* and *M. javanica*, have been frequently reported in Brazil (Inomoto, 2011) and can reach 50 to 90 % (Simão *et al.*, 2005). *Meloidogyne incognita, M. javanica* and *M. arenaria* are the most common root-knot species associated with *P. vulgaris* causing extensive losses in the Americas, Africa and Asia (Di Vito et al., 2005, Sikora et al., 2005; Sikora & Greco, 1990). Besides these species, *M. paranaensis* and *M. enterolobii* can also occur in the crop (Machado, 2011). Recently, novel root-knot species were detected in bean growing areas in Brazil, *M. inornata* (Machado *et al.*, 2013) and *M. luci* (Machado *et al.*, 2016).

Di Vito *et al*. (2004, 2007) reported losses in bean crops associated with *M. incognita* and *M. javanica* in the Americas, Europe, Africa and Asia. In Brazil, damage reports are lacking, but the majority of bean growing areas show favorable conditions to nematode multiplication, as sand soils, well drained and with mean temperatures of 25 to 30 ^{o}C (Agrofit, 2010). The occurrence of root-knot nematodes in bean crops causes typical symptoms in the root system of plants, the galls, besides the underdevelopment of plants that shows abnormal pigmentation of leaves, similar to nutritional deficiencies (Agrofit, 2010).

The management of phytonematodes is based mainly in the use of resistant varieties, crop rotation and nematicides (Ferraz *et al*., 2010). However, as there are no nematicides registered for bean in Brazil, the use of resistant genotypes has a great importance. Resistance is an efficient tool to manage nematodes which improve the yields in infested fields, does not increase the total costs for production and is environmentally friendly.

The development of resistant cultivars to root-knot nematodes is extremely important for the sustainability of the bean production chain. Due this, IAPAR has been included this trait in his bean breeding program for several years. Annually, at least 40 genotypes developed by the breeding program are tested to resistance to the root-knot nematodes *M. incognita, M. javanica* and *M. paranaensis*, the most important phytonematodes in our conditions.

Genotypes are selected by their yield and grain qualities and sent to the Nematology Laboratory to procced the tests. Plants are cultivated under greenhouse controlled conditions and inoculated with nematodes. Evaluations taking into account the reproduction of nematodes, based on the reproduction factor (RF), and the number of nematodes per gram of roots. The more resistant genotypes are tested twice more, in order to ensure the characteristic and to maintain the quality for management recommendations.

Results obtained until now showed that all genotypes tested to *M. incognita* and *M. javanica* were susceptible to both nematodes, although great phenotypic variation was observed. This variation is highly desirable in the breeding program, since genotypes can be used as

progenitors to develop more resistant genotypes. For *M. paranaensis*, several lineages were considered resistant in the tests, which can be released as resistant cultivars.

Unfortunately, more efforts are necessary in order to select and develop resistant cultivars to the most important nematodes. The germplasm bank under the hold of IAPAR will be tested looking for resistant genotypes that could be used as progenitors in the breeding program. Besides, the change of genotypes among different countries and germplasm banks could improve the search for resistant genotypes. All these efforts are needed to better manage the phytonematodes present in agricultural areas and to ensure high yield quality to growers.

REFERENCES

AGROFIT. Manejo de doenças do feijoeiro comum. 2010. Available at http://extranet.agricultura.gov.br/agrofit_cons/principal_agrofit_cons. Accessed 10 August 2013.

DI VITO, M.; PARISI, B.; CATALANO, F. Effect of population densities of *Meloidogyne incognita* on common bean. Nematologia Mediterranea, 32: 81-85, 2004.

DI VITO, M.; PARISI, B.; CATALANO, F. Pathogenicity of *Meloidogyne javanica* on common bean (*Phaseolus vulgaris* L.) in pots. Nematropica, 37: 339-344, 2007.

FERRAZ, S.; FREITAS, L.G.; LOPES, E.A.; DIAS-ARIEIRA, C.R. Manejo sustentável de nematoides. Viçosa: UFV, 2010. 306 pp.

INOMOTO, M.M. 2011. Nematoides do gênero Pratylenchus em algodoeiro e feijoeiro no Brasil. In: CONGRESSO BRASILEIRO DE NEMATOLOGIA, XXIX, Brasília. Resumos, p.147-149.

MACHADO, A.C.Z. Nematoides em feijão: perdas de 10% podem chegar a 50%. Portal do agronegócio, 2011. Available at http://nematologia.com.br/2011/12/nematoides-em-feijao-perdas-de-10-podemchegar-a-50/. Accessed 10/08/2013.

MACHADO, A.C.Z.; DORIGO, O.F.; MATTEI, D. First report of the root knot nematode *Meloidogyne inornata* parasitizing common bean in Paraná State, Brazil. Plant Disease, 97: 431, 2013.

MACHADO, A.C.Z.; DORIGO, O.F.; CARNEIRO, R.M.D.G.; ARAÚJO FILHO, J.V. *Meloidogyne luci*, a new infecting nematode species on common bean fields at Paraná State, Brazil. Helminthologia, 53: 207-210, 2016.

MAPA. Ministério da Agricultura, Pecuária e Abastecimento. Brasil: projeções do agronegócio 2011/2012 a 2021/2022. Brasília, DF, pp. 1–50, 2013. Available at http://www.agricultura.gov.br/vegetal/culturas/feijao.

SIKORA, R.A., GRECO, N., SILVA, J.F.V. Nematode parasites of food legumes. In: LUC, M., SIKORA, R.A., BRIDGE, J. (Eds) Plant parasitic nematodes in subtropical and tropical agriculture. CAB Publishing, Wallingford, USA, pp. 259 – 318, 2005.

SIKORA R.A.; GRECO N. Nematode parasites of food legumes. Pp. 181-235. In: Plant Parasitic Nematodes in Subtropical and Tropical Agriculture (Luc M., Sikora R.A. and Bridge J. eds). CAB International, Wallingford, U.K, 1990.

SIMÃO, G.; HOMECHIN, M.; SANTIAGO, D.C.; SILVA, R.T.V.; RIBEIRO, E.R. Comportamento de duas cultivares de feijoeiro em relação à *Meloidogyne javanica*. Ciência Rural, 35:266- 270, 2005.

ANALYSIS OF PHASEOLIN GENE COPY NUMBER BY QUANTITATIVE PCR

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INTRODUCTION: The 7S globulin phaseolin is the most abundant seed protein in common bean (*Phaseolus vulgaris*) accounting for close to 50% of total seed protein in cultivated varieties. Two different types of phaseolin genes encode two phaseolin subtypes, α- and β-. Their coding sequence is distinguished by the presence of a 27 bp repeat in α-phaseolin (Slightom et al. 1985; Kami and Gepts 1994). The α- and β-phaseolin genes are more dissimilar in their promoters and 5'-untranslated regions (Slightom et al. 1983; Anthony et al. 1990). Phaseolins are encoded by a single complex locus in the genome, predicted to contain multiple genes in tandem. Southern hybridization studies showed that the phaseolin multigene family consists of approximately seven members (Talbot et al. 1984). It is notably difficult to assemble next generation sequencing read data for loci with highly repetitive gene sequences such as those of storage protein genes (Dong et al. 2016). The first assembly of the reference genome G19833 contained unique copies of α- and β-phaseolin on chromosome 7, on opposite strands approximately 120 kb apart (Schmutz et al. 2014). A newly released version (v2.1) contains three α- and two β-phaseolin genes (Fig. 1). We used the quantitative polymerase chain reaction (qPCR) according to Ingham et al. (2001), in the genetic stock SARC1 which contains both α- and β-phaseolin (Osborn et al. 2003), to determine the number of copies of these genes.

MATERIALS AND METHODS: Genomic DNA was extracted from leaf tissue using a QIAGEN DNeasy Plant Mini Kit (Toronto, ON). DNA samples were quantified using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Burlington, ON), and quality evaluated from A_{260}/A_{280} ratio and by agarose gel electrophoresis. Gene-specific primers were designed using Primer-3 (Rozen and Skaletsky 2000). Primers were designed for single copy genes as follows: for β-cyanoalanine synthase (Phvul.008g06110.1), qBSAS3.1-F23, 5'-GCATCATCCTCAAGCAAAGGT-3'; and qBSAS3.1-R172, 5'-CAGGGAGGTCCTTAGGCAAC-3'; for lipoxygenase (Phvul.005g15700.1), qLox-F19, 5'-GGTCTCATCAATAGGGGCCA-3'; and qLox-R117, 5'-ACGACCTAAGAAGGCAGTGA-3'; for phytoene desaturase (Phvul.001g264200.1), qPDS-F141, 5'-CGACCCACTTCATTTCGTGC-3' and qPDS-R306, 5'-CTTCAGGGGTTGTAGCGGAC-3'. For phaseolin, a primer pair amplified both phaseolin genes: qPhs-F66, 5'-ATTTGCCACTTCACTCCGGG-3' and qPhs-R246, 5'-CCTGAACTCCACAAGACGGT-3'; a second one was specific for β-phaseolin: qβPhsF, 5'-CCTTTCTTGGTATGTAAGTCCG-3' and qβPhsR-372, 5'-GCCAGTGACTAATTTGAGTTGGTTG-3'. Quantitative PCR was performed with SsoFast EvaGreen Supermix using a CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Mississauga, ON). Reactions contained 15 ng of DNA and primers at a concentration of 0.5 µM. They were carried out in Hard-Shell 96-well clear PCR plates (Bio-Rad) in a final volume of 15 µl. In the same plate, controls without template were performed in duplicate. The PCR program consisted of an initial step of 3 min at 95°C, followed by 40 cycles of 15 seconds at 95°C and 30 seconds at 53.0 to 63.0°C. Reactions were run at different annealing temperatures as follows: 53.0, 53.7, 55.0, 56.9, 59.4, 61.3, 62.5 and 63.0°C. Data were expressed as the cycle number necessary to reach a threshold fluorescence value (C_q). The reported values are the means of two biological replicates consisting of independent DNA

extracts, with each biological replicate the average of three technical replicates. The specificity of primer pairs was confirmed by melt curve analysis in comparison with controls without template, and by agarose gel electrophoresis of PCR products. PCR efficiency was calculated from a standard curve of C_q versus the logarithm of starting template quantity. Each assay was optimized so that the efficiency ranged between 95% and 105%, with a coefficient of determination (R^2) > 0.99.

RESULTS: The C_q of *phaseolin* was low compared to the single copy genes (*BSAS3.1*, *PDS* and *Lox*) at any temperature of annealing (T_a) tested. The average C_q for single copy genes was similar (ca. 22.5 cycles). The value was intermediate for β-phaseolin (20 cycles), and lower for the non-specific phaseolin primers (19). Based on exponential accumulation, the copy number of phaseolin genes is estimated to be equal to eight, with four copies of β-phaseolin and four copies of α-phaseolin genes. The present results are consistent with the above noted hybridization results of Talbot et al. (1984). A possible reason to explain the discrepancy between the number of genes in the genome assembly and that measured by empirical methods may be the presence of additional, non-functional gene copies, which may not be annotated in the reference genome.

REFERENCES
Anthony JL, Vonder Haar RA et al. (1990) Nucleotide sequence of an α-phaseolin gene from *Phaseolus vulgaris*. Nucleic Acids Res 18:3396.
Dong J, Feng Y et al. (2016) Analysis of tandem gene copies in maize chromosomal regions reconstructed from long sequence reads. Proc Natl Acad Sci U S A 113:7949-7956.
Ingham DJ, Beer S et al. (2001) Quantitative real-time PCR assay for determining transgene copy number in transformed plants. BioTechniques 31:132-140.
Kami JA, Gepts P (1994) Phaseolin nucleotide sequence diversity in *Phaseolus*. I.Intraspecific diversity in *Phaseolus vulgaris*. Genome 37:751-757.
Osborn TC, Hartweck LM et al. (2003) Registration of *Phaseolus vulgaris* genetic stocks with altered seed protein compositions. Crop Sci 43:1570-1571.
Rozen S, Skaletsky H (2000) Primer3 on the WWW for general users and for biologist programmers. Methods Mol Biol 132:365-386.
Schmutz J, McClean PE et al. (2014) A reference genome for common bean and genome-wide analysis of dual domestications. Nat Genet 46:707-713.
Slightom JL, Drong RF et al. (1985) Nucleotide sequences from phaseolin cDNA clones: the major storage proteins from *Phaseolus vulgaris* are encoded by two unique gene families. Nucleic Acids Res 13:6483-6498.
Slightom JL, Sun SM et al. (1983) Complete nucleotide sequence of a French bean storage protein gene: phaseolin. Proc Natl Acad Sci USA 80:1897-1901.
Talbot DR, Adang MJ et al. (1984) Size and organization of a multigene family encoding phaseolin, the major seed storage protein of *Phaseolus vulgaris* L. Mol Gen Genet 198:42-49.

INTROGRESSION OF A POWDERY MILDEW RESISTANCE GENE INTO MARKET CLASS FABADA

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Powdery mildew (PM) is being one of the most serious diseases affecting bean crops in the north of Spain. Two independent resistance genes were reported in the cultivar Porrillo Sintetico, one of them located on linkage group (LG) Pv11, conferring partial resistance, and other located on LG Pv04 conferring a total resistance response (Campa and Ferreira 2017). The objective of this work was the introgression of the gene conferring total resistance from Porrillo Sintetico into the line X2776. Genomic regions introgressed after the breeding program were studied using 'genotyping by sequencing' (GBS).

MATERIAL AND METHODS

X2776 is a breeding line developed at the SERIDA having fabada seed phenotype (white and very large seeds), determinate growth habit (*finfin*) and two introgressed resistance locus against virus (*I* gene) and anthracnose (*Co-2* cluster) (Ferreira et al. 2016). The X2776 line shows a partial resistance response against PM.

Total resistance in X2776 was introgressed using a backcrossing method including six backcross generations followed by four self-pollinated generations with individual plant selection using resistance tests. Seed and plant phenotype were also considered in the selection process of each generation. Resistance tests were carried out according to Trabanco et al. (2012) using a local isolate of PM maintained on plants of susceptible bean cv. Xana in spore-proof chambers.

The parental lines X2776, Porrillo Sintetico and four resistant plants derived from F_4BC_6 families were genotyped through GBS using the *ApeKI* restriction enzyme. Physical positions of the SNP markers were established using the *Phaseolus vulgaris* genome v1 (GenBank Assembly accession GCF_000499855.1).

RESULTS AND DISCUSSION

Four homozygous resistant plants against PM (4562-1, 4562-2, 4562-3, and 4562-4) having white and large seeds (fabada seed phenotype) were obtained in the breeding process. GBS analysis revealed a total of 8763 polymorphic SNPs between the parental lines, which were distributed along the eleven bean chromosomes. The average number of SNPs per chromosome was 796.2 with a minimum of 290 SNPs in chromosome Pv10 and a maximum of 1214 SNPs in chromosome Pv02. The four resistant plants showed the genotype of the parental line X2776 for all the SNPs except for a maximum of 36 SNPs on chromosome Pv04 that showed the Porrillo Sintetico allele (Table 1). These 36 SNPs tag a region between 91,481 and 2,338,026 bp of chromosome Pv04. Individuals 4562-3 and 4532-4 showed the most extensive introgressed region. Individual 4562-1 had a high level of heterozygosity, from 235,860 to 2,338,026 bp, suggesting that this chromosome region is not involved in the resistance. The four individuals showed a common introgressed region between 91,481- 226,281 bp which should contain the gene or genes responsible of the total resistance to PM. Molecular markers that tag this introgressed genomic region can be useful for marker assisted selection.

This result agrees with the genetic analysis conducted in the $F_{2:3}$ population X2776 x Porrillo Sintetico in which a single gene conferring total resistance was identified in Porrillo Sintetico, in a physical position between 84,202 bp and 218,664 bp of chromosome Pv04 (Campa and Ferreira 2017).

Table 1. Genomic profile of four F$_4$BC$_6$ homozygous resistant individuals and the parental lines X2776 (recurrent) and Porrillo Sintetico (donor) for 36 SNPs located on chromosome Pv04. The tag sequence and the physical position of each SNP are indicated. -: missing value. R, Y, S, W, K, and M: heterozygous

SNP physical position (bp)	Tag sequence	X2776	Porrillo S	4562-1	4562-2	4562-3	4562-4
				F$_4$BC$_6$ individuals			
91481	CAGCAGGCTAGGGTGAATATT(A/C)ACTGTTCCTGAGGTATTTGCAAATGAAAAGGTGTCACAGAAG	C	A	A	A	A	A
91507	CAGCAGGCTAGGGTGAATATTAACTGTTCCTGAGGTATTT(G/A)CAAATGGAAAGGTGTCACAGAGG	G	A	A	A	A	A
91522	CAGCAGGCTAGGGTGAATATTAACTGTTCCTGAGGTATTTGCAAATGAAAAGGTGTCACAG(A/G)AG	G	A	A	A	A	A
95873	CTGCAAAACGTGTTTGAGCTTTGGTACGGCGGAATAGTTCGTATTA(C/T)TGTTCTGCGTCGGAGAA	T	C	C	C	C	C
100292	CTGCCCTAACTATTA(C/T)GAGATCATTATTTGTAATGTGATTTATTCTTTAACTCGTGTATTTGTA	T	C	C	C	C	C
226281	CAGCCTTAACACTACT(A/T)CCTTGCACTACTTCGTTCCTTATGCTGTTGGAGCTTCAGCAAGGTTT	T	A	A	A	A	A
235860	CTGC(A/T)GTCACGGCTTCTAGCTTGGATTTCGCTCTCAACACCTTGTAACTCAAGTTTCTAACCTT	T	A	W	T	A	A
235905	CTGCAGTCACGGCTTCTAGCTTGGATTTCGCTCTCAACACCTTGTAAC(T/C)CAAGTTTCTAACCTT	T	C	Y	T	C	C
262296	CTGCATGACGTTGT(G/C)AATGACTTCAGTTCTTGACCAAAACTTTGTTTCCTCTTACCATGCAGAA	G	C	S	G	C	C
262491	CAGCGAATTTGAACCATAATAAAAAACTGTCTTATTTTGTTACATAGAAACTAAAATAAGAAG(C/G)	G	C	S	G	C	C
365640	CTGCACAGAAAACAAATCCACTCCCAAGTTAATT(C/T)ATTGCTCAAATACCTTACATGCATCACAG	T	C	Y	T	C	C
514500	CAGCCGGACTGCATCAAACCGTC(G/A)TGCTTCACGCCGCGCTTTTTATCCGGAAAATCCAAGAAAG	G	A	R	G	A	A
564897	CTGCTTTCGCATGCTAGTTGGCTGGCAAAGGATATTTATTTGG(C/T)TTGTGGCTGATTCACAATATG	C	T	C	C	T	T
565024	CAGCACTTTTG(G/A)TGGACTTGGACAGGGTCTTTATTTGGATGGAAAGTTAAATGGTGATTTTAGA	G	A	R	G	A	A
592704	CTGCAGTTGTCTTCAGAAAACATCAACAACACCAAAC(A/T)ATGTCGAGCAAAGACTCAAAGGTGAA	A	T	W	A	T	T
592710	CTGCAGTTGTCTTCAGAAAACATCAACAAC(A/G)CCAAACAATGTCGAGCAAAGACTCAAAGGTGAA	A	G	R	A	G	G
1058766	CTGCAA(C/G)TAGCTGGGGACGCATTGGTGGATGATGATGAATTACAGGACCTTTCAGAGAAGGAAG	G	C	S	G	C	C
1104710	CAGCACGCGCCGCCTTC(A/G)AGCGACTTCCCGTTCTCAATCCCATCGTCGAAATGGACGGTAACTT	G	A	R	G	A	A
1143859	CAGCTGAG(G/C)TCGATGGTCTCTCATACTGGGTGACATTGGTCTCGGGATTCCAATAGTAAAGATA	C	G	S	C	G	G
1143891	CAGCTGAGGTCGATGGTCTCTCATACTGGGTGACATTGGT(C/A)TCGGGATTCCAATAGTAAAGATA	A	C	M	A	C	C
1216747	CAGCAACTGAA(C/T)TTGTATGTGATGCTTTAACTTTTCTGGATACTTCAAGGGATTCTGCTTCAAT	C	T	Y	C	T	T
1216789	CTGCGGTTCTGCAATATCCTCCAGTTTATATGGG(T/G)AGGCTTTTTGACAGCTCAAATTACCAAAC	T	G	K	T	-	G
1216801	(C/T)AGCTCAAATTACCAAACCGAGGAAGATGATGGGAAAAAGGTTATTGATCCAAAGAAAATCCAG	C	T	Y	C	-	T
1485452	CAGCACCA(G/A)ATACAAGATTTATCAACACAAAACAAAAAATAAAAGACATTTTTAGATGGTACAA	G	A	R	G	A	A
1485455	CAGCA(C/T)CAGATACAAGATTTATCAACACAAAACAAAAAATAAAAGACATTTTTAGATGGTACAA	C	T	Y	C	T	T
1826541	CTGCCAATGAAATCAAAAAATCCAATAAATCA(T/C)CCTCGGGAGAACAAAGAGATGGGAAATTCTC	T	C	Y	T	C	C
1838570	CTGCGG(A/C)GGCGGCGGCGCGTTGAGGTTGCGTTCGCCGCCGGTAGAATCGGAGAGCTCCATGATC	C	A	M	N	A	A
1854518	CTGCATCAAAGAT(A/G)TCAGAGCAGTTGATCCATACTGTTCCAACTCGCAATGCCCGCATCAAGGT	G	A	-	G	A	A
1919879	CAGC(A/G)TAGTTGGAGCCATAATAGTATCAATTGGCCTTTATGTTGTATTGTGGGGCAAGGCAACA	A	G	R	A	G	G
1919890	CAGCATAGTTGGAGC(C/T)ATAATAGTATCAATTGGCCTTTATGTTGTATTGTGGGGCAAGGCAACA	C	T	Y	C	T	T
2121101	CTGCGTTGTCCAGAAAGTGTTTGTGGAAATGCTCACTCACTAAAT(C/T)CTGAAATTCCGAGTTTTT	C	T	Y	C	T	T
2138790	CTGC(C/T)TTAACAGTCGGTCAAATCAGTAATACAATCAATATAAAACTGATAGTGGATCTTTGAAT	T	C	Y	T	C	C
2138801	CTGCCTTAACAGTCG(G/A)TCAAATCAGTAATACAATCAATATAAAACTGATAGTGGATCTTTGAAT	A	G	R	A	G	G
2199473	CTGCATTAAAAT(A/G)TCGCTTCCCTGTAATCATTTGAAGGACCATGTTGAATATCAGATGAGAAAA	A	G	R	A	G	G
2338021	CTGCCTTTTAATGATTTTTTCTTTTTCCGTTTTTGCAGATTACTGTTATATTACAA(T/C)ATCATGG	T	C	Y	T	C	C
2338026	CTGCCTTTTAATGATTTTTTCTTTTTCCGTTTTTGCAGATTACTGTTATAT(T/C)ACAATATCATGG	C	T	Y	C	T	T

The new breeding line was obtained from individual 4562-2 after two more self-pollinated generations (F$_6$Bc$_6$). This new line (X4562) has fabada seed phenotype, determinate growth habit (*finfin*), resistance against virus (*I* gene) and anthracnose (*Co-2* cluster) like the recurrent parental line X2776, and it has incorporated a gene conferring total resistance against PM derived from the donor line Porrillo Sintetico.

REFERENCES

Campa A, Ferreira JJ. 2017. Theor Applied Genet, in press

Ferreira JJ, Murube E, Campa A. 2016. The Plant Genome, doi:10.3835/plantgenome2016.08.0081

Trabanco, Pérez-Vega E, Campa A, Rubiales D, Ferreira JJ. 2012. Euphytica 86:875–882

THE RELATIVE IMPORTANCE OF THE CHARACTERS IN THE STUDY OF THE GENETIC DIVERSITY OF COMMON BEAN

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INTRODUCTION

The genetic diversity present in local and improved bean cultivars allows exploring an existing variability already adapted to the specific climatic conditions, contributing a lot to breeding programs. Morphoagronomic characteristics are considered of great importance, since they allow the direct evaluation of agronomic interest characters. The analyze of the relative importance of the characters makes it possible to discard characteristics that contribute less to the discrimination of the evaluated materials, reducing costs and labor in the next experiments, being that the minor importance characteristics may be those that show less variability or that are represented by other one (Cruz, Regazzi e Carneiro, 2012). The present work had the objective of accessing the genetic variability of common bean cultivars and identify the characteristics of greater relative importance.

MATERIAL AND METHODS

Thirty-nine bean cultivars were used for the characterization, 20 belonging to the carioca commercial group and 19 to the black commercial group (tests were performed independently for each commercial group). All cultivars come from breeding programs of public or private institutions from Brazil. The trials were established in four environments in the state of Paraná-BR, two in the 2014/2015 rainy season, in Ponta Grossa and Guarapuava, and two in the dry season of 2015, in Ponta Grossa and Santa Tereza do Oeste. The experimental design was a randomized complete block with three replicates and plots consisting of four rows of 4 meters spaced 0.5 m, with a population of 12 plants per linear meter, considering the two central lines as a useful plot. The quantitative descriptors evaluated are on Table 1. The evaluations for the descriptors were carried out in a sample of ten plants of each experimental plot (except PROD), and for the statistical analysis the average of each plot was used. The relative importance of the quantitative variables studied was analyzed by the Singh method (Singh, 1981) using the Genes computational program (Cruz, 2013).

Table 1. Characteristics evaluated.

Abbreviation	Quantitative characteristics
LPL	Length of primary leaf
WPL	Width of the primary leaf
PLI	Primary leaf index
LCL	Length of central leaflet
WCL	Width of the central leaflet
CLI	Central leaflet index
LMS	Length of the main stem
IFP	Insertion of the first pod
NN	Number of stem nodes
LP	Length of the pod
SPOD	Number of seeds per pod
LP	Number of locules per pod
PP	Number of pods per plant
SP	Number of seeds per plant
TMS	Thickness of the main stem
LS	Length of seed
SW	Seed width
ST	Seed Thickness
WSP	Total weight of seeds in the plant
W1000	Weight of 1000 seeds
COEF J	Coefficient J=length/width (seed)
COEF H	Coefficient H=thickness/width (seed)
PROD	Production

RESULTS AND DISCUSSION

The characteristics that had greater relative importance according to the method proposed by Singh method in the carioca group were LMS, LS, LPL, WPL and WCL with 12.23, 11.24, 10.90, 10.11 e 9.05%, respectively (Figure 1). For the black group the most important were SW, COEF J, LP and ST with 24.70, 17.44, 11.39 e 10.60%, respectively.
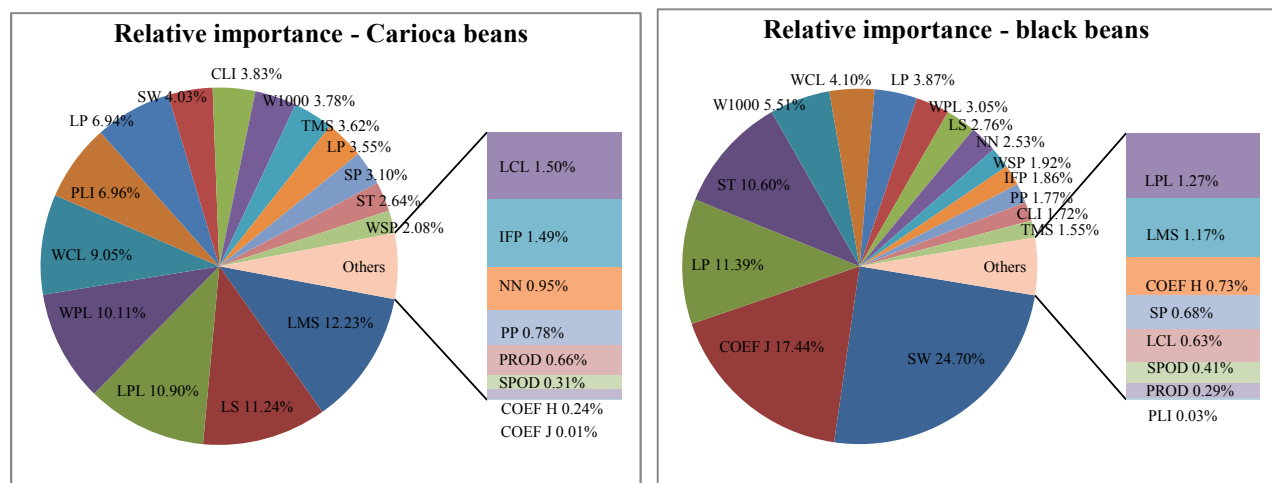
Figure 1 – Relative importance of the characters for carioca beans and black beans group.

The LMS characteristic was the most important variable in the Carioca group, probably due to the presence of plants of determined habit (Type I) among the studied cultivars, providing greater variability for this characteristic. In the black commercial group, the characteristics related to seed size were the most important, and have often been used to distinguish cultivars. Hegay et al., (2014) observed that the size of the seed was the characteristic that most contributed in the distinguishing of the genotypes studied by them. The PROD had low discriminant power in both groups, despite being an important commercial character. An explanation for this result is that the productivity averages did not show much variation. Other characteristics classified as not important also presented similar values between the cultivars evaluated or were correlated with characteristics of greater importance. It can be observed that few characteristics were more important, however these more important characteristics are not the same for the two commercial groups. The most important variables for the genetic divergence of cultivars of the black group are related to the seed, while in the carioca group the most important characteristics are related to the plant morphology. These results show that the most important characteristics for genetic divergence in one group of cultivars are not the same when evaluated in another group, suggesting that the results obtained cannot be extrapolated to different groups.

REFERENCES

CRUZ, C.D. Genes - a software package for analysis in experimental statistics and quantitative genetics. Acta Scientiarum, Maringá, v.35, n.3, p.271-276, 2013.

CRUZ, C. D.; REGAZZI, A. J.; CARNEIRO, P. C. S. Modelos biométricos aplicados ao melhoramento genético. 4.ed. Viçosa: UFV, 2012, V. 1, 514p.

HEGAY, S. et al. Genetic diversity analysis in *Phaseolus vulgaris* L. using morphological traits. Genetic Resource and Crop Evolution, Gatersleben, v. 61, p. 555-566, 2014.

SINGH, D. The relative importance of characters affecting genetic divergence. Indian Journal of Genetic and Plant Breeding, New Delhi, v. 41, p.237-245, 1981.

STRATEGIES FOR USE OF GERMPLASM COLLECTIONS OF BEANS – THE CASE OF *PHASEOLUS VULGARIS* AT EMBRAPA TEMPERATE CLIMATE

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INTRODUCTION

The genetic resources of plants have historically constituted the basis for the development of world agriculture comprising in such way the instrument of greater significance to the food safety of human populations. Germplasm collections make up the building for the conservation of these resources, realizing from the more traditional forms, such as are found at the level farmer's production units, where are found the guardians of seeds, up to the most complex and institutional germplasm banks as of national and international organizations. The latter have significant numbers of entries obtained through specific expeditions for collection or through donations made by farmers, predominantly guardians of seeds. The International Centre for Tropical Agriculture-CIAT, located in Colombia, for example, has more than 36,000 entries. The uses that are given to material stored in Germplasm Banks are usually limited to the development of new cultivars in breeding programs, meaning that an extremely low number of accesses has been exploited. The present work presents the structure of the common bean (*Phaseolus vulgaris* L.) collection at Embrapa Clima Temperado and reveals adopted strategies aiming to dynamize the use of this collection.

MATERIAL AND METHODS

The current collection of common bean (*Phaseolus vulgaris* L.) from Embrapa Clima Temperado began to be constituted in 1987, when the program of genetic improvement of this species in Embrapa at Pelotas, Rio Grande do Sul State, was reactivated. As from that year, the number of introductions at the end of 2016 reached the number of 868 coming from donations from farmers, technicians from the rural extension area and technical assistance, collection expeditions and donations from other professionals related to agriculture. Among these accesses, 700 are considered as landraces. It is to be considered that in this number, the sources of germplasm developed in research institutions are not included.

In order to make the use of existing germplasm more dynamic, strategies were developed that reach different dimensions of the agricultural research universe.

The first dimension is the use in the breeding program. Landraces have been integrated, through artificial crosses with germplasm of the breeding program, in the development of new cultivars according to favorable characteristics found in them. The second dimension is the use of a participatory breeding program that includes three different approaches: 1. Selection by farmers of individual plants in segregating populations, in loco, that is, in the farmer's production unit. This approach means that the selections that the farmer performs are tested in later stages until the identification or not of a population adapted to that environment. 2. The second approach involves the submission of a collection of cultivars developed by research

organizations and registered for cultivation with the Agriculture Ministry, to the selection by the farmers in a process identified as Common Bean Demonstration Units System (SUDF) (Villela et al., 2016). 3. The third approach is the submission of a collection of landraces to farmers' selection, a process identified as a Biodiversity Score - PBio (Antunes and Bevilaqua, 2009). Both the Demonstration Units and PBios consist of four-rows of 4m plots of each material and farmers are allowed to keep some of the seeds of the selections they make.

RESULTS AND DISCUSSION

The adoption of the reported strategies resulted in the development of *Phaseolus vulgaris* Genetic Pools I and II within the Embrapa Temperate Climate breeding program, which, under participatory breeding programs, generated seven cultivars for the farmers' own use, which later entered into seed exchange fairs from which they were disseminated. Simultaneously, individual plant selections conducted in the Embrapa breeding program generated breeding lines that have participated in the agronomic evaluation phases.

From the SUDF, started in 1990/91, more than 2,000 UDs were submitted, and 90% of the farmers who participated in the SUDF selected for own use at least one of the cultivars that integrated the System.

In relation to the PBios, more than 200 PBios were conducted by farmers and by this process about 140 landraces were distributed, allowing an increase of the genetic diversity of the bean crop, with this reducing its vulnerability.

Briefly, the use of the bean germplasm collection according to the strategies pointed out, led to a greater dynamism of the existing collection.

REFERENCES

VILLELA, A. T.; ANTUNES, I. F.; PIANA, C. F. de BRUM; SILVA, J. G. C. da; BEVILAQUA, G. A. P. The Common Bean (*Phaseolus vulgaris* L.) Demonstration Unity System – SUDF – Applicability potential for family farming. Annual Report of The Bean Improvement Cooperative. v. 57, p.165-166. 2016.

ANTUNES I. F.; BEVILAQUA G. A. P. Partituras de Biodiversidade – PBio – Uma nova alternativa para ampliar a base genética de espécies cultivadas e promover a segurança alimentar. SIMPOSIO DE RECURSOS GENETICOS PARA AMÉRICA LATINA Y EL CARIBE, SIRGEALC, 7, 2009. 1 CD-ROM., 2009.

COMMON BEAN (*Phaseolus vulgaris* L.) CULTIVAR EVALUATION BY FAMILY FARMERS

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INTRODUCTION

In Rio Grande do Sul (RS) State, in Southern Brazil, family farming is responsible for about 70% of common bean production (Villela et al., 2016). Common bean production in the State can be referred to according to the twelve Emater, the official extension institution, administrative regions. In order to get common bean farmers acquainted with new released cultivars, in the early 90s, the Embrapa Temperate Climate with the contribution of Emater put in place the Common Bean Demonstration Units System – SUDF (Villela et al., 2016). Simultaneously, the SUDF had as additional goal to minimize the effects of genotype x environment interaction, since the evaluation was carried out at the farmers' fields, from which the most suitable cultivars could be selected.

This paper, which comprehends the results from experiments conducted at Emater's Pelotas region and sums up to the results obtained from the experiments developed at the Ematers' Soledade region in the State of Rio Grande do Sul, contributes to the understanding of the behavior of the tested cultivars under different environmental conditions under farmers' management, according to their adaptation to these conditions.

MATERIAL AND METHODS

Methodology follows the description by Villela et al.(2016), where the Demonstration Unities (UD) were composed of seventeen cultivars already recommended by research institutions located in Southern Brazil, as well as by the cultivar in use by the farmer, as check. In the region of Pelotas, located in the South of Rio Grande do Sul, 31.7654° S, 52.3376°, the Emater's administrative region comprises 21 municipal offices, from which 14 carried out 49 UDs. The testing period spread from 1993/94 to 2011/12. UD's, for the most part, were installed in properties of farmers selected by Emater / RS employees. Statistical analysis involved the analysis of variance for the variable grain yield and the Dunnett's test mean comparison having the farmer's cultivar as term of comparison. For Pelotas' region, fourteen of the seventeen cultivars presented the required amount of data for statistical analysis.

RESULTS AND DISCUSSION

As shown in Table 1 for the fourteen SUDF cultivars tested, no significantly differences were detected for the cultivars and the farmer's cultivar (check) comparison. Since the Embrapa Temperate Climate headquarters is located at Pelotas, it might be thought that farmers had the opportunity of adoption of the new cultivars at a high speed rate, what would result in one of the UD's cultivars becoming a check cultivar (farmer's cultivar), for which the differences in yield would appear of low magnitude. At the same time, it can be seen that the cultivars with seed coat colors that no black namely Carioca, Iraí, Iapar 31 and Pérola, can be adopted by famers with no significant yield losses as compared to the black seeded ones.

Table 1. Mean grain yield (kg.ha^{-1}) and releasing year of SUDF cultivars in comparison to farmer's cultivar. Emater /RS' Pelotas region, RS, Brazil.

Cultivar	Grain yield (kg.ha^{-1})	Releasing year
Farmer's cultivar (check)	1,738.3	variable
Rio Tibagi	1,724.0	1976
Carioca+	1,811.1	1976
Guateian 6662	1,973.2	1979
Iraí+	1,869.1	1981
Macanudo	1,962.3	1989
FT 120	2,017.4	1989
Minuano	1,943.1	1991
Iapar 31+	1,835.6	1994
Macotaço	1,867.7	1994
Iapar 44	1,553.7	1994
Guapo Brilhante	1,689.3	1995
FT Nobre	1,803.1	1996
Pérola+	1,854.5	1999
Diamante Negro	1,414.9	1999

* Cultivar differs from the check by Dunnett's test at α=0,10.
+ Cultivar with no black seed coat

REFERENCES

Villela, A.T.; Antunes, I. F.; Piana, C. F. de Brum; Silva, J. G. C. da; Bevilaqua, G. A. P. The Common Bean (*Phaseolus vulgaris* L.) Demonstration Unity System –SUDF – Applicability potential for family farming. Annual Report of the Bean Improvement Cooperative, v.59, p.165-166, 2016.

SEED MANAGEMENT SYSTEMS OF *Phaseolus* USED BY FARMERS FROM OAXACA, MÉXICO

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The management and conservation of landraces and wild germplasm of *Phaseolus* have influenced the diversity levels reported in previous studies (Worthington *et al*., 2012; Soleri *et al*., 2013; Chávez-Servia *et al*., 2016). Oaxaca is located at southern Mexico and it is a complex state due its accidental geography and variable climatic composition influenced by the Gulf of México and the Pacific Ocean (García-Mendoza *et al*., 2004). The state of Oaxaca is divided into eight ethno-cultural regions (INEGI, 2017; http://www.inegi.org.mx) clearly differentiated by the presence of ethno-linguistic groups and a wide variety of landscapes, weather, wildlife and vegetation. The main linguistics families in Oaxaca are 'Zapotecos', 'Mixtecos', 'Chinantecos', 'Mixes', 'Triquis', and 'Mazatecos'; they summarize sixteen different groups each one divided in linguistic variations (Ordóñez, 2000). Agriculture is the leading activity of these ethnical groups and they maintain landraces that have been inherited (Espinosa-Pérez *et al*., 2014) by his ancestors including the involved knowledge for the conservation and agronomic management. The aim of this work was to analyze and characterize seed management systems by farmers from Oaxaca, México

Surveys (398) were conducted from October to November, 2016 into 10 communities from Oaxaca: two communities from 'Valles Centrales' (Zapotecos) region; six from 'Sierra Norte' (four of them from Chinantecos and two Mixes); and two communities at 'Mixteca' (Mixtecos). Survey included 18 questions divided into five sections: 1 (3 questions; no. of landraces and traits for identification among populations); 2 (5, agronomic management); 3 (3, utilization and preferences); 4 (5, management, conservation and seed provisioning); 5 (1, if it's a threatened genotype or accession).

This work detected 76 different local names to *Phaseolus* germplasm throughout three regions and four ethnic groups from Oaxaca. Farmers from Valles Centrales are the older bean producers (56 years-old), while chinantecos showed 53 years, mixes 52 and mixtecos 47. The most common bean local names were 'frijol delgado' (thin bean), frijol 'de milpa amarillo' (yellow milpa bean) and 'frijol de ejote bejuco' (bejuco pod bean). Chinantecos use 67 different names to their beans while mixtecos uses 57, zapotecos 50 and mixes 45. In addition, survey data indicated that is more frequent that each family to conserve their own bean germplasm generation by generation. This strategy is preferred over preserve germplasm near communities or local markets or helping by neighbors. According farmer answers, the more threatened germplasm belongs to *P. coccineus* (ayocote beans). Farmers from Valles Centrales (zapotecos) and Sierra Norte (chinantecos and mixes) identified germplasm mainly based on seed traits (color, brightness, mottled patterns, size or shape) while Mixtecos prefer identification of outstand germplasm based on flower color (Table 1). Farmers from Oaxaca have capacity to recognize intra and inter-specific variation in beans as well as the range of adaptation and capability of environmental exploitation of their own germplasm (Worthington *et al*., 2012; Espinosa-Pérez *et al*., 2015). Farmers from Oaxaca

exhibit empirical basis to classify bean germplasm without stringent rules (Soleri *et al.*, 2013) but performing broad morphological and genetic patterns. Farmer classification can result in both synonyms and homonyms. Farmer classifications appears to form a bean version that best fits their own needs and circumstances. Thus, same-named seed lots could be redundant units of diversity (Soleri *et al.*, 2013; Espinosa-Pérez *et al.*, 2015). Local bean germplasm in Oaxaca should be the basis of *in situ* diversity assessment, collections for *ex situ* conservation, and on-farm improvement programs due such accessions play a major role in sustainable agriculture due their adaptation to local environmental conditions and consumer tastes (Worthington *et al.*, 2012; Soleri *et al.*, 2013; Espinosa-Pérez *et al.*, 2015).

Table 1. Traits for identification of bean germplasm by four ethnic groups from Oaxaca, México.

Identification traits	Valles Centrales	Sierra Norte Chinantecos	Sierra Norte Mixe	Mixteca
Seed	42	46	70	
Pod	6	22		2
Plant	6	6	4	11
Consumption		3		
Origin (criollo)		1	2	2
Time to yield		4	5	
Sowing place				4
Tree or more traits	2			2
Seed + pod	2	4	4	
Flower color				67
Two seed traits	34	9	9	2
Seed + plant	3	1	5	2
Seed+ sowing frequency	6			
Pod + plant		1	2	
Flower color + plant				7
Total	101	97	101	99

REFERENCES:

García-Mendoza A. *et al.* (2004). Biodiversidad de Oaxaca. UNAM. México.

Chávez-Servia JL *et al.* (2016). Grain Legumes, InTech, Rijeka, Croatia. doi:10.5772/63439.

Espinosa-Pérez E.N. (2015) Rev. Fitotec. Mex. 29:29-38.

Ordóñez M.J. (2000) Invest. Geogr. UNAM (México) 42:67-86.

Soleri, D. *et al.* (2013). Econ. Bot. 67:283-298.

Worthington M. *et al.* (2012) Crop Sci. 52:1721-1735.

DRY BEAN CULTIVAR IAC ALVORADA UNDER DIFFERENT RATES OF *RHIZOBIUM* INOCULANT IN THE PLANTING FURROW

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INTRODUCTION: No publications were found regarding liquid inoculation in the planting furrow of dry bean, which indicates a demand for investigations to assist the producer in deciding which method of inoculation to adopt. Thus, the aim of this study was to evaluate the viability of liquid inoculation in the planting furrow and determine the best application rate of inoculant, using the dry bean cultivar IAC Alvorada.

MATERIALS AND METHODS: A field experiment was conducted in a no-till planting system in the 2014/2015 crop season in a *Latossolo Vermelho distrófico* in the municipality of Lambari, MG, Brazil. The experimental design was randomized blocks with three replications and eight treatments, involving five application rates of liquid inoculant in the planting furrow (0, 120, 240, 480, and 720 mL ha^{-1}), liquid seed inoculation (40 mL kg^{-1}), and two controls without inoculation: one with N-urea (80 kg ha^{-1} N, ½ at sowing and ½ in topdressing, between the V3 and V4 stages of the crop cycle) and another without mineral N.

The experimental unit (14.4 m^2) consisted of six four-meter-length rows, spaced at 0.6 m, and the area effectively used was the four central rows. All the plots received base fertilization of 110 kg ha^{-1} of P$_2$O$_5$ (simple superphosphate) and 40 kg ha^{-1} of K$_2$O (potassium chloride), mechanically applied during furrowing. In addition, all levels of inoculation received 20 kg N-urea ha^{-1}, so as to meet the recommendations of Soares et al. (2016). Sowing was manual at the density of 15 seeds per meter and the cultivar used was IAC Alvorada, of semi-upright plant architecture and moderate resistance to anthracnose (Carbonell et al., 2008).

The strain used was CIAT 899 of *Rhizobium tropici*. The inoculant was prepared in liquid medium "79" (Fred & Waksman, 1928). Inoculant quality was monitored by counting the number of colony forming units (CFU), respecting the legal minimum of 10^9 CFU per mL of inoculant. Seed inoculation occurred shortly before sowing, whereas application in the planting furrow was immediately after sowing. In the latter case, distribution in the furrow was performed with a manual backpack sprayer and the spray volume was equivalent to 20 L ha^{-1}.

At full flowering (R6 stage) a sample of 10 plants (rows 2 and 3) was removed at random from each plot for determination of the number of nodules (NN) and nodule dry matter (NDM), shoot dry matter (SDM), and shoot nitrogen concentration (SNC) and shoot nitrogen accumulation (SNA). All the data were subjected to analysis of variance through use of the software Sisvar 4.0, after having been subjected to tests of normality and homocedasticity of variances, using the R software. The variables NN and NDM were first transformed into $(x+1)^{0.5}$. In the cases of significant effect of treatments, grouping of means was performed by the Scott-Knott test at the level of 5% probability.

RESULTS AND DISCUSSION: Contrary to expectations, the application rate of 80 kg N-urea ha^{-1} did not have a negative effect on dry bean nodulation (Table 1), indicating that other factors are involved in this effect and that the definition of application rates cannot be generalized. Moreover, the expressive nodulation of the non-inoculated treatments (Table 1) also indicates the

presence of native rhizobia populations efficient in nodulating dry bean, which certainly contributed to obtaining SNC and SNA values similar to those obtained from the maximum nitrogen fertilization (Table 1). The good performance of these symbionts on these and other variables of dry bean had already been reported in the studies of Figueiredo et al. (2016) and Oliveira et al. (2016). Regardless of the application rates, inoculation in the planting furrow had the same effect as seed inoculation. This result, however, should be carefully analyzed because there was good activity of the native population. New studies are recommended, above all in areas with native rhizobia less efficient in biological nitrogen fixation.

Table 1. Number of nodules (NN) and mean nodule dry matter (NDM), shoot dry matter (SDM), and shoot nitrogen concentration (SNC) and shoot nitrogen accumulation (SNA) of dry bean cv. IAC Alvorada under different treatments. Lambari, MG, Brazil, spring/summer crop season 2014/2015.

Treatment	NN[1] (plant unit $^{-1}$)	NDM[1] (mg plant^{-1})	SDM (g plant^{-1})	SNC (%)	SNA (mg plant^{-1})
No inoculation in furrow (0 mL ha^{-1})	27 a	400 a	9.6 a	2.7 a	256 a
½X rate inoculation in furrow - ISu (120 mL ha^{-1})	30 a	361 a	10.4 a	2.6 a	268 a
1X rate ISu (240 mL ha^{-1})	22 a	310 a	10.4 a	2.6 a	268 a
2X rate ISu (480 mL ha^{-1})	17 a	337 a	10.2 a	2.5 a	256 a
3X rate ISu (720 mL ha^{-1})	23 a	378 a	9.9 a	3.3 a	318 a
Seed inoculation	36 a	645 a	10.9 a	2.5 a	274 a
Control with N	27 a	362 a	11.1 a	3.4 a	369 a
Control without N	19 a	397 a	4.8 b	3.1 a	160 a
Overall mean	249	399	96.8	2.8	271

Mean values followed by the same lowercase letters in the columns belong to the same group by the Scott-Knott test ($P \leq 0.05$). [1]Mean values compared according to transformed data $(x+1)^{0.5}$.

It should also be noted that both the inoculated treatments (furrow and seed) and the treatment without inoculation in the furrow that received 20 kg N-urea ha^{-1} exhibited growth (SDM) that did not differ from the control fertilized with 80 kg N-urea ha^{-1} (Table 1). The only treatment that proved to be inferior was the control without inoculation and without N, confirming that, in this soil, the application of mineral N, even at a small rate, was fundamental for the initial start and growth of dry bean.

REFERENCES

CARBONELL et al. IAC-Alvorada and IAC Diplomata: new common bean cultivars. *Crop Breeding and Applied Biotechnology*, v. 8, n. 2, p. 163-166, 2008.

FIGUEIREDO, M. A. Nitrogen and molybdenum fertilization and inoculation of common bean with Rhizobium spp. in two oxisols. *Acta Scientiarum. Agronomy*, v. 38, n. 1, p. 85-92, 2016. doi:http://dxdoiorg/104025/actasciagronv38i126661

FRED, E. B.; WAKSMAN, S. A. *Laboratory manual of general microbiology.* New York: McGraw-Hill, 1928. 143 p.

OLIVEIRA, D. P. et al. Seed treatment with fungicides does not affect symbiosis between common bean (Phaseolus vulgaris L) and rhizobia. *Agronomy Journal*, v. 108, p. 1–8, 2016. doi:102134/agronj2016020105

SOARES, B. L. et al.. The agronomic and economic efficiency of commom-bean inoculation with rhizobia and mineral nitrogen fertilization. *Revista Brasileira de Ciência do Solo*, v. 40, p. 1–13, 2016. doi: 10.1590/18069657rbcs20150235

APPLICATION RATES OF *RHIZOBIUM* INOCULANT IN THE PLANTING FURROW IN DRY BEAN cv. BRS ESTILO

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INTRODUCTION: One of the limitations of liquid inoculation with rhizobia in the planting furrow in the dry bean crop is the small number of research studies involving this type of inoculation and the establishment of application rates suitable for good performance of biological nitrogen fixation (BNF). Thus, the aims of this study were to evaluate the viability of liquid inoculation in the planting furrow in dry bean BRS Estilo and certify if the increase in the inoculation rate benefits its symbiosis with *Rhizobium* sp.

MATERIALS AND METHODS: A field experiment was carried out in a no-till planting system in the 2014/2015 crop season in a *Latossolo Vermelho distrófico* in the south of Minas Gerais, Brazil, at 986 m altitude, 21º58'S latitude and 45º20'W longitude. The experimental design was randomized blocks with three replications and eight treatments, involving five application rates of liquid inoculant in the planting furrow, seed inoculation, and two controls without inoculation, one without N and the other fertilized with 80 kg ha^{-1} of mineral N.

There were no records of prior inoculation in the dry bean crop in the area. Each experimental unit (14.4 m^2) consisted of six four-meter-length rows, spaced at 0.6 m, and the unit area effectively used was the four central rows. All the plots received base fertilization at the rate of 110 kg ha^{-1} of P$_2$O$_5$ and 40 kg ha^{-1} of K$_2$O mechanically applied during furrowing for planting. In addition, the inoculated plots received 20 kg N-urea ha^{-1} at planting so as to meet the recommendations of Soares et al. (2016). Sowing was manual at the density of 15 seeds per meter, and the cultivar used was BRS Estilo of high yield potential and intermediate resistance to common bacterial blight and to rust (Melo et al., 2010).

The strain used was CIAT 899 of *Rhizobium tropici*. The inoculant was prepared in liquid medium "79" (Fred & Waksman, 1928). Inoculant quality met the minimum legal requirement of 10^9 viable cells of *Rhizobium* per mL of inoculant. The rate of inoculant applied on the seed was 40 mL kg^{-1}, and the seeds were inoculated shortly before sowing. The application rates in the furrow were determined to obtain rhizobia populations of 0, ½, 1, 2, and 3 times the rate of 240 mL ha^{-1} (corresponding to rates of 0, 120, 240, 480, and 720 mL ha^{-1}, respectively). Distribution of the inoculant in the furrow was performed with a manual backpack sprayer, and the spray volume was equivalent to 20 L ha^{-1}.

At flowering, 10 plants were collected from each plot (rows 2 and 3) for determination of the number and dry weight of nodules and shoot dry matter, as well as N concentration and accumulation in the shoots. To meet the requirements of analysis of variance, the nodulation data were first transformed into $(x+1)^{0.5}$. The data were subjected to analysis of variance and the effects of the treatments evaluated by the F test (p≤0.05). In the cases of significant effect of the treatments, grouping of means was performed by the Scott-Knott test (p≤0.05).

RESULTS AND DISCUSSION: The treatments significantly affected plant dry matter and shoot N accumulation in dry bean (Table 1). There was good experimental precision in the estimates of the characteristics evaluated, even in relation to the number and dry matter of nodules, the values of which normally prove to be higher in the literature.

Seed inoculation and inoculation in the planting furrow exhibited equivalent nodulations, regardless of concentration of the inoculant. However, the highest values of dry bean growth were

observed in the control fertilized with 80 kg of N-urea ha^{-1} and in some concentrations of liquid inoculation in the planting furrow, even without expressing direct proportionality between concentration and growth. The control without N and without inoculation also had good performance under most of the variables, probably as a result of the expressive nodulation observed, which is indicative of the efficiency of native rhizobia populations. Shoot N accumulation, for its part, followed the shoot dry matter (SDM) (Table 1).

Likewise, other authors, also for soils of the south of Minas Gerais (Fonseca et al., 2013; Figueiredo et al., 2016; Oliveira et al., 2016; Soares et al., 2016), found native rhizobia with nodulating ability and BNF similar to the those of the strain CIAT 899, already recommended by the Brazilian Department of Agriculture (Ministério da Agricultura, Pecuária e Abastecimento) for dry bean. These results are encouraging and represent significant savings on nitrogen fertilizers.

Table 1. Number of nodules (NN) mean nodule dry matter (NDM), shoot dry matter (SDM), shoot nitrogen concentration (SNC), and shoot nitrogen accumulation (SNA) in dry bean cv. BRS Estilo. Spring-summer crop season 2014/2015.

Treatment	NN[1] (plant unit $^{-1}$)	NDM[1] (mg plant^{-1})	SDM (g plant^{-1})	SNC (%)	SNA (mg plant^{-1})
No inoculation in furrow (0 mL ha^{-1})	20 a	248 a	6.96 b	2.90 a	204 b
½X rate inoculation in furrow - ISu (120 mL ha^{-1})	31 a	445 a	6.75 b	2.73 a	184 b
1X rate ISu (240 mL ha^{-1})	19 a	275 a	8.55 a	3.27 a	279 a
2X rate ISu (480 mL ha^{-1})	20 a	345 a	8.09 a	3.03 a	248 a
3X rate ISu (720 mL ha^{-1})	24 a	345 a	6.56 b	3.47 a	221 b
Seed inoculation	20 a	224 a	5.39 b	2.93 a	158 b
Control with N	17 a	209 a	10.70 a	3.03 a	325 a
Control without N	24 a	323 a	8.28 a	3.07 a	257 a
Overall mean	22	302	7.66	3.05	234
Coefficient of variation	19.38	4.21	17.21	10.47	20.69

Mean values followed by the same lowercase letters in the columns belong to the same group by the Scott-Knott test ($P\leq0.05$).
[1]Mean values compared according to transformed data $(x+1)^{0.5}$.

At the application rate of 240 mL ha^{-1}, the method of inoculation in the planting furrow proved to be viable, showing efficiency similar to or even greater than that of seed inoculation, and providing for plant growth and N nutrition equivalent to the values obtained from fertilization with 80 kg ha^{-1} of mineral N. In spite of these results, new studies should be carried out aiming at use of this technique in dry bean, above all in areas without native rhizobia efficient in this symbiosis.

REFERENCES

FIGUEIREDO, M. A. Nitrogen and molybdenum fertilization and inoculation of common bean with *Rhizobium* spp. in two oxisols. *Acta Scientiarum. Agronomy,* v. 38, n. 1, p. 85-92, 2016.

FONSECA, G. G. et al.; Resposta de cultivares de feijoeiro-comum à inoculação das sementes com duas estirpes de rizóbio. *Bioscience Journal*, v. 29, n. 6, p. 1778-1787, 2013.

FRED, E. B.; WAKSMAN, S. A. *Laboratory manual of general microbiology.* New York: McGraw-Hill, 1928. 143 p.

MELO, L. C. et al. BRS Estilo: common bean cultivar with Carioca grain, upright growth and high yield potential. *Crop Breeding Applied Biotechnology.* v. 10, n. 4, p. 377-379, 2010.

OLIVEIRA, D. P. et al. Seed treatment with fungicides does not affect symbiosis between common bean (*Phaseolus vulgaris* L) and rhizobia. *Agronomy Journal,* v. 108, p. 1–8, 2016.

SOARES, B. L. et al. The agronomic and economic efficiency of commom-bean inoculation with rhizobia and mineral nitrogen fertilization. *Revista Brasileira de Ciência do Solo*, v. 40, p. 1–13, 2016.

TESTING A DIFFERENTIAL SET OF ANDEAN AND MESOAMERICAN COMMON BEAN GENOTYPES TO CHARACTERIZE *RHIZOBIUM* ISOLATES

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INTRODUCTION

Nitrogen (N) deficiency is present in common bean (*Phaseolus vulgaris* L.) fields in most production regions in Central America and the Caribbean (CA/C), especially on small hillside farms where no or sub-optimum levels of fertilizer are used. Under these conditions, *Rhizobium* inoculants could represent a cheap and effective input to increase bean yield. Despite the potential benefits from increased biological nitrogen fixation, *Rhizobium* inoculation is rarely used by farmers in CA/C. Nodulation and nitrogen fixation (N2) of bean cultivars in response to *Rhizobium* inoculation is quite variable due to several biological and edaphic factors (Graham *et al.* 2003). On the other hand, the response of different bean cultivars and germplasm accessions to different *Rhizobium* species and strains that nodulate and fix N2 is often difficult to measure on farms. A better understanding of specific bean host-plant-*Rhizobium* strain interactions would facilitate the selection of superior symbionts with greater response to inoculation and increase bean productivity in N-limited soils.

MATERIALS AND METHODS

The nodulation response of 13 local rhizobia isolates and two commercial strains of *Rhizobium* were evaluated using a differential set of 12 common bean genotypes. This differential set of genotypes was selected based on contrasting nodulation among the genotypes and in agreement with the findings of Racancoj *et al.* (2014). Six Andean, 'ICA Quimbaya', G05686, 'Ervilha' (ADP 512), 'Mantega' (ADP 516), CAL 143 and G06727, and six Mesoamerican 'Macuzalito', G21212, 'Bribri', 'Tio Canela 75', 'Carioca' and 'Tacana', bean genotypes were included. The study was conducted in a screenhouse at Zamorano, Honduras using experimental units of 90 x 20 x 13 cm PVC rain gutters containing a sand substrate previously autoclaved at 120°C and 15 PSI for 30 min. Two day old seedlings of the different bean genotypes were planted and inoculated two days after planting (DAP) with 1 mL liquid inoculant (1×10^5 cfu/mL). Inoculant treatments were isolates from nodules collected in 13 farm fields from five departments in Honduras and the check strains CIAT 899 (*R. tropici*) and CIAT 632 (*R. etli*) used for commercial inoculant production. The study was conducted using a split plot arrangement of a randomized complete block design with four replications. *Rhizobium* isolates/strain treatments were assigned to main plots and bean genotypes to sub-plots. Plants were irrigated alternatively one day with tap water and the other with a nutrient solution free of N (Somasegaran and Hoben, 1995). At 15 DAP, plant samples were taken to measure the response to inoculation using a 1 to 9 nodulation scale (1= absence or very few small, <1mm diameter, ineffective nodules; 9= >20 large, >3mm diameter, effective nodules). Plants with scores 1-3 were considered as having a negative (-) and 4-9 as having a positive (+) response to inoculation. Binary numbers assigned to the 12 bean genotypes were used to characterize the *Rhizobium* isolates and strains.

RESULTS AND DISCUSSION

The nodulation response of the 12 differential bean genotypes to the inoculation with 13 *Rhizobium* isolates varied. Based on the 1-9 nodulation scale, bean genotypes varied from ineffective (negative responses to most isolates) as in the Tatumbla isolate (binary number 40-0), to very effective (positive responses to most isolates) as in the Cantarranas isolate (binary number 61-63) (Table 1). The check strains CIAT 632 presented high nodulation (score >6) with all 12 bean genotypes (binary number 63-63), and CIAT 899 with 11 genotypes (binary number 61-63). These two strains have been used for nodulation studies and inoculant production in Central America and the Caribbean (Hungria *et al*. 2003). The genotype Bribri had good nodulation with the largest number (11/12) of isolates. Further characterization of the rhizobium isolates from other bean production regions will demonstrate the usefulness of the bean differential set for studying bean-rhizobium interaction and to select efficient native strains for inoculant production.

Table 1. Evaluation of *Rhizobium* isolates from nodules collected at 13 sites and check strains CIAT 899 (*R. tropici*) and CIAT 632 (*R. etli*) using a differential nursery of 12 common bean genotypes. Zamorano, Honduras, 2016.

Isolate	Andean						Mesoamerican						Binary number
	A (1)	B (2)	C (4)	D (8)	E (16)	F (32)	G (1)	H (2)	I (4)	J (8)	K (16)	L (32)	
Guinope	+	+	+	+	-	+	-	+	+	-	+	-	47-22
El Ocotal	-	+	-	+	+	+	+	+	+	+	+	+	58-63
Araulí	+	-	+	+	-	+	+	+	+	+	+	+	45-63
Las Acacias	+	+	-	+	+	+	+	+	+	+	+	-	59-31
Santa Ana	+	-	+	+	-	+	-	+	+	+	+	-	45-30
SJuan Flores	-	-	-	-	-	+	+	-	+	+	+	-	32-29
Tatumbla	-	-	-	+	-	+	-	-	-	-	-	-	40-0
Zamorano	-	-	+	+	-	+	+	+	+	+	+	-	44-31
Cantarranas	+	-	+	+	+	+	+	+	+	+	+	+	61-63
El Espino	-	-	-	+	-	+	+	+	+	+	+	+	40-63
Río Bonito	+	-	-	+	-	-	-	-	+	+	+	-	9-28
Agua Dulce	-	-	-	-	+	-	+	+	+	+	+	+	16-63
Zacapa	+	+	-	+	-	+	+	+	+	+	-	-	43-15
CIAT 899	+	-	+	+	+	+	+	+	+	+	+	+	61-63
CIAT 632	+	+	+	+	+	+	+	+	+	+	+	+	63-63

A= ICA Quimbaya, B= G05686, C= Ervilha, D= Mantenga, E= CAL 143, F= G06727, G= Macuzalito, H= G21212, I= Bribri, J= Tio Canela 75, K= Carioca and L= Tacana. Isolate names are from locations where nodules were collected. Positive (+) response correspond to 1-3 and negative (-) to 4-9 scores from a 1-9 nodulation scale.

REFERENCES

Graham *et al*. 2003. Addressing edaphic constraints to bean production: Bean/Cowpea CRSP perspective. Field Crops Research 82: 179-192.

Hungría *et al*. 2003. Benefits of inoculation of the common bean (*Phaseolus vulgaris*) crop with efficient and competitive *Rhizobim tropici* strains. Biology and Fertility of Soils 39: 88-93.

Racancoj *et al*. 2014. Response of Andean and Mesoamerican common bean genotypes to inoculation with *Rhizobium* strains. Ann. Report. Bean Improv. Coop. 57:245-246.

RHIZOBIUM INOCULATION IN DRY BEAN CV. BRSMG MADREPÉROLA SUBJECTED TO FUNGICIDE SEED TREATMENT

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INTRODUCTION: Fungicide seed treatment is an important strategy for plant pathogen control in the dry bean crop. However, there are reports that it may hinder biological nitrogen fixation (BNF). The present study was carried out for the purpose of verifying the compatibility of fungicide treatment and BNF, so as to assist producers in making decisions in regard to selection of products with the least impact on biological processes and, consequently, on yield.

MATERIALS AND METHODS: A field experiment was carried out in the 2014 dry season in a *Latossolo Vermelho distroférrico típico* in the municipality of Lavras, MG, Brazil. There was no record of inoculation prior to the dry bean crop in the area, and the population of native rhizobia capable of nodulating dry bean was approximately 10^3 colony forming units per gram of soil. The statistical design used was randomized blocks with three replications and a $(5\times3) + 1$ factorial arrangement, involving five fungicides in seed treatment (*Captan, Carboxin + Thiram, Difenoconazole, Fluazinam + Thiophanate-methyl*, and *Fludioxonil + Metalaxyl-M,* all at application rates recommended by the manufacturers) and three levels of inoculation (without inoculation, seed inoculation, and inoculation in the planting furrow), plus an additional treatment with 80 kg ha^{-1} of N (40 kg ha^{-1} N at sowing + 40 kg ha^{-1} N at topdressin inoculant containing $\pm10^9$ viable cells of the strain CIAT 899 (*Rhizobium tropici*) per mL was applied at the rates of 40 mL kg^{-1} on seeds or 300 mL ha^{-1} in the planting furrow. Seed inoculation occurred one hour before sowing. Sowing was manual, at a density of 15 seeds per meter. The cultivar BRSMG Madrepérola was used, with high yield potential and good level of resistance to the main diseases that occur in Minas Gerais. Plants are prostrate, of type III growth habit, with low tolerance to lodging (CARNEIRO et al., 2012). Distribution of the inoculant in the furrow was performed after sowing with the aid of a manual backpack sprayer, and the spray volume was equivalent to 20 L ha^{-1}.

The experimental unit consisted of six four-meter-length rows, spaced at 0.6 m, and the area effectively used was the four central rows. All the plots, except for those of the additional treatment, received base fertilization at the rate of 20 kg N-urea ha^{-1}, 110 kg P_2O_5 ha^{-1} (simple superphosphate source), and 40 kg K_2O ha^{-1} (potassium chloride). The application of a low application rate of N at sowing followed the recommendations of Soares et al. (2016).

The following evaluations were made: number of nodules (NN) and nodule dry matter (NDM), shoot dry matter (SDM), shoot nitrogen concentration (SNC), and shoot nitrogen accumulation (SNA), and grain yield, as well as grain N concentration (GNC) and grain N accumulation (GNA). All the data were subjected to analysis of variance after having been previously subjected to tests of normality and homoscedasticity of variances. The variables NN and NDM were first transformed into $(x+0.5)^{0.5}$. In the cases of significant effect of treatments, grouping of means was performed by the Scott-Knott test ($P \le 0.05$). For comparison between the additional treatment and the mean of the factorial, the *F* test was used, since there were only two means.

RESULTS AND DISCUSSION: The manner of application of the inoculant did not affect nodulation, growth, or BNF, inferring from the N content in the plant (SNC and SNA); this likewise occurred in evaluations at maturity (Table 1). The presence of nodules in the treatment without inoculation and without mineral N indicates the occurrence of native strains of rhizobia

in the soil, capable of nodulating as much as the inoculated bacteria. Similar results were found by Figueiredo et al. (2016) and Oliveira et al. (2016). The good activity of these rhizobia, just as the introduced rhizobia, was certainly essential for providing the nutrition necessary for obtaining the good yields observed, around 1400 kg ha^{-1}.

Apparently, the excess of mineral N did not hurt the establishment of symbiosis of the native rhizobia, providing NN, NDM, and SDM equivalent to those of the factorial treatments. The higher SNC and SNA of the factorial, possibly coming from the greater input of mineral N (40 kg ha^{-1} of N at sowing and 40 kg ha^{-1} of N at topdressing), did not result in differences in yield or in nutrition of the grain of the contrast treatments (Table 1).

Table 1. Number of nodules (NN) and mean nodule dry matter (NDM), shot dry matter (SDM), shoot nitrogen concentration (SNC), and shoot nitrogen accumulation (SNA), grain nitrogen concentration (GNC) and grain nitrogen accumulation (GNA), and grain yield (GY) of dry bean cv. BRSMG Madrepérola according to inoculation with rhizobium and fungicide seed treatment.

Treatment	NN[1]	NDM[1]	SDM	SNC	SNA	GY	GNC	GNA
Inoculation level	(plant unit $^{-1}$)	(mg plant^{-1})		(%)	(mg plant^{-1})	(kg ha^{-1})	(%)	(kg ha^{-1})
Absence of inoculation	29 a	360 a	581 a	3.0 a	176 a	1379 a	3.0 a	41. 1 a
Seed inoculation	20 a	290 a	617 a	3.2 a	224 a	1416 a	3.1 a	44.2 a
Inoculation in planting furrow	27 a	290 a	730 a	3.1 a	196 a	1479 a	3.2 a	47.2 a
Fungicide								
Captan	25 a	320 a	741 a	2.8 b	206 a	1459 a	3.0 a	43.9 a
Carboxin + Thiram	24 a	350 a	656 a	3.4 a	218 a	1484 a	2.9 a	43.3 a
Difenoconazole	28 a	340 a	601 a	3.1 a	183 a	1406 a	3.3 a	46.2 a
Fluazinam + Thiophanate-methyl	22 a	270 a	618 a	3.2 a	195 a	1418 a	3.2 a	45.6 a
Fludioxonil + Metalaxyl-M	28 a	310 a	599 a	3.2 a	193 a	1356 a	3.1 a	41.7 a
Factorial	26 a	320 a	643 a	3.1 b	199 b	1425 a	3.1 a	44.2 a
Additional	18 a	200 a	765 a	3.8 a	293 a	1605 a	2.8 a	45.4 a

The mean values followed by the same lowercase letters in the columns belong to the same group in the Scott-Knott test ($P \leq 0.05$). [1]Mean values compared according to transformed data $(x+0.5)^{0.5}$.

The fungicides used in the seed treatment did not exhibit any phytotoxic effect on dry bean in the sense of reducing or delaying its growth. Not even the rhizobia appear to have been affected by the fungicides (see high nodulation, Table 1). As no problems related to diseases were observed in the experiment, it was also not possible to detect any effect of the fungicides on improving plant health conditions in such a way as to alter dry bean growth. Only the active ingredient Captan reduced the SNC, but this decrease does not appear to be related to the activity of BNF since nodulation was not affected.

Just as in the studies of Oliveira et al. (2016), which aimed at seed inoculation, the fungicides applied on seeds at the rates recommended by the manufacturers appear not to have compromised the symbiosis of native or introduced rhizobia. However, to confirm this, new studies should be developed in the laboratory and in the field.

REFERENCES

CARNEIRO, J.O.S. et al. BRSMG Madrepérola: common bean cultivar with late-darkening Carioca grain. *Crop Breeding Applied Biotechnology*, v. 12, p. 281-284, 2012.

FIGUEIREDO, M. A. Nitrogen and molybdenum fertilization and inoculation of common bean with *Rhizobium* spp. in two oxisols. *Acta Scientiarum. Agronomy*, v. 38, n. 1, p. 85-92, 2016.

OLIVEIRA, D. P. et al. Seed treatment with fungicides does not affect symbiosis between common bean (*Phaseolus vulgaris* L.) and rhizobia. *Agronomy Journal*, v. 108, p. 1–8, 2016.

SOARES, B. L. et al. The agronomic and economic efficiency of commom-bean inoculation with rhizobia and mineral nitrogen fertilization. *Revista Brasileira de Ciência do Solo*, v. 40, p. 1–13, 2016.

COMPARISON OF TWO CULTIVARS OF *Phaseolus vulgaris* L. INOCULATED WITH A STRAIN OF NITROGEN FIXING RHIZOBACTERIA

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INTRODUCTION: A whole spectrum of soil microorganisms in the rhizosphere, may affect the bean (*Phaseolus vulgaris* L.) yield as well as the interaction among the different microorganisms which might exist in the soil. The bean plant has also the opportunity to interact with other microorganisms as in the case of the bacteria (Gherbi *et al.*, 2008; Marsh *et al.*, 2008; Oldroyd *et al.*, 2009). The nitrogen fixing bacteria such as a strain of *Rhizobium tropici* CIAT 899, are capable of fixing the atmospheric nitrogen when it occurs in symbiosis with its host plant, in this case the bean plant.

MATERIALS AND METHODS: The experiment was carried out in a greenhouse of Colegio de Postgraduados at Montecillo, state of Mexico in July of 2016. Two cultivars of determinate growth habit were employed: OTI and Cacahuate 72. A group of seedlings in the simple leaf stage were inoculated with a suspension of strain *Rhizobium tropici* CIAT 899 bacteria. Another group was not inoculated. The plants were grown in plastic containers with 7 kg of sandy loam soil. The experimental design was a complete randomized with five replications. The following treatments were evaluated: 1) cv. OTI inoculated; 2) cv. OTI non inoculated; 3) cv. Cacahuate 72 inoculated and 4) cv. Cacahuate 72 non inoculated. Sixty nine days after planting, the rates of photosynthesis and transpiration were determined. At physiological maturity there were registered: the number of normal pods, seeds and seed yield (all per plant). The statistical analysis was performed with the SAS program (SAS, 2012).

RESULTS: The number of normal pods per plant was the same for all the treatments. However, differences occurred in the number of seeds per plant, with higher values for cv. OTI inoculated and OTI non inoculated, as compared to Cacahuate 72 both inoculated and non inoculated. There were differences for the seed yield per plant between the cv. OTI inoculated and Cacahuate 72 non inoculated. The other treatments were equal to OTI inoculated (Table 1). The rate of photosynthesis was higher for cv. OTI both inoculated and non inoculated as compared to cv. Cacahuate 72. The transpiratory rates were higher for OTI inoculated with respect to the other three treatments (Table 2).

Table 1. Seed yield and its components in two cultivars of *Phaseolus vulgaris* L. non inoculated and inoculated with a suspension of a strain *Rhizobium tropici* CIAT 899 bacteria.

Treatment	Normal pods per plant	Number of seeds per plant	Seed yield (g per plant)
OTI inoculated	13.4 a*	69.4 a	19.48 a
OTI non inoculated	11.8 a	60.6 a	17.76 ab
Cacahuate 72 inoculated	10.6 a	43.6 b	17.06 ab
Cacahuate 72 non inoculated	9.0 a	37.8 b	15.14 b
*Different letters within each column indicate statistical difference between treatments (Tukey test P≤0.05).			

Table 2. Photosynthesis and transpiration rates in two cultivars of *Phaseolus vulgaris* L. non inoculated and inoculated with a suspension of a strain *Rhizobium tropici* CIAT 899 bacteria.

Treatment	Photosynthesis rate $\mu mol\ CO_2\ m^{-2}\ s^{-1}$	Transpiration rate $mmol\ m^{-2}\ s^{-1}$
OTI inoculated	21.28 a*	1.86 a
OTI non inoculated	19.64 a	1.22 b
Cacahuate 72 inoculated	11.78 b	0.98 bc
Cacahuate 72 non inoculated	10.82 b	0.84 c
*Different letters within each column indicate statistical difference between treatments (Tukey test P≤0.05).		

REFERENCES

Gherbi H, Markmann K, Svistoonoff S, Estevan J, Autran D, Giczey G, Auguy F, Peret B,Laplaze L, Franche C, Parniske M, Bogusz D (2008) SymRK defines a common genetic basis for plant root endosymbioses with arbuscular mycorrhiza fungi, rhizobia, and Frankia bacteria. Proc Nat Acad Sci USA 105:4928–4932.

Marsh JF, Gobbato E, Schultze M, Oldroyd GED (2008) Ram1 and Ram2: signaling specificity in mycorrhizal symbiosis. Abstract book of 8th European nitrogen fixation conference, Gent, Belgium, August 30–September 3, 2008, p 26.

Oldroyd GED, Harrison MJ, Paszkowski U (2009) Reprogramming plant cells for endosymbiosis. Science 324:753–754.

Statistical Analysis System (SAS Institute). 2012. SAS version 9.4 for Windows. SAS Institute Inc. Cary, N.C., USA.

NITROGEN FERTILIZATION AND INOCULATION OF COMMON BEAN WITH *Rhizobium tropici*

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) has a great importance as a food crop worldwide and Brazil has a production about 3.29 million tons (FAO, 2014). Nitrogen (N) is the nutrient most absorbed and extracted by common bean and its use has a significant influence on yield. Due to the high cost of nitrogen fertilizers and the losses of this nutrient in the soil, which contributes to environmental pollution, it is of great interest to search for techniques that can maximize its efficiency. Although bean plants have the capacity to establish mutual symbiosis with bacteria, biotic and abiotic factors can act to reduce the efficiency of this relationship. This study aimed at evaluating the efficiency of inoculation practice with rhizobia and doses of nitrogen on production components of common bean.

MATERIAL AND METHODS

The experiment was conducted at Experimental Farm of the Vale do Rio Verde University situated in Três Corações city (21°42'S, 45°15' and altitude of 855 m), in the Minas Gerais State, Brazil, during the 2015 dry growing season. The soil of the experimental area was classified according to Brazilian classification as a Dystroferic Red Latosol (EMBRAPA, 2013). A randomized block experimental design was implemented, with three replicates. Treatments were arranged as a factorial 2x7 (presence/absence of inoculant x 7 doses of nitrogen). Seeds from 'Pérola' cultivar (Carioca grain) was inoculated with the commercial product Total Nitro® which is constituted by *Rhizobium tropici* strain SEMIA 4077 and *Rhizobium tropici* strain SEMIA 4080. Nitrogen fertilization was performed using urea at the planting furrow and topdressing. Each plot consisted of four 4-m-long rows spaced 0.5 m apart (8 m^2). The seed density was 18 seeds m^{-1} at a depth of 3-4 cm, and it was thinned out to 15 plants 20 d after sowing. Regarding the fertilization and irrigation, the plants were managed according to the technical recommendations for the crop. Weeds were controlled using manual weeding whenever necessary and pest and disease control was not required. At 100 DAE, the plants were collected from each plot to measure the number of pods per plant, number of grains per pod, 100-seed weight, final stand, and grain yield. The data were subjected to analysis of variance and, in case of significance, the means were compared by the Scott-Knott's test ($P \le 0.05$), using the R software (R Development Core Team, 2011).

RESULTS AND DISCUSSION

According to the Table 1, significant differences ($P \le 0.05$) were not observed for the interaction inoculation x doses of N for all evaluated variables, with exception of number of pods per plant. For this characteristic, the inoculated plants presented superiority over the non-inoculated ones, without affecting the final yield. Several authors point out that this characteristic is closely related to the plant genetics. According to Arf et al. (2008), this item is a feature of high heritability and, therefore, closely related to the cultivar.

For the other evaluated characteristics, no statistical difference was observed between treatments. Many factors may have contributed to the achievement of these results since nitrogen fixation is closely related to the climatic variables and genetic characteristics of each cultivar as reported by Araújo et al. (1996) and Lemos et al. (2003). These authors evaluated the nodulation in bean cultivars and reported that the cultivar 'Carioca Precoce' presented better symbiotic performance in relation to other cultivars, and it is probable that the results found in this study are related to the cultivar used.

Table 1 Values obtained for 100-seed weight (SW, in g), number of pods per plant (NPP), number of grains per pod (NGP), final stand (FS, in 1,000 plants per ha) and grain yield (GY, in kg ha^{-1}) of common bean cultivars grown at the 2015 dry growing season at Três Corações, Minas Gerais, Brazil.

Treat.	SW[ns]		NPP*		NGP[ns]		FS[ns]		GY[ns]	
Doses of N	I	NI	I	NI	I	NI	I	NI	I	NI
0	29.1a	28.9a	12.8a	10.8a	4.4a	4.5a	165a	215a	1242.7a	1158.7a
20	27.8a	28.3a	11.9a	13.3a	4.5a	4.4a	171a	177a	1228.7a	1399.3a
40	30.6a	29.6a	13.3a	10.5a	4.4a	4.5a	142a	219a	1184.7a	1697.3a
60	30.4a	31.5a	15.3a	10.4a	4.6a	4.1a	159a	193a	1428.7a	1469.3a
80	31.0a	28.4a	16.0a	12.6a	4.3a	4.2a	185a	170a	1580.0a	1396.7a
100	28.6a	29.7a	15.3a	10.6a	4.6a	4.3a	185a	178a	1755.3a	1029.3a
120	30.6a	32.3a	15.0a	13.8a	4.5a	4.3a	177a	139a	1657.3a	1350.0a
Means	29.7A	29.8A	14.2A	11.7B	4.5A	4.3A	169A	184A	1439.6A	1357.2A
CV(%)	9.0		17.8		5.8		21.47		25.03	

Means followed by the same lowercase letter in the columns and uppercase letter in the rows are not significantly different at $P \leq 0.05$ using Scott-Knott's test. CV = coefficient of variation; I = inoculated plants; NI = non-inoculated plants

In this study, we found satisfactory value for soil organic matter (2.91 dag kg^{-1}). The mineralization of this organic matter by the bacteria present in the soil probably caused an availability of N for the plants that received lower doses of N, then showing no statistical differences for doses.

REFERENCES

ARAÚJO, F.F.; MUNHOZ, R.V.; HUNGRIA, M. (1996). Início da nodulação em sete cultivares de feijoeiro inoculadas com duas estirpes de *Rhizobium*. *Pesquisa Agropecuária Brasileira*, 31(6): 435-443.

EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA. (2013). *Sistema Brasileiro de classificação de solos* (3ª ed.). Brasília, DF: Embrapa-SPI.

FOOD AND AGRICULTURE ORGANIZATION (FAO). *FAOSTAT statistical database*. 2014. Available at: <http://faostat.fao.org/>. Access on: Aug. 2016.

LEMOS, L.B.; FORNASIERI FILHO, D.; CAMARGO, M.B.; SILVA, T.R.B.; SORATTO, R.P. (2003). Inoculação de rizóbio e adubação nitrogenada em genótipos de feijoeiro. *Agronomia*, 37(1):26 31.

R Development Core Team. (2011). *R: A language and environment for statistical computing*. Vienna, AT: R Foundation for Statistical Computing.

ANTIOXIDANT ACTIVITY IN RELATION TO THE SIZE OF POD IN DIFFERENT SNAP BEAN GENOTYPES

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INTRODUCTION: The snap bean (*Phaseolus vulgaris* L.) is a legume that is widely consumed on the world scenario due to its biochemical composition and nutritional quality (Furlan et al., 2016). Besides the nutritional compounds, the snap bean presents antioxidants properties, inhibiting or retarding the oxidative damage, which avoids the propagation of oxidative reactions and can prevent diseases caused by free radicals. (Silva et al., 2009).
The aim of this study was to evaluated the interaction between the antioxidant activity and pod size of several snap bean genotypes cultivated in the sowing season spring/summer and autumn/winter.

MATERIAL AND METHODS: The experiment was conducted in an organic system with protected cultivation in the municipality of Londrina, Paraná, Brazil. The study was conducted in a completely randomized design, in a factorial scheme (8 *vs.* 2), with four replications, being eigth snap bean genotypes with indeterminate growth pattern (Teresópolis Ag 481, HAV 69, HAV 41, Preferido Ag 482, Macarrão Brasília, Trepador Top Seed, HT 30 e Favorito Ag 480) and tow sowing season: spring/summer of 2014 and autumn/winter of 2015. Each experimental plot was composed by 10 plants spaced at 0.20 m in the line and 1.00 m between lines.

Samples of pods were collected from each treatment, being measured: the average pod mass, length and diameter of pods - measured in 10 pods per plant; And antioxidant activity (RUFINO et al., 2007). The data were submitted to analysis of variance by the F test (p <0.05) and compared by the principal component analysis (PCA), using software R (R, 2012).

RESULT: According to the analysis of variance (Table 1), it was found significant differences for the interaction genotypes *vs.* sowing seasons for all variables. In both seasons, the principal components analysis (PCA) (Figure 1) showed positive correlations for the phytometric characteristics of the pods and the absence of correlation of these variables with the antioxidant activity, suggesting that characteristics related to the size and mass of pods do not govern the antioxidants accumulation.

In the spring/summer season it was possible to separate the genotypes into three groups (Group I : Preferido, Brasília, HT 30 and Favorito; Group II: Trepador, HAV 41 and HAV 69; Group III: Teresópolis). In the second season four groups was separated (Group I: Trepador, HAV 69 and Brasília; Group II: Preferido, HAV 41 and Favorito; Group III: HT 30; Group IV: Teresópolis). The differences found in the grouping of genotypes are explained by the high environmental participation in the expression of these characteristics. However, the cultivars Brasilia and Teresópolis showed high capacity *per se* (in the antioxidant accumulation and pod size).

Table 1. Mean squares values for phytometric characteristics of pods and antioxidant activity of eight snap bean genotypes grown at two sowing seasons. Londrina, Brazil, 2015.

Sources of variation	Average of pod mass (g)	Pod length (cm)	Pod diameter (cm)	Antioxidant (mg DPPH g^{-1})
Season (S)	73.859**	46.342**	0.001^{NS}	0.353^{NS}
Genotypes (G)	20.871**	32.945**	0.075**	1.219**
S *vs.* G	5.644**	6.730**	0.018**	1.112**
Residue	0.496	0.456	0.005	0.263
CV (%)	8.29	4.44	7.47	22.68
Mean	8.49	15.2	1.00	5.11

** Significative ata 1% and ^{NS} Non significative by F test ($p < 0.05$).

Figure 1. Principal component analysis (PCA) obtained by the phytometric characteristics and antioxidant activity of pods of eight snap bean genotypes sowing in spring/summer (SS1) and autumn/winter (SS2).
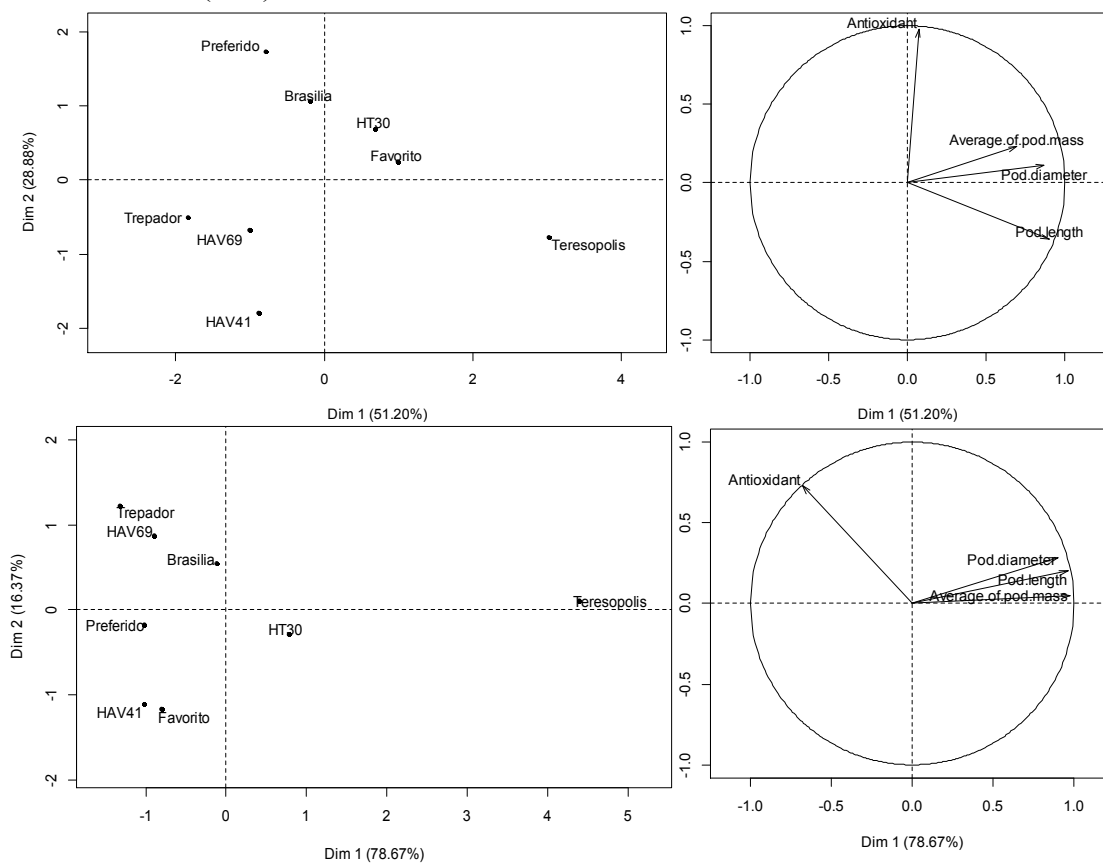
Furlan, FF, Freiria, GH, Alves, GAC, Gomes, GR, Almeida, LHC, Furlan, MF and Takahashi, LSA. (2016). The use of various strains of *Rhizobium tropici* for inoculation of snap bean cultivars with a determinate growth pattern. *Semina: Ciências Agrárias,* 37(6): 3965-3972.

R Development Core Team. (2012). *R: a language and environment for statistical computing.* Vienna: R Foundation for Statistical Computing.

Silva, AG, Rocha, LC and Canniatti Brazaca, SG. (2009). Physico-chemical characterization, protein digestibility and antioxidant ativity of commun bean (*Phaseolus vulgaris* L.). *Alimentos e Nutrição Araraquara,* 20 (4): 591-598.

EXPORTATION RATES OF NUTRIENTS IN SNAP BEAN WITH INDETERMINATE GROWTH HABIT

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INTRODUCTION: The potential exportation of macronutrients in the harvested part of each crop is an important aspect to consider for programming the restitution of these mineral elements, avoiding their exhaustion in the soil (RAIJ, 2011). The aim of this study was to evaluate the potential exportation rates of nutrients by the harvested pods in a snap bean genotype with indeterminate growth habit, providing theoretical basis for the recommendations of restitution fertilization in the production systems with the cultivation of this vegetable crop.

MATERIAL AND METHODS: The experiment was carried in a greenhouse covered with polyethylene (150 μm thickness) in State University of Londrina – UEL, Londrina, PR, Brazil (23º23'S, 51º11'W and 566 m of altitude). Seeds of snap bean, Topseed® cv. "Líder" with indeterminate growth habit and cylindrical pods, sown at May 15, 2016, in plastic pots filled with sand as substrate and spaced 0.6 m between lines and 0.3 m between plants. Each pot received five seeds and thinning performed when the seedlings reached V1 stage (emergence). In total, 200 plants grown in four rows of pots. The application of nutrients was performed with a fertigation system for a better control of their supply. This system, the substrate and preparation of nutrient solution was the same utilized by Almeida et al, (2016).

The macronutrients concentrations in the vegetative phase were: 72; 10; 62.5; 112; 12 and 16 mg L^{-1} of N, P, K, Ca, Mg and S, respectively; and for micronutrients: 1.374; 0.507; 0.372; 0.315; 0.054 and 0.0054 mg L^{-1} of Fe, Zn, Mn, B, Cu and Mo, respectively. These concentrations resulted in 1.0 dS m^{-1} of electrical conductivity (EC) of nutrient solution. In the reproductive phase, all the nutrients had doubled concentrations, providing an EC of 2.0 dS m^{-1}.

The harvests of fresh pods manually performed at 56, 63 and 72 days after emergence (DAE). The fresh pods were weighed (FPW, g pot^{-1}) at each harvest and then dried in ventilated oven at 55 ºC for 72 h, obtaining the dry weight (DPW, g pot^{-1}). The pods humidity (PH, %) was obtained by the equation 1: PH = ((FPW – DPW)/FPW) x 100

The dried pods were ground in Willey mill, submitted to acid digestion and the nutrients contents in the dry matter (g kg^{-1}) analyzed according to the methods contained in Silva (2009). The results of the contents of nutrients in dry weight basis (NDWB, g kg^{-1}) together with the pods humidity (PH, %), enabled to obtain the nutrients contents in fresh weight basis (NFWB, g kg^{-1}), using the equation 2: NFWB = NDWB x ((100 - PH)/100).

This represents the rate of nutrient exportation, which was expressed in g ton^{-1} of fresh pods harvested (mean of the three harvests), multiplying NFWB by 1000.

RESULTS AND DISCUSSION: Among the macronutrients, higher rates of exportation were observed for N followed by K. In the sequence, P and Ca, which were not different, while S and Mg had the lowest exportation rates (Figure 1).
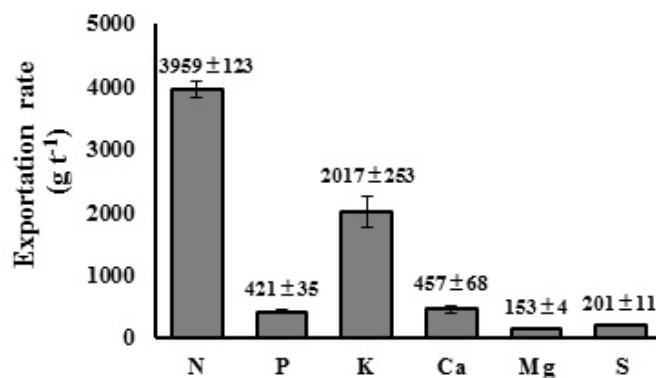
Figure 1. Exportation rates of macronutrients nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S) in fresh pods of snap bean with indeterminate growth habit. Bars represent the standard deviation.

Soratto et al. (2013), studying common bean cultivated for grain harvest (dry bean), observed exportation rates averaging from 25 (N), 4 (P), 15 (K), 2 (Ca), 2 (Mg) and 2 (S) kg t^{-1} of grains. Results regarding the exportation rates of nutrients are very scarce for snap bean, but the data obtained in this study demonstrates that they are quite lower than dry bean. Faquin and Andrade (2004) presented values of exportation rates for snap bean with flattened pods close to the obtained in this study for cylindrical pods.

For other vegetable crops, such as potato (Fernandes et al., 2011) and carrot (Cecílio Filho and Peixoto, 2013), the exportation rates are also lower in magnitude than dry bean, as seen in the present study for snap bean. This is due to the major yield of these crops (several tons per hectare), especially because of their higher moisture content, which dilutes the content of nutrients in the fresh weight basis. For example, the mean of pods humidity obtained in this study was 90.87 ± 1.34 %.

CONCLUSION: Considering that snap bean with indeterminate growth habit may attain high yields of fresh pods, such as 20 to 35 ton ha^{-1}, the exportation of nutrients with the harvest, especially for N and K, may represent significant amounts and must be considered for managing of the fertilization of production systems in which this crop is inserted.

REFERENCES

ALMEIDA, L.H.C.; FRATONI, M.M.J.; FREGONEZI, G.A.F.; TAKAHASHI, H.W.; (2016). Nutritional assessment of potassium in tomato (*Lycopersicon esculentum Mill.*) by direct reading of fruit sap. Afr. J. Agric. Res. Vol. 11(14), pp. 1245-1256.

CECÍLIO FILHO, A.B.; PEIXOTO, F.C. 2013. Acúmulo e exportação de nutrientes em cenoura 'forto'.Revista Caatinga, Mossoró, v.26, n.1, p.64-70.

FAQUIN, V.; ANDRADE, A.T. 2004. Nutrição mineral e diagnose do estado nutricional das hortaliças. Lavras: UFLA/FAEPE. 88p.

FERNANDES, A.M.; SORATTO, R.P.; SILVA, B.L. 2011. Extração e exportação de nutrientes em cultivares de batata: I – Macronutrientes. Revista Brasileira de Ciência do Solo, Viçosa, v.35, p.2039-2056.

RAIJ, B. 2011. Fertilidade do solo e manejo de nutrientes. Piracicaba: IPNI. 420p.

SORATTO, R.P.; FERNANDES, A.M.; SANTOS, L.A.; JOB, A.L.G. 2013. Nutrient extraction and exportation by common bean cultivars under different fertilization levels: I – Macronutrients. Revista Brasileira de Ciência do Solo, Viçosa, v.37, p.1027-1042.

RESISTANCE LINES OF SNAP BEAN TO FUSARIUM WILT
(*Fusarium oxysporum* f. sp. *phaseoli*)

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INTRODUCTION

The genus *Phaseolus* is attacked by soil fungus, *Fusarium oxysporum* f. sp. *Phaseol*, which causes wilt or yellowing (Nunes, 2003), and once introduced to crops, has its most viable and effective control with the use of resistant cultivars (Sartorato & Rava, 1994). Considering that obtaining disease-resistant cultivars is one of the most efficient ways to avoid considerable losses of productivity (Pereira & Ribeiro, 2012), this work aimed to evaluate the performance of snap bean of determined growth as resistance to *Fusarium Oxysporum* f. sp. *Phaseoli* during the development of plants.

MATERIAL AND METHODS

Four strains of bean pods of determined growth habit were evaluated, using as a control the cultivar UEL 1. The sowing occurred on 04/03/2011, with five seeds per pot. For sowing, ceramic vessels with a capacity of five liters were used, with spacing of 0.1 m x 0.6 m between vessels. The soil used consisted of a clay soil with a clay texture, corrected through the formulated fertilizer 08-28-16 at the dose of 0.05 kg per pot. After the emergency, thinning was performed, maintaining one plant per pot. In the preparation of the inoculum the modified methodology of inoculation with colonized sorghum grains was used (Gasperi, 2000; Klingelfuss et al., 2002). Following the methodology, maize grains colonized with *Fusarium* were used for inoculation of the bean-pod strains, performed on 01/04/2011, when the plant reached the V4 development stage. In this procedure, two colonies of corn colonized on the substrate of each vessel were introduced. At the end of the crop cycle the yellowing symptoms caused by *Fusarium* were evaluated, and there were no lesions in the plant colon. In this evaluation, grades were assigned according to Gasperi's methodology (2000): resistant 0-1,0; Moderately resistant 1.1-1.5; Moderately susceptible 1.6-2.0; Susceptible 2.1-2.5 and highly susceptible 2.6-3.0. In the harvest performed on 06/05/2011, pods, roots and aerial part of the plants were weighed. The experimental design was completely randomized, in a 5x2 factorial scheme (five lines and two treatments, inoculated with *Fusarium* and without inoculation), with four replications. The data were submitted to analysis of variance and the means of the treatments compared by the Tukey test at 5% probability.

RESULTS AND DISCUSSION

As expected, there was a significant difference between the treatments in the severity question, being the treatment that did not receive inoculation, superior, compared to all inoculated lines. As for the latter, it is noted that the HAV 8 line obtained the highest mean severity, statistically considered to be the worst among the lineages and taking into account the scale of notes adopted in this study, was considered susceptible. The HAV 11 and HAV 21 strains obtained statistically similar results and were classified as moderately resistant. On the other hand, the HAV 34 and UEL 1 (control) strains obtained the best results regarding the analyzed item, being classified as resistant to *Fusarium* wilt, as shown in table 1. The susceptibility or resistance of (Table 1). In the present study, the pathogenic and pathogenic

interactions of pathogenic and pathogenic pathogens (Simão et al., 2010) were observed in all inoculated strains, with emphasis on the UEL-1 control and the HAV 8 and HAV 34, which, although originating from the same region, were shown to be opposite in terms of disease resistance, with HAV 8 being classified as susceptible and HAV 34 and UEL-1 as resistant. The results obtained in this work, in which different levels of resistance of lines from the same region were observed.

Table 1. Notes and degrees of resistance attributed to the roots of the bean lineages inoculated with *Fusarium oxysporum* f. sp. *Phaseoli,* based on the scale adapted from Gasperi (2000).

Treatments	Notes of symptoms	Degree of resistance
HAV 8	2,525	Susceptible
HAV 11	1,575	Moderately susceptible
HAV 21	1,3	Moderately tough
HAV 34	0,425	Resistant
UEL1	0,425	Resistant

CONCLUSION

The snap bean strains showed differences regarding resistance to *Fusarium oxysporum* f. sp. *Phaseoli*, with UEL-1 and HAV 34 being considered resistant. Because resistance is a characteristic of high heritability, it is possible to succeed in a breeding program for selection of resistant plants.

REFERENCES

GASPERI, A.C. Variabilidade de isolados, reação de cultivares e danos causados por *Fusarium solani* f.sp. *glycines* em soja. Passo Fundo, Dissertação (Mestrado) – Faculdade de Agronomia e Medicina Veterinária, Universidade de Passo Fundo, 91 p. 2000.

KLINGELFUSS, L.H., YORINORI, J.T., ARIAS, C.A.A. & DESTRO, D. Reaction of soybean cultivars to sudden death syndrome and disease scoring methods for screening resistance. Crop Breeding and Applied Biotechnology, p. 257-264. 2002.

NUNES, W. Manejo de fungos do solo na cultura do feijão irrigado. In: FANCELLI, A.L.; DOURADO NETO, D. (Ed.) Feijão Irrigado: Tecnologia e produtividade. Piracicaba: ESALQ, 2003. p. 127-151.

PEREIRA, R.B; PINHEIRO, J.B. (2012) Manejo integrado de doenças em hortaliças em cultivo orgânico. Circular Técnica, Brasília- DF. 12p

SARTORATO, A. & RAVA, C. A. Murcha ou amarelecimento de *Fusarium*. In: _____ (eds.). Principais doenças do feijoeiro comum e seu controle. Brasília, EMBRAPA-SPI. p. 175-190, 1994.

SIMÃO, G; ORSINI, I. P; SUMIDA, C. H; HOMECHIN, M (*in memorian*); SANTIAGO, D. C; CIRINO, V. M. Reação de cultivares e linhagens de feijoeiro em relação à *Meloidogyne javanica* e *Fusarium oxysporum* f. sp. *phaseoli*. Ciência Rural, Santa Maria, v.40, n.5, p.1003-1008, 2010.

INOCULATION AND LEAF FERTILIZATION OF MOLYBDENUM AND COBALT ON SNAP BEAN YIELD

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INTRODUCTION: The snap bean (*Phaseolus vulgaris* L.) is a vegetable consumed around the world. The physiological and morphological quality of pods are important characteristics for determinate the commercial pattern, so techniques must to be used for increase the production of pods. Inoculation and application of nutrients on the leaves are management practices with great response and low cost.

The aim of this work was to evaluate the seed inoculation with *Rhizobium tropici* strains and the leaf application of molybdenum and cobalt on the yield of snap bean genotypes with determinate growth pattern.

MATERIAL AND METHODS: The experiment was conduct in a greenhouse and was used the genotype UEL 2. The experimental design was complete randomized with four replications, resulting in a 2 *vs.* 4 factorial (2- with or without inoculation; 4- doses of commercial product) . The seeds were inoculated with a mix of *Rhizobium tropici* strains (SEMIA 4077, SEMIA 4080 and SEMIA 4088) following the method of Furlan et al. (2016) and the doses used were 0, 50, 100 and 150% of the ML71 recommended dose (mix of molybdenum and cobalt). Were evaluated number of pods per plant, fresh mass of pods and potential yield of snap bean culture. The data was submitted to analysis of variance and the averages were compared by the Tukey test ($p < 0.05$) and adjusted by polynomial regression equations.

RESULTS: The results showed that in the treatments with inoculation of the strain mix was observed an increase in all characteristics, independent the dose applied (Table 1). This phenomena can be attributed to the gain in the process of biological nitrogen fixation, obtained by the symbioses between bean plant and bacteria.

Table 1. Averages of fresh mass of commercial pods (FMCP), number of commercial pods (NCP) and potential yield of UEL 2 snap bean genotype inoculated or no with *Rhizobium tropici* strains mix.

	FMCP (g)		NCP		YIELD (kg)	
Inoculation	With	Without	With	Without	With	Without
Averages	30.11 a	8.96 b	5.45 a	1.99 b	3010.88 a	895.39 b
CVs (%)	8.20		12.78		8.19	

Different letters are significant at Tukey test ($p < 0.05$)

It was observed a significant effect between the sources of variation leaf fertilization (molybdenum plus cobalt) and inoculation (with or without) for all variables. The performance of FMCP, NCP and YIELD was similar (Figure 1), reaching a maximum point with further decrease. This fact can be explained by the toxicity caused by the high doses of molybdenum and

cobalt. Romanini Jr. et al. (2007) observed high levels of yield in the inoculated treatments as well as Jesus Jr. et al. (2004) achieved increase in the productivity of bean plant after leaf fertilization of molybdenum.

Figure 1. Doses of leaf fertilization of molybdenum and cobalt associated with inoculation of *Rhizobium tropici* strains mix in UEL 2 snap bean genotype. A- fresh mass of commercial pods (FMCP) (g), B- number of commercial pods (NCP) and C- potential yield (kg).
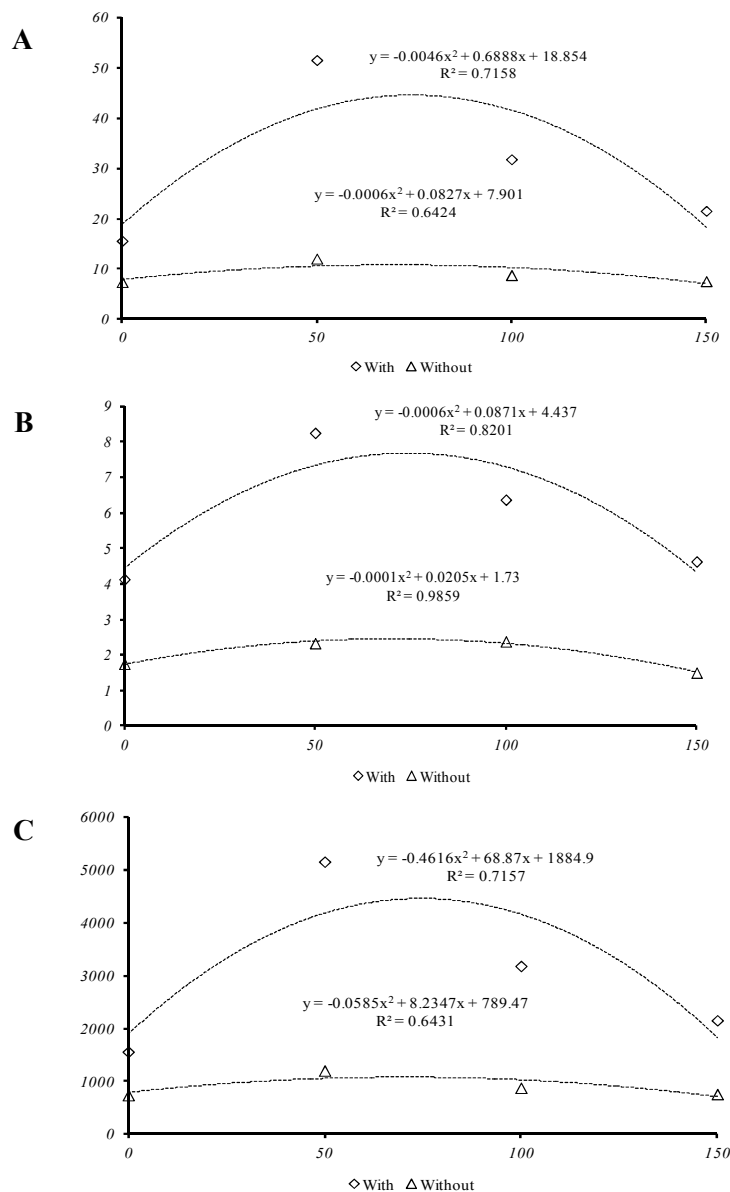
Furlan, FF, Freiria, GH, Alves, GAC, Gomes, GR, Almeida, LHC, Furlan, MF and Takahashi, LSA. (2016). The use of various strains of *Rhizobium tropici* for inoculation of snap bean cultivars with a determinate growth pattern. *Semina: Ciências Agrárias,* 37(6): 3965-3972.

Jesus Júnior, WC, Vale, FXR, Coelho, RR, Hau, B, Zambolim, L, Berger, R.D. (2004). Management of angular leaf spot in common bean (*Phaseolus vulgaris* L.) with molybdenum and fungicide. *Agronomy Journal,* 96 (3): 665-670.

Romanini Junior, A., Arf, O, Binotti, FFS, Sá, ME, Buzetti, S, Fernandes, FA. (2007). Avaliação da inoculação de rizóbio e adubação nitrogenada no desenvolvimento do feijoeiro, sob sistema plantio direto. *Bioscience Journal,* 23 (4): 74-82.

SNAP BEAN PRODUCTIVITY UNDER DIFFERENT POPULATION DENSITIES

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INTRODUCTION

The greatest expression of the productive potential of crops is the result of the combination of a set of factors, including the population of plants, by having strong influence in various morphological and physiological characteristics and grain yield. The correct choice of the population should take into consideration information such as time of sowing, region, history of disease in the area, growth habit, growing and soil fertility and thus outlining the proper spacing for the culture. For each cultivar, there is an optimum range of population where plants take advantage of maximum radiation, water and nutrients, providing increased production, which should be associated with the ease of implementation of cultural management and cost of inputs. The objective of this work was to evaluate the influence of the density of snap bean of determined growth on commercial production, aiming at management for high yields.

MATERIAL AND METHODS

The experiment was carried out at the State University of Londrina, with an altitude of 566 m, latitude 23º 23 'S and longitude 51º 11' W, in a soil classified as a dystrophic Red Latosol, the climate in the region classified according to Köeppen as subtropical moist mesothermic (Cfa) (Almeida et al., 2016). For the study were used the cultivars HAV 8 and UEL-1, both of determined growth. The experimental design was a randomized block design, with four replicates for each treatment. In each experimental unit, consisting of four rows, plant densities of 8, 10, 12, 14 and 16 seeds per linear meter were seeded. The line spacing was 0.50 m and, thus, the populations used as treatments for each material, were: T1 - 160000 plants ha^{-1}, T2 - 200000 plants ha^{-1}, T3 - 240000 plants ha^{-1}, T4 - 280000 plants ha^{-1}, T5 - 320000 plants ha^{-1}. The plants were harvested 65 days after planting, when approximately 75% of pods have reached the commercial point. There are evaluated the components: average weight of 10 commercial pods for treatment and productivity (kg ha^{-1}).For the classification of commercial and non-commercial in pods, the pods with a length of less than 10 cm, which were in the range of 10-15 cm long and with a diameter of less than 7 mm, and all the fibrous pods, were considered to be non-commercial. The rest were considered commercial. The data were subjected to analysis of variance and averages compared by Scott-Knott test, the 5% probability.

RESULTS AND DISCUSSION

For cultivar HAV 8, treatments 1, 4 and 5 provided higher yields. In general, for treatment 5, despite productivity similar to previous treatments, the average weight of commercial pods was lower, indicating that the higher the number of plants per area, the higher the competition for photoassimilates and the lower translocation of nutrients to the grains filling, as shown in table 1. Pereira et al. (2003) working with the snap beans cultivars of determined growth 'Cororalina' and' Talmalina ', in the densities of 66666 and 133333 plants ha^{-1}, obtained similar results regarding the average weight of pods, being verified larger Weights at the lowest density. These

authors also verified that there was no difference between the yields for the cultivar "Turmalina", in agreement with the results obtained in this work for the cultivar HAV 8.

Didonet and Costa (2004) also found similar results in work with common bean. As for UEL-1, Table 1 shows that the highest yields were obtained at the lowest densities, in treatments 1 and 2, showing that a smaller number of plants per area causes less competition between them, with higher availability of Nutrients, water and light, corroborating with the previous authors, who obtained higher productivity (17700 kg ha-1) in lower plant density for the 'Coralina' cultivar.

It was verified that the increase of the density of plants converged to lower yields, corroborating with Santos (2013) that, working with cowpea, also obtained a similar result. Comparing the cultivars, we can observe that both showed similar maximum yields, evidencing a greater compensatory capacity for the cultivar UEL 1, because the maximum productivity reached by this cultivar occurred when was used a lower plant density.

Table 1. Average weight and yield of cultivars HAV 8 and UEL-1.

Treatments (plants ha^{-1})	HAV8 Average weight of commercial pods (g)	HAV8 Productivity (kg ha^{-1})	UEL-1 Average weight of commercial pods (g)	UEL-1 Productivity (kg ha^{-1})
T$_1$ - 160000	60.90 a	2.189.00 a	72.05 a	2.259.00 a
T$_2$ - 200000	46.44 b	1.370.80 b	58.24 a	2.443.80 a
T$_3$ - 240000	48.91 b	1.426.80 b	48.46 b	1.719.80 b
T$_4$ - 280000	64.88 a	2.253.00 a	40.29 b	1.653.40 b
T$_5$ - 320000	50.97 b	2.476.60 a	46.01 b	1.488.40 b
C.V* (%)	14.74	26.21	23.51	21.68

Means followed by the same letter in the column do not differ in 5% level of probability by Scott-Knott test. * CV = coefficient of variation

CONCLUSION

Both cultivars had higher productivity and average weight of pods in the lowest population density.

REFERENCES

ALMEIDA, L.H.C.; FRATONI, M.M.J.; FREGONEZI, G.A.F.; TAKAHASHI, H.W.; (2016). Nutritional assessment of potassium in tomato (*Lycopersicon esculentum Mill.*) by direct reading of fruit sap. **Afr. J. Agric. Res.** Vol. 11(14), pp. 1245-1256.

DIDONET, A.D; COSTA, J.G.C. População de plantas e rendimento de grãos em feijoeiro comum de ciclo precoce. **Pesquisa Agropecuária Tropical**, 34 (2): 105-109, 2004 – 107.

PEREIRA, A.V; OTTO, R.F; REGHIN, M.Y. Respostas de feijão-vagem cultivado sob proteção com agrotêxtil em duas densidades de plantas. **Horticultura Brasileira**, v .21 n .3 jul/set. 2003.

SANTOS, J.F. Resposta do feijão-caupi a diferentes densidades de plantas em Neossolo Regolítico no Agreste Paraibano. **Tecnologia e Ciência Agropecuária**, v.7, n.4, p. 37-41, dez. 2013.

SENSORY AND INSTRUMENTAL ANALYSIS IN SNAP BEAN

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INTRODUCTION

Snap Bean (*Phaseolus vulgaris* L.) belongs to the family of legumes, for the production of this species is important vigor, yield, be resistant to diseases and pests; produce light green pods of shape and size that meet the requirements of the market; possess pleasant taste; have few wires or fibers, besides being a good source of protein for human nutrition (FILGUEIRA, 2013). Based on the economic importance of this activity in Brazil and that can make viable small farms, this work had the objective of sensory and instrumental evaluation of snap bean genotypes in order to verify which would have good acceptance with the consumer and could compete with the cultivars the market.

MATERIAL AND METHODS

The work was conducted at 23° 23' south latitude and 51° 11' west longitude and average altitude of 566m. Five genotypes of snap bean (HAV 06, HAV 11, HAV 28, HAV 50 and HAV 69) were sown from CIAT (International Center for Tropical Agriculture) and a standard cultivar, TORINO, already Indeterminate growth, with cylindrical shaped pods, as well as lineages. Harvesting was performed when the pods were tender and reached their ideal point 70 days after sowing or 20 days after flowering. After the pods were harvested, the sensorial analyzes were started.

RESULTS AND DISCUSSION

The HAV 69 lineage obtained the highest mean score (5.7) between the lines, on a scale varying from 1 to 7, being between "moderately liked" and "liked very much" and did not differ from Standard TORINO. In relation to the purchase intention, the HAV 69 line was again the one that obtained the highest average grade (4.1) among the lineages, on a scale ranging from 1 to 5, being close to "possibly buy" and did not differ from the control. It can be observed that there is a strong influence of the appearance of the pods on the intention of purchase by the consumers, since the treatments with greater sensorial notes presented greater intention to buy (Table 1).

Table 1. Sensory evaluation of appearance and purchase intention of pods

Genotypes	Appearance note	Intention to purchase note
TORINO	5,1 [a, b] ± 1,0	3,7 [a, b] ± 0,9
HAV 06	4,9 [b] ± 1,1	3,5 [b] ± 0,9
HAV 11	4,9 [b] ± 1,5	3,3 [b, c] ± 1,3
HAV 28	4,6 [b, c] ± 1,3	3,1 [b, c] ± 1,0
HAV 50	3,9 [c] ± 1,6	2,8 [c] ± 1,2
HAV 69	5,7 [a] ± 0,9	4,1 [a] ± 0,9

Means followed by [a,b,c] do not differ by Dunnett test at 5% probability.

The L* value measures the luminosity contained in the sample, and the higher the L* value the clearer the sample, the HAV 69 genotype has the lighter pods and does not differ from the TORINO standard. It turns out that the higher the brightness, the better the note for appearance and the greater the purchase intention for the pods. The variation of the value of a* shows the intensity of the green color in the sample, and the smaller the value of a*, the greater the intensity of green contained in the sample. The HAV 6 lineage has less green intensity than the other pods. There was no difference between the TORINO standard, which obtained the lowest value of a*, therefore it has the highest green intensity in its pods, and the other strains (HAV 11, HAV 28, HAV 50, HAV 69). The variation of the value of b* shows the intensity of the yellow color contained in the sample, the higher the value of b*, the greater the yellow color intensity in the sample. It can be observed that the cultivar TORINO has a higher yellow intensity than the others (Table 2).

Table 2. Color parameters (L *, a *, b *) of the cooked pods.

Genotypes	L*	a*	b*
TORINO	38,5 [a] ± 2,8	-7,3 [a, b] ± 1,2	19,9 [c] ± 2,1
HAV 06	34,3 [d] ± 2,1	-6,7 [b, c] ± 1,9	16,4 [a] ± 2,1
HAV 11	35,4 [c, d] ± 2,6	-7,0 [a, b, c] ± 1,0	17,8 [b] ± 2,6
HAV 28	38,5 [a] ± 4,6	-7,2 [a, b, c] ± 1,6	21,3 [d] ± 3,9
HAV 50	37,6 [a, b] ± 3,0	-7,6 [a] ± 1,5	19,4 [c] ± 2,2
HAV 69	36,9 [b, c] ± 2,2	-6,5 [c] ±1,7	18,9 [b, c] ± 2,0

Means followed by [a,b,c] do not differ by Dunnett test at 5% probability.

After cooking some changes in the coloring characteristics of the pods occurred, such as the decrease in the L* value, and that each pod behaved differently from the raw pod. The Torino, HAV 28 and HAV 50 are the clearest and do not differ statistically from each other, and HAV 6 and HAV 11 are the darkest ones. However, the HAV 28, HAV11, HAV 6 and HAV 69 obtained lower results, therefore a greater intensity of the green color, and did not differ significantly from the standard.

The cooking had an influence on the coloring parameters of the pods, it is observed that within the same line the cooking decreases the value of L*, and the raw pods are lighter than the cooked pods, that is, they have a higher value of L*. However, the value of a*, did not change significantly between raw and cooked pods. However, for the value of b*, significant changes occurred, and the raw pods have a higher yellow content, and when they pass through the cooking process this value is decreased, except for HAV 28 lineage that did not present difference in the value of b*.

CONCLUSION

Appearance of pods has a direct influence on the intention of purchase by consumers. The HAV 69 genotype has good appearance and intent to purchase grades, with the potential to compete with traditional market cultivars and a new option for family farmers.

REFERENCES

FILGUEIRA, F.A.R. Novo Manual de Olericultura: Agrotecnologia moderna na produção e comercialização de hortaliças, 3ª ed. Viçosa; UFV, 2013. 319 p.

GENETIC DISSIMILARITY AMONG GENOTYPES OF SNAP BEANS BASED ON YIELD AND PRODUCTION COMPONENTS

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INTRODUCTION: The snap bean belong to the same botanical Family and species of common bean (*Phaseolus vulgaris* L.), however is classified as a vegetable because its immature pods with tenuous grains are the consumed part, thus is essential the obtaining of genotypes that ally high yields and great pod quality. An alternative, which has been used to direct new improvement programs, is the study of genetic dissimilarity (Freiria et al., 2016).

The aim of this study is to evaluate the genetic dissimilarity of tem snap beans genotypes based on yield and production components, with the intent to identify the most promising crosses.

MATARIAL & METHODS: The rehearsal was conducted at Universidade Estadual de Londrina (UEL), located in latitude of 23º19'41.00" S, longitude of 51º12'18.19" W and altitude of 590 meters (Londrina, Parana state, Brazil). The design was completely randomized blocks with three repetitions, being each treatment composed by the tem genotypes of snap beans: Feltrin Vicenza Amarelo Baixo, UEL 1, UEL 2, T1, T3, T13, T24, T25, T39 and T41. Each parcel was composed by four lines (4m length) with a spacing of 0.50m between lines and were considered as borders the two external and 0.50m from the extremities of the central lines.

The seedling was in March 18th 2016 and the harvest realized in June 10th of the same year (fall/winter cultivation). Were measured the characteristics: Yield of pods (kg ha^{-1}), unitary pod´s fresh mass (g), number of pods per plant, pods diameter (mm) and length of pods (cm). For the dissimilarity analysis, the Mahalanobis generalized distance was estimated and was proceeded the cluster of UPGMA. The relative contribution of the characters was estimated based on Singh (1981). Also was made the main compounds analysis.

RESULTS: Based on Figure 1A it can be noted that the pods yield was the least contributor to dissimilarity (3.30%) and the pods length was the characteristic that presented the highest contribution, followed by pods diameter (40.49% e 24.73%, respectively). Based on UPGMA cluster (Figure 2B) is possible to make the separation of the genotypes into two groups, in 30% of genetic divergence: Group I – T25, T39, T3, T24, T1 and T13; and Group II – T41, Feltrin Vicenza Amarelo Baixo, UEL 1 and UEL 2. Results proximal to the ones obtained with the main compounds analysis (MCA) (Figure 2).

The Group I presented higher relation with the quality of pods, producing bigger pods in length and diameter, and with a higher unitary weight, consequently, pods with higher commercial appeal. Group II by the MCA was broken down into two new groups (Group IIa – T41 and Feltrin Vicenza Amarelo Baixo; and Group IIb – UEL 1 and UEL 2). The Group IIa presented higher association with the production vectors and number of pods per plant, which is, was constituted by more outputs genotypes. There was not observed any negative correlation among the vectors of the variables permitting to obtain productive genotypes and with a higher

pods quality (Figure 2). Thus, the cross between the individuals of Group I with the ones from Group IIa showed promising to the obtainment of new materials.
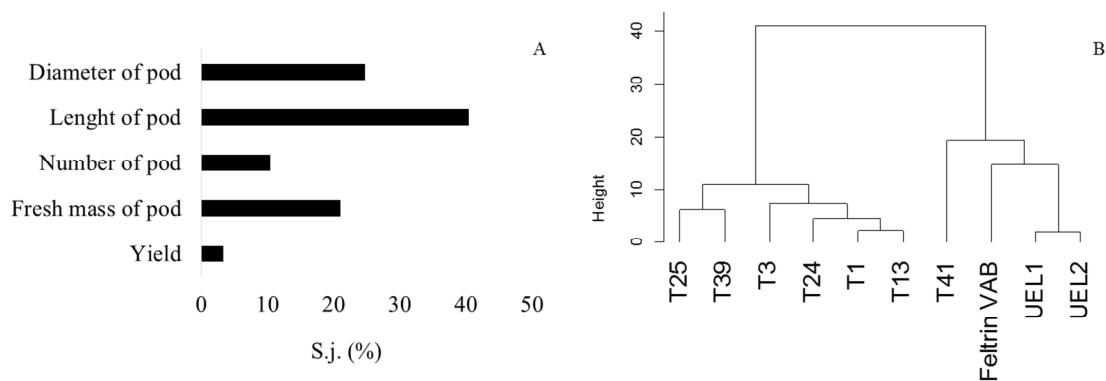
Figure 1. Relative contribution of the characters to the genetic dissimilarity according to the method proposed (A) and representative UPGMA dendrogram of the genetic divergence of the tem snap beans genotypes, obtained based on the Mahalanobis dissimilarity matrix (B).
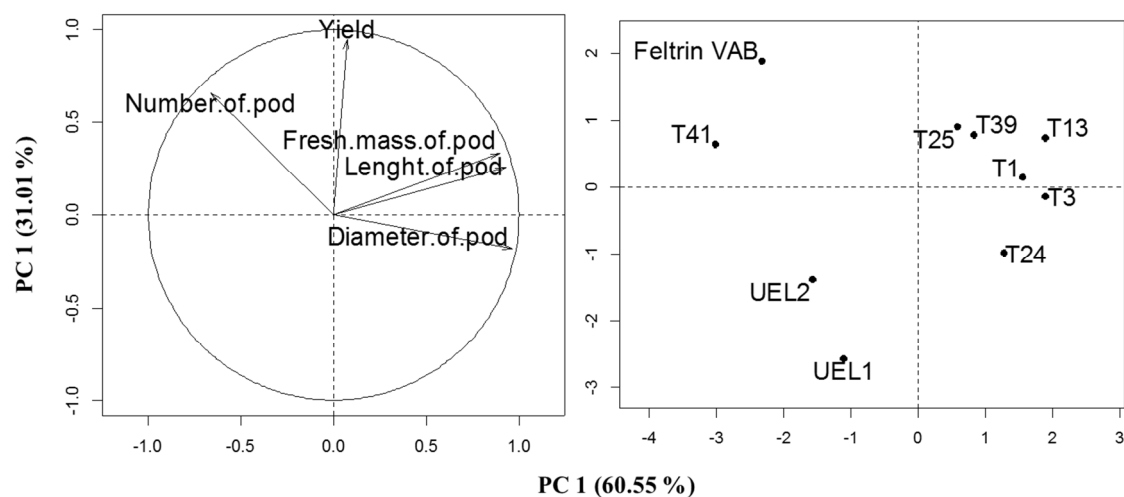
Figure 2. Main Compounds Analysis (MCA) of ten genotypes of snap beans to yield and pods production compounds.

REFERENCES

Freiria, GH, Bortolato, KS, Dias, FMV, Furlan, FF, Almeida, LHC, Gomes, GR, Bertoncelli, DJ, Alves, GAC, Omura, MS and Prete, CEC. (2016). Genetic divergence and correlations between characters of transgenic soybean cultivars. International Journal of Current Research, 8 (8): 36875-36880.

Singh, D. (1981). The relative importance of characters affecting genetic divergence. The Indian Journal of Genetics and Plant Breeding, 41: 237-245.

CORRELATIONS AND PATH ANALYSIS UNDER MULTICOLINEARITY IN THE ASSOCIATION OF VEGETATIVE CHARACTERISTICS WITH THE YIELD OF SNAP BEANS

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INTRODUCTION: The comprehension of the plant vegetative attributes in the yield is of a fundamental importance to the management, as well as to the genetic improvement. The main tool used in this study are the estimation of correlations, however, because they do not provide the relative importance of the direct and indirect influence of other characters in the yield, they do not determine the cause effect relation among them (Furtado et al., 2002).

The best comprehension of the evolved causes in this association can be obtained by the path analysis. On the other hand, multicolinearity conditions could produce inconsistent values or with any congruence with the biological phenomenon studied (Moreira et al., 2013). With the intention to study strategies to contour this effect it was aimed to evaluate different methods to sidetrack the multicolinearity, as well as study the simple correlation among the studied characters.

MATERIAL & METHODS: The experiment was conducted in the city of Londrina, Parana state, Brazil (23º19'41.00"S, 51º12'18.19"W and altitude 590m), in the period of March 18th until June 10th of 2016, in field conditions. Were evaluated three cultivars (Feltrin Vicenza Amarelo Baixo, UEL 1 e UEL 2) and seven accesses of snap beans from the germplasm bank of State University of Londrina (T1, T3, T13, T24, T25, T39 and T41). The evaluations were divided at the development stages R1 (plant´s height, total dry mass, foliar area, specific leaf area index and leaves dry mass) and R7 (plant´s height, total dry mass and yield of pods).

Were estimated the Pearson correlations and path analysis, in which the yield of pods was considered the basic variable and the other characters were considered the explicative variables. In conditions of high multicollinearity (Cruz; Carneiro, 2003) were proceeded the disposal of variables of high interrelation, as well as the crest path analysis.

RESULTS: Was observed in table 1 high interrelations among the vegetative characteristics of the snap bean plant, which had propitiate a multicolinearity considered severe (CN = 18526.29). To contour the adverse effects of multicolinearity, was applied at first, the exclusion of the leaf area and dry mass foliar variables in the development stage R1, which presented higher contribution to that fact, rendering the multicolinearity moderate (CN = 121.98). However, the effects of multicolinearity still yet observed (Table 2), as the presence of inconsistent values.

The second alternative to sidetrack this effect was the crest path analysis, with the value of k = 0.125 and with the utilization of all (Table 3). The coefficients of the path analysis obtained by this methodology show that the total dry mass of plants in the development stage R7 presented higher association with the yield of pods (0.74) with low participation of indirect effects.

Table 1. Pearson simple correlation values among the characteristics plant´s height (PH), total mass dry (TMD), foliar area (FA), index specific foliar area (ISFA), dry mass foliar (DMF) and yield of pods (YIELD) in snap beans.

	PH (R1[1])	TMD (R1)	FA (R1)	ISFA (R1)	DMF (R1)	PH (R7)	TMD (R7)	YIELD
PH (R1)	1	0.79**	0.80**	0.34	0.75*	0.64*	0.78**	0.27
TMD (R1)		1	0.95**	0.24	0.98**	0.55	0.47	-0.04
FA (R1)			1	0.42	0.93**	0.43	0.42	-0.09
ISFA (R1)				1	0.27	0.01	0.18	-0.27
DMF (R1)					1	0.58	0.46	-0.07
PH (R7)						1	0.83**	0.58
TMD (R7)							1	0.74*
YIELD								1

[1]At the development stages R1 and R7. **, * significant at 1% and 5%, by the t test, respectively.

Table 2. Estimative of the direct and indirect effects of the characteristics plant´s height (PH), total dry mass (TMD), index of specific foliar area (ISFA), under the yield of pods (YIELD) in snap bean.

Effect	PH (R1[1])	TMD (R1)	ISFA (R1)	PH(R7)	TMD (R7)
Direct on YIELD	-0.45	-0.15	-0.33	-0.18	1.37
Indirect via PH (R1)	-	-0.35	-0.15	-0.29	-0.35
Indirect via TMD (R1)	-0.12	-	-0.04	-0.08	-0.07
Indirect via ISFA (R1)	-0.11	-0.08	-	-0.01	-0.06
Indirect via PH (R7)	-0.12	-0.1	-0.01	-	-0.15
Indirect via TMD (R7)	1.06	0.64	0.25	1.13	-
Total (Pearson correlation)	0.27	-0.04	-0.27	0.58	0.74

[1]At the development stages R1 and R7.

Table 3. Crest path analysis with the estimative of the direct and indirect effects of the characteristics plant´s height (PH), total dry mass (TMD), index of specific foliar area (ISFA), dry mass foliar (DMF) under the yield of pods (YIELD) in snap bean.

Effect	PH (R1[1])	TMD (R1)	FA (R1)	ISFA (R1)	DMF (R1)	PH (R7)	TMD (R7)
Direct on YIELD	-0.08	-0.11	0.11	-0.27	-0.35	0.2	0.77
Indirect via PH (R1)	-	-0.07	-0.07	-0.03	-0.06	-0.05	-0.07
Indirect via TMD (R1)	-0.09	-	-0.11	-0.03	-0.11	-0.06	-0.05
Indirect via FA (R1)	0.08	0.1	-	0.04	0.1	0.05	0.04
Indirect via ISFA (R1)	-0.09	-0.07	-0.11	-	-0.07	-0.01	-0.05
Indirect via DMF (R1)	-0.26	-0.35	-0.33	-0.1	-	-0.21	-0.16
Indirect via PH (R7)	0.13	0.11	0.08	0.01	0.12	-	0.16
Indirect via TMD (R7)	0.59	-0.36	0.32	0.14	0.35	0.63	-
Total (Pearson correlation)	0.27	-0.04	-0.09	-0.27	-0.07	0.58	0.74

[1]At the development stages R1 and R7.

REFERENCES

Cruz, CD and Carneiro, PCS. (2006). Modelos biométricos aplicados ao melhoramento genético. Viçosa: Editora UFV.

Furtado, MR, Cruz, CD, Cardoso, AA, Coelho ADF and Peternelli, LA. (2002). Análise de trilha do rendimento do feijoeiro e seus componentes primários em monocultivo e em consórcio com a cultura do milho. Ciência Rural, 32 (2): 217-220.

Moreira, SO; Gonçalves, LSA, Rodrigues, R, Sudre, CP, Amaral Júnior, AT and Medeiros, AM. (2013). Correlações e análise de trilha sob multicolinearidade em linhas recombinadas de pimenta (*Capsicum annuum* L.). Agrária, 8(1): p. 15-20.

CENTERS OF DOMESTICATION FOR CHINESE, SPANISH, AND BEANCAP SNAP BEAN POPULATIONS

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INTRODUCTION: Snap beans were primarily developed in Europe after the Columbian Exchange through selection for low fiber pods, thicker pod walls, and pod stringlessness (Myers & Baggett, 1999). Abundant evidence supports separate domestications of dry beans in the Andean and Mesoamerican centers, and the best available evidence suggests that snap beans were derived from dry bean from both centers, although the majority descend from the Andean gene pool (Gepts, 1998). While most snap beans were developed in Europe, they are not exclusively found there. Snap beans may have also been developed in China, and there is evidence that at least one bean with low fiber pod traits may have been developed by Native Americans, viz. 'Trail of Tears.' Three unique bean populations from China, Spain, and North America have the potential to shed light on the broader development of snap beans and their dissemination pathways out of the Americas using modern molecular tools. The first of these populations is an uncatalogued collection of Chinese snap beans assembled from a trip in 1991 by Michael Dickson (Cornell Univ.) consisting of 58 genotypes. The second consists of a selection of 11 Spanish genotypes from the Misión Biológica de Galicia – CSIC (Pontevedra, Spain) collection. These are a subset of lines selected from this collection that possess edible pod traits (de Ron, personal communication). The last population, the BeanCAP diversity panels, consists 149 snap beans mostly from commercial bean lines in North America and Europe.

MATERIAL AND METHODS: Genomic DNA was extracted from young trifoliate leaves using a CTAB method and genotyped by the Soybean Genomics and Improvement Laboratory (Beltsville, MD) using the Illumina Infinium Genechip BARCBEAN6K_3 platform. The 'adegenet' R package was used to perform principal coordinate analysis, visualize the analysis, and then perform a discriminate principal component analysis with two clusters.

RESULTS AND DISCUSSION: Three axis of the principal coordinates analysis accounted for 50% of the variation with the first axis consisting of the Mesoamerican by Andean split. The Mesoamerican grouping consists mainly of heirloom pole bean accessions (Table 1). Notable Mesoamerican lines within the BeanCAP were 'Trail of Tears' and several Pole Blue Lake lines. Two old American heirloom snap beans were also of Mesoamerican origin, namely 'Aunt Hattie' and 'Grandma Nellie's Yellow Podded Mushroom Pole Bean.' Eighty-six percent of the Chinese genotypes were Mesoamerican whereas only 8% of the snap bean BeanCAP were Mesoamerican in origin (Table 1).

 The plot showed Andean groupings for European Extra Fine with Bush Blue Lake snap beans on the second axis distinct from Romanos and phaseolin type 'C' snap beans. Both European Extra Fine and Bush Blue Lake types represent substantial mixing between centers of domestication. Intermediate to these two extremes were the majority of commercial bush snap bean cultivars. Very few of Spanish accessions were of Mesoamerican origin (Table 1), with the majority of these grouping with Romano types. Refugee types formed a distinct group within the Andean center, but may have had some introgression from Mesoamerican germplasm distinct from what occurred with Bush Blue Lake snap beans.

Considering that the BeanCAP is representative of North American and European commercial lines and commercial breeding materials, the high number of Mesoamerican lines within the Chinese population could be a source of new germplasm and new traits for breeders and geneticists.

Table 1. Categorization of genotypes by center of origin based on a discriminate analysis of principle components with 2 clusters. An Andean origin is indicated by a "1" and a Mesoamerican origin is indicated by a "2".

Accession	Origin	Accession	Origin	Accesson	Origin	Accession	Origin	Accession	Origin	Accession	Origin	Accession	Origin
Chinese genotypes		91-3008	2	Benton	1	Gallatin_50	1	Paloma	1			Tapia	1
91-1009	2	91-3013	2	Black_Valentine	1	Galveston	1	Panama	1			Tendercrop	1
91-1028	2	91-3110	1	Blue_Peter_Pole	2	Gina	1	Paulista	1			Tendergreen	1
91-1033	2	91-3225	2	Bogota	1	Gold_mine	1	Pix	1			Teseo	1
91-1073	2	91-3255	2	Booster	1	Goldrush	1	Polder	1			Thoroughbred	1
91-1096	2	91-3346	2	Brio	1	Green_Arrow	1	Pole_Blue_Lake	2			Titan	1
91-1098	2	91-3389	2	Brittle_Wax	1	Grenoble	1	Pole_Blue_Lake_S7	2			Top_Crop	1
91-1104	2	91-3405	2	Bronco	1	Hayden	1	Pretoria	1			Trail_of_Tears	2
91-1145	2	91-3436	2	Cadillac	1	Hercules	1	Profit	1			True_Blue	1
91-1215	1	91-3588	1	Calgreen	1	Hialeah	1	Prosperity	1			Ulysses	1
91-1285	2	91-3594	2	Carlo	1	Hystyle	1	Provider	1			Unidor	1
91-1309	1	91-3709	2	Carson	1	Idaho_Refugee	1	Redon	1			US_5_Refugee	1
91-1443	1	91-3736	2	Castano	1	Igloo	1	Renegade	1			Valentino	1
91-1542	2	91-3857	2	Catania	1	Impact	1	Rocdor	1			Venture	1
91-1555	2	91-3915	2	Celtic	1	Jade	1	Rockport	1			Warrior	1
91-1574	1	91-3918	2	Charon	1	Kentucky_Wonder	2	Roller	1			Widusa	2
91-1613	2	91-3921	2	Cherokee	1	Koala	1	Roma_II	1			Zeus	1
91-1643	2	91-3982	2	Coloma	1	Kylian	1	Romano_118	1			Zodiac	1
91-1664	2			Contender	1	Labrador	1	Romano_Gold	1				
91-1672	2	**Spanish genotypes**		Corbette_Refugee	1	Landmark	1	Royal_Burgundy	1	**BeanCAP dry beans**			
91-1728	2	PHA0008	1	Cyclone	1	Landreths_Stringless	1	Saporro	1	Montcalm	1		
91-1738	1	PHA0112	1	Dandy	1	Magnum	1	Scorpio	1	Olathe	2		
91-1748	2	PHA0192	1	Derby	1	Masai	1	Seabiscuit	1	Seafarer	2		
91-1750	2	PHA0224	2	Doral	1	Matador	1	Secretariat	1	Gloria	2		
91-1755	2	PHA0272	1	Dusky	1	McCaslan_42	2	Selecta	1				
91-1759	2	PHA0315	2	Dutch_Double_White	2	Medinah	1	Serengeti	1	**Heirloom genotypes**			
91-1768	2	PHA0319	1	Eagle	1	Mercury	1	Serin	1	Aunt_Ada	1		
91-1772	2	PHA0385	1	Ebro	1	Minuette	1	Seville	1	Aunt_Hattie	2		
91-1892	2	PHA0401	1	Embassy	1	Navarro	1	Shade	1	Cosse_Violette	2		
91-1940	2	PHA0402	1	Envy	1	Nicelo	1	Sirio	1	Grandma_Nellies	2		
91-1976	2	PHA0453	1	Espada	1	Nomad	1	Slenderella	1	Guatemalan_hierloom	2		
91-1989	2			Esquire	1	Normandie	1	Slenderpack	1	Hidatsa_Shield_Figure	1		
91-2093	2	**BeanCAP snap beans**		EZ_Pick	2	NY6020_5	1	Sonesta	1	New_Mexico_Cave	2		
91-2094	2	Acclaim	1	Ferrari	1	Opus	1	Spartacus	1	Swiss_Landfrauen	1		
91-2095	2	Angers	1	Festina	1	Oregon_1604M	1	Speedy	1				
91-2096	2	Astun	1	Flavio	1	Oregon_2065	2	Stallion	1	**Other genotypes**			
91-2097	2	Balsas	1	Flavor_Sweet	1	Oregon_5402	1	Stayton	1	FM1_Pole_Blue_Lake	2		
91-2099	2	Banga	1	Flo	1	Oregon_5630	1	Storm	1				
91-2100	2	BBL156	1	Fortex	2	Oregon_91G	1	Strike	1				
91-2101	2	BBL274	1	FR_266	1	Oregon_Giant_Pole	2	Stringless_French	1				
91-2102	1	Benchmark	1	Fury	1	Palati	1	Summit	1				

REFERENCES

Gepts, P. (1998) Origin and Evolution of Common Bean: Past Events and Recent Trends. HortScience 33: 1124-1130.

Myers, J.R., and J.R. Baggett. 1999. Improvement of snap beans. p. 289-329. *In:* Singh, S. (ed.) Common Bean Improvement for the 21st Century. Kluwer Acad. Publ., Boston.

BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY IN GRAINS OF LIMA BEAN GENOTYPES

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INTRODUCTION: Lima bean (P*haseolus lunatus*) plays a relevant role, mainly in the Northeast region of Brazil, where it is an alternative source of income and food source for the population, which consumes it in the form of mature or green grains, decreasing dependence on common bean (*Phaseolus vulgaris*). The dry grains have nutritional value similar to common bean, containing approximately 63% of carbohydrates, 25% of protein and 6% of fibers (AZEVEDO et al, 2003). Grain consumption contributes synergistically to its medicinal properties as antioxidant, diuretic, anti-inflammatory, antitumor and antimicrobial, with a positive effect against some chronic diseases (DÍAZ et al, 2010). The cowpea (*Vigna unguiculata*) has nutraceutical properties quite pronounced and increasingly consumed in human food, the beneficial effects of its bioactive compounds remains unexplored (BECKER; SIDDHURAJU, 2007). In the literature there is little data available on Lima bean and its potential antioxidant capacity for which the grains of several varieties were analyzed.

MATERIAL AND METHODS: Whole grains of Lima bean genotypes were analyzed from bean germplasm bank of Embrapa Clima Temperado. As witnesses, two cultivars of cowpea, from Rio Grande do Sul State, were used. All the varieties analyzed had predominantly red color, except Baio control. The genotypes were cultivated at the Terras Baixas Experimental Station in plansoil with drainage deficiency and low fertility. Fertilization was performed with a mixture of avian bed, rock powder and natural phosphate in the same proportion using a dose of 2 t.ha^{-1}. After being harvested, they were dried to 12% water content and transported to the Embrapa Clima Temperado Food Nucleus.

For the analyzes of the raw and whole grains, the samples were ground in a knife mill for the purpose of obtaining flour. The flour was stored in capped polyethylene bottles and kept at room temperature. Analyzes were performed using the following methodologies: total anthocyanins (Fuleki and Francis, 1968); total carotenoids (Talcott and Howard, 1999, adapted from Swain and Hillis, 1959) and antioxidant activity for DPPH method (adapted from Brand-Williams et al., 1995).

RESULTS AND DISCUSSION: Among the varieties analyzed, G 195 and G 349 presented the best results for anthocyanins, differing significantly from the controls used. Segundo Puertas-Mejía et al. (2013) in addition to having a relatively high protein content, this grain also presents antioxidant substances, among them anthocyanins, a type of polyphenols present naturally in food and of great importance for health. Although beans are not considered sources of carotenoids, the varieties G 195A and cv. Baio were outstanding in relation to the others, with respectively 7.38mg/100g and 7.23mg/100g of total carotenoids.

The range in concentration of total phenolic compounds was reduced, and varied from 5,445mg/100g, in G 198, at 7,545mg/100g in the cv. Baio, which obtained the highest

concentration of total phenolic compounds and the highest antioxidant activity . Silva et al. (2009) to characterize physically and chemically raw bean cultivars also found correlated results in total phenolic compounds contents and antioxidant activity.

For antioxidant activity, there was also a statistical difference between the analyzed varieties. G 195 and cv Baio (check) presented the best antioxidant capacity but without statistical difference.

Table 1. Anthocyanins and carotenoids concentrations in Lima bean genotypes from germplasm bank of Embrapa Clima Temperado.

Genotypes	Total anthocyanins (mg equivalent cianidina-3-glicosídeo/100mg)	Total carotenoids (mg equivalent β-caroteno/100mg)
G 198	15.22+2.45 a*	6.42+0.25 ab
G 195A	2.99+0.33 c	7.38+0.29 a
G 349	15.92+1.15 a	1.76+0.51 c
Baio (C)	8.84+1.55 b	7.23+0.68 a
Amendoim (C)	2.60+0.22 c	6.59+0.18 ab

* Different letters in the column show significant difference of means by the Tuckey test at the 5% probability level

Table 2. Concentrations of phenolic compounds and antioxidant activity in Lima bean genotypes from germoplasm bank of Embrapa Clima Temperado.

Genotypes	Total phenolic compounds (mg equivalent chlorogenic acid/100mg)	Antioxidant activity (µg equivalent Trolox/g)
G 198	5445.14+499.83 a*	13670.33+70.33 c
G 195A	5750.35+572.57 a	20529.64+1341.59 ab
G 349	6108.38+369.98 a	16192.68+912.93 b
Baio (C)	7545.22+644.97 a	23411.81+1200.53 a
Amendoim (C)	6137.44+140.03 a	4775.36+309.57 d

* Different letters in the column show significant difference of means by the Tuckey test at the 5% probability level

CONCLUSIONS: There was variability among the cultivars regarding anthocyanins, carotenoids and antioxidant activity, but similar results for phenolic compounds. The genotypes G 198 and G 349 stood out for anthocyanins. Baio and G 349 for phenolic compounds while G195A and the Baio had the high antioxidant activity.

REFERENCES

AZEVEDO, J.N. et al. Composição química de sete variedades de feijão-fava. Teresina, PI: Embrapa Meio Norte, 2003. 4p. (Embrapa Meio Norte. Comunicado Técnico, 152.)

BECKER, K.; SIDDHURAJU, P. The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) Walp.) seed extracts. Food Chemistry, v. 101, p. 10-19, 2007.

DÍAZ, A.M. et al. Concentrations of condensed tannins and anthocyanins in common bean seed coats. Food Research International, v. 43, n.2, p. 595-601, 2010.

PUERTAS-MEJÍA, M.A. et al. Determinación de antocianinas mediante extracción asistida por radiación de microondas en frijol (*Phaseolus vulgaris* L.) de alto consumo en Antioquia-Colombia. Revista Cubana de Plantas Medicinales, v. 18, n. 2, p. 288-297, 2013.

SILVA, A.G. et al. Caracterização físico-química, digestibilidade protéica e atividade antioxidante de feijão comum (P*haseolus vulgaris* L.). Alimentos e Nutrição, Araraquara, v.20, n.4, p. 591-598, 2009.

MAPPING AND PREDICTING COLOR RETENTION AND OTHER QUALITY TRAITS IN BLACK BEAN POPULATIONS

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INTRODUCTION

Dry edible beans (*Phaseolus vulgaris* L.) provide an economical, nutritious food source for millions of people around the world and are grown in a diverse variety of sizes, colors, shapes, and agronomic traits. In the United States, dry beans are commonly consumed as ready-to-eat canned products, which makes canning quality important to industry standards and consumer preferences. When black beans are processed for canning, they dramatically lose their dark coloration, which results in an undesirable, faded-brown, canned product. Although phenotyping is time and labor-intensive, genotypes with superior color retention have been identified that can enable genetic improvement of this key trait. This research seeks to improve color retention and canning quality via complementary improvements in genotyping and phenotyping, bypassing the current onerous phenotyping procedures. Mapping color retention and canning quality traits to the dry bean genome may allow marker assisted selection, while refining predictive models may be able to efficiently screen dry seed.

MATERIALS AND METHODS

Several recombinant inbred line (RIL) populations were developed from parental genotypes that showed extreme differences in color retention (Table 1). Once canned, breeding line B14311 has abysmal color retention, resulting in light brown beans, while B12724 and B10244 'Zenith' have outstanding color retention, resulting in dark black beans. Color and appearance scores of canned beans are determined by a trained sensory panel using separate 1-5 hedonic scales, where 1 is very unfavorable (light brown color or split beans, respectively) and 5 is very favorable (dark black color or fully intact beans, respectively). B14311 was selected as a parent common to both populations due to the combination of poor color score and relatively high appearance score. The high appearance scores reflect good seed coat integrity, demonstrating that color loss in this genotype is not necessarily due to mechanical breakdown of the seed coat. Recurrent parents B12724 and 'Zenith' have good appearance scores and excellent color scores, despite having different genetic backgrounds and potentially different mechanisms of color retention. Selecting only parents with similar appearance scores minimizes any potential confounding effect between color and appearance ratings.

Approximately 150 RILs from each population were advanced to the $F_{4:6}$ generation before being canned using a modified protocol developed by Hosfield and Uebersax (1980). In addition to panelists' evaluation of color and appearance, canned RILs will be weighed, photographed, and analyzed for texture. At submission of this article, DNA from each RIL is being extracted via a modified CTAB protocol for genotyping on the BARCBean6K_3 microarray (Song et al., 2015). Genotypic data will be used to create linkage maps, which will then be combined with canning data to map quantitative trait loci (QTL) for color retention and appearance. This study will attempt to identify new QTL and validate previous findings by Wright and Kelly (2011) and Cichy et al. (2014).

Table 1. Phenotypic comparison of parents used in the mapping populations.

	Agronomic Traits					Canning Traits	
	Yield	**100 Seed Wt.**	**Days to**	**Lodging**	**Agron.**	**Appearance Score†**	**Color Score***
Parent	**(cwt/acre)**	**(g)**	**maturity**	**(1-5)**	**Score**	**(1-5)**	**(1-5)**
B14311	24.2	18.7	96	1.0	5.0	3.7	1.7
ZENITH	23.3	22.4	96	1.0	4.8	4.2	5.0
B12724	22.0	21.2	101	1.0	3.5	3.5	4.8

Parental lines have similar agronomic traits, but contrasting color retention. *Zenith and B12724 have excellent color retention, while B14311 has abysmal color retention. †Appearance scores measuring degree of splitting are similar among parents. This minimizes the confounding effect of seed coat mechanical failure as the cause of leaching. Data from MSU 2015 Standard Black Yield Trials (Wright and Kelly, 2015)

FUTURE DIRECTIONS

Imaging data of the RILs not only provides objective measurements of color components for QTL analysis, but also permits the generation and refinement of predictive models. Images of canned beans will produce models for color and appearance, which can then be compared to actual color and appearance scores of the panelists. Dry beans from each RIL will be subjected to visible and near-infrared reflectance spectroscopy (Vis/NIR) and hyperspectral imaging to refine predictive models for color, appearance, and texture that were previously developed by Mendoza et al. (2014, 2017). These models can then be validated by imaging dry seed of additional black bean lines. Select lines will be canned and phenotyped to determine the potential for phenomic selection of color retention and other canning quality traits in black beans.

REFERENCES

Cichy, K.A., A. Fernandez, A. Kilian, J.D. Kelly, C.H. Galeano, S. Shaw, M. Brick, D. Hodkinson, and E. Troxtell. 2014. QTL analysis of canning quality and color retention in black beans (*Phaseolus vulgaris* L.). Mol. Breed. 33(1): 139–154.

Hosfield, G.L., and M.A. Uebersax. 1980. Variability in physico-chemical properties and nutritional components of tropical and domestic dry bean germplasm. J. Amer. Soc. Hort Sci 105(2): 246–252.

Mendoza, F.A., K. Cichy, R. Lu, and J.D. Kelly. 2014. Evaluation of Canning Quality Traits in Black Beans (*Phaseolus vulgaris* L.) by Visible/Near-Infrared Spectroscopy. Food Bioprocess Technol. 7(9): 2666–2678.

Mendoza, F.A., J.D. Kelly, and K.A. Cichy. 2017. Automated prediction of sensory scores for color and appearance in canned black beans (*Phaseolus vulgaris* L.) using machine vision. Int. J. Food Prop. 20(1): 83–99.

Song, Q., G. Jia, D.L. Hyten, J. Jenkins, E.-Y. Hwang, S.G. Schroeder, J.M. Osorno, J. Schmutz, S.A. Jackson, P.E. McClean, and P.B. Cregan. 2015. SNP Assay Development for Linkage Map Construction, Anchoring Whole Genome Sequence and Other Genetic and Genomic Applications in Common Bean. G3 Genes|Genomes|Genetics 5(11): 2285-2290.

Wright, E.M., and J.D. Kelly. 2011. Mapping QTL for seed yield and canning quality following processing of black bean (*Phaseolus vulgaris* L.). Euphytica 179(3): 471–484.

Wright, E.M., and J.D. Kelly. 2015. MSU 2015 Standard Black Yield Trial.

COMPARISON OF COOKING TIME OF COMMERCIAL BEAN VARIETIES AND NEW BREEDING LINES DEVELOPED IN EASTERN AFRICA

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INTRODUCTION

Long cooking time is a major constraint to domestic bean consumers and the processing industry in eastern Africa because it requires more energy, time and increases the cost of production of processed dry bean products, and reduces their competitiveness with other grain legumes. Cooking time of dry beans can vary from 1½ to 8 hours depending on variety (Miles and Sonde, 2004; Kimani et al, 2005). However, little has been done to develop fast cooking bean varieties in eastern Africa (Kimani et al, 2005). Breeding fast cooking bean is now critical due to strong preference for fast cooking and processed foods especially in urban communities. Cooking time of commercial bean varieties grown in eastern Africa under controlled or comparable conditions is not known. Such information will provide a baseline against which improvement in cooking time can be measured. Our objective was to compare the cooking time of commercial varieties and new advanced bean lines selected for fast cooking at the University of Nairobi.

MATERIALS AND METHODS

Cooking time of 34 bean genotypes were determined using a Mattson cooker in the Food Science laboratory, Upper Kabete campus, University of Nairobi. The genotypes included 10 popular commercial varieties, seven recently released biofortified varieties and 17 advanced lines of diverse market classes selected for fast cooking (Table 1). The study genotypes represented the Andean and Mesoamerican gene pools and the major market classes grown in east, central and southern Africa. Beans were for soaked for 16 hours before cooking. Each trial was replicated three times. Data was analysed using Genstat software (v15).

RESULTS AND DISCUSSION

Results showed highly significant (P<0.001) differences in cooking time among the test genotypes (Table 1). Cooking time varied from 26 to 106 minutes for all genotypes. This indicated adequate genetic variation for cooking time among these genotypes. Cooking time of commercial varieties varied from 47. 3 minutes (Mex 142) to 105.9 minutes (KAT 56). Among the red mottled market class, three biofortified varieties and three advanced lines cooked significantly faster (23 to 40 minutes) than the corresponding commercial check varieties, Rosecoco and KAT B69. Three red kidney lines cooked significantly faster (71 to78 minutes) than Canadian Wonder (GLP 24) and KAT56. A similar trend was observed for small red, navy, pinto and specked sugar market classes. Among the new biofortified bean varieties, cooking time varied from 26 minutes (*Kenya Afya*, *Kenya Cheupe* and *Kenya Majano*) to 45 minutes (*Kenya Maua*). Cooking time of ten advanced canning bean lines varied from 26 minutes (KCB 13-10) to 36 minutes (KCB 13-01). Industry reference canning variety, Mex 142, cooked in 47.3 minutes. This indicates that the new canning bean varieties cooked much faster than any of the

popular commercial varieties. Results indicated that selection for fast cooking was effective and represent significant advances in development of fast cooking beans in east, central and southern Africa. Utilisation of the fast cooking varieties and advanced lines can save time, energy and promote bean consumption in urban and rural consumers and bean processing industry.

Table 1. Cooking time of advanced lines, biofortified and commercial bean varieties.

Advanced lines			Biofortified varieties		
Genotype	Market class	Cooking time (minutes)	Variety	Market class	Cooking time (minutes)
BCB11-144	Red mottled	30.6	Kenya Afya	Yellow	28.3
BCB11-204	Sugar	28.0	Kenya Almasi	Brown	40.7
BCB11-245	Small red	30.1	Kenya Cheupe	Navy	40.9
BCB11-327	Red kidney	27.0	Kenya Majano	Yellow	26.5
BCB11-62	Navy	28.6	Kenya Maua	Red mottled	36.3
KCB11-01	Red mottled	29.3	Kenya Madini	Red mottled	26.6
KCB13-02	Red mottled	36.1	Rosecoco Madini	Red mottled	40.0
KCB13-03	Red kidney	33.9			
KCB13-04	Red kidney	34.8			
KCB13-05	Sugar	34.6	**Commercial varieties**		
KCB13-06	Sugar	32.1	Variety	Market class	Cooking time (minutes)
KCB13-07	Small red	30.2	Mex 142	Navy	47.3
KCB13-08	Small red	33.8	GLP1104	Mwezi Moja	71.0
KCB13-09	Navy	26.5	GLP 2	Red mottled	67.1
KCB13-10	Navy	25.9	GLP 24	Red kidney	70.9
KCB13-11	Navy	31.4	GLP 585	Small red	81.3
KCB13-12	Navy	28.3	GLP 92	Pinto	57.0
			KAT56	Red kidney	106.0
			KAB B1	Yellow	48.0
			KAT B69	Red mottled	56.7
			KAT B9	Medium red	100.5
Mean (all genotypes)		43.6	**LSD $_{0.05}$**		12.6

REFERENCES

Kimani, P.M., R.A. Buruchara, K. Ampofo, M. Pyndji, R. Chirwa, R. Kirkby. 2005. Breeding bean for smallholder farmers in Eastern, Central and Southern Africa: Constraints, achievements and potential. *Proc. Pabra Millenium Workshop*, 28 May – 2 June 2001, Arusha, Tanzania, p. 11-28.

Miles, C.A. and M. Sonde. 2004. Evaluation of water absorption rate in niche market dry beans. *Annu. Rep. Bean Improv. Coop*. 47:219-220.

COOKING TIME AND SENSORY ANALYSIS OF A DRY BEAN DIVERSITY PANEL

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INTRODUCTION

Cooking time and sensory quality are two important traits when selecting dry beans for consumption, but have largely been overlooked by breeders in favor of yield and other traits. Dry beans are an affordable, nutrient-rich food, but often require long cooking times, particularly without prior soaking. They also display a range of sensory characteristics, with consumers preferring cooked beans that are sweet and soft[1]. Increased interest in dry beans to make new products necessitates studies assessing the diversity of sensory traits in beans, which would allow beans to be selected for specific products. In this study, the Andean Diversity Panel[2] (ADP) was assessed for cooking time and sensory characteristics in order to identify diversity for these traits.

MATERIALS AND METHODS

Cooking Time Evaluation: 398 genotypes of the ADP were harvested in Hawassa, Ethiopia in 2015, six months prior to evaluation. Prior to cooking, each sample was soaked for 12 hours in 250 ml distilled water after ensuring moisture content was between 10-14%. Two replicates per genotype of 25 seeds each were cooked in random order in boiling distilled water using the Mattson cooker method for determining cooking time[3]. The Mattson cooker uses twenty-five 85g stainless steel rods with 2mm diameter pins that pierce beans loaded in wells when sufficiently cooked. For this study, the 50% and 80% cooking times were recorded, and the 80% cook time is regarded as the time required to cook each genotype to completion. The cooking time data was analyzed using the MIXED procedure in SAS with genotype as a fixed effect and rep as a random effect.

Sensory Analysis: Before sensory evaluations were made, a panel of reviewers was trained to rate boiled beans using the determined hedonic scales (Table 1). Once training was complete, sensory evaluations of 388 genotypes from the ADP commenced. The beans were prepared by boiling to the length of time determined using the Mattson cooker, and lemon water was used as a palette cleanser between genotypes. Four panelists tasted twenty genotypes per session and evaluated them according to their training. Each genotype was tasted in two separate sessions for a total of eight evaluations per genotype. Following collection of sensory ratings of the diversity panel, the data was analyzed using the MIXED procedure in SAS, with genotype as a fixed effect. Random effects included rep, reviewer, session, reviewer*seed type, and session*seed type. Seed type was also evaluated as a fixed effect with the random effect genotype(seed type) included in the model.

RESULTS AND DISCUSSION

The genotypes and seed types investigated in this panel exhibited a diversity of cooking times and sensory characteristics (Table 2). The cooking times ranged from 16.7 to 68.9 minutes across the for the 2015 harvest. The distribution of cooking times by seed type covered a narrower range, but cooking time differences among seed types are still apparent. ANOVAs at the genotype and seed type levels revealed significantly different cooking times among genotypes and seed types at $\alpha = 0.05$.

For sensory characteristics, the genotype level showed statistical significance at $\alpha = 0.05$ for all traits but beany, cooked, and earthy. This reflects a range of sensory characteristics present in the ADP and provides information regarding which beans may perform well as ingredients, as

extremes for future sensory evaluations, or even as potential breeding material. As the seed type level showed statistical significance in few sensory characteristics, it appears that a range of sensory characteristics exist within each seed type. This suggests that currently, seed type does not define the flavor or texture of a dry bean, but presents an opportunity to target consistent, desirable sensory profiles when breeding dry beans for current and new market classes.

Future work involves a second year of data collection and association mapping to reveal genomic loci that influence cooking time and sensory traits. This information can enable breeders to target faster cooking times and specific sensory profiles and allow for improvement of agronomic traits without sacrificing desirable cooking time and sensory quality.

Table 1: 5-point hedonic scales used by reviewers for evaluation of sensory characteristics.

5-Point Hedonic Sensory Evaluation Scales	
Flavor Intensity	1-5, bland to strongly flavored
Beany	1-5, no/very little bean flavor to very bean flavored
Cooked	1-5, very raw flavor to very cooked flavor
Vegetative	1-5, no vegetative flavor to very strong vegetative flavor
Earthy	1-5, no earthy flavor to very strong earthy flavor
Starchy	1-5, no starchy flavor to very strong starchy flavor
Sweet	1-5, no sweetness to very sweet
Bitter	1-5, no bitter flavor to very bitter
Seed Coat	1-5, no perceptible seed coat to very tough seed coat
Texture	1-5: mushy (1), smooth (2), grainy (3), thicker grainy quality (4), or chunky (5)

Table 2: Cooking time and sensory data for the ADP 2015 harvest. Number of genotypes (N), or seed types for "Cook Time (S)", mean, median, range, and p-values from the analyses of variance of the cooking time and sensory evaluation data of the diversity panel.

	N	Min	Median	Mean	Max	St. Dev	CV%	P-value
Cook Time	398	16.70	30.30	31.48	68.88	7.14	10.37	<.0001
Cook Time (S)	26	23.05	30.69	30.87	39.77	3.23	8.14	<.0001
Flavor Intensity	388	1.75	2.88	2.87	3.88	0.44	15.28	<.0001
Beany	388	1.75	2.75	2.78	4.00	0.41	14.71	0.1731
Cooked	388	3.63	4.50	4.50	5.00	0.30	6.56	0.0814
Vegetative	388	1.13	2.00	2.02	3.38	0.38	18.99	<.0001
Earthy	388	1.13	1.88	1.97	3.14	0.34	17.49	0.1808
Starchy	388	1.88	3.13	3.10	4.38	0.37	12.07	<.0001
Sweet	388	1.00	1.63	1.65	3.25	0.39	23.55	<.0001
Bitter	388	1.00	1.63	1.69	3.88	0.37	21.99	<.0001
Seed Coat	388	1.75	3.00	2.97	8.38	0.48	16.13	<.0001
Texture	388	1.25	2.63	2.61	4.00	0.42	16.24	<.0001

REFERENCES:
    1. Mkanda, A. V., Minnaar, A. & De Kock, H. L. Relating consumer preferences to sensory and physicochemical properties of dry beans (Phaseolus vulgaris). *J. Sci. Food Agric.* **87,** 2868–2879 (2007). **2.** Cichy, K. A. *et al.* A Phaseolus vulgaris diversity panel for andean bean improvement. *Crop Sci.* **55,** 2149–2160 (2015). **3.** Wang, N., Daun, J. K. & Malcolmson, L. J. Relationship between physicochemical and cooking properties, and effects of cooking on antinutrients, of yellow field peas (Pisum sativum). *J. Sci. Food Agric.* **83,** 1228–1237 (2003).

DARKENING OF THE SEED COAT IN DRY BEAN GENOTYPES DURING EARLY DAYS AFTER HARVEST

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INTRODUCTION

As it is a center of origin of dry beans (*Phaseolus vulgaris* L.), in Mexico there are a wide variety of colors and shapes that are consumed. Post harvest, darkening of the seed coat is a problem in light colored beans, which decreases its marketability. In the lab, coat color is evaluated by reflectance spectrophotometry and it is used as a parameter for selection in genetic improvement. Although this trait exhibits a clear genotype-environment interaction, it is possible to select genotypes with greater color stability during storage, which is associated with increased in shelf life. In previous studies (Jacinto *et al.* 2006, 2007) polyphenol oxidase activity has been associated with the proneness of genotypes to darkening during storage. To select genotypes less prone to darkening of seed coat, accelerated aging is induced in the beans by increasing the temperature and relative humidity, this process implies time and work. The objective of this study was to assess the tendency of a group of eleven genotypes to darken during the first 44 days after harvesting.

MATERIALS AND METHODS

During PV 2014, eleven dry bean genotypes were sown at Santa Lucía de Prías, Texcoco, estado de México. The experimental plot was one 4 m- long row. Except for one black seed coat variety, the other 10 genotypes were light colored either with pattern or one single color. Upon reaching maturity, the plants of each plot were hand threshed. The first day after threshing color was measured using a CM-5 spectrophotometer (Konica Minolta, Inc., Osaka, Japan). Color reflectance was recorded in the CIE Lab color coordinate system, with D65 Illuminant and 10º observer. Samples were then left at room temperature in glass cases for 44 days. During 28 days color measurements every two to three days were taken; then it was measured at 44 days. Data was processed through an analysis of variance and a correlation test.

RESULTS AND DISCUSSION

Genotypes exhibited significant differences in color variables L*, a*, and b* (P≤0.01). With the passing of the days, genotypes of clear coat darkened. The L* value decreased in those with clear coat, and increased in the black: Negro Perla. After 44 days, the diminishing in L* value compared to its control (0 days after harvesting), was up to -6.7 units. Data of six improved varieties are shown on figure 1. Genotypes increased in different magnitude of reddish tones (maximum Δa*=2.9 units) while the b* variable had a tendency to decrease. In general the color change was associated with an increase in the reddish colors in light colored beans and in some cases, as Flor de Mayo, also yellowish tones increased; while in yellow beans intensity of yellow decreased. In Negro Perla the more days the less red (a*) tones and slightly increased blue (b*) Within the light colored varieties Pinto Saltillo and Bayo Azteca showed low proneness to darken. While Flor de Mayo M-38, Flor de Durazno and Bayomex showed higher level of darkening. Two accessions of native bean called vaquita-rojo (V. rojo) and vaquita negra (V. negra) (figure 2) were distinguished for minimum color change. By comparing the color (L*) of

the eleven genotypes 7, 15 and 44 days after harvest, correlations between L* (7 days AH) and L* (15 days AH) were detected r =0.92. While L* (15 days AH) and L* (44 days AH) r = 0.91. Between L* (7 days AH) and L* (44 days AH) r= 0.84

CONCLUSION

The results suggest that the reflectance spectrophotometry may be used in the early days after harvest to select the genotypes less prone to postharvest darkening without performing accelerated aging tests.
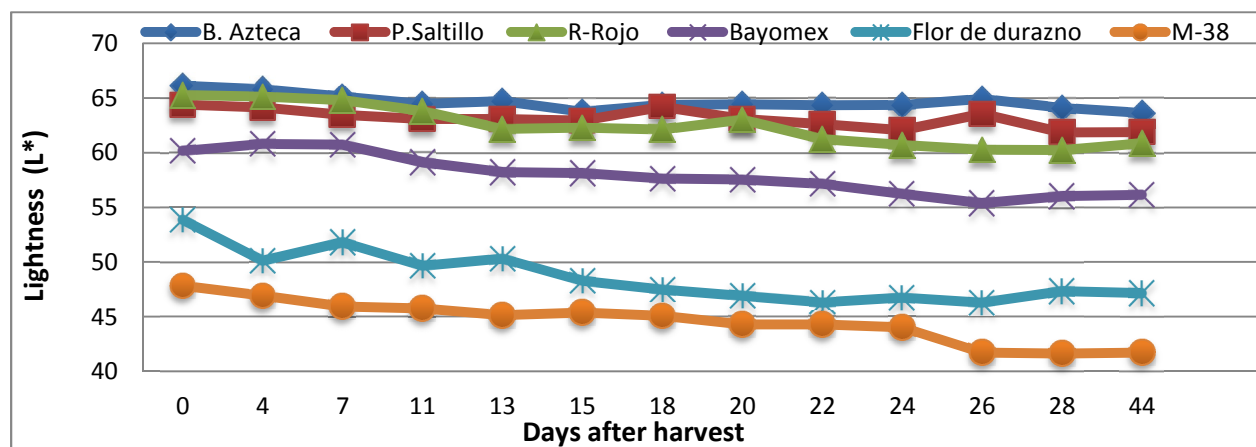
Figure 1. Color changes of the seed coat of 6 improved varieties of *Phaseolus vulgaris* L. measured during 44 days at room temperature after harvest
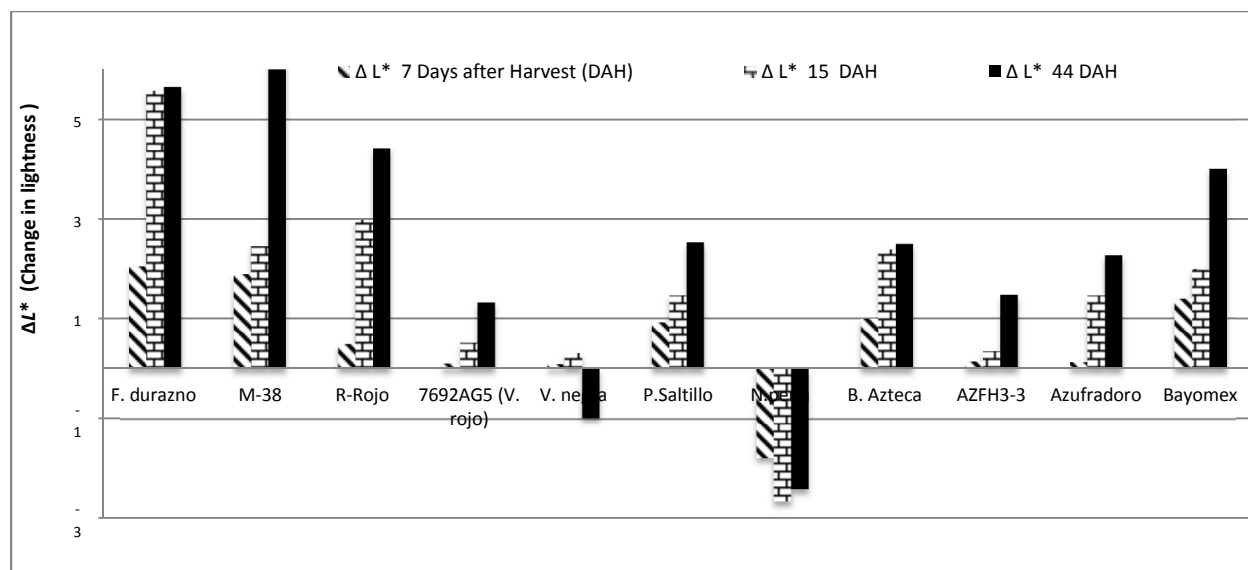
Figure 2. Change in lightness (ΔL*) of seed coat color of 11 improved and native varieties of *Phaseolus vulgaris* L.

REFERENCES

Jacinto-Hernández, C., Garza-García, R., Campos Escudero, A., Bernal-Lugo, I. 2006. Annual Report of the Bean Improvement

Jacinto-Hernández, C., Garza-García, R., Campos Escudero, A., Bernal-Lugo, I. 2007. Annual Report of the Bean Improvement

GENOTYPIC VARIATION IN FLATULENCE CAUSING OLIGOSACCHARIDES IN BIOFORTIFIED AND COMMERCIAL DRY BEAN VARIETIES

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INTRODUCTION

Elimination of flatulence is a challenging practical problem associated with consumption of legumes. The problem is compounded by the variability in susceptibility among individuals. Rackis (1981) established that the oligosaccharides-verbascose, stachyose and raffinose are major causes of flatulence. They escape digestion and are fermented by intestinal micro floral to form excessive amounts of carbon dioxide and hydrogen. Little has been done to develop bean varieties that combine agronomic superiority with nutritional quality, fast cooking and low flatulence levels in eastern Africa. The objectives of this study were to: (i) determine if there is genotypic variation in concentration of oligosaccharides associated with flatulence in commercial bean varieties, recently released biofortified bean and advanced breeding lines, and (ii) the effect of cooking on oligosaccharide concentration.

MATERIALS AND METHODS

Study materials were 10 commercial dry bean varieties and seven recently released biofortified cultivars representing the Andean and Mesoamerican gene pools and the major market classes grown in east, central and southern Africa. Verbascose, stachyose and raffinose were extracted twice from ground raw and cooked bean milks using a 3:7 v/v methanol-water mixture and quantified on a high performance chromatography system using analytical grade standard reagents. The oligosaccharides sugars were quantified using a high performance liquid chromatography (Chromatography Systems model 750, Shimadzu, USA) with a differential refractometer. The precipitated material was removed by centrifugation and the supernatant filtered prior to analysis. The column used (250mm*4.6mm i.d) was packed with spherisorb-5-amino, as a slurry in propan-2-ol. Sample injection valve, model 7120 was used. The eluting solvent was acetonitrile/water (67:33, v/v) with a flow rate of 2.0 ml min^{-1} at a temperature of 40^0C. Quantification was carried out by peak area comparisons of sample and standards of known concentration (Pinthong et al, 1980). Standards were obtained from SIGMA-Aldrich, USA. Data was analyzed using Genstat statistical software (v15).

RESULTS AND DISCUSSION

There were significant differences in total oligosaccharide, raffinose and stachyose concentration among the genotypes (Table 1). However, differences in verbascose concentration were not significant. Cooking significantly reduced concentration of total oligosaccharides, raffinose and stachyose. Total oligosaccharide concentration varied from 4.1 % (*Kenya Almasi*) to 5.89% (*Kenya Madini*) with a mean of 4.96%. Raffinose concentration varied from 0.40% (KCB13-08) to 0.05% (GLP2). Stachyose concentration varied from 2.3% (*Kenya Almasi*) to 4.2% (*Kenya Madini*). Most genotypes showed only traces of verbascose except KCB13-05 (0.028%), KCB 13-06 (0.47%), *Kenya Majano* (0.049%) and GLP 1004 (0.15%). Mex 142, which is known to be low in flatulence causing factors, had total oligosaccharide concentration

of 5.43. Except for *Kenya Afya*, most of the genotypes with higher levels of oligosaccharides were red mottled, small red and red kidneys. However, there were coloured genotypes with low oligosaccharide concentration. This implied that ability to induce flatulence may be a genotype specific trait, which may not be associated with grain colour or size. Flatulence in the study genotypes was largely due raffinose and stachyose. The results indicated that there is adequate variation for oligosaccharide concentration to facilitate selection for low flatulence beans of diverse market classes, which can promote consumption of dry beans.

Table 1. Cooking time and grain oligosaccharide concentration of new biofortified bush bean varieties and commercial varieties.

Variety	Cooking time (minutes)	Total oligosaccharide (%)		Raffinose (%)		Stachyose (%)		Verbascose (%)	
		Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked
Kenya Maua	44.9	5.030	4.848	0.1885	0.0670	3.342	3.281	0.0000	0.0000
Kenya Afya	26.5	6.938	4.143	0.1840	0.0945	5.254	2.549	0.0000	0.0000
Kenya Madini	40.9	6.835	4.953	0.3395	0.1240	4.995	3.329	0.0000	0.0000
Kenya Almasi	26.6	4.307	4.577	0.1995	0.0810	2.607	2.996	0.0000	0.0000
Kenya Cheupe	26.5	4.362	4.579	0.2935	0.2355	2.569	2.843	0.0000	0.0000
Kenya Majano	36.3	4.845	5.706	0.0770	0.0830	3.171	4.123	0.0970	0.0000
Rosecoco Madini	40.0	4.307	4.577	0.1995	0.0810	2.607	2.996	0.0000	0.0000
Checks									
Mex 142	47.3	5.716	5.147	0.2030	0.1810	4.013	3.466	0.0000	0.0000
GLP1004	71.1	4.356	4.797	0.1870	0.1200	2.369	3.177	0.3000	0.0000
GLP 2	67.1	4.394	4.753	0.0485	0.0815	2.846	3.172	0.0000	0.0000
GLP 24	70.9	4.551	5.235	0.0810	0.1025	2.970	3.633	0.0000	0.0000
GLP 585	81.3	5.157	4.761	0.1225	0.1265	3.534	3.135	0.0000	0.0000
GLP 92	57.0	3.971	4.760	0.1790	0.1425	2.292	3.117	0.0000	0.0000
KAT B1	48.0	5.308	4.659	0.1205	0.0905	2.887	3.447	0.0000	0.0000
KAT 69	56.7	5.371	5.879	0.0530	0.0690	3.818	4.310	0.0000	0.0000
KAT 56	105.9	4.922	4.826	0.0875	0.0870	3.335	3.240	0.0000	0.0000
KAT B9	100.5	4.878	4.474	0.1260	0.0900	3.252	2.885	0.0000	0.0000
Mean		5.132	4.788	0.2404	0.1169	3.375	3.171	0.0161	0.0000
LSD$_{0.05}$		0.0868		0.00728		0.0838		0.01817	
CV(%)		14.5		0.7		0.1		0.00732	

CONCLUSIONS: Results of this study indicate that there was considerable genetic variation in flatulence causing factors in the genotypes studied. Cooked samples of six of the new varieties had low oligosaccharide concentration compared with industry standard, Mex 142 and most of the commercial varieties. Raffinose and stachyose were the main oligosaccharides in these varieties, with only traces of verbascose. In addition, *Kenya Cheupe* showed excellent canning characteristics. Dissemination and utilization of the new bean varieties can increase productivity, incomes, reduce micronutrient malnutrition, save time and costs of cooking, reduce incidence of flatulence, promote value addition and consumption in eastern Africa.

REFERENCES: Rackis, J.J. 1981. Flatulence caused by soya and its control through processing. JAOCS (1981): 503-509

GENETIC VARIABILITY ASSOCIATED TO TECHNOLOGICAL QUALITY OF BEAN ELITE LINES OF SPECIAL GRAINS

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INTRODUCTION: Special grains beans have gotten more space and generally reach higher prices in the national and international markets, due to their great diversity of types, sizes, shapes, colors and brightness. In the breeding, the large demand to obtain more uniform and productive cultivars can lead to loss of some characters, such as the technological quality of the grains (COELHO *et al*., 2007). Thus, in order to meet the demands of producers and acceptance by consumers, it is necessary to consider the selection of genotypes with reduced cooking time, without hard grains and with a lower percentage of shell, since these characteristics have a wide variability and are influenced by the genotypic constitution and environmental conditions. Thus, the objective of this work was to evaluate the genetic variability associated to characteristics related to the technological quality of genotypes of special grains common bean.

MATERIALS AND METHODS: The experiment was carried out at the Bean Research Laboratory of the Federal University of Viçosa, in Viçosa-MG. The field essay from which origin to the grains used was conducted in Coimbra-MG, in the 2012 fall-winter. We used 12 elite lines and four common bean cultivars of special grains components of the value for cultivation and use (VCU) test. The experimental design was in randomized blocks, with three replications. The technological quality of the grains was evaluated by means of the cooking time using the Mattson cooker, with samples of 25 grains, according to the method adapted by Proctor and Watts (1987). The percentage of hard grains was determined with samples after immersion of one hundred grains in 200 mL of distilled water for 16 hours at ambient temperature. The grains that did not soak were counted being identified by the wrinkling of the shell. The percentage of shell was performed with samples of 5 cooked grains. From these grains, the shell and the cotyledons were separated; dry in an oven, at 105°C until constant weight. After that, the shell and cotyledons were weighed to determine the percentage of shell, according to the following formula: % Shell = (shell weight (g) + cotyledon weight (g) x 100). The data obtained were submitted to analysis of variance and, when significant, the effects were studied by the Tukey test at 5% of significance.

RESULTS AND DISCUSSION: Genetic variability among the evaluated genotypes was observed for all traits analyzed. The VR-18 line showed the same cooking time as the PT-65 line, which was 25.05 and 30.30 minutes, respectively, and the shortest cooking times, amongst the other genotypes (Table 1). Bean genotypes with shorter cooking times have consumer preference, as it means energy and capital savings, in addition to reducing the time to prepare the meal. The VR-14 line showed the highest percentage of hard grains, followed by BRS Timbó and Jalo EEP genotypes. However, most genotypes evaluated did not present hard grains (Table 1). Hard grains are undesirable, as they can increase the cooking time, and reflect in the commercial depreciation of the product. The Ouro Vermelho, BRS Timbó, VR-18 and BRS

Vereda genotypes showed the lowest percentage of shell, which is required, since the lower the value obtained the lesser the remnants of shell at the end of chewing, and so the cultivar will have greater acceptance (OLIVEIRA *et al.*, 2013).

TABLE 1: Mean values of cooking time (COOKING), percentage of hard grains (HG) and percentage of shell (SHELL) of common bean genotypes of "special grains" grown in the 2012 winter in Coimbra-MG.

GENOTYPES	COOKING (min)	HG (%)	SHELL (%)
OURO VERMELHO	30,35 d[1]	0,00 c	1,87 d
BRS TIMBÓ	31,15 d	1,00 b	2,57 d
BRS VEREDA	40,95 a b	0,00 c	4,65 c d
BRS RADIANTE	36,55 b c	0,00 c	5,60 c
VR-18	25,05 e	0,00 c	2,19 d
PT-65	30,30 d e	0,00 c	10,72 b
JALO EEP	32,25 c d	0,33 b c	6,67 c
PT-68	33,55 c d	0,00 c	11,98 b
CNFJ 15288	33,90 c d	0,00 c	11,80 b
RAD/E550-284	34,00 c d	0,00 c	10,36 b
VR-14	34,45 c d	29,00 a	11,79 b
VR-15	35,45 c d	0,00 c	17,25 a
CNFRx 15275	35,45 c d	0,00 c	16,79 a
RC2RAD-155	36,50 b c	0,00 c	10,17 b
VR-16	36,70 b c	0,00 c	6,16 c
VR-17	42,70 a	0,00 c	5,71 c
CV(%)	3,81	14,84	11,28

[1] Means followed by the same letter in the column do not differ by Tukey test at 5% significance.

CONCLUSIONS: There is genetic variability for all traits associated with the technological quality evaluated in the genotypes of special grains. The VR-18 line stands out among the other genotypes evaluated for having all technological characteristics favorable to the consumer market.

REFERENCES

COELHO, C. M. M. *et al*. Diversidade genética em acessos de feijão (*Phaseolus vulgaris* L.). Ciência Rural, Santa Maria, v. 37, n. 5, p.1241-1247, 2007.

PROCTOR, J. R.; WATTS, B. M. Development of a modified Mattson bean cooker procedure based on sensory panel cookability evaluation. Canadian Institute of Food Science and Technology Journal, Apple Hill, v. 20, n. 1, p. 9-14, 1987.

OLIVEIRA, V. R. *et al*. Perfil sensorial de cultivares de feijão sob diferentes tempos de cozimento, Alimentos e Nutrição, Araraquara, v. 24, n. 2, p. 145-152, 2013.

GENETIC VARIABILITY FOR GRAIN TECHNOLOGICAL TRAITS IN ANDEAN COMMON BEAN GENOTYPES

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INTRODUCTION

Andean common bean group presents tegument coloration and grain size variability. In this group, Dark Red kidney, Cranberry, Great Northern, Light Red kidney, Pinto, among others are the main commercial bean classes (Blair et al., 2010). High hydration rates, grain integrity and low cooking time are grain technological traits demanded by consumers and processors. These traits favor the fast prepare and also grain freezing and canning (Della Corte et al., 2003). Since there is genetic variability for grain technological traits among the Andean genotypes, the development of promising lines is possible by bean breeding programs to reach market demands. The aim of this study was to evaluate grain technological traits of Andean common bean genotypes in order to use them as variability source in the development of new cultivars.

MATERIAL AND METHODS

Fourteen bean genotypes belonging to the Andean origin center (Table 1, Figure 1) were grown in the experimental field of the Agronomic Institute of Paraná, Santa Tereza do Oeste, PR, Brazil (lat 25° 23', long 53° 38', 749 m asl), from August to November 2016, corresponding to the rainy season. The experimental design used was randomized blocks with two replications. After the harvest, a sample of 300 grams of the grains of each experimental plot was taken to evaluate the following grain technological traits: water retention capacity before cooking (WRCbc), water retention capacity after cooking (WRCac), solids content in the sauce after cooking (SC) and cooking time (CT) according to the methodology used by Della Corte et al. (2002). All determinations were performed in duplicate. The data were submitted to hierarchical grouping analysis (HGA) using XLSTAT software.

RESULTS AND DISCUSSION

The genotypes showed variability for the grain technological traits (Table 1). The mean values of WRCbc ranged from 96.28 to 108.78 g 100g^{-1} and for WRCac from 130.30 to 151.92g 100g^{-1}. All genotypes showed low cooking time (13 to 18.5 minutes) and low SC in the sauce (0.77 to 1.09 g 100g^{-1}). Other groups of Andean beans presented CT between 22 to 27 minutes and WRCbc from 106 to 107 g 100g^{-1} (Cichy et al., 2015). The low time for cooking the grains is an important trait, since it implies a reduced cooking time and low energy expenditure, facilitating its preparation, as well as the high capacity of water absorption provides greater yield after cooking, these traits make the beans an easy food processing and attractive to consumption. HGA employed in the study of grain technological traits classified the genotypes into three groups (G1, G2 and G3). All the genotypes of the Great Northern commercial class were inserted in the G1 group, except IPR Garça cultivar. This group presented high WRCac and SC content. KID44 (Light Red Kidney) genotype also showed similar traits to G1. The Cranberry genotypes (BRS Realce and BRS Radiante) and Pinto bean (LPSIA09-07 and LPSIA09-38) are in the G2 group and their main traits are low WRCbc, WRCac and CT. In G3 group were joined the Dark Red Kidney genotypes (BRS Embaixador, LP15-04 and G6416) and IPR Garça (Great Northern) that are associated with high WRCbc. The genotypes showed diversity for grain

technological traits and the possibility of development of new cultivars with desirable grain traits for consumer market.

Table 1. Average values of the grain technological traits and HGA groups of Andean bean genotypes, cultivated in the 2016 rainy season, in Experimental Field of Santa Tereza do Oeste, PR, Brazil.

Genotypes	Group	TC[1]	WRCbc[2]	WRCac[3]	SC[4]	CT[5]
BRS Ártico	1	Great Northern	96.29	150.87	0.89	14.75
LP05-06	1	Great Northern	103.38	144.48	0.90	15.75
LP05-07	1	Great Northern	98.07	148.05	0.93	18.00
LP05-17	1	Great Northern	99.18	147.08	0.78	15.00
LP06-01	1	Great Northern	105.14	151.92	1.09	16.25
KID44	1	Light Red kidney	101.32	140.75	0.89	15.50
		G1 Mean	**100.56**	**147.19**	**0.91**	**15.88**
BRS Radiante	2	Cranberry	100.05	136.25	0.88	16.25
BRS Realce	2	Cranberry	98.36	139.94	0.77	15.00
LPSIA09-07	2	Pinto	96.28	137.81	0.91	15.75
LPSIA09-38	2	Pinto	98.98	130.30	0.88	14.00
		G2 Mean	**98.42**	**136.08**	**0.86**	**15.25**
BRS Embaixador	3	Dark Red Kidney	108.78	142.01	0.91	13.00
G 6416	3	Dark Red Kidney	104.74	136.24	0.82	18.25
LP15-04	3	Dark Red Kidney	107.98	138.92	0.91	13.25
IPR Garça	3	Great Northern	107.38	139.62	0.84	18.50
		G3 Mean	**107.22**	**139.20**	**0.87**	**15.75**

[1]Tegument color, [2]water retention capacity before cooking, [3]water retention capacity after cooking, [4]solids content in the sauce after cooking, [5]Cooking time.
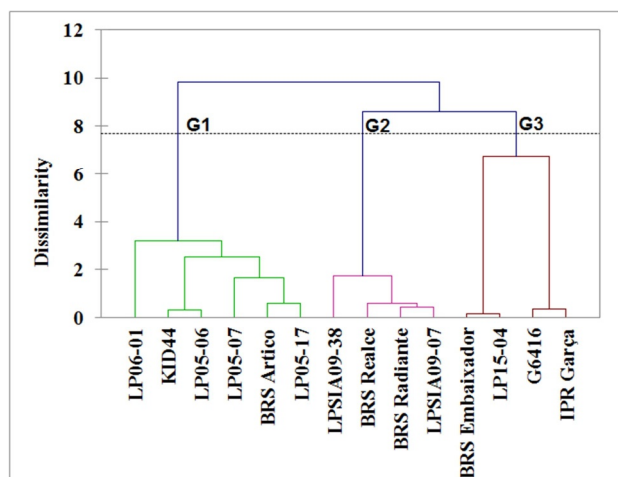

Figure 1. Grouping from 14 Andean common bean, based on grain technological traits analyses of genotypes cultivated in the 2016 rainy season, in Experimental Field of Santa Tereza do Oeste, PR, Brazil.

REFERENCE

Blair, M. W. et al. 2010. Genetic diversity, inter-gene pool introgression and nutritional quality of common beans (*Phaseolus vulgaris* L.) from Central Africa. Theor. Appl Genet, 121: 237-248.

Cichy, K. A. et al. 2015 Genetic diversity and genome-wide association analysis of cooking time in dry bean (*Phaseolus vulgaris* L.). Theor. Appl Genet, 128: 1555-1567.

Della Corte, A. Et al. 2003. Environment effect on grain quality in early common bean cultivars and lines. Crop Breed. Appl. Biotechn.3:193-202.

IMPACT OF INTERACTION P AND Zn ON BIOFORTIFICATION OF BEAN

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INTRODUCTION: A large part of the world population has low levels of micronutrients, especially pregnant women and children in developing countries. Zinc (Zn) is a micronutrient, performing many functions in the organism. Biofortifcation aims to increase the concentration of certain mineral in parts consumed of the cultivated plants. The crops of most interest are those that constitute the basic diet for the majority of the population, including beans.

Two main strategies are used in biofortification, selection of cultivars more favorable to biofortification and increase the micronutrient dose. Biofortification of bean with Zn is difficult due to interaction with phosphorus (P). The decrease in zinc absorption caused by phosphorus depends on soil atributes such as pH, cation exchange capacity and direct reaction with Zn and subsequent precipitation. The increase of the dose of Zn can reduce the absorption of iron by plants by competitve inhibition.

This study aimed to evaluate the interaction between P and Zn about biofortication in two bean cultivars, evaluating the concentration of Zn and iron in the grain and production.

MATERIALS AND METHODS: The experiment was carried out in a greenhouse of the Department of Soil Science at the Federal University of Lavras, Lavras, Minas Gerais, Brazil. Treatments were arranged in a completely randomized design with two replicates and a factorial scheme 2x2x4 involving two bean cultivars (Alvorada and Estilo), two doses of P (200 and 400 mg kg^{-1}) and four dose of Zn 4 (0, 25, 50 and 100 mg kg^{-1}).

Pots were filled with 3 Kg of soil, the soil used were classified as dystroferric Red Latosol (LVdf), with a clay texture that contained the following chemical attiributes: pH in water = 5; P = 1,13 mg dm^{-3}; K = 54,0 mg dm^{-3}; Ca = 1,5 cmol$_c$ dm^{-3}; Mg = 0,2 cmol$_c$ dm^{-3}; Al = 0,4 cmol$_c$ dm^{-3}; H+Al = 6,3 cmol$_c$ dm^{-3}; SB = 1,84 cmol$_c$ dm^{-3}; t = 2,24 cmol$_c$ dm^{-3}; T = 8,14 cmol$_c$ dm^{-3}; m = 18%; cation exchange capacity at pH 7 = 23%; organic matter = 2,87 dag kg^{-1}; P-rem = 12,93 mg L^{-1}. Was previously performed liming on the soil, increasing the saturation by base to 70 %, the levels of Ca^{2+} and Mg^{+2}. The experiment was conducted with two plants per pot until the formation of the grains. At the end of the experiment was determinated dry gain mass (MSG) per pot and the levels of Zn and Fe in grains.

For the application of the respective treatments it was used NH$_4$H$_2$PO$_4$ p.a. as source of phosphorus and for Zn, was used ZnSO$_4$.7 H$_2$O p.a. The other nutrients were apllied in the following amounts, three N aplications were performed during the cultivation, totaling 300 mg N kg^{-1}, K= 150 mg kg^{-1}, S= 40 mg kg^{-1}, B=0,81 mg kg^{-1}; Cu= 1,33 mg kg^{-1}; Fe= 1,55 mg kg^{-1}; Mn= 3,66 mg kg^{-1}; Mo= 0,15 mg kg^{-1}. Data were submitted to an analysis of variance (p <0.05) and regression models that fit the data.

RESULTS AND DISCUSSION: Triple interaction was observed for the effect on MSG and Zn content, for the Fe content, only interaction between P and Zn dose and between P and Cultivar dose were noticed, comparing the arrangements with each variable analyzed. For the Zn doses, regression models were tested, and the second and first degree models adjusted the data.

The greatest responses in grain production (Fig. 1) was observed in cultivar Estilo at the lowest dose of P, being increased the MSG according to the increase of the dose of Zn apllied. At the highest P dose, the cultivar Estilo showed a decrease in MSG, showing a possible toxicity due to higher doses of Zn (50 and 100 mg kg-1), compromising the production. The cultivar Alvorada was more tolerant to this Zn toxicity. In the lowest dose of P, did not respond to the aplication of Zn, was only observed at the highest dose where there were the MSG decrease linearly with the increase of the Zn dose. This result showed the antogonism between P and Zn, that the increase of available Zn limits the adsorption of P, with consequent loss in productivity. Higher levels of Zn increased the Zn content in the grain (Fig. 1), being favorable to the biofortification process, this increase was not verified for the cultivar Estilo in the dose of

400 mg P kg^{-1}, because in higher doses of Zn (50 e 100 mg kg^{-1}), the plants could not reach the reproductive stage.
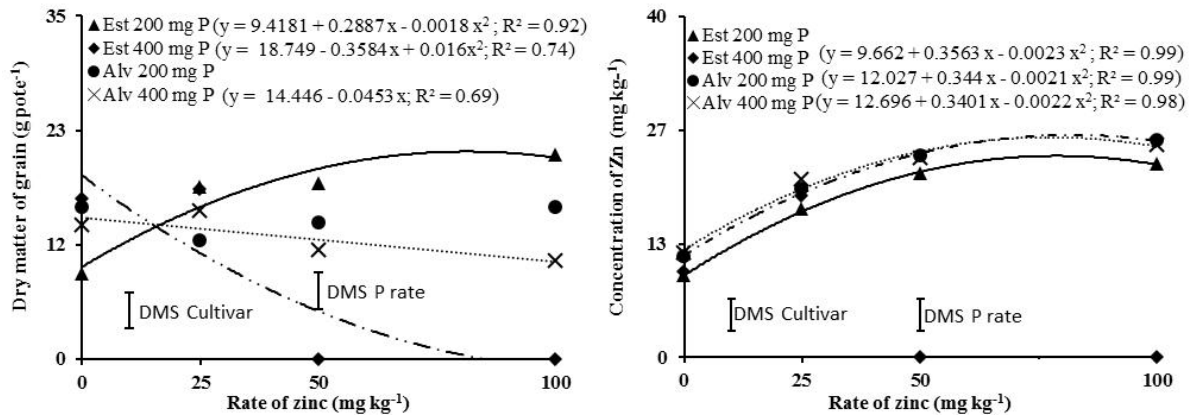

Figure 1: Effect of the interaction Cultivar x Phosphorus x Zinc on grain production and concentration of Zn, Est - Cultivate Style, Alv - Cultivate Dawn, 200 mg P - dose of 200 mg kg - 1 of P, 400 mg P - dose of 400 mg Kg-1 of P, DMS Cultivar - minimum significant difference between cultivars, DMS P rate, minimum significant difference between doses of P. Coefficient of variation: 13.95% and 17.35% respectively for grain harvest and Zn concentration.

The Fe content in the bean grains decreased with the increase of the Zn dose, being this reduction was more expressive when it was used as higher doses of P (Fig. 2). This shows that there is an inhibition of Fe absorption due to the increase in the amount of Zn in the soil derived from the application of a larger dose, suggesting possible competition between both. A lower dose of P had higher Fe content in the grain, with increases of 160 % and 112 % respectively. P to doses of 50 and 100 mg kg $^{-1}$ of Zn was not observed for other doses.

Zn together with P may decrease the uptake of Fe by plants. It is observed in figure 1 that with the increase of the dose of P, the cultivar Estilo showed a decrease in the Fe content in the grains, fact not observed for the cultivar Alvorada. Among the tested cultivars Alvorada showed higher Fe content for both doses of P.

The interaction P and Zn directly influences the biofortification with Zn in the beans, and adjusting fertilizations with P does not compromise biofortification. It is also worth mentioning that the beans serve as a source of Fe for the population, and this interaction can depreciate the Fe content in the grains, thus compromising their nutritional potential.
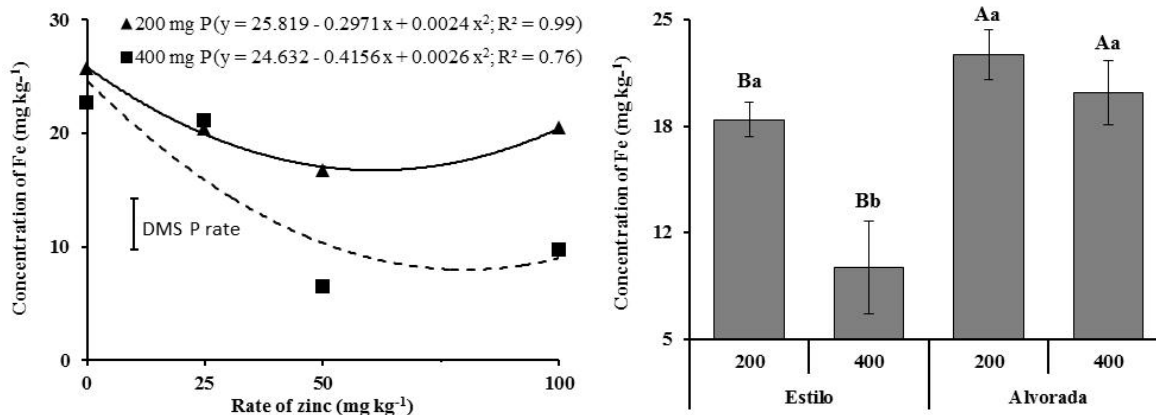
Figure 2: Concentration of Fe in the grains as a function of the interaction P and Zn, and P and Cultivar, DMS - Minimum significant difference between doses of P, upper case letters compare the cultivars within the same dose of P, lower case letters compare the doses of P within the same Cultivar, upper and lower case letters are not differentiated by the F test (p <0.05), coefficient of variation: 21.66%.

CONCLUSIONS: The increase in the application rates of Zn increases concentration of Zn in grain bean. The concentration of Fe in grain of bean reduce with increases application rates of Zn in bigger magnitude to rate of P of 400 mg kg^{-1}.

The cultivar Alvorada is more tolerant to fertilization with Zn.

THE MANTECA YELLOW BEAN: A GENETIC RESOURCE OF FAST COOKING AND HIGH IRON BIOAVAILABILITY PHENOTYPES FOR THE NEXT GENERATION OF DRY BEANS (*Phaseolus vulgaris* L.)

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Dry beans (*Phaseolus vulgaris* L.) are a nutrient dense food produced globally as a major pulse crop for direct human consumption. Despite being rich in protein and micronutrients, long cooking times limit the use of dry beans worldwide, especially in regions relying on wood and charcoal as the primary sources of fuel for cooking, such as Sub-Sahara Africa and the Caribbean. Coincidently, these same regions also have high densities of women and children at risk for micronutrient deficiencies [1]. There is need for a fast cooking bean, which can positively impact consumers by reducing fuel cost and preparation time, while simultaneously complementing the nutritional quality of house-hold based meals [2].

To help accelerate a reliable increase in dry bean production for Sub-Saharan Africa, the Andean Bean Diversity Panel (ADP; http://arsftfbean.uprm.edu/bean/) was assembled as a genetic resource in the development of fast cooking, nutritional improved, biotic/abiotic resistant varieties. A germplasm screening for atmospheric cooking time (100°C) of over 200 bean accessions from the ADP identified only five fast cooking entries [3]. Two entries were white beans from Burundi (Blanco Fanesquero) and Ecuador (PI527521). Native to Chile, two of the six fast cooking entries were collected from Angola, and had a pale lemon 'Manteca' yellow seed color (Cebo, Mantega Blanca). Traditional knowledge from Chile suggests Manteca yellow beans are low flatulence and easy to digest [4].

Yellow beans of various shades are important in Eastern and Southern Africa. Their popularity has increased in recent years and they often fetch the highest prices at the marketplace. There is evidence to suggest that Manteca yellow beans have a unique nutritional profile when compared to other yellow seed types; with more soluble dietary fiber, less indigestible protein and starch, and are also free of condensed tannins. The hypothesis was tested that this unique composition would also have a positive influence on the bioavailability of iron in an *in vitro* digestion/Caco-2 cell culture bioassay.

The three fast cooking Manteca entries were compared to the white beans (Blanco Fanesquero, PI527521), and to a subset of 11 yellow bean entries selected from ADP that varied in seed type and appearance with geographic origins from North and South America, as well as East and South Africa (Table 1). This model set of 16 entries is identified as the Yellow Bean Panel (YBP). The YBP was planted in a Randomized-Complete-Block Design with 2 field replicates at the Michigan State University, Montcalm Research Farm near Entrican, MI for 2 field seasons (2015, 2016) and evaluated 3 months after harvest. Moisture-equilibrated raw seed (10%) were soaked for 12 hours in distilled water prior to determining the number of minutes to reach 80% cooking time in boiling distilled water using an automated Mattson pin-drop device. Iron concentration and *in vitro* iron bioavailability in cooked seed were measured according to the methods previously describe in Wiesinger et al., 2016 [5].

Table 1 shows the significant variations in cooking times measured in YBP entries, ranging from 16 minutes for Blanco Fanesquero to 69 minutes in Middle American yellow– Amarleo. Values are combined means of field replicates from 2015 and 2016 (entry x year interaction *P* = 0.258). All three Manteca yellow entries had significantly faster cooking times when compared to the other yellow entries of the YBP. Table 1 also shows significant variations in iron concentrations measured in the cooked/lyophilized/milled entries of the YBP ranging from as little as 56 ppm to as high as 70 – 84 ppm in the two Canary seed types and the

Michigan State high yielding yellow breeding line Y11405. There was no relationship between cooking time and the concentration of iron in cooked seed (r = 0.165 P = 0.282).

The y-axis of Figure 1 depicts the significant variations (P < 0.0001) in iron bioavailability measured in cooked/lyophilized/milled entries of the YBC. Iron bioavailability is expressed as the percentage score of Caco-2 cell ferritin formation (ng ferritin / mg total cell protein) that is relative to a high bioavailable iron white navy bean (cv. Merlin) control, which is run with each assay. Figure 1 shows significantly greater iron uptake was observed in all three Manteca entries when compared to the other – especially slower cooking – yellow YBP entries. Figure 1 also illustrates the strong relationship between the cooking time and iron bioavailability scores of the YBP. Although having high iron concentrations, the two Canario entries and Y11405 did not have the highest iron bioavailability (Table 1, Figure 1). These findings add further support to existing research that suggests Manteca seed type has enhanced nutritional and fast cooking properties that can be promoted to populations who rely on beans as a major source of protein and minerals. An opportunity arises for a hybrid between the fast cooking, high iron bioavailability characteristics exhibited by the Manteca yellow bean with the qualities of the high yielding, high iron Y11405 (Figure 1).

1⌉ McLean et al. *Public Health Nutr.* 2009 **12**; 444 **2⌉** Rebellow et al. *J. Agric. Food Chem.* 2014 **62**; 7029
3⌉ Cichy et al. *Theor. Appl. Genet.* 2015 **128**; 1555 **4⌉** Leakey *Ann. Rpt. Bean Improv. Coop.* 1992 **35**; xiii
5⌉ Wiesinger et al. *J. Agric. Food Chem.* 2016 **64**; 8592

Table 1. Atmospheric cooking times and iron concentrations of entries in the Yellow Bean Panel.

Entry/*Seed Type*	Cooking Time (*min*)[1]	Iron (*ppm*)[2]
Blanco Fanesquero *white*	16[k]	65[c]
PI527521 *white*	18[k]	56[f]
Ervilha *Manteca*	18[jk]	61[de]
Mantega Blanca *Manteca*	19[jk]	58[ef]
Cebo *Manteca*	19[jk]	67[bc]
Uyole 04 *Yellow*	22[ij]	60[e]
Chumbo *Njano*	24[hi]	62[de]
Uyole 98 *Yellow*	26[fgh]	64[cd]
ACC Y012 *Yellow*	28[efg]	64[cde]
Canario, Cela *Canary*	29[efg]	70[b]
CDC-Sol *Yellow*	30[def]	56[f]
Patron *Yellow*	31[de]	66[bcd]
Y11405 *Yellow*	33[d]	84[a]
Canario *Canary*	38[c]	70[b]
PI527538 *Njano*	39[c]	66[bcd]
Amarelo *Dark Yellow*	69[a]	67[bc]

[1]Means of two field replicates per entry, field seasons 2015 & 2016. [2]Means of two field replicates per entry, field season 2015. Means sharing the same superscript in each column are not significantly different at $P \le 0.05$.
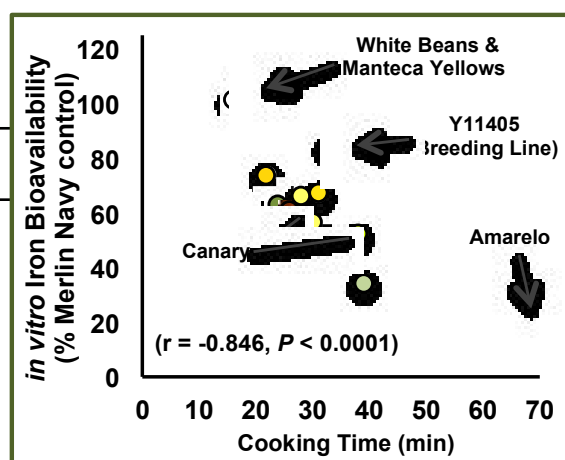

Figure 1. Scatter plot depicting the relationship between the cooking time of the YBP and iron bioavailability. Values are two field replicates per entry for field season 2015.

SEED ABORTION IN NATURALLY POLLINATED FLOWERS OF MEXICAN NATIVE PLANTS OF *Phaseolus coccineus* L.

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INTRODUCTION: *Phaseolus coccineus* L. plants require insects or hummingbirds to pollinate their flowers and set pods. The number of pods that can be produced by a plant is set by the number of flowers while the number of seeds is set by the number of ovules within the flowers (Stephenson, 1981). The arrest of the development of the seed after its partial differentiation, - seed abortion-, also determines the number of seeds per plant. The objective of this work is to determine the percentage of seed abortion per plant of two Mexican native varieties of *Phaseolus coccineus* L.

MATERIALS AND METHODS: Two varieties (accessions no. 8446 and 8448) were selected from the Mexican bean collection of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP). Seeds harvested in November 2014, were sown in pots on April 5, 2015 and seedlings were transplanted outdoors on April 20, distant 2 meter apart. The plants were of indeterminate growth, climbing type. There were a natural occurrence of bees, bumblebees, and hummingbirds pollinators during the flowering period. The experiment was a complete randomized design, with two treatments (varieties), five replications (one plant per replication) and five sampling dates (Oct. 14, Nov. 4, Nov. 25, Dec. 9, and Dec. 16). At each sampling date, the mature pods per plant were harvested and opened. The following data were registered: a) the number of normal seeds per pod; b) the number of aborted seeds per pod including early abortions detected with the stereoscopic microscope. The sum of (a) and (b) = c, which represented the *potential number of seeds per POD* in each sampling date. It was evident at this point that practically all the pods *in a variety had the same potential number of seeds. Therefore, the potential number of seeds per PLANT* in each sampling date represented by $Y = c*n$, where n represents the number of pods per plant in each sampling date. Following when applicable, the similar procedure for seed abortion: Z = total number of aborted seeds per plant in each sampling date. The *percentage of seed abortion* $(Z/Y)*100$ (total number of aborted seeds per plant in each sampling date/potential number of seeds per plant in each sampling date).

Finally, the *percentage of seed abortion per plant for a variety* is the average of the five values obtained for each one of the sampling dates. The means were compared by the t-student test ($p<0.05$) using the SAS program (SAS, 2012).

RESULTS AND DISCUSSION: Both varieties showed 97 % of pods with 4 potential seeds and 3 % of pods with 5 potential number of seeds, while Rocha and Stephenson for the Scarlet variety found pods with 6 potential number of seeds. Therefore, the representative number of potential number of seeds per pod is characteristic for a variety.

The representative *seed abortion percentage* was different between varieties. In the accession 8446 was 48, and the accession 8448 was 33% in average ($p<0.05$) (Fig. 1). It can be concluded that under natural conditions, these two varieties differ in the abortion percentage.
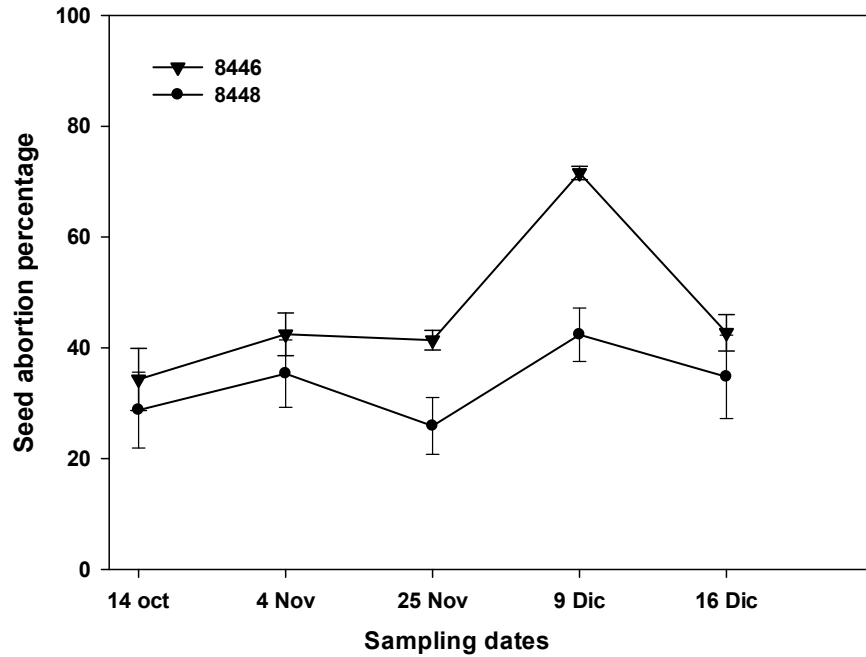
Figure 1. Seed abortion percentage in two native varieties of *Phaseolus coccineus* L.

REFERENCES

Rocha, O.J., and A. G. Stephenson. 1991. Effects of nonrandom seed abortion on progeny Performance in *Phaseolus coccineus* L. Evolution (45) (5): 1198-1208.

Statistical Analysis System (SAS Institute). 2012. SAS version 9.4 for Windows. SAS Institute Inc. Cary, N.C., USA.

Stephenson, A.G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. Ann. Rev. Ecol. Syst. 12:253-79.

BEAN SEED STORED FOR 12 YEARS USING SILICA GEL AT COMMON REFRIGERATION TEMPERATURE SHOWED HIGH VIABILITY AND GERMINATION PERCENTAGE

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INTRODUCTION

Bean seed storage requires specific control of temperature and humidity. Different research institutions have expensive equipment and controls to maintain and preserve germplasm viability. However, not all laboratory facilities have the recommended equipment to guarantee long term storage with no adverse effects on seed germination and vigor.

During my research dealing with bacterial pathogens on the common bean (*Phaseolus vulgaris* L.), tepary bean (*P.acutiflius* A.Gray), the scarlet runner bean (*P.coccineus* L.) and other grain legume species, it has been necessary to maintain small quantities of seeds originating from experiments conducted at the greenhouse located in the Mayagüez campus of the University of Puerto Rico.

Over these years, seeds harvested in the greenhouse have been stored in a laboratory refrigerator. Some bean seeds have been stored directly in paper bags or envelopes enclosed in a plastic bag and some others were stored in the same way but using silica gel packages. This paper describes an easy and low cost method of storing bean seed for at least 12 years while maintaining a high germination percentage.

MATERIAL AND METHODS

Fourteen different genotypes which included *P.vulgaris* and *P.acutifolius* were harvested under greenhouse conditions in June 24, 2004. Four envelopes (6 x 11cm) of each genotype were prepared depending on seeds available. For each genotype group a seven gram packet of silica gel was added and placed in a plastic sandwich bag. Each bag was sealed using an impulse sealer (model AIE 300 of American Int.NL Electric. All genotypes were placed in another plastic bag in which three silica packet were added, sealed and placed at 4C in a Raetone refrigerator (Model AIE 300). Seeds were stored for at least 12 years under the conditions described above and were used on November 3, 2016. Seeds were pre-germinated for three days under laboratory conditions using sterile petri dishes containing a wet kimwipe paper (EX-L, Kimberly-Clark, USA) and then planted using the soil mixture PRO-MIX® (BX) and watered by drip irrigation. Germination was recorded seven days after planting. All plants showed normal growth.

Another group of germplasm lines stored without silica gel packets, but following the same methods described above, was also evaluated.

RESULTS AND DISCUSSION

All genotypes evaluated in which silica gel was used were viable with a range in germination between 80-100%. Of 14 genotypes evaluated, nine had 100% germination, four had 90% or more, and one had 80% **(Table 1).** None of the genotypes that were stored in the refrigeration in envelopes and sealed in plastic bags without silica gel were viable. Other separate assays were conducted and have shown similar results. Low temperature and humidity are important factors in seed preservation. Under our conditions electrical outages are common especially during the hurricane season. Thus, storage temperatures were subjected to changes, indicating humidity as

the main factor to control to maintain high seed viability and germination percentages. Salcedo (2013) described for post-harvest management of bean seeds sealed storage rooms at 15C and 10% relative humidity or to reduce seed humidity using silica gel at 2:1 or 3:1 ratio in closed cabinets or desiccation jars for small volumes. NCGRP recommends -20C for long term and 4C and 20-30% relative humidity for medium-term storage (Dierig et al., 2014). The method presented here is more simple, easy, inexpensive, while it overcomes temperature variability due to loss of electrical power and it is recommended for medium-long term-storage of small quantities of seeds.

Table 1. Germination percentage of *P.vulgaris* and *P.acutifolius* seeds preserved with silica for 12 years under refrigeration at 4C.

Genotype[1]	No. Seed planted	No. Seeds Germinated 3 days[2]	No. Seeds Germinated 10 days[2]	Germination Percentage 10 days[2]
Tepary 1*	10	6	10	100.0
Tepary 2*	10	4	9	90.0
Tepary 4*	7	0	7	100.0
ICTA Ostua	11	9	10	90.9
ICTA Santa Gertrudis	12	11	11	91.6
Pecho Amarillo	10	10	10	100.0
MUG 132	10	8	8	80.0
MAR 2	11	11	11	100.0
MAR 309	11	11	11	100.0
DOR 364	11	11	11	100.0
Porrillo Sintético	10	9	10	100.0
212	10	10	10	100.0
DOR Pinto	10	3	9	90.0
11B	4	3	4	100.0
Total	137	106	131	
Total Germination %		**78%**	**96%**	

[1]Genotype ***** = refers to *P.acutifolius*, otherwise = *P. vulgaris*. [2]Germination percentage was recorded at 3 days in petri dishes and 7 days after planting in the greenhouse.

REFERENCES

Dierig, D., Blackburn, H., Ellis, D., and Nesbitt, M. 2014. Storage Protocols for Seeds at the National Centre for Genetic Resources Preservation (NCGRP) page 114. In: Curating seeds and other genetic resources for ethnobiology. Chapter 8, ISBN: 978-1-84246-498-4.

Salcedo JM. 2013. Regeneration guidelines for Common Bean. Biodiversity International (SGRP), Regional Office for the Americas, Cali, Colombia.

INTRA - AND INTER-SPECIES VARIABILITY AND GENETIC RELATIONSHIPS IN WILD AND CULTIVATED BEANS FROM MEXICO

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Beans (*Phaseolus* spp.) and maize are major staples in Mexican food. Studying the genetic diversity of wild and cultivated *Phaseolus* species is a major challenge for conservation and exploitation. We suggest that new information should contribute to improving our knowledge of intra and inter-species genetic variability as well as the genetic relationships among domesticated species of *Phaseolus* in México. In addition, the increased knowledge could improve the conservation of *Phaseolus* genetic resources throughout Mexico and provide a global overview of the importance of an integrative use of *Phaseolus* in common bean breeding (Hernández-Delgado *et al*., 2015). This work had two aims: to characterize the genetic variability among and within four domesticated species of *Phaseolus*, and to estimate the relationship and genetic structure of populations in germplasm of *P. acutifolius, P. coccineus, P. lunatus* and *P. vulgaris*.

The intra- and inter-species variability and genetic relationships in germplasm from *P. acutifolius, P. coccineus, P. lunatus,* and *P. vulgaris* was analyzed. Twelve accessions of each species were collected from throughout Mexico and compared with the following controls: *P. albescens, P. coccineus* subsp. *striatus* var. *purpurascens, P. parvifolius*, and *P. xolocotzii* as well as the bred cultivars (*P. vulgaris*) Negro Jamapa, Negro Papaloapan, Pinto Centauro, and Pinto Coloso. Germplasm was analyzed with 15 simple sequence repeat (SSR) markers, six genic and nine inter-genic, which amplified 292 alleles (225 intergenic and 67 genic markers). Values of expected (H_e) and observed (H_o) heterozygosity per accession and SSR were calculated, and Molecular Analysis of Variance (AMOVA) was performed. Genetic structure of populations and coancestry values were determined using STRUCTURE V 2.3.3 (Pritchard *et al*., 2010) and STRUCTURE HARVESTER V 0.6.92 (Earl and vonHoldt, 2011).

No associations among genetic and geographic distances were identified. In total, 74% of the molecular genetic variance was identified within species with the other 26% identified among species; genetic differentiation among species was high ($F_{ST} = 0.26$). Population structure analysis (Fig. 1) indicated robust clustering by each species and controls; however, species *P. lunatus* and *P. vulgaris* were more closed as *P. acutifolius* and *P. coccineus*. Between these two groups were located the controls and (*P. xolocotzii, P. albescens, P. coccineus* subsp. *striatus* var. *purpurascens, P. parvifolius*, and bred controls). Hamann *et al*. (1995) reported increased intra-specific variation in *P. vulgaris* and *P. lunatus* compared with *P. coccineus* and *P. acutifolius*. Angioi *et al*. (2009) used chloroplast simple sequence repeats (cpSSR) to analyze the domesticated species of *Phaseolus* (*vulgaris, coccineus, lunatus, acutifolius* and *dumosus*) and found a clear separation of each species, particularly *P. coccineus* from all others with a particularly close relationship between *P. vulgaris* and *P. dumosus*. Our results confirmed the hypothesis that *P. vulgaris* exhibits the highest values of genetic variability compared with the

other three species, *P. coccineus, P. acutifolius* and *P. lunatus*, based on allele range and heterozygosity values. In addition, wild *Phaseolus* germplasm collected throughout México exhibits increased genetic variability than bred *P. vulgaris* germplasm.
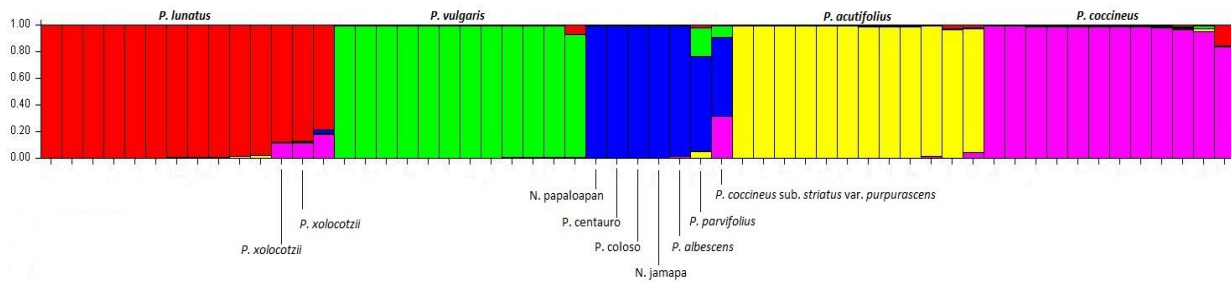

Fig. 1. Graph of the Bayesian population structure of Mexican *Phaseolus* species (*K* = 5), where each bar represents an individual accession. Each color represents membership coefficient groups determined using the Structure software package, version 2.3.1 (Pritchard *et al.,* 2010).

REFERENCES

Angioi S.A. *et al.* (2009) Plant Biol. 11:598-612.

Earl D. A. and B. M. Van Holdt (2012) Conserv. Genet. Resour. 4:359-361.

Hamann A. *et al.* (1995) Genome 38:507-515.

Hernández-Delgado S. *et al*. (2015) Advances in Genetic Diversity Analysis of *Phaseolus* in México. In: Molecular Approaches to Genetic Diversity. Intech. Rijeka, Croatia.

Pritchard J.K. *et al.* (2010) Documentation for Structure software: ver. 2.3. Univ. Chicago, USA.

CHARACTERIZATION OF ENVIRONMENTS WHERE WILD BEANS (*Phaseolus* spp.) ARE DISTRIBUTED IN MEXICO

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The characterization of germplasm based on environmental conditions of each collecting site by using GIS may help to understand the genetic variability of germplasm collections as well as associations with ecological adaptation. Ecogeographic analysis is needed to develop any conservation plan regarding distribution and representativeness. The genetic variability of domesticated species of *Phaseolus* spp. is well represented in germplasm banks. However, there is a deficit of seed from wild species and these accessions are poorly documented. The objective of this study was to determine the climatic adaptation of wilds species of *Phaseolus* throughout México.

The germplasm included 29 species and two subspecies of *Phaseolus* belonging to the germplasm bank of the Centro de Biotecnología Genómica-IPN at Reynosa, México. Sites of collection were geo-referenced by calculating latitude and longitude coordinates based on passport collection data. Data included 101 site coordinates matrix describing (i) climatic variables: monthly average temperature and precipitation; elevations (WorldClim, Hijmans *et al*., 2005); (ii) photoperiod (NOAA Solar calculator, http://www.esrl.noaa.gov/gmd/grad/solcalc/index.html); and (iii) climatic type (Medina-García *et al*., 1998). The environment information was obtained with the DIVA-GIS software ver. 7.1.7 (Hijmans *et al*., 2004; http://www.diva-gis.org).

Sites of collection (Table 1) represent the greatly natural geographic range of adaptation and distribution of the genus. The general sort of environmental features included photoperiod of 11.68 to 14.23 h; 8 to 3083 masl; mean annual temperatures ranged from 12.07 to 26.96 ºC; mean annual rainfall of 10.33 to 202.68 mm. The species show preferences for subtropical and tropical climates with arid to humid conditions. Subtropical sub-humid temperate climate included the most of species (11) followed by subtropical arid temperate (9 species). The ecogeographical analysis wild bean collection indicated the great adaptive variability of *Phaseolus* in México which also serves to represent the potential distribution of species, to assist and planning future collection expeditions and perform efficient strategies to acquire, manage, and conserve wild bean genetic resources.

REFERENCES

Hijmans, R. J. *et al.* (2005) Int. J. Climatol. 25:1965-1978.

Hijmans, R.J. *et al.* (2004) DIVA-GIS. Vsn. 5.0. A geographic information system for the analysis of species distribution data. 83 p.

Medina-García, G. et al. (1998) Los Climas de México: Una Estratificación Ambiental Basada en el Componente Climático. Libro técnico núm. 1. INIFAP-CIRPAC, Guadalajara, México. 103 p.

Table 1. Environmental descriptors obtained from GIS data bases for 29 *Phaseolus* species from México.

Taxon	Photoperiod (h)†			Altitude (m)‡			Mean annual temperature (ºC) §			Mean annual rainfall (mm) ¶			Climate types #
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	
P. acutifolius	11.5	14.5	13.0	9	2102	1206	12.1	29.2	20.2	4.5	184.7	54.4	10, 11, 13, 14, 15, 18, 26
P. albescens	11.8	14.1	12.9	1535	2125	1830	9.9	23.5	17.0	12.0	194.5	71.3	11, 15
P. albiviolaceus	11.6	14.4	12.9	322	971	647	15.4	28.5	21.7	12.5	191.5	71.6	13, 19
P. coccineus **subsp.** *coccineus*	11.8	14.1	12.9	1578	2825	1942	9.9	24.3	17.3	7.8	234.5	83.4	9, 10, 11, 14, 15
P. coccineus **subsp.** *striatus*	11.8	14.1	12.9	2861	2949	2905	4.9	19.3	12.4	9.5	236.5	86.6	11
P. esperanzae	11.7	14.2	12.9	971	2459	1715	9.7	24.7	17.2	6.0	115.0	43.1	9, 13
P. filiformis	11.4	14.5	13.0	31	2117	1227	12.0	29.3	20.2	2.5	60.3	20.5	9, 13
P. glabellus	11.7	14.2	12.9	170	1470	661	16.4	28.3	22.2	39.3	331.3	128.6	11, 19, 27
P. gladiolatus	11.6	14.3	12.9	2023	2023	2023	9.5	25.3	17.4	10.0	95.0	42.7	9
P. laxiflorus	11.8	14.1	12.9	1691	1691	1691	11.9	26.7	19.7	4.0	206.0	74.6	15
P. leptostachyus	11.8	14.1	12.9	2020	2165	2093	8.3	20.9	15.0	15.0	249.0	90.4	11
P. lunatus	11.7	14.1	12.9	8	358	133	19.4	30.3	24.6	25.0	261.0	103.8	26, 27, 28
P. maculatifolius	11.5	14.5	13.0	1386	1386	1386	10.9	26.8	18.6	14.0	111.0	46.5	9
P. maculatus	11.6	14.3	12.9	741	3083	2010	8.3	24.9	16.6	6.5	108.3	43.4	9, 11, 13
P. macvaughii	11.9	14.0	12.9	14	14	14	21.3	33.3	27.4	1.0	253.0	74.2	27
P. micranthus	11.8	14.2	12.9	530	1031	707	17.1	30.0	23.6	5.3	320.7	105.1	27
P. microcarpus	11.5	14.5	13.0	1334	1420	1377	12.6	29.8	20.9	2.5	107.5	32.7	13, 14
P. nodosus	11.8	14.1	12.9	1716	1716	1716	11.1	24.7	18.2	3.0	173.0	58.8	15
P. novoleonensis	11.4	14.6	13.0	1350	1639	1543	10.5	25.4	17.6	16.0	170.7	61.7	10
P. oligospermus	11.9	13.9	12.9	1203	1203	1203	15.4	28.1	21.9	12.0	248.0	98.0	23
P. palmeri	11.4	14.5	13.0	2356	2356	2356	7.4	27.1	16.8	18.0	75.0	38.0	9
P. parvifolius	11.9	14.0	12.9	2280	2280	2280	8.1	19.0	13.9	28.0	337.0	134.7	11
P. pedicellatus	11.7	14.2	12.9	2238	2238	2238	7.4	20.6	14.4	19.0	229.0	85.5	11
P. pluriflorus	11.7	14.2	12.9	2165	2165	2165	7.5	19.1	13.7	23.0	320.0	111.2	11
P. purpusii	11.6	14.4	12.9	2157	2157	2157	8.5	25.1	16.8	2.0	69.0	28.1	9
P. rotundatus	11.8	14.1	12.9	1705	2072	1908	9.1	25.9	17.7	5.7	193.3	59.2	10, 11, 15
P. vulgaris	11.8	14.1	12.9	964	2038	1538	12.6	27.2	20.2	10.4	236.0	87.3	11, 15, 19, 23, 27
P. xanthotrichus	11.9	13.9	12.9	1386	1386	1386	13.3	26.6	20.2	8.0	294.0	110.3	23
P. xolocotzii	11.9	14.0	12.9	1567	1567	1567	14.5	27.7	21.6	2.0	208.0	74.7	15
P. zimapanensis	11.6	14.3	12.9	1342	1768	1555	11.3	26.5	18.9	10.0	124.5	47.4	9, 10
Mean	**11.7**	**14.2**	**12.9**	**8**	**3083**	**1453**	**12.1**	**26.9**	**19.5**	**10.4**	**204.6**	**73.2**	

†, Mean monthly calculated on NOAA Solar Calculator (National Oceanic and Atmospheric Administration, http://www.esrl.noaa.gov/gmd/grad/solcalc/index.html).
‡, Hijmans *et al*. (2005).
§, ¶, Mean monthly based on series from 1950 to 2000 (Hijmans *et al*., 2005).
Climate types description (Medina-García *et al*., 1998).

GENETICS OF YIELD VARIATION AND GENOTYPE BY ENVIRONMENT INTERACTIONS IN THE COOPERATIVE DRY BEAN NURSERIES

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Common bean (*Phaseolus vulgaris*) yields have improved in multiple long-term breeding efforts across the world. An important long-term trial that supports these breeding efforts is the Cooperative Dry Bean Nursery (CDBN), an ongoing 60+ year collaboration across the United States and Canada. However, large genotype-by-environment interactions (GxE) persist in common bean (Figure 1). Though genomics assisted breeding tools and analyses to study GxE are rapidly improving (Heffner *et al.,* 2009; Perez & de los Campos 2014), accurate phenotyping in relevant field conditions remains a major limitation of these analyses. Major phenotyping efforts such as the CDBN, when combined with genomic data, offer unparalleled opportunities to determine how major genetic factors affect genotype by environment interactions.

Figure 1. Examples of genotype by environment interactions for yield of four CDBN varieties in response to a precipitation related principal component.

To characterize the genetics of phenotypic variation in common bean, phenotypes and fitness in a wide range of environments must be connected to the alleles that influence them. In collaboration with current common bean sequencing efforts, we are sequencing 320 varieties and breeding lines from the CDBN to establish a genome-wide association (GWA) mapping population. We will use this panel to determine the genomic regions associated with phenology, yield, and other traits phenotyped by the CDBN. We will also use weather data associated with each location and year to determine the genetics of, and the abiotic factors leading to, GxE interactions between these phenotypes and climate.

Variety, phenotype and weather collection

117 varieties in the CDBN have single nucleotide polymorphism (SNP) data through genotyping by sequencing (GBS), as part of the Mesoamerican Diversity Panel (Moghaddam *et al.*, 2016). Breeders with ties to the CDBN generously provided seed that allowed GBS sequencing of an additional 203 varieties from the CDBN, currently underway.

Collaborators in the CDBN have collected phenotypic data for a suite of agronomic traits for over 500 common bean varieties grown at various subsets of 84 locations. There are between
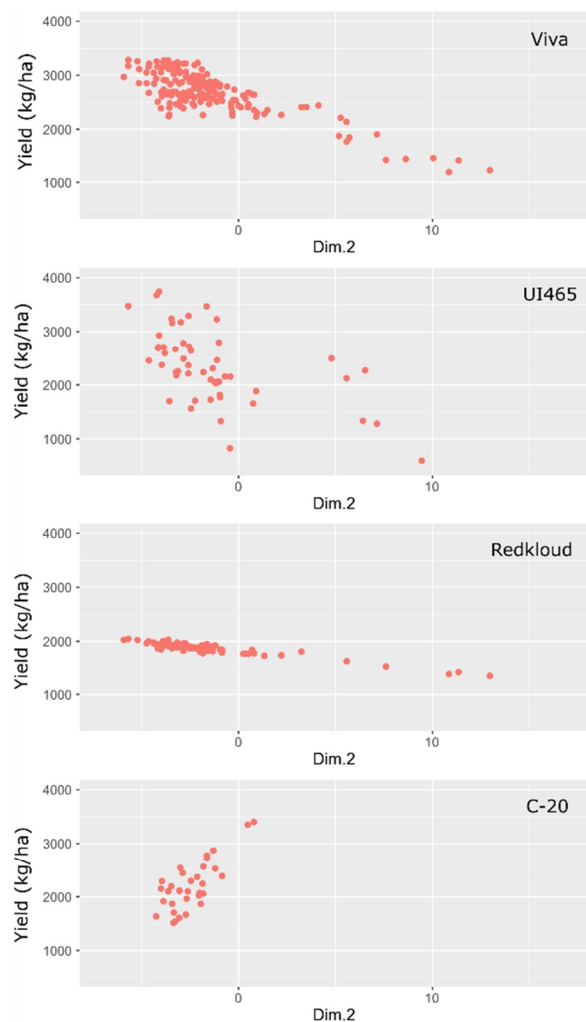
nine and twelve phenotypes for which we will have sufficient data for an analysis of GxE through environmental PCA (ePCA) and GWA. The dataset also includes monthly weather data associated with each trial year at each site. We are working to add and compare daily weather data from NOAA to environmental PCA derived from monthly weather data.

Planned Analyses

We will perform a PCA on all monthly and daily weather variables. In a preliminary analysis with monthly weather variables, the first two PCs explained 39.1% and 22.6% of the variance, and loaded strongly with temperature and precipitation variables, respectively. We will determine the slopes and variance for variety yields in response to these environmental PCs. This will allow us to identify varieties in two categories: "yield stable", with small slopes and low variance, and "yield labile" varieties, with significant positive or negative slopes (reaction norms) and low variance. Preliminary results indicate that reaction norms and environmental sensitivity differ by race: we observe more stable varieties than expected for PC2 in Andean lines, and more negative reaction norms than expected for PC2 in Durango lines.

Work is currently in progress to link datasets containing phenotypic, weather, pedigree, and genetic variables. When the genotype data is complete, we will screen for genomic regions associated with yield variation by using the GEMMA software to conduct genome-wide association (GWA) for yield and other phenotypes in multiple environments simultaneously while controlling for population structure (Zhou and Stephens 2014). Multivariate analyses substantially increase power to detect pleiotropic variants and variants that affect only one of multiple correlated phenotypes (Stephens 2013). The kinship matrix correction applied by GEMMA will be essential to account for breeding relationships among lines and past introgression events between varieties. Factors such as domestication clade, market class, ePCAs, and pedigree information will be included as additional cofactors in the models.

REFERENCES

Heffner EL *et al.* (2011) Genomic selection accuracy using multifamily prediction models in a wheat breeding program. *Plant Genet.* **4**:65-75.

Moghaddam SM *et al.* (2016) Genome-Wide Assocation Study Identifies Candidate Loci Underlying Agronomic Traits in a Middle American Diversity Panel of Common Bean (*Phaseolus vulgaris* L.). *Plant Gen.* **9**(3): 1-21.

Perez P & de los Campos G (2014) Genome-Wide Regression and Prediction with the BGLR Statistical Package. *Genetics* **198**:483-495.

Stephens M (2013) A Unified Framework for Association Analysis with Multiple Related Phenotypes. *PloS One* 8.

Zhou X, Stephens M (2014) Efficient multivariate linear mixed model algorithms for genome-wide association studies. *Nat Methods* **11**:407–409

EFFECT OF PLANT POPULATION IN GREAT NORTHERN AND PINTO BEAN PRODUCTION IN WESTERN NEBRASKA

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INTRODUCTION

In this project we explored the effect of plant population and row spacing on the yield and quality of great northern and pinto beans grown in Nebraska. This project builds on the findings from a preliminary non-replicated great northern variety trial conducted at Morrill, NE in 2014. That trial included four great northern cultivars with different plant architecture. In general, yields were reduced 18.8% (795 kg ha^{-1}) when plant population increased from 251,152 to 300,715 plants ha^{-1}. Yield reduction was greatest in '6107' (24.7%) followed by 'Marquis' (20.1%), 'Beryl-R' (15.5%) and 'Coyne' (14.0%). In the current project we used replicated trials to evaluate the impact of plant population on two great northern and two pinto bean cultivars. Within each market class, one cultivar had a prostrate (III) and the other had an upright (II) growth habit. Our goal was to identify the optimal plant population and row spacing for each cultivar.

MATERIALS AND METHODS

This study was conducted during 2015 at the PREC-Scottsbluff, NE. Two great northern, 'Marquis' (III) and 'Draco' (II), and two pinto cultivars, Montrose (III) and Sinaloa (II) were planted in separate experiments at two row spacing (15 and 30 inches) and four plant populations. Target populations for the 30-inch row spacing were 45,000, 80,000, 100,000, and 120,000 plants/acre. Target populations for the 15-inch row spacing were 80,000, 100,000, 120,000, and 150,000 plants/acre. Four and seven rows were planted for the 30- and 15-inch row spacing experiments, respectively.

RESULTS AND DISCUSSION
- Target and actual plant populations were similar.
- Within each market class, yield differed significantly among cultivars at both 15- and 30-inch row spacing.
- Plants matured significantly earlier under high plant populations (generally 1 day earlier).
- 100-seed weight was reduced by 8% for both pinto cultivars at high plant populations.
- At the 15-inch row spacing, cultivars with growth habit III (Montrose and Marquis) reached their highest yield at a plant population of 100,000 plants/acre, however yield decreased at a plant population of 150,000 plants/acre.
- In general, dry beans planted at the 15-inch row spacing had higher yields than those planted at the 30-inch row spacing

CONCLUSIONS
- These results are insufficient to identify the optimal plant population and row spacing combination for these great northern and pinto cultivars.
- Growing conditions were atypical during 2015, therefore, this study will be repeated in 2016 and 2017.

Great northern and Pintos at 15 inch row spacing

Great northern and Pintos at 30 inch row spacing

180
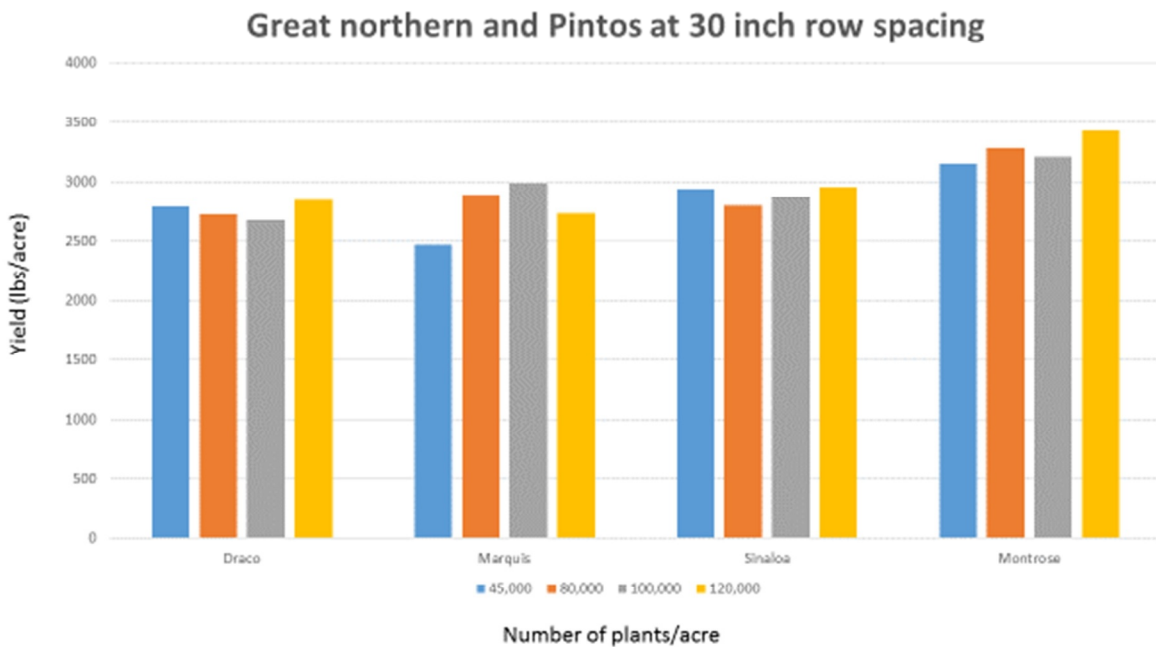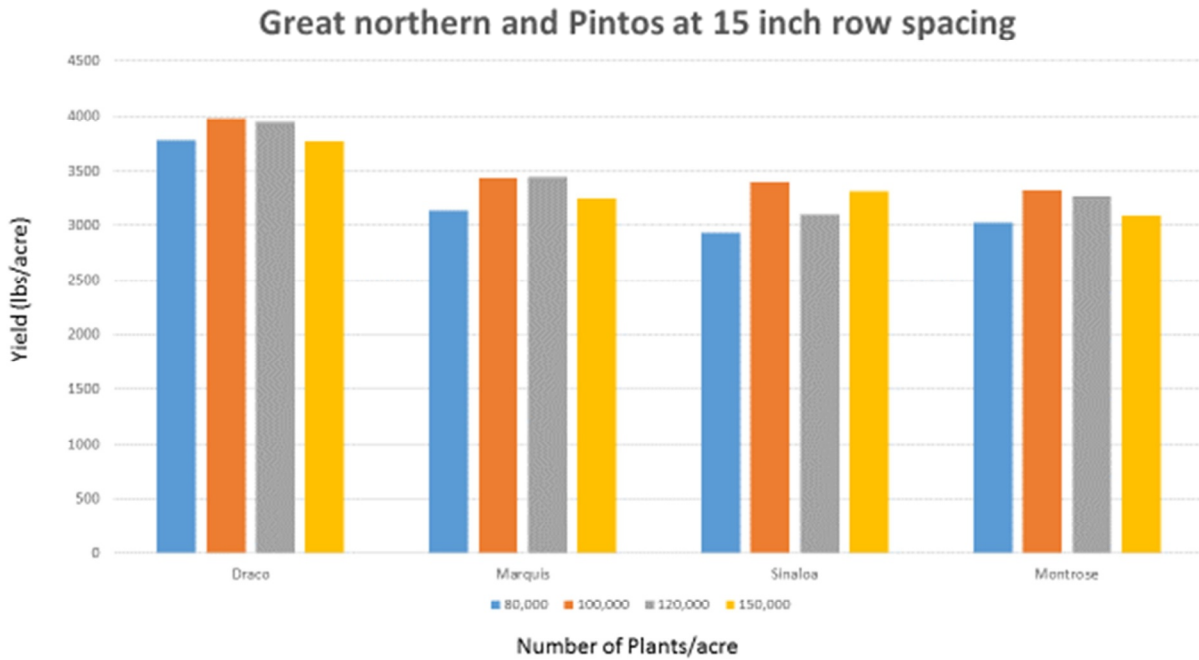
SHADE EFFECTS IN PINTO SALTILLO COMMON BEAN CULTIVAR GROWN UNDER TWO CROPPING SYSTEMS IN DURANGO, MÉXICO

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INTRODUCTION: In Durango, forest plantations with woody species reached 1,866 ha in 2013 (SEMARNAT, 2013). Forest plantations have been recommended under irrigation and agricultural lands for the intensive production of wood, pulp and firewood, as well as for Christmas trees cultivation. In 2015, intensive production of forest biomass was supported using different pine species such as *Pinus greggii*, *P. cembroides* and *P. engelmannii*. High yield potential has been also detected in the same production areas for agricultural crops such as common bean (*Phaseolus vulgaris*), which is considered as a productive and profitable option by farmers in order to obtain food and economic benefits during pine timber production. Decrements have been observed in grains per pod, grains per plant and harvest index, as percentage of shade increased (Hadi *et al*., 2006). The objective of this study was to evaluate shade effects on yield of Pinto Saltillo common bean cultivar grown under two cropping systems in Durango, México.

MATERIALS AND METHODS: From 2014 to 2016, an agroforestry (alley cropping) system was implemented at INIFAP´s experiment station located in Durango, México. The agroforestry system included an 8 to 10 year old *Pinus greggii* plantation and a common bean cultivar (Pinto Saltillo), showing intermediate maturity (96 days after planting: DAP). Common bean cultivar was sown when the rainy season started (June 24th to August 4th) using strips between pine tree lines, which was planted 3 m apart and 1.5 m between plants. In 2014, pine trees showed average values of 3.3 m for plant height, 11.0 cm of stem basal diameter and 2.0 m of crown diameter.

Pinto Saltillo, common bean cultivar was sown in alternate strips using two rows, 130 m in length and 0.81 m apart. A traditional cropping system was established in an adjacent plot without pine plantings to be used as a control. Fertilizer was applied by hand at the dose of 25-35-00 (N-P_2O_5-K_2O). Weed control included two mechanical weeding complemented with one chemical application (Fomesafen) and two hand weeding. Supplemental irrigation was applied in order to avoid crop loss caused by intermittent drought registered across years during the cropping season, which was associated with low rainfall. In 2014, two pyranometers (Kipp & Zonen SP Lite 2) were installed over 80 cm from the soil surface in order to evaluate global solar radiation (watts/m^2) and the shade effect in common bean plants. In 2015 and 2016, readings were taken during the reproductive period using an AccuPAR (Decagon LP-80, Decagon Devices, Inc.) ceptometer for light interception measurements in both cropping systems.

The experimental plot consisted in one strip with two rows and three (2014 and 2015) to five (2016) replications. Phenological data were registered for days to flowering and physiological maturity. At maturity, three equidistant plant samples were taken from each crop strip for yield determination. Samples consisted of two rows with 5 m in length by 0.81 m in width (8.1 m^2). Analysis of variance was obtained under randomized complete block design combined over years with six to 15 replications (samples) and mean comparisons were performed by Tukey´s test ($P \leq 0.05$).

RESULTS AND DISCUSSION: Increments in light interception (shade) were registered across years averaging 24 % in 2014 to 33 % in 2016 (Figure 1). Variation was observed for days to flowering and maturity across years. Early flowering (33 DAP) and maturity (82 DAP) was observed in 2016, mainly due to delayed plantings and photoperiod sensitivity registered in Pinto Saltillo. A significant reduction of seed yield was observed between cropping systems. The highest seed yield was observed at traditional

system during 2016 (2,647.4 kg ha^{-1}). Highest yield reduction (67 %) was registered under the highest shade level (33 %) observed in 2016 (Figure 1). Lowest yield average was also observed in the agroforestry system (880.3 kg ha^{-1}) compared to traditional system (2,647.4 kg ha^{-1}).
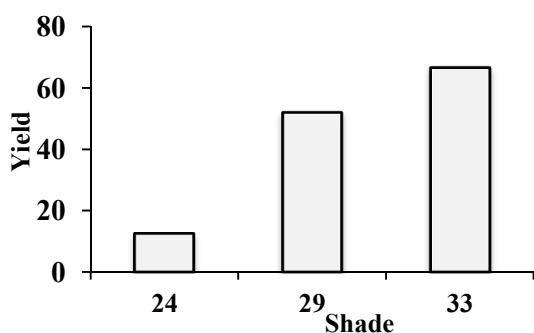

Figure 1. Shade effect on common bean seed yield registered in an agroforestry system implemented in Durango, México. (2014-2016).

Effect on yield reduction was related to increments in light interception (shade) caused by tree growth and the lack of tree pruning. Similar values for 100 seeds weight (32.6 to 34.0 g/100 seeds) were observed across cropping systems. Higher yield and low reduction in seed weight are desired traits in common bean cultivars grown under pine-common bean intercropping system. In despite of yield reduction, common bean represents an important option in agroforestry systems and the farmers would have food production and an additional income during the early growth of the trees.

Table 1. Traits evaluated in Pinto Saltillo common bean cultivar grown under two cropping systems.

Year		Days to Flowering	Days to Maturity	Yield kg ha^{-1}	100 seeds Wt (g)
		Agroforestry System			
2014		46	105	1,577.5[b]	33.5
2015		40	105	754.8[b]	32.9
2016		33	82	880.3[b]	34.9
	Average	**40**	**105**	**1,070.9[B]**	**33.7**
		Traditional System			
2014		45	104	1,804.0[a]	34.3
2015		40	104	1,463.6[a]	32.7
2016		34	82	2,647.4[a]	32.6
Average		**40**	**105**	**1,971.7[A]**	**33.2**

Letters in columns indicate significant differences according to Tukey´s test ($P \leq 0.05$) between cropping systems ([A-B]) and years[a-b].

REFERENCES

Hadi, H.; K. Ghassemi-Golezani; F. R. Khoei; M. Valizadeh; M. R. Shakiba. 2006. Response of common bean (*Phaseolus vulgaris* L.) to different levels of shade. Journal of Agronomy 5: 595-599.

SEMARNAT. 2015. Consulta temática. Plantaciones Forestales Comerciales. http://dgeiawf.semarnat.gob.mx:8080/ibi_apps/WFServlet?IBIF_ex=D3_RFORESTA09_02&IBIC_user= dgeia_mce&IBIC_pass=dgeia_mce

Rosales S., R.; J. A. Sigala R.; M. Torres M.; H. Sarmiento L. 2015. Common bean and low cost inoculation as components of sustainable agroforestry systems in Durango, México. Annual Report of the Bean Improvement Cooperative 58: 125-26.

YIELD GAINS IN DRY BEANS IN THE U.S.

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INTRODUCTION

Yield Gains can be estimated by comparing On-Farm yields vs. Potential yield (a.k.a., realized yield) or measured in common trials. The difference between On-Farm yield and Potential yield is known as the Yield Gap. Plant breeders primarily focus on increasing Potential yield while also attempting to optimize the interaction between genotype, environment, and agronomic practices to increase On-Farm yield and reduce the Yield Gap. In recent years, scientists in developing countries and the U.S. have made major advances in dry bean disease resistance, stress tolerance, and increased yield (Kelly, 2004). Agronomic and biotechnological tools have contributed to these achievements. The objective is to estimate Yield Gains in dry beans in the U.S.

MATERIALS AND METHODS

Yield gains in dry bean were estimated as: 1) Comparing On-Farm yields *vs* potential yield: Production data from the National Agricultural Statistical Service (USDA-NASS, 2013) was used to estimate of On-Farm yields, and data obtained from the Cooperative Dry Bean Nursery (CDBN) grown every year since 1950 with an average of 10 locations each year across the most important dry bean production regions in the U.S. was used to estimate Potential yield. For our comparisons, only data between 1981 and 2012 have been used from these trials to provide estimates of Potential yields during the last 30 years. The earliest year in which On-Farm yield values are available from the National Agricultural Statistics Service (NASS) is 1909 (USDA-NASS, 2013). **2) Yield gains as measured in common dry bean trials:** Common trials using cultivars released over time to specifically measure the genetic contribution of yield gain over time without confounding environmental or cultural effects. These trials were conducted by four participants in the Bean Coordinated Agriculture Project (http://www.beancap.org/) project during 2011 and 2012. They included cultivars released since 1956 for 16 navy bean and since 1965 for 20 pinto bean. Analysis of variance was used to determine interactions between location and cultivar, and linear regression was used to estimate genetic progress over time by regressing the response variables on year of cultivar release.

RESULTS AND DISCUSSION

Overall, On-Farm yield across all market classes of dry bean grown in the U.S. show a seed yield increase of 12.9 kg ha-1 yr-1 between 1909 and 2012. Potential yield data from the CDBN also shows a positive trend with and average seed yield increase of 7.3 kg ha-1 yr-1 during the last 31 years. Interpreting these results is difficult because of the large genetic diversity among market classes, yield potential, disease resistance, seed characteristics, and other traits. Therefore, we evaluated individual yield gains among four market classes, namely pinto, navy, black, and kidney beans. These four market classes account for approximately 95% of the total U.S. production.

Yield gain from selection in dry beans tested in common trials ranged between 13.9 to 17.4 kg ha^{-1} yr^{-1} for navy and pinto beans, respectively. These results are similar to the yield increases reported for soybean (Specht et al., 1984; Voldeng et al., 1997), wheat (Lopes et al., 2012), and dry beans (Singh et al., 2007) in previous studies. They are also similar to findings in this report that evaluated On-Farm and Potential yield data from the USDA-NASS and the CDBN, respectively. The fact that similar gains are found by using different sources of data suggest that the results provided here are robust and accurate. The results also suggest that yield increases have been linear since 1956 and suggest that dry bean cultivars have not reached a yield plateau for most market classes. Continued introgression of germplasm from other races of common bean should provide new sources of germplasm to enhance yield into the foreseeable future. For navy bean, seed size, plant height and maturity have not changed since 1956. Plant height for pinto bean cultivars increased from 34 to 57 cm over time due to introgression of Mesoamerican germplasm with upright Type II architecture. Seed size increased slightly in pinto bean but did not change in navy bean cultivars over time. In addition to breeding efforts and to reduce the Yield Gap found in all market classes, there is a need to keep improving management practices that allow for higher production efficiency.

Kidney beans showed a yield gain increase between 19.1 and 39.9 kg ha^{-1} yr^{-1}. This is the highest gain among all market classes, which is surprising given the challenges associated with production and genetic improvement of kidney beans. Yield gains in kidney beans have been a challenge because they have a narrow genetic base and high susceptibility to biotic and abiotic factors (Beaver and Osorno, 2009). In addition, kidney beans have the smallest yield gap among those compared. The small difference between On-Farm and Potential yield in kidney beans may result from the fact that producers of kidney beans tend to manage the crop better than for other market classes because market prices are typically higher.

REFERENCES

Beaver, J.S., and J.M. Osorno. 2009. Achievements and limitations of contemporary common bean breeding using conventional and molecular approaches. Euphytica 168:145-176.

Kelly**,** J.D. 2004. Advances in Common Bean Improvement: Some Case Histories with Broader Applications. Acta Hort. (ISHS) 637:99-122.

Lopes, M.S., M.P. Reynolds, Y. Manes, R.P. Singh, J. Crossa, and H.J. Braun. 2012. Genetic yield gains and changes in associated traits of CIMMYT spring bread wheat in a "Historic" set representing 30 years of breeding. Crop Sci. 52:1123-1131.

Singh, S.P., H. Terán, M. Lema, D.M.A. Strausbaugh, P.N. Miklas, H.F. Schwartz and M.A. Brick. 2007. Seventy-five years of breeding dry bean of the Western USA. Crop Sci. 47:981-989.

Specht, I.E., and J.H. Williams. 1984. Contribution of genetic technology to soybean productivity - Retrospect and prospect. p. 49-74. In W.R. Fehr (ed.) Genetic contributions to yield gains of five major crop plants. CSSA Spec. Publ. 7. CSSA, and ASA, Madison, WI.

Voldeng, H.D., E.R. Cober, D.J. Hume, C. Gillard and M.J. Morrison. 1997. Fifty-eight years of genetic improvement of short-season soybean cultivars in Canada. Crop Sci. 37: 2: 428-431.

GENETIC DIVERGENCE IN COMMON BEAN GENOTYPES CULTIVATED IN NORTH OF MINAS GERAIS, BRAZIL

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INTRODUCTION: Common bean (*Phaseolus vulgaris* L.) presents a very great diversity of grains forms and color. It is a culture of great relevance for the Brazilian economic scenario, since its great importance and preference in the Brazilians food. The advance of genetic breeding has increased the number of cultivars with ideal characteristics of crop. However, the extensive use of one or more genetically similar cultivars provides narrowing of the genetic basis. Genetic diversity provides strategies that avoid the use of genetically similar cultivars and thereby avoid problems of origin mainly of biotic character. Thus, it is required that cultivars are not only adapted and productive, but also genetically divergent. Therefore, the objective of this work was to evaluate the genetic diversity of 25 common bean genotypes evaluated in the VCU tests in the north of Minas Gerais.

MATERIAL AND METHODS: The test carried out in Janauba were composed of 25 common bean genotypes of the carioca commercial group, selected among the breeding programs of UFV, UFLA, EPAMIG and EMBRAPA Rice and Bean. Soil preparation was conventional one, with a plowing and two harrowing. The area was then grooved and fertilized using a mechanized seeder adjusted for 0.5 m spacing between rows with a planting density of about 15 plants m^{-1}. The plots consisted of four rows of plants, 4 m in length, and additional irrigation was used. Grain yield, mean number of pods per plant (PPL), average number of grains per pod (GPP) and mass of 100 grains (M100) were evaluated. The data were submitted to analysis of variance for all the studied characteristics. The effect of the genotypes when significant were compared by the Scott-Knott test, at 5%. The generalized distance of Mahalanobis was used for the determinate genetic divergence among genotypes (SINGH, 1981). Tocher optimization method was used for the clustering. The analyzes were performed using the computational application in GENES genetic and statistical.

RESULTS AND DISCUSSION: Analysis of clustering using the Tocher method, based on the dissimilarity matrix, made it possible to distribute the genotypes in 11 different groups (Figure 1). Group I was composed of the genotypes CNFC 11965, RCII-2.19, BRSMG Majestoso, CVIII-2, MAIV-15.204 and MAIV-18.259. The genotypes P-18,163, Pérola, BRSMG Talismã, CVIII-5 and VC-17 represented respectively by groups 6, 7, 8, 9, 10 and 11 formed groups with only one genotype each one. These genotypes showed high diversity in relation to the others.
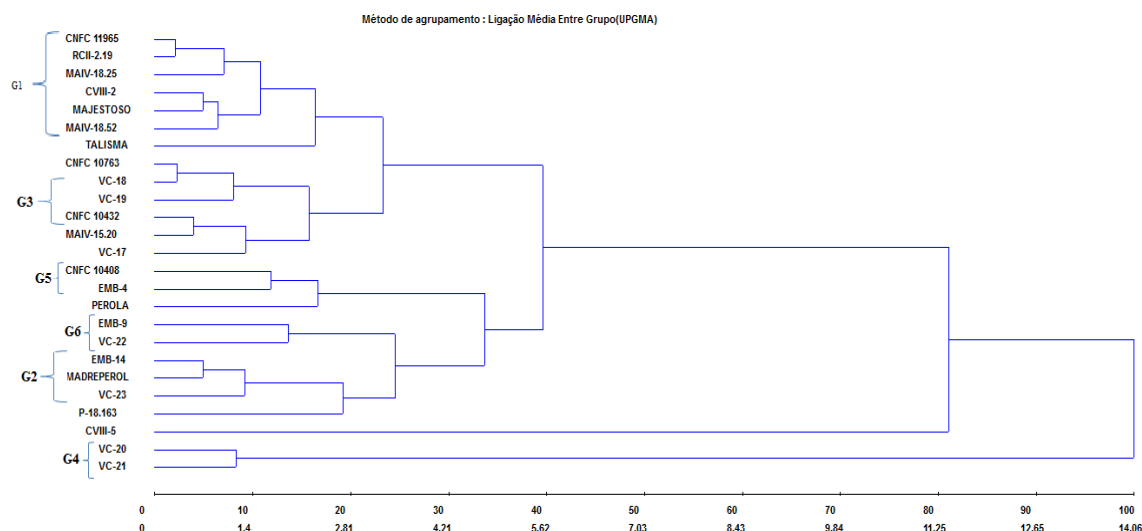
Figure 1. Representative dendrogram of the clustering of 25 common bean genotypes by the UPGMA method, based on the dissimilarity estimated from grain yield and yield components (Janaúba-MG, 2017).

The Table 1 shows the contribution of the characteristics to the divergence, it is observed that from the four evaluated characteristics, it was the number of pods per plant (35.85%) that more contributed to the divergence, while the mass of one hundred grains (12.77%) presented the smallest contribution.

Table 1. Relative contribution of the characters average number of pods per plant (PPL), average number of grains per pod (GPP) and mass of 100 grains (M100) and yield (PROD) for diversity by Singh method (1981) generalized distance of Mahalanobis.

Caracteres	S.j	Value in %
PPL	673.475392	35.8505
GPP	666.935651	35.5023
M100	239.928674	12.7719
PROD	298.228379	15.8753

CONCLUSION: There is genetic divergence among the genotypes studied in the VCU tests conducted in the north of Minas Gerais. The P-18.163, Pérola, BRSMG Talismã, CVIII-5 and VC-17 genotypes are the most divergent.

REFERENCES
CRUZ. C. D. GENES - a software package for analysis in experimental statistics and quantitative genetics. Acta Scientiarum. Agronomy. Maringá. v. 35. n. 3. p. 271-276. 2013.

SINGH D. The relative importance of characters affecting genetics divergence. The Indian Journal of Genetics & Plant Breeding. New Delhi, v. 41. n. 2. p. 237-245. 1981.

GENETIC PROGRESS EVALUATION IN BEAN FAMILIES, BASED ON THE POOLED ANALYSIS WITH COMMON CONTROLS

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INTRODUCTION

In the a breeding program leading, various strategies can be used and they are capable to yield good results. However, hybridization is a routine in current breeding programs and it has been the main new bean lines source (Menezes Júnior et al. 2013). The big difficulty in the autogamous plants breeding is finding two parents who bring together all the interest phenotypes. In this case, the alternative would be to promote successive cycles of selection and interbreeding of the best individuals or the best families (Geraldi 1997). The genetic progress periodic estimation is fundamental to guide the plant breeders about the selective strategies used and the alternatives that could be adopted to increase their efficiency. Thus, the procedures commonly used to compare selective cycles are the different cycles families or lines evaluation, which can be done using common witnesses (Ramalho 1996). The work objective was to estimate the genetic progress of two recurrent selection cycles based on the evaluation of families with common controls.

MATERIAL AND METHODS

The experiments were carried out at the Experimental Station of Coimbra, belonging to the Department of Plant Sciences of the Federal University of Viçosa (UFV). The base population, zero cycle (C_0), it was obtained by the combination of 20 carioca type grains, with favorable phenotypes for several agronomic interest characters. The parents were recombined in a circulating diallel design, with each parent participating in two crosses, generating 20 populations. From these populations, the families were derived and evaluated for three generations, in the generations $F_{2:4}$ and $F_{2:5}$. The same recombination procedure and C_0 evaluation were performed in cycle one (C_I).

The genetic progress for productivity was estimated based on the $F_{2:3}$ generations families performance (2007 and 2010 winter crops, being 380 families), $F_{2:4}$ (2008 and 2011 drought crops, 160 families) And $F_{2:5}$ (2008 and 201 winter harvests, 40 families) of both C_0 and C_I cycles, respectively. Initially, a pooled variance analysis was performed and the family means adjusted by the control effect of the two experiments. Using the adjusted averages, the genetic progress was estimated. Statistical analyzes were performed with the help of the GENES program (Cruz, 2006).

RESULTS AND DISCUSSION

Table 1 shows the adjusted productivity averages for families $F_{2:3}$, $F_{2:4}$ and $F_{2:5}$ and controls evaluated in the C_0 and C_I cycles, and the respective genetic gains obtained. In all generations, the C_I families were higher than the C_0 families. This superiority was also verified in relation to controls. Estimates of genetic progress (GP) were obtained considering the families of each generation. Genetic gains for grain yield were 23.7%, 14.2% and 16.6% for the generations $F_{2:3}$, $F_{2:4}$ and $F_{2:5}$, respectively (Table 1). There was an overestimation of genetic progress when families were used in each cycle with the use of common controls when compared with other studies with simultaneous evaluation progress (Alves et al, 2015).

Table 1 - Average grain productivity (kg.ha^{-1}) and their respective genetic progress from the pooled analysis with common controls in the $F_{2:3}$, $F_{2:4}$ e $F_{2:5}$ generations.

Generation	Cycle	Grain Productivity Averages
$F_{2:3}$	C_0	3021
	C_I	3736
	Test	3044
	GP(%)	**23.7**
$F_{2:4}$	C_0	3295
	C_I	3762
	Test	3189
	GP(%)	**14.2**
$F_{2:5}$	C_0	3639
	C_I	4242
	Test	3491
	GP(%)	**16.6**

REFERENCES

Cruz CD (2006). Programa Genes: Biometria. Editora UFV, Viçosa, 382p.

Geraldi IO (2005). Por que realizar seleção recorrente. In: Simpósio de atualização em genética e melhoramento de plantas, 9., 2005, Lavras. Anais... Lavras: UFLA, Programa de Pós-Graduação em Agronomia/Genética e Melhoramento de plantas, p. 1-8.

Menezes Júnior JAN, Rezende Júnior LS, Rocha GS, Silva VMP, Pereira, AC, Carneiro PCS, Peternelli LA and Carneiro JES (2013) Two cycles of recurrent selection in red bean breeding. Crop Breeding and Applied Biotechnology 13: 41-48.

Ramalho MAP (1996) Seleção recorrente. In: Reunião nacional de pesquisa de feijão, 5., 1996, Goiânia. Anais... Goiânia: EMBRAPA-CNPAF, p. 153-165.

VARIABILITY AMONG AND WITHIN COMMON BEAN PROGENIES FROM SEGREGATING POPULATIONS

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INTRODUCTION

Bulk within progenies derived from F_2 plants (Frey 1954) is one of the most used breeding methods for common bean in Brazil. The original proposal was that from F_2 generation, individual plants would be harvested and sowed by lines, $F_{2:3}$ progenies. At harvesting time, these progenies would also be handled individually. A bulk from each progeny is used to generate the next generation. This means that each progeny is conducted by bulk method. This process is repeated until $F_{2:6}$ or further generations, when the progenies are evaluated in experiments with replications. Many common bean breeding programs in Brazil use bulk within F_2 progenies (Bulk/ F_2). However, the progenies are evaluated in experiments with replication from $F_{2:3}$ generation. When applying this procedure, very often no selection is applied within the progenies, even at final stages, before releasing the new cultivars for farmers. Therefore, this new cultivar might be a multiline. In this way, this variability within progenies is not well explored. In our work the variation between and within progenies conducted by Bulk/ F_2 method was estimated aiming to guide future works that also uses this method.

MATERIAL AND METHODS

The segregating population used came from the Universidade Federal de Lavras (UFLA) recurrent selection program for grain yield. Details about obtainment of original population and conduction of selection cycles are presented by Silva et al. (2010). Twenty progenies were recombined to generate the fourteenth cycle (CXIV). In July 2014, 100 $S_{0:2}$ progenies were evaluated using 10x10 latice with tree replications. Plots consisted of two rows of two meter length. Seeds from ten progenies, taken at random, were divided into two sub-samples. The first sub-sample was stored in a cold chamber; however, the second sub-sample was proceeded further using the Bulk/F_2(S_0 progenies)method. A scheme of how the process was conducted is shown in Figure 1: Inbreeding Generations Scheme.
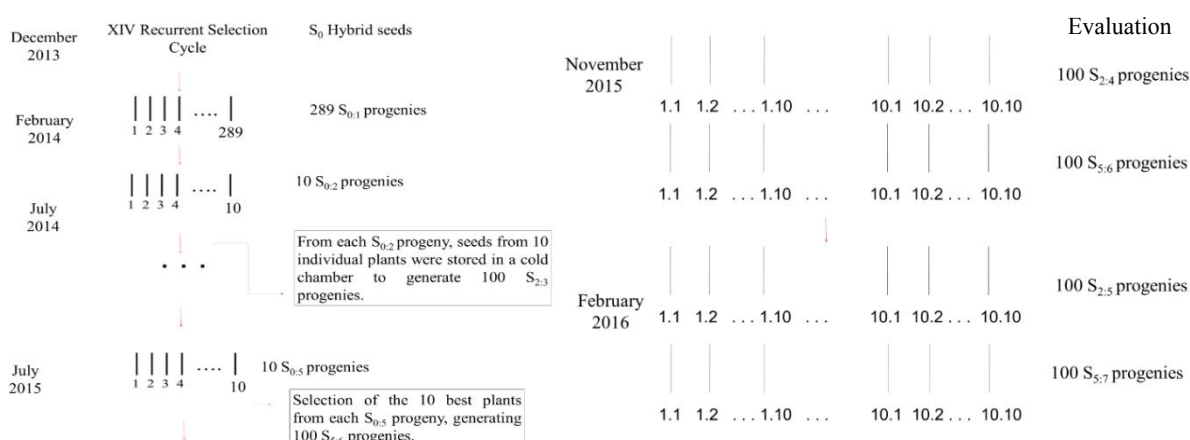
In November, 2015 and February 2016 lattice 10x10 design was used, but in the first case with two replications and in the second case, with tree replications. All seasons were conducted at Lavras, Minas Gerais, Brazil. The trait under evaluation was grain yield. Genetic parameters were estimate according to Ramalho et al. (2012).

RESULTS AND DISCUSSION

Considering the adopted method, each "$S_{2:4}$" progeny represent the variation within S_0 progenies after four inbreeding generations. For the same reason, "$S_{5:6}$" progenies represent the variation within S_0 after six inbreeding generations.

Heritability estimates among progenies were higher than the heritability estimates within progenies (Table 1). However, as expected, as inbreeding increased, the difference between the estimates decreased. It is also important to point that some within heritability estimates can be considered as null, since their lower limit estimates are negative.

When few plants were used to represent the variation within progenies, it is possible to infer that expressive variability was released. Not selecting within progenies derived from F_2 plants at final stages, as it has been done sometimes, leads to a new cultivar that is actually a multiline. If there are advantages or disadvantages when adopting this strategy for a trait such as grain yield, should still be more studied and explored.

Table 1- Heritability (h^2) and grain yield (GY) mean estimates and its confidence intervals (lower limit - LL - and upper limit - UL).

	$S_{2:4}$ /$S_{2:5}$			$S_{5:6}$ /$S_{5:7}$		
	LL	h^2	UL	LL	h^2	UL
Between	0.115	0.67	0.885	0.56	0.81	0.945
Within	-0.93	0.25	0.74	-0.185	0.48	0.845
	LL	GY (Kg.ha^{-1})	UL	LL	GY(Kg.ha^{-1})	UL
	2452.5	2711.0	3025.0	2479.5	2881.5	3325.5

* The heritability estimate, grain yield and confidence intervals are based on November 2015 and February 2016 estimates means.

REFERENCES

FREY, K.J. The use of F_2 lines in predicting the performance of F_3 selections in two barley crosses. Agronomy Journal, v. 46, p.541-544, 1954.

RAMALHO, M.A.P.; ABREU, A.F.B.; SANTOS, J.B.; NUNES,J.A.R. Aplicações da Genética Quantitativa no Melhoramento de Plantas Autógamas.(Ed.)UFLA. 522p.2012.

SILVA, G.S.; RAMALHO, M.A.P.; ABREU, A.F.B.; NUNES, J.A.R. Estimation of genetic progress after eight cycles of recurrent selection for common bean grain yield. Crop Breeding and Applied Biotechnology 10: 351-356, 2010.

SELECTION STRATEGIES OF PROGENIES IN A COMMON BEAN RECURRENT SELECTION PROGRAM

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INTRODUCTION

Breeders biggest challenge is to identify the best progenies/lines or hybrids that will remain the best ones in future years and under different conditions from the experimental stations ones. The interaction progenies x environment is expressive for common beans in Brazil (MENDES; RAMALHO; ABREU, 2009; LIMA; RAMALHO; ABREU, 2012; LIMA et al., 2015), consequently the selection success can be hampered. Therefore, the aim with this work was to evaluate a progeny selection strategy that considers not only the progenies means, but also the mean from the population from which the progeny came. It uses several inbreeding generations information in order to mitigate the interaction effect.

MATERIAL AND METHODS

Progenies used in this work came from Universidade Federal de Lavras (UFLA) recurrent selection program for grain yield, XV cycle. 439 $S_{0:1}$ progenies, 322 $S_{0:2}$ and 79 $S_{0:4}$ progenies were evaluated in 2015/2016. All progenies were obtained by a recombination system that is similar to a "*top cross*". 20 $S_{0:3}$ progenies are selected in each cycle and used as female in crossing with the 19 other ones.

Grain yield was the trait under evaluation. The data were analyzed by mixed model approach. BLUP's effects were estimate considering the original population effect and also progenies of previous inbreeding generations effect. The genetic variance among progenies (V_P), the progenies x environments interaction variance (V_{PA}), and heritability (h^2) among progenies were estimated. Coincidence index was also estimated among the 10% best progenies considering one or more generation and the reference generation, $S_{0:4}$.

RESULTS AND DISCUSSION

The coincidence of the best progenies when considering the generation of selection and the reference generation $S_{0:4}$was low at most cases (Table 1).It was low even when considering all the evaluation generations ($S_{0:1}$, $S_{0:2}$ e $S_{0:3}$) in relation to $S_{0:4.}$ In this case, the coincidence of the best progenies was only 20%.

Tabela 1 - 10% best $S_{0:4}$ progeniescoincidenceselectedbasedononeor more generations, genetic variance among progenies (V_P), progenies x environment interaction variance (V_{PA}) and heritability among progenies(h^2), for grain yield (Kg.ha^{-1}).

Generation	Coincidence	V_P	V_{PA}	h^2 (%)
$S_{0:1}$	0	60642.00	-	21
$S_{0:2}$	66.67	98994.00	-	41
$S_{0:3}$	0	25150.00	-	13
$S_{0:1}$, $S_{0:2}$ e $S_{0:3}$	20.00	23711.00	38677.00	23

The interaction progenies x environments is the most probable explanation to this low coincidence. This expressive progenies x environments interaction effect indicates that progenies grain yield was not coincident in all environments (generations/ seasons) evaluated. The interaction variance component (V_{PA}), was 1.6 times higher than the variance between progenies (V_P), for grain yield, considering the sequential analysis (Table 1). This emphasizes the high interaction effect.

Resende et al. (2016)proposed a new selection strategy by which the progeny merit is evaluated by an index that takes into account not only the progeny "per se" performance, but also the original population performance and the inbreeding generation's relationship coefficient. They showed that when adopting this index, the selection efficiency is higher. However, in this work, as commented previously, this index didn't improve the selection efficiency, probably due to the high progenies x environments interaction effect.

REFERENCES

MENDES, F. F.; RAMALHO, M. A. P.; ABREU, A. F. B. Índice de seleção para escolha de populações segregantes de feijoeiro-comum. **Pesquisa Agropecuária Brasileira**, Brasília, v. 44, n. 10, p. 1312-1318, out. 2009.

LIMA, L. K.; ABREU, A. F. B.; RAMALHO, M. A. P. Implications of the progeny x environment interaction in selection index involving characteristics of the common bean. **Geneticand Molecular Research**, Ribeirão Preto, v. 11, p. 4093-4099, 2012.

LIMA, D. C.; ABREU, A. F. B.; FERREIRA, R. A. D. C.; RAMALHO, M. A. P. Breeding common bean populations for traits using selection index. **Scientia Agricola,** v. 72, p. 132-137, 2015.

RESENDE, M. D. V.; RAMALHO, M. A. P.; CARNEIRO, P. C. S.; CARNEIRO, J. E. S.; BATISTA, L. G.; GOIS, I. B. Selection index with parents, populations, progenies and generations effects in autogamous plant breeding. **Crop Science**, v. 56, p. 530-546, n. 2016.

CORRELATION BETWEEN GENOTYPE DIFFERENCES IN YIELD AND CANOPY TEMPERATURES IN WYOMING DRY BEAN

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INTRODUCTION

Breeders and physiologists continue to seek phenotypic and genetic markers that are easy to measure and help predict yield.

METHODS

In 2015, 49 dry bean genotypes from varying market classes were sown on 19 June 2015 on a Haverson and McCook loam at Lingle (WY). Experimental design was a split-plot with irrigation level the main plot and genotypes (one row only, 6 m, 76-cm spacing) assigned to subplots. Irrigation levels were "unstressed" (for the season) vs. "partial drought." Partial drought consisted of full irrigation pre-bloom but was followed by approximately irrigation at 50% potential evapotranspiration post-bloom. There were two replicates per genotype per water regime. The fully irrigated plot received 6.09 inches of supplemental water while the limited plot received 2.38 inches of supplemental water (irrigation was performed weekly). Other details of the methods are provided in Heitholt and Baumgartner (2016). Canopy temperatures were recorded on 9 August with a Spectrum Technologies IR Temp Meter.

A second and similar study was sown on 27 May 2016 at Lingle (WY) with 23 genotypes on a Haverson, McCook loam and a Heldt silty clay. Plots (four rows) were 5 m long with 76-cm rows. Differential watering (0.75 inches vs. 0.50 inches) was employed at each irrigation post-bloom with a split-plot arrangement (three replicates per genotype per irrigation regime). Canopy temperature was recorded mid-morning and mid-afternoon on 23 July with an Apogee MI-2H0 infrared thermometer several days after a differential watering. Other methodological details for this second study are provided in Heitholt et al. (2017). A hail storm on 27 July terminated the crop and no yield data was collected.

A third study was conducted at Powell, WY. The study was sown on 25 May 2016 at PREC using a split-plot arrangement with two irrigation rates and 36 genotypes replicated three times per irrigation regime. Plots were three rows (56-cm spacing) wide and 4.6 m long. Irrigation rate (full vs. less-than-full) was the main plot and genotype the subplot. Canopy temperature was recorded on 23 July (mid-morning and mid-afternoon) with an Apogee MI-2H0. Other methods information for this third study are provided in Heitholt et al. (2017).

RESULTS AND DISCUSSION

At Lingle during 2015, yield was negatively correlated with canopy temperature across both watering regimes (Fig. 1). At Lingle in 2016, canopy temperatures were significantly different among the 23 genotypes (data not shown) and there was the expected trend for the canopies in the drought treatment to be warmer than the well-watered treatment (32.6 vs. 29.5°C in the am and 33.8 vs. 31.4°C in the pm). At Powell 2016, yields were unaffected by drought treatment but yields (averaged across irrigations) were again negatively related to canopy temperatures (Fig. 2). These results showed that canopy temperature may provide some indication of relative yield potential and this trait may be an important screening option for breeders.
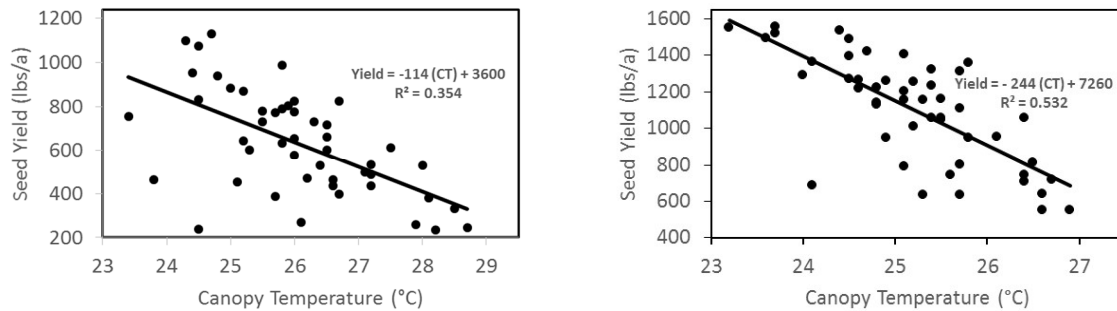
Figure 1. Relationship between grain yield among 49 genotypes and canopy temperature at Lingle (WY) on 9 August 2015. Drought treatment (left) and well-watered (right).
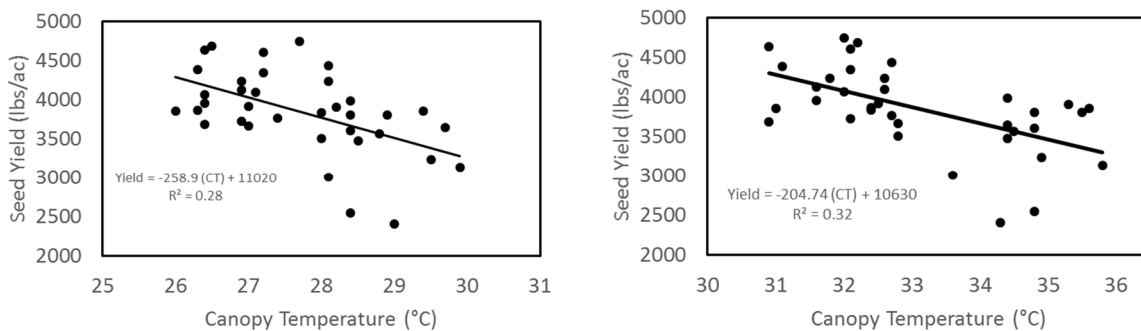

Figure 2. Relationship between grain yield among 36 genotypes and canopy temperatures on 18 July at Powell in 2016. Morning measurements can be found on the left and afternoon measurements can be found on the right.

REFERENCES

Heitholt, J., and B. Baumgartner. 2016. Drought susceptibility index and canopy traits of 49 dry bean genotypes subjected to water stress. Univ. Wyoming Agr. Exp. Stn. Field Days Bull., p. 99-100. http://www.uwyo.edu/uwexpstn/_files/docs/2016-field-days-bulletin.pdf.

Heitholt, J. and V. Sharma. 2017. Variation in canopy temperature (CT) and normalized difference vegetation index (NDVI) for 23 dry bean genotypes grown under well-watered and water stress conditions. Univ. Wyoming Agr. Exp. Stn. Field Days Bull., p. xx-yy. http://www.uwyo.edu/uwexpstn/_files/docs/2017-field-days-bulletin.pdf.

Heitholt, J., V. Sharma, and A. Pierson. 2017. Yield in 36 dry bean genotypes and its correlations with agronomic traits. Univ. Wyoming Agr. Exp. Stn. Field Days Bull., p. xx-yy. http://www.uwyo.edu/uwexpstn/_files/docs/2017-field-days-bulletin.pdf.

ADAPTABILITY AND STABILITY OF COMMON BEAN CULTIVARS WITH CARIOCA GRAIN TYPE USED IN BRAZIL

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INTRODUCTION

In Brazil beans are of great economic and social importance, as it is cultivated by small and large producers in all Brazilian regions. Diversified production systems and sowing times are used, therefore, the crop is subject to the most diverse environmental conditions. The presence of the interaction genotypes x environments hinders the work of the breeders, as it results in the variable behavior of the genotypes on the different environments, making selection difficult, especially for quantitative characteristics (CARGNIN et al., 2006). Several studies have reported that G x E interactions can be reduced using either specific cultivars in particular environments or cultivars with wide adaptability and stability (RAMALHO et al., 2012). Thus, the aim of this study was to evaluate the influence of G x E interaction on grain yield of common bean cultivars with carioca grain type.

MATERIAL AND METHODS

Twenty common bean cultivars belonging to the carioca commercial group were used. All cultivars come from breeding programs of public or private institutions from Brazil, and indicated for cultivation in the state of Paraná. The trials were established in four environments in the state of Paraná-BR, two in the 2014/2015 rainy season, in Ponta Grossa and Guarapuava, and two in the dry season of 2015, in Ponta Grossa and Santa Tereza do Oeste. The experimental design was a randomized complete block design with three replicates and plots consisting of four rows of 4 meters spaced 0.5 m, with a population of 12 plants per linear meter, considering the two central lines as a useful plot. The grain yield was obtained by weighing grams of the two central rows of the plot, adjusted to 13% of humidity and extrapolated to kg/ha. The results were submitted to analysis of individual variance of each environment and the test of homogeneity of error variance, followed by analysis of joint variance, considering the effects of genotype and environment as fixed. For the study of adaptability and stability, the methodology proposed by EBERHART & RUSSELL (1966) was used. All analyzes were processed in the Genes software (CRUZ, 2013).

RESULTS AND DISCUSSION

The estimate obtained by the ratio between the largest and the smallest mean square of the residues was less than seven (5.76), indicating homogeneity of the residual variances, allowing the joint analysis, which revealed a significant effect for all the sources of variation tested (Table 1). The significant effect of the cultivar x environment interaction reveals that cultivars have a differentiated response to environmental changes.

When analyzing the factors of adaptability and phenotypic stability by the criterion of EBERHART & RUSSELL (1966), 80% of the cultivars presented wide adaptability ($\beta_{li}=1$) (Table 2). The cultivars Dama, Pérola and IPR Maracanã showed adaptability to favorable environments ($\beta > 1$), that is, they respond favorably to environmental improvements. On the other hand, the cultivar IPR Curió presented $\beta < 1$, evidencing to be adapted to unfavorable environments, not presenting increase in grain yield with the improvement of the environment. For phenotypic stability, 90% of the cultivars showed regression

Table 1. Summary of the joint analysis of variance for grain yield (kg/ha) evaluated in 20 common bean cultivars of carioca grain type.

SV	Df	Mean Square	
Blocks/Environment	8	411440	
Cultivars (C)	19	1076498	**
Environments (E)	3	23377149	**
C x E	57	470051	*
Error	152	47444411	
Average		2564,31	
CV (%)		21,79	

**/* Significant at 1 and 5% levels, respectively, by the F test

deviation equal to zero ($\sigma^2_{di}=0$), indicating a high predictability of behavior (Table 2). In contrast, the cultivars IPR Campos Gerais and IPR Curió presented significant regression deviations ($\sigma^2_{di} \neq 0$), evidencing unpredictable behavior in the environments.

Cultivars	Mean (kg/ha)	β_1	σ^2_{di}		R^2 (%)
IPR Bem-te-vi	3127.91	1.163	80926.255	NS	81.03
FT-65	2895.21	0.573	-87162.797	NS	91.90
BRS Notável	2873.06	1.023	-66496.279	NS	94.21
Dama	2869.01	1.642*	-71132.046	NS	97.95
Bola Cheia	2867.68	1.089	-67232.933	NS	94.96
Pérola	2771.34	1.722*	-36626.765	NS	96.25
IPR Campos Gerais	2739.88	0.729	222159.813	*	48.75
Carioca	2627.55	0.886	115548.841	NS	67.60
IPR Tangará	2620.77	1.162	91742.530	NS	80.11
IAC Formoso	2572.59	0.836	-70153.840	NS	92.33
IPR Andorinha	2542.51	0.704	-49975.461	NS	84.25
IAPAR 81	2517.36	1.078	-59704.009	NS	93.87
Gol	2514.98	1.079	123513.902	NS	74.94
BRS Estilo	2432.09	0.827	-85983.744	NS	95.67
IAC Alvorada	2394.17	1.308	48297.248	NS	86.79
IPR Maracanã	2387.01	1.651*	74648.408	NS	89.92
IPR Quero-quero	2300.96	0.797	-41209.440	NS	85.53
IPR Curió	2196.96	-0.006**	290793.355	*	0.005
IAC Imperador	2142.66	0.706	-4765.221	NS	74.59
IPR Eldourado	1892.66	1.034	177340.547	NS	68.93

Table 2. Mean grain yield (kg/ha) and estimates of the parameters (regression coefficient (β_1), regression deviation (σ^2_{di}) for adaptability and stability according to EBERHART & RUSSELL (1966), in 20 common bean cultivars of carioca grain type, at four experimental sites.

NS, * e **: non significant, significant at 5 e 1%, by t test (h_0: $\beta_{1i} = 1,0$) and the F test F (h_0: σ^2_{di}).

Considering the results obtained by the analysis of EBERHART & RUSSEL simultaneously with the average grain yield of the cultivars, it is verified that the majority of the genotypes showed high predictability of behavior and wide adaptability. In addition, the IPR Bem-te-vi, FT-65 and BRS Notável cultivars deserve to be highlighted, as they presented, together with these parameters, high grain yield. In addition, these genotypes showed high determination coefficients ($R^2 > 75\%$), indicating that most of their total variations are explained by the adopted model (Table 2).

REFERENCES
CARGNIN, A. et al. Interação entre genótipos e ambientes e implicações em ganhos com seleção em trigo. Pesquisa Agropecuária Brasileira, v.41, n.6, p.987-993, 2006.

CRUZ, C.D. Genes - a software package for analysis in experimental statistics and quantitative genetics. Acta Scientiarum, Maringá, v.35, n.3, p.271-276, 2013.

EBERHART, S. A.; RUSSELL, W. A. Stability parameters for comparing varieties. *Crop Science*, Madison, v. 6, n. 1, p. 36- 40, 1966.

RAMALHO, M. et al. Aplicações da genética quantitativa no melhoramento de plantas autógamas. Editora UFLA, Lavras, 2012.

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2016 FINANCIAL STATEMENT
BEAN IMPROVEMENT COOPERATIVE

BALANCE AS OF January 1, 2016   **$ 15,739.89**

INCOME

		2016
2016 Dues	$	4755.00
Extra Articles for 2015 Report	$	25.00
2017 Dues prepaid	$	80.00
Back Issues	$	0.00
Bank Interest	$	119.00
TOTAL INCOME	$	4979.00

EXPENSE

Labor Charges	$	975.00
Postage, Copy Charges and Office Supplies	$	1,266.00
Printing and shipment – Volume 59	$	2,367.65
PayPal Fees	$	217.79
TOTAL EXPENSE	$	4826.44

BALANCE AS OF December 31, 2016   **$ 15,892.45**