
ANNUAL REPORT OF THE
BEAN IMPROVEMENT COOPERATIVE
BIC
A VOLUNTARY AND INFORMAL ORGANIZATION
TO EFFECT THE EXCHANGE OF INFORMATION AND MATERIALS
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THE LVI

Report of The

BEAN IMPROVEMENT COOPERATIVE

No. 56 March 2013

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Coordinating Committee

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http://www.css.msu.edu/bic
SITE REGULARLY UPDATED

Note: It will be assumed that interested individuals may freely cite (including author credit) any report or note in this BIC report, unless the author indicates to the contrary. As a professional courtesy, individuals citing BIC notes should notify the authors of his or her intentions. The BIC Coordinating Committee approved this statement on November 5, 1975.

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Cover: Snap bean pods courtesy of Jim Myers

THE 56th 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-seventh Biennial Meeting from October 28 through October 30, 2013, in Portland, Oregon. The local BIC meeting organizer is Jim Myers myersja@hort.oregonstate.edu. In addition, the associated meetings with our colleagues in the North American Pulse Improvement Association will be from October 31 to November 1, 2013 [NAPIA hosts: Clare Coyne or Rebecca McGee (rebecca.mcgee@ars.usda.gov)]. The Phaseolus Crop Germplasm Committee, BIC Genetics Committee and the Regional W-2150 Committee are scheduled for October 30. A field trip is also planned for October 30th. Please refer to the information provided by the local organizing committee in the current report, and look for additional information and updates posted on the BIC web site www.css.msu.edu/bic. A call for abstracts will be posted on line.

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, Howard Schwartz (howard.schwartz@colostate.edu) by May 31, 2013. We will continue to recognize our founding members through the **Frazier-Zaumeyer Distinguished Lectureship**. The Lectureship will be awarded at the meeting in Portland and nominations should be sent to Howard Schwartz. 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 this continued support of Dr. Kelly for maintaining the website. Note that some Research Technology sections on the website have been updated while others await new contributions. 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.

To reduce mailing costs and expedite communications, the BIC continues to conduct business by email and through postings on the web page. 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 and suggestions to make the BIC a more versatile and effective organization and any thoughts can be shared with members of the Coordinating Committee. See you in Portland, Oregon in October…….

Dr. Phillip Miklas, BIC President

BIC COMMITTEE MEMBERSHIP - 1957 to 2012

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, Beaver, **Kelly**, Kotch, Miklas, Myers, Park, Riley, Schwartz (ex officio), Singh, Vandenberg
2001 Antonius, Beaver, **Kelly**, Kotch, Miklas, Myers, Park, Riley, de Ron, Schwartz (ex officio), Vandenberg
2003 Beaver, **Kelly**, Kmiecik, Kurowski, Miklas, Myers, Park, Riley, de Ron, Schwartz (ex officio), Vandenberg
2006 Beaver, **Kelly**, Kmiecik, Miklas, Myers, Park, Riley, de Ron, Schwartz (ex officio), Shellenberger, Vandenberg
2008 Beaver, **Kelly**, Kmiecik, Miklas, Myers, Pauls, Riley, de Ron, Schwartz (ex officio), Shellenberger, Vandenberg
2010 Beaver, Kelly (ex officio), Kmiecik, **Miklas**, Myers, Pauls, Riley, de Ron, Schwartz, Shellenberger, Vandenberg
2012 Bett, Kelly (ex officio), Kmiecik, **Miklas**, Myers, Osorno, Pastor-Corrales, Pauls, Riley, de Ron, Wahlquist

Awards Committee:

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

Genetics Committee

2008: Bett, Blair, Gepts, McClean, Miklas, **Porch**, Urrea, Welsh (ex officio).
2011: Bett, Blair, Gepts, Kelly, McClean, **Porch**, Urrea, Welsh (ex officio).
2012: Bett, Blair, Gepts, Kelly, McClean, **Porch**, Urrea, Welsh (ex officio).

Phaseolus Genetics Committee Minutes
2012 meeting in Niagara Falls, Canada

Meeting location: Niagara Falls Marriott Gateway Hotel, Niagara Falls, Canada
Date: November, 5, 2012
Time: 5 PM

Attendance (committee members present)

Kirstin Bett	University of Saskatchewan, Saskatoon	Committee member
James Kelly	Michigan State University	Committee member
Tim Porch	USDA-ARS, Puerto Rico	Chairperson
Carlos Urrea	University of Nebraska	Committee member

Note: There was insufficient attendance of the committee to establish a quorum, thus these minutes will be considered as suggestions for the commmittee to consider for approval.

Old Business:

1. The minutes were approved from the 2011 meeting on Nov. 2 in San Juan, Puerto Rico.

New Business:

1. Anthracnose nomenclature and genetics of highly variable pathogens (J. Kelly, Michigan State U.): Several suggestions have been made in a letter from J. Kelly and J Ferreira (10/2012). The anthracnose Co genes are essentially resistance gene clusters and not separate major genes, thus a new system for naming Co genes is proposed. This new system should include relative position on the genetic map, genotype of the pathogen, and the bean genotype in which the resistance gene was identified. An additional superscript would be helpful for identifying the original genotype and pathogen from which the gene was identified (e.g., $Co\text{-}3^{38\ WD}$; for race 38 (CL18 isolate) on Widusa bean genotype in the Co-3 cluster). In addition, potentially flawed results can result from allelism tests in highly variable pathogens. Therefore, progeny testing with multiple races is proposed for addition to the requirements for the naming of new genes.
2. BCMV nomenclature (J. Hart, Cornell U.): A proposal to replace the *cyv* gene symbol with the $bc\text{-}3^2$ gene symbol was presented based on evidence that indicates that *cyv* appears to be another allele of the *bc-3* locus. A literature review as well as phenotypic and molecular evidence was presented supporting the single locus hypothesis. The committee will consider this new nomenclature.
3. Membership: Several individuals were nominated as new members on the Genetics Committee including: Juan M. Osorno, Juan Jose Ferreira, and Venu (Kal) Kalavacharla.

Meeting adjourned at 6:14 PM.

RECIPIENTS of BIC MERITORIOUS SERVICE & ACHIEVEMENT AWARDS

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 James D. Kelly- Michigan State University, Plant Breeder [Achievement Award]

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]

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

2013 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 56-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 Technical Merit 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-seventh Anniversary of the BIC Biennial Meeting in Portland OR, Oct. 29, 2013.

BIC AWARD NOMINATION

Return by May 31, 2013 to:

Dr. Howard F. Schwartz howard.schwartz@colostate.edu
BIC Awards Committee Chairman
Dept. of Bioagricultural Sciences and Pest Management
Colorado State University
Fort Collins, CO 80523-1177, USA

The other Awards Committee members are Drs. Steve Noffsinger and Shree P. Singh.

Nominee: 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]

Second announcement for the Biennial BIC/NAPIA 2013 meeting in Portland, Oregon

BIC = 28-30 October, 2013 and NAPIA = 30 Oct – 01 Nov, 2013

The meeting will be at the DoubleTree by Hilton at 1000 Northeast Multnomah Street, Portland, OR 97232. To make reservations, call their toll-Free number at 800-996-0510, or direct line at (503) 281-6111. To receive the negotiated rate, provide the **group code BPC**. **You may also book through the hotel's Personalized Online Group Website:** http://doubletree.hilton.com/en/dt/groups/personalized/R/RLLC-DT-BPC-20131027/index.jhtml?WT.mc_id=POG. Hotel cutoff date is **06-October, 2013**

Room rates: 119 USD/Night Queen bed nonsmoking
129 USD/Night Queen balcony nonsmoking

Transportation from the Portland International Airport (PDX) is by Light Rail (red line, approx. 25 minutes). The station nearest the hotel is the Lloyd Center/NE 11th Ave MAX Station.

BIC hosts contact: James Myers myersja@hort.oregonstate.edu or
Phillip Miklas phil.miklas@ars.usda.gov

NAPIA hosts contact: Clare Coyne clarice.coyne@ars.usda.gov or
Rebecca McGee rebecca.mcgee@ars.usda.gov

Schedule

Day	*Date*	*Activity*	*Time*
Monday	28-Oct	BIC Board meeting	7-8A
		BIC talks and posters	8A-5P
Tuesday	29-Oct	BIC talks and posters	8A-5P
		BIC Banquet	6-8P
Wednesday	30-Oct	W2150/PCGC	8A-12P
		Tour (BIC/NAPIA)	1-5P
		BIC Genetics Committee	7-9P
		NAPIA Board meeting	7-9P
Thursday	31-Oct	NAPIA	8A-5P
Friday	1-Nov	NAPIA	8A-5P

Registration cost for BIC will be approximately $160 (full) and $100 (for students); final registration costs will be provided closer to the registration deadline. Registration forms and call for presentations will be emailed in April/May 2013.

IN MEMORY OF DOUGLAS W. BURKE

Douglas W. Burke died December 26, 2012. He was 92 years old. He was born in Lovell, Wyoming on April 27, 1920. He served in the U.S. Army during WWII. Soon after the war, he married Rachel Smith and together they raised five sons. Doug received his B.S. and M.S. degrees from the University of Wyoming in 1947 and 1948. He earned a Ph.D. in Plant Pathology from Washington State University in 1955. Soon after graduation Dr. Burke began a storied career with the USDA-ARS in 1955. His first position was in Greely, CO, but after one year transferred to Prosser, WA in 1956, where he initiated the dry bean research and breeding program primarily in support of the bean seed industry but served the commercial dry bean industry just as well. His career with USDA-ARS spanned 30 years. During the latter years, in addition to being a Research Plant Pathologist, he served as the Research Leader for the entire Vegetable and Forage Crop Research Unit.

Doug made many important contributions during his career. His most well known work was to define the Fusarium root rot problem in beans. He conducted numerous ecological studies which revealed the importance of cultural practices, soil temperature, soil compaction and aeration, and other factors in management of Fusarium root rot in beans. The review article "Control of Fusarium root rot with resistant beans and cultural management' published in Plant Disease 67:1312-1317 (1983) is still a widely cited and useful resource by today's standards. Doug was not afraid of hard work or getting dirty as most of his root rot research was field based and required shoveling to sample roots. His root rot research earned him a sabbatical at the University of Wisconsin, Madison in 1967-1968 to conduct similar research on the ecology of Aphanomyces root rot.

The other legacy that Dr. Burke left us with was a full coffer of important cultivars and germplasm lines in the major dry bean market classes. These materials including Royal Red dark red kidney, Kardinal light red kidney, NW-410 pinto, Roza pink, and Rufus red incorporated resistance to *Bean common mosaic virus* (BCMV) and *Beet curly top virus* (BCTV) in addition to tolerance to Fusarium root rot. Many of the materials were tolerant of drought, low fertility, and soil compaction as well. Cultivars like Othello pinto, Viva pink, and NW63 red, are still widely grown today across the U.S. and Canada. Some of these offered early maturity and wide adaptation in addition to the above disease resistance traits which contributed to their longevity as sought after cultivars. The cultivars he released continue to outperform most other cultivars and breeding lines in stress trials conducted today. Although not trained as a plant breeder, his accomplishments as a breeder are quite remarkable and long lasting. Doug was very considerate, freely shared his germplasm lines and cultivars, and collaborated with other researchers worldwide.

Doug served on the BIC Coordinating Committee from 1971 to 1975, was Chairman of the BIC Awards Committee in 1970-1971, and in 1977 received the BIC Meritorious Service Award. He was also a contributing member of the American Phytopathological Society. After retirement he spent a year in Botswana with USAID helping with cowpea production. In 1985-1987, Doug and Rachael went on an LDS church mission to India. He was a genealogy enthusiast. He took care of Rachael for nine years as her health failed. He is survived by a sister, his 5 sons, and many grandchildren and great-grandchildren.

IN MEMORY OF JOHN J. KOLAR

John J. Kolar, 90, Emeritus Professor at University of Idaho in the Plant, Soil and Entomological Sciences Department died on Monday, August 20, 2012, at St. Luke's Magic Valley Medical Center, Twin Falls, Idaho of natural causes. A graveside memorial service was held on Friday, August 24, at Sunset Memorial Park, with military honors by the Magic Valley Honor Guard and Idaho National Guard. John held the University's dry bean breeding position at the Kimberly Research and Extension Center from the early 1960s to the mid 1980s. He was involved in the development of many important dry bean varieties grown in the Pacific Northwest.

Born on June 14, 1922, in Raynesford, Montana, John graduated from St. Mary's High School in Great Falls, Montana, and worked the family cattle ranch from 1940 to 1943. He was drafted into the Army during the World War II and proudly served his country from 1943 to 1947. He returned to Montana State University on the GI bill, where he received his Bachelor of Science in 1950 and his Master of Science in 1952. He then entered the Ph.D. program at Iowa State and earned his doctorate in Agronomy in 1955.

John accepted a position as a Research Agronomist first at the University of Idaho-Aberdeen Research and Extension Center, Idaho, then in 1960, he moved to Twin Falls, Idaho where he worked at the Kimberly Research and Extension Center until his retirement in 1985. John was in the bean breeding program at Kimberly Research and Extension Center from 1960 to 1985, and headed the program from about 1970, when Dr. L.L. (Bill) Dean left the program, until his retirement in 1985.

He had an extensive breeding program developing varieties in pinto, pink, great northern Mexican red, and navy market classes. He developed or assisted in development and release of numerous bean varieties for Idaho and the Pacific Northwest. His direct releases included UI 60, UI 61, and UI 425 great northern; UI 114, UI 126, and UI 129 pinto. He initiated the crosses from which were developed many other varieties released by his successor, Dr. Jim Myers. These included UI 320 pinto; UI 465 great northern; UI 228, UI 239, and UI 259 Mexican red; UI 537 pink; UI 125 and UI 137 small white/navy; UI 906 and UI 911 small black; UI 722 dark red kidney; and UI 686 cranberry.

John had an excellent eye for selecting breeding lines varieties that that met market class standards and for choosing parents for productive crosses. He selected on the basis of quality and productivity without regard to origins. For example, UI 129 pinto was discovered as a single plant off type with unknown pedigree in his breeding nursery, but the lack of breeding history did not stop John from releasing what he considered to be a top notch variety at the time. John worked closely with colleagues at the USDA Snake River Soil and Water Conservation Center, and with many private and public breeders nationwide, especially those in the Pacific Northwest, in the exchange of germplasm and variety testing. He coordinated the national Cooperative Dry Bean Nursery from about 1980 until his retirement in 1985.

John is survived by his two sons, Steve of Twin Falls and Randy of Norman, Oklahoma and their families.

IN MEMORY OF DONALD R. WOOD

Donald R. Wood, a fifty-year resident of Fort Collins, died May 27, 2012 at the age of 91. Don was born in Keats, Kansas to Jay Roy and Stella Blain Wood on April 17, 1921. He and his four brothers survived the dustbowl and depression in southern Kansas. He went on to obtain his degrees from Kansas State University, Colorado State University and the University of Wisconsin. He was a Captain in the Marine Corp during World War II. Don was married to Marcile Norby for 45 years until her death in 1989, and in 1991, he married Annette Davison, who is also deceased. He was a generous, loving and loyal father to Chester and Marilyn Wood, and enjoyed his five grandchildren and three great grand children. His passions in life included his family, service to his church locally and globally, fly fishing, photography, flying small planes, and travelling the world.

Don was a professor, plant breeder and geneticist at Colorado State University from 1949 to 1987. He emphasized bean protein biochemistry and human nutrition, and at one point one of his pinto varieties occupied more than 30 percent of Colorado's bean acreage. Some of his releases included Bill Z, Olathe, and Ouray. His germplasm resources have provided a long-lasting legacy for continued improvement of dry beans for the Central High Plains. He was recognized internationally as an invited speaker at various symposia, and was a consultant to the FAO, UN and US-AID on food crop research. He received the BIC Meritorious Service Award in 1982 in recognition of his service to the BIC since its inception, and he also served as leader on regional technical committees such as the W-150 (now W-2150) for bean improvement and the industry.

INFLUENCE OF BASAL SALT SOURCES ON REGENERATION OF COMMON BEAN

Gerardine Mukeshimana and James D. Kelly

Department of Plant, Soil and Microbial Sciences, Michigan State University,
1066 Bogue Street, East Lansing, 48824 MI

INTRODUCTION: The successful production of genetically engineered common bean has not been achieved due to their recalcitrance to *in vitro* regeneration. Common bean regeneration has been extensively studied using Murashige and Skoog (MS) medium. However, a recent study demonstrated that Gamborg's (B5) medium was more efficient for direct organogenesis and regeneration of the embryo axis of common bean than MS (Quintero-Jimenez et al., 2010). This suggests the potential importance of the source of basal salts as a factor that might enhance the regeneration of common bean. The present study was conducted to evaluate the effect of various basal salts on the regeneration of common bean.

MATERIALS AND METHODS: The effects of basal salts on regeneration of common bean were tested using embryo axis from 'Merlot' bean cultivar. Mature, dry seeds were surface-sterilized with 3% sodium hypochlorite with continuous shaking for 10 min followed by four rinses with sterile distilled water, and then soaked in sterile distilled water for approximately 16 h. The seed coats were removed and the embryos were excised using a sterile scalpel. Embryo axis explants were obtained by cutting off radicles and leaflets.

Six basal salts (Sigma-Aldrich, St. Louis, MO, USA), consisting of MS, KAO (Kao and Michayluk, 1975), QL (Quorin and Lepoivre, 1977), woody plant medium- WPM (Lloyd and McCown, 1980), Chu's (N6) (Chu et al., 1975) and White's (White, 1963), were tested. Regeneration media were prepared by mixing each basal salt at recommended concentrations, 1 mg L^{-1} B_5 vitamins, 44.4 µM 6-benzyl-aminopurine (BAP). All media contained 3% sucrose, pH adjusted to 5.6, solidified with 0.8% (w/v) Bacto agar and autoclaved for 20 min.

Two experiments were conducted in Petri dishes (100 x15 mm). Ten embryo axes were placed on each medium replicated three times for the first experiment for a total of 30 explants per medium. The second experiment was conducted only with MS, QL, and WPM media because these were the best performing media as determined by the first experiment. Ninety embryos axes were used for each regeneration medium. In all experiments, dishes containing explants were kept in the dark for 2 wk and placed under a 16 h photoperiod of 30 µmol $m^{-2}s^{-1}$ from cool white fluorescent tubes for two additional wk. Regeneration was recorded as the number of embryo axes regenerating at least one shoot. The regeneration frequency was calculated as the number of regenerated explants/ total number of explants x 100. Shoot number per explant were counted on four randomly chosen explants at the end of evaluation period. Data were analyzed using SAS 9.2 (SAS institute, Cary, NC) and the means were separated by the Duncan's Multiple Range test.

RESULTS AND DISCUSSION: Results of the result regeneration frequencies and the shoot number per explant are presented in Table1. The highest percentage of regeneration was observed in WPM and QL media while MS resulted in the lowest regeneration frequencies (Table1). MS, QL, and WPM resulted in higher average numbers of shoots/explant than the other

media (Table1). Lower numbers and unhealthy shoots/explant were regenerated on KAO, White's, and Chu's media.

Table1. Effect of different basal salts on regeneration of common bean embryo axes and number of shoots per explant in two experiments

	Experiment 1		Experiment 2	
Media	Regeneration frequency (%)	Average number of shoots	Regeneration frequency (%)	Average number of shoots
WPM	66.7±28.8[a]	6.7±2.2[a]	69.8± 22.8[a]	N/A
QL	56.7±11.5[ab]	8.0±3.8[a]	76 ± 25.0[a]	6.2[a]
KAO	50.0±17.3[abc]	3.2± 1.5[b]	N.A	N/A
White	30.0±10[bc]	1.5±0.57[b]	N.A	N/A
Chu's	23.3±25.1[bc]	3.0±1.41[b]	N.A	N/A
MS	20.0±10[c]	8.3±1.7[a]	46.2±19.2[b]	4[b]

Numbers within the same column followed by the same letter are not significantly different (α=0.05); N/A: Not Applicable

In these experiments, two media, WPM and QL, appeared to have the greatest potential for inducing adventitious shoot regeneration. WPM was able to induce multiple shoot regeneration at the highest frequency, but these shoots were weak and chlorotic compared to shoots regenerated from QL and MS media. The major differences in macronutrients among these media are ammonium, nitrate, and calcium ion and total ion concentrations. While WPM contains lower concentrations of both ammonium and nitrate ions than MS and QL media, MS medium has the highest concentrations of both ammonium and nitrate ions, and QL has a medium content of ammonium ions with an increased level of calcium ions. The unhealthy looking shoots regenerated from WPM medium might be due to a low concentration of nitrogen that could not support healthy bean growth. The inability of WPM to sustain strong shoot growth has been reported in other plant species (Canli and Tian, 2009). Bean shoots regenerated on WPM would need to be quickly transferred to a different medium before they start to regenerate to keep them alive or the WPM medium would need to be supplemented with nitrogen to produce healthy regenerated shoots. MS medium induced low regeneration frequencies than other media suggesting that an intermediate level of nitrogen content may be useful in promoting bean regeneration. The high concentration of ammonium ions in MS may have reduced regeneration due to the toxic effect of ammonium ions. The toxic effect of ammonium in tissue culture might be due to pH changes and acidification of the medium or to the toxic effect of free ammonium ions.

The high calcium ion concentration in QL medium may have been responsible for the high regeneration frequency and the number of shoots per explant observed on this medium. Calcium ions play an important role in cell wall and membrane formation and they are important in young and actively growing tissues since they are needed in mitotic spindle microtubules assembly and breakdown and thus cellular division.

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INHERITANCE STUDY OF THE "EMBRYO ABORTION" TRAIT IN *PHASEOLUS VULGARIS* L. EMBRYO DEFECTIVE LINE FROM EMS MUTAGENESIS

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INTRODUCTION

A better understanding of *Phaseolus* embryogenesis is needed to overcome incompatibility barriers in interspecific hybrids. We isolated a *Phaseolus vulgaris* mutant line defective in seed development within the second generation of an ethyl methanesulfonate (EMS) TILLING population of *P. vulgaris* cv. BAT93. This study was undertaken to elucidate the inheritance of the "embryo abortion" trait in *Phaseolus vulgaris* L. embryo defective line.

MATERIALS AND METHODS

EMS selected mutagenized plants, defective in embryo development (Silué *et al.*, 2006), and wild-type plants of *P. vulgaris* genotype BAT93 constitute the plant material. Plants were grown under the following conditions: 27°C/23°C (day/night), 75% relative humidity and 12 hours photoperiod. For this study, the two materials were reciprocally crossed to produce F_1 seeds. Seven F_2 populations (137 total individuals) using wild-type plants as female parents were screened for seed setting to determine the proportions of plants defective in seed development and plants with normal seeds. Seeds from sixteen F_2 plants (F_3 progenies, $F_{2:3}$) derived from one of the seven F_1 plants were sown to evaluate the rate of homozygous and heterozygous among these sixteen F_2 plants. The presence of at least one mutagenized plant with aborting embryos in an offspring demonstrates the heterozygocity of the F_2 plant. The observed ratios were tested against the expected ratio according to Mendel laws using chi-square test.

RESULTS AND DISCUSSIONS

Analysis of the reciprocal crosses reveals that the "embryo abortion" trait is maternally inherited: if a plant defective in seed development is pollinated with wild-type pollen, the F_1 seeds abort before maturity; if a wild-type plant is pollinated with pollen from mutant plant, the F_1 seeds have the normal phenotype (embryo without abnormalities) and germinated to produce normal F_1 plants. The F_1 plants, from the cross wild type (♀) x mutagenized (♂), were self-pollinated to produce F_2 seeds. These F_2 seeds were sown to generate F_2 plants and a total of 137 plants were scored for seed development: 106 plants have wild-type phenotype and produce normal seeds while 31 plants produce abnormal seeds with embryos which all abort before maturity. These data fit a mendelian 3:1 (wild-type phenotype:mutant phenotype) segregation ratio, indicating that the "embryo abortion" trait is controlled by a single recessive gene (Table 1; $\chi^2 = 0.41$, df = 1, $P > 0.05$). To confirm this hypothesis, the progenies of sixteen F_2 plants from one F_1 were analysed to determine the ratio of homozygotes and heterozygotes among the F_2 plants with normal phenotype. A 2:1 ratio of heterozygotes and homozygotes was observed within these 16 F_2 plants, confirming that the mutation trait is monogenic (Table 2).

Identical results, i.e. maternal inheritance of embryo abortion trait and its monogenic recessive control, were obtained with several traits following induced mutations. In *Arabidopsis*, Léon-Kloosterziel et al. (1994) isolated from an EMS treated population a *testa* mutant in which the heart-shaped phenotype was maternally inherited and controlled by a single recessive gene. The following induced mutants were also under the control of single recessive genes: *sus* and *rsy* mutants obtained by T-DNA mutagenesis and EMS mutagenesis in *Arabidopsis* (Apuya et al., 2002; Schwartz et al., 1994), *poc* mutants obtained by EMS mutagenesis in tomato (Al-Hammadi et al., 2003) and *sic* mutants obtained by EMS mutagenesis in pea (Liu et al., 1999).

Table 1. F_2 segregation of seed development from crosses between wild type plants and mutant plants

Cross	F_2 plants segregation for seeds setting					
	Observed ratio					
Wild-type x Mutagenized (♀ x ♂)	Total F_2 plants	Plants with normal seeds	Plants with abnormal seeds	Expected ratio[a]	χ^2	P-value
	137	106	31	3 : 1	0.41	52.14

[a]: according to Mendel laws

Table 2. F_3 plants segregation from sixteen F_2 plants derived from crosses between wild type plants and mutant plants

Cross	F_3 plants segregation for seed setting					
	Observed ratio					
Wild-type x Mutagenized (♀ x ♂)	Total F_2 progenies screened	Progenies with mutant plants	Progenies without mutant plants	Expected ratio[a]	χ^2	P-value
$F_{2:3}$ line 4	16 (193 F_3 plants)	13	3	2 : 1	1.53	21.65

[a]: according to Mendel laws

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VARIABILITY OF COMMON BEAN RESISTANCE (*Phaseolus vulgaris* L.) TO LINURON

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Herbicides are an integral part of modern agriculture because they provide cost-effective increase in agricultural productivity. In this respect the selectivity to varieties of the applied herbicides is of primary importance. Therefore breeding for resistance to herbicides is focus of our attention. The stage of plant development affects the ability of the herbicides to enter the plant. Plants in the germination or young seedling stage (0 to 30 d) are more susceptible to a soil-applied herbicide than in the later stages (Rao 2009).

Selectivity of an applied herbicide is influenced by several environmental factors under which the plant grows. The various environmental factors that affect plant growth and hence herbicide activity and selectivity are temperature, rainfall (water), humidity, light and wind. They largely influence absorption and translocation of herbicides by plant (Rao 2009).

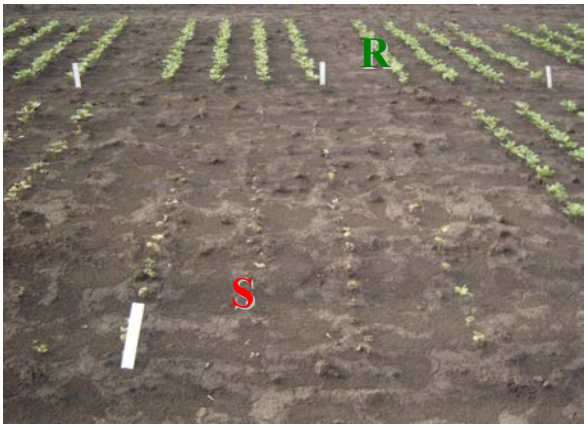


Fig. 1 Resistant (R) and susceptibel (S) reaction of breeding bean lines to the soil-applied herbicide linuron

Linuron is a herbicide widely used on common bean in Bulgaria.

When, however, at seedling stage till first trifoliolate leaf there are rainfalls or sprinkling is applied at norm ≥ 15 mm, the active substance of this herbicide, due to its water solubility (63.8 mg/L, CAPL, 2013) reaches to the roots of the bean plant and is absorbed there; chlorosis on the whole leave is observed in the susceptible plants followed later by leaf necrosis from the periphery and the tip, until the plants die entirely (Tonev, 2000).

MATERIAL AND METHODS

The competitive varietal trial in 2012 was planted on 26.04.2012. Linuron [*N'*-(3,4-dichlorophnyl)-*N*-methoxy-*N*-methylurea] was applied on 28.04.2012 at dose 1.5 kg/ha active substance. The mean date of germination of the cultivars involved in the varietal trial was 6.05.2012. On 10th May there was a rainfall of 23 mm, and on 15th May – of 17.8 mm. As a result a distinct response of the breeding materials to the selective toxic activity of linuron was observed. Such a breeding environment can be imitated by artificial sprinkling at norm 20 mm. On 28th May the resistance reaction of the selection lines and varieties to linuron was read according to a five-degree scale (Table 1).

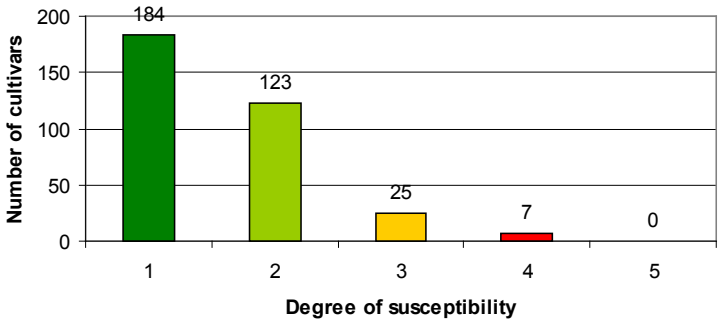
RESULTS AND DISCUSSION

Under natural field conditions the resistance of 339 common bean varieties and breeding lines (IIa growth habit) to linuron was evaluated.

Table 1
Scale for reading the resistance reaction of bean to the soil-applied herbicide linuron.

Evaluation		Characterization
1		No signs of susceptibility of the bean plant to linuron
2		First and second true leaves are heavily deformed and chlorotic, with single or no necrotic spots. The other leaves have no symptoms.
3		Deformation combined with chlorosis on up to ½ of the leaf surface.
4		Heavy chlorosis and necrosis on the entire leaves of all plants and of the vegetation tips of ½ of the plants, which die within a few days.
5		Over 90 % of the plants necrotize and die.

Considerable genetic variability was found among the breeding lines included in the competitive varietal trial (**Figure 2**). One hundred eighty-four accessions had resistant reaction 1, and 123 accessions demonstrated resistant reaction 2, which allows breeding of varieties resistant to linuron. Breeding lines evaluated with 3 and 4 were discarded. **Figure 1** shows common bean varieties, which are susceptible (S) or resistant (R) to linuron.



groups according to their degree of resistance to the soil herbicide linuron.

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BREEDING HIGHLY PRODUCTIVE RED FLOWERED LINES OF SNAP BEAN FOR BELARUS CONDITIONS

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Snap bean (*Phaseolus vulgaris L.*) cultivation became more and more popular in Belarus from year to year and in south regions it has a significant impact to the set of crops which farmers grow. Snap bean is also popular in the countryside and in the summer cottage owners. This wide popularity of snap bean is supporting by national and foreign breeders, and as a result there are 22 varieties in National List which slightly differ in pod color, pod texture, time of maturity, but green podded bush varieties with white flowers are the most spread. Thus, there are only 3 varieties with wax pods, and no varieties with purple pods or climbing type of snap bean in National List (2012) of Belarus. Generally, only 4 (18%) varieties from registered were bred in Belarus by national breeders. This can be caused by domination of the private sector in the snap bean growing in Belarus, thus, the attention to snap bean breeding was low. However, interest to new varieties of snap bean increased in recent years, also in connection with the expansion of the cultivation of this crop in larger areas for sale of fresh beans in supermarkets. This is the reason for our increase in activity in snap breeding for Belarus conditions.

Our common bean and also snap bean breeding program has started in 1996 from accumulation of germplasm collections from different germplasm banks, markets, breeders etc. For example, more than 2 000 accessions from USDA collections were tested since 2003 in field conditions of Belarus and 1 320 from them are maintained in our collection now because of their normal seed productivity, early maturing and other useful properties. Also the exotic germplasm samples were available for us, and we have used such samples as during research of the variations of the characters, traits and adaptability, so as parental components for breeding programs. As a result the common bean germplasm collection of Belarus State University consist now 2 835 accessions of different origination, also including own breeding material. Approximately 39 % of germplasm samples are snap bean.

Annual field tests include the estimation of growth characteristics, yield potential and pod quality using the randomized complete block design with four replications. Data is collected on growth habit, days to 50% flowering, plant height by measuring the distance from the stem of each plant to the end of the main stem (on the day of the harvest), height of first pod, pod clearance, days to green pod maturity, number of pods per plant, marketable green pod yield (calculated for t/ha), different pod characteristics (color, shape, length, width and snap-ability), average weight of pods, average number of seeds per pod, 100 seed weight, plants seed productivity, and grain yield (calculated for kg/ha with 13% humidity). All collected annual results of field tests were used for supplement of the databases for statistical analysis.

The normal dates of sowing for common bean in Belarus are 15-25 May, and optimal period for seeds harvesting is 20 August – 10 September. Thus, the possible full period of vegetation for common bean is no longer than 100-110 days. Early sowing could solve the problem of increasing periods of plant growth and increase the mass of the plant and its general productivity, but late frosts in May do not allow it. Thus, low cold tolerance at early stage of plants development, susceptibility to Halo blight and Anthracnose, and late maturity are the mine

limiting factors for high pod productivity of many investigated samples of bush snap bean. Many years of versatile study of germplasm samples allowed us to select the sources of important and useful traits for creating and implementing breeding programs. The exotic germplasm also used. Crosses were made in the greenhouse and in field conditions between high productive samples (Delinel, Olga, Carson, Polka, Tara, Purple Teepee, Purple Queen, Nekarkonigin, Goldmarie, Laurina, Pation, Claron and others) and Halo blight and Anthracnose resistant material, cold tolerant samples, and among high productive samples, also with exotic germplasm samples for general population improvement. Generation advance was continued with lines in all stages of development. In the field, single plants were selected from all crosses. Selection of candidate lines was based on improved plant architecture combined with high yield potential and good pod quality.

As a result we have developed many different lines with high yield potential (up to 30 t/ha for bush lines), with different pod color, also with Halo blight and Anthracnose resistance confirmed in laboratory conditions.

Lines with red flowers resulting from backcrossing of hybrids between best snap varieties and P.vulgaris x P.coccineus (like PI 638666, PI 638667 and PI 638668, also own hybrids) and different snap varieties are of great interest due to the unusual for common bean color of flowers. All these lines inherited the red color of flowers from P.coccineus and at the same time have the high quality pods. Some of them have a great prospect to become a candidate variety after few years of seed multiplication and additional tests for yield. We also look forward to their popularity (especially small growers) due to heavy (sometimes large) beautiful and unusual flowers.

A challenge problem is the description of these lines during preparing for DUS-test under UPOV rules. According to UPOV TG 12/9 (http://www.upov.int/edocs/tgdocs/en/tg012.pdf) the flower color vary:

UPOV No.	Characteristic	Example Varieties	Note
16.	Flower: color of standard		
	white	Tuf (D)	1
	pinkish white	Mira (D)	2
	pink	Maxi (D), Vilbel (D)	3
	violet	Delinel (D), Purple Teepee (D)	4
17.	Flower: color of wing		
	white	Tuf (D)	1
	pinkish white	Signal (D)	2
	pink	Maxi (D), Vilbel (D)	3
	violet	Delinel (D), Purple Teepee (D)	4

But specified in Table pink color is not corresponding to red-pink hue of our lines flowers color and is a lighter shade of purple or lilac. Thus, in case of the confirmed success of our lines and their next transfer to the state variety testing system it is necessary to make a decision in relation to addition of new characteristic of flower color to the national DUS test guideline, and, may be, to international TG.

ENVIRONMENTAL INFLUENCE ON SEED BIOACTIVE COMPOUNDS IN NEGRO SAN LUIS CULTIVAR GROWN IN DURANGO, MÉXICO

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INTRODUCTION: In North-Central México improved economic benefits need to be achieved in common bean (*Phaseolus vulgaris*) production by increasing yields and adding value by the obtaining of bioactive compounds from seeds. Specific selection for industrial use is actually performed among common bean cultivars planted in Durango. In 2012, Negro San Luis seed has been supplied by bean traders due to scarcity of Pinto Saltillo seed, caused by drought registered in 2011. In Northern-México black bean cultivars are only used to supply markets in Central and Southern México, and low prices (<5.0 MXN pesos per kilogram) are commonly paid to bean producers. Flavonoids, saponins and other bioactive-nutraceutical compounds are actually used in pharmaceutical industry and could be obtained from common bean black seeded cultivars (Guajardo *et al*., 2012). This options need to be explored in order to generate added value to the Negro San Luis production obtained in Durango and Zacatecas. Significant variation has been observed for seed protein and bioactive components in plants, caused by environmental conditions (Jeffery *et al*., 2003). Stability in flavonoids and other bioactive components need to be characterized in Negro San Luis cultivar, in order to evaluate possibilities of this cultivar as a raw material in pharmaceutical and food industry. The objective was to evaluate flavonoids and saponin content registered in Negro San Luis seeds harvested in 13 sites of Los Llanos de Durango, common bean producing area.

MATERIAL AND METHODS: Negro San Luis plant samples were taken at 13 locations of the common bean producing area known as Los Llanos in the State of Durango, Méx. Samples were harvested in farmer plots when plants showed physiological maturity, using polyethylene bags for plants transportation and then sun dried before threshing. Plant samples were threshed using an Almaco® stationary plot thresher. After homogenization of the flour obtained from milled seed sub-samples, 2.5 g were accurately weighed and extracted with 15 mL of 80 % aqueous methanol (DEQ Monterrey, México). Extraction was carried out in a Vortemp 1550 (Labnet International, Inc. Edison, NJ) for 30 min at 2.87 g and 25 °C, using eight 50 mL centrifuge tubes. The resulting extract was filtered using a No. 1 Whatman filter paper and the solids were washed with 80 % methanol to adjust to a final volume of 15 mL. Saponins and flavonoids were quantified using an HPLC–DAD–ELSD (Agilent Technologies, Santa Clara, CA) system. Separation was performed in a Zorbax SB-Aq 4.6 mm ID x 150 mm, 3.5 µm reverse column (Agilent Technologies, Santa Clara, CA) with a flow of 0.5 mL/min. Elution was conducted with (A) HPLC-grade water adjusted to pH 2 with trifluoroacetic acid (Sigma, St. Louis, MO) and (B) HPLC-grade acetonitrile (100 %). Separation was achieved with 20 % B for the first 6 min, increasing the B concentration to 50 % at 12 min and to 100 % at 30 min. This last solvent concentration was maintained for the next 10 min. Chromatograms were acquired at

220, 280 and 320 nm and the peaks integrated by the HP-Agilent Software (Chemstation for LC Copyright Agilent Technologies, 1990–2003). Peak identification of flavonoids was based on retention time and UV spectra. To confirm the detection of the DDMP-conjugated saponins their absorption at 295 nm was obtained (Guajardo *et al*., 2012).

RESULTS AND DISCUSSION: Variations were observed among locations for six bioactive components detected in Negro San Luis seeds (Figure 1), but significance was observed ($p<0.01$) only for quercetin, myricetin and kaempferol. Higher average extraction values per gram of flour were observed for quercetin (2,837 µg/g) and soyasaponin Soy αg (945 µg/g); and the lowest values were registered for kaempferol (8 µg/g). Maximum value for quercetin (4,265 µg/g) was registered in San José Nazareno, resulting similar to those observed for other four locations across Los Llanos producing area. Different amounts of three soysaponins were also observed, such as Soy Af (603 µg/g), Soy αg (945 µg/g) and Soy βg (51 µg/g). Values observed for bioactive components resulted lower compared to those obtained in previous reports using Negro San Luis (Guajardo *et al*., 2012), where values for quercetin were 6,432 µg/g, myricetin 1,157 µg/g and kaempferol 66 µg/g. Environmental factors showing strong influence on bioactive compound level in common bean seeds need to be identified in order to manipulate the accumulation of important extracts for food and pharmaceutical industry.

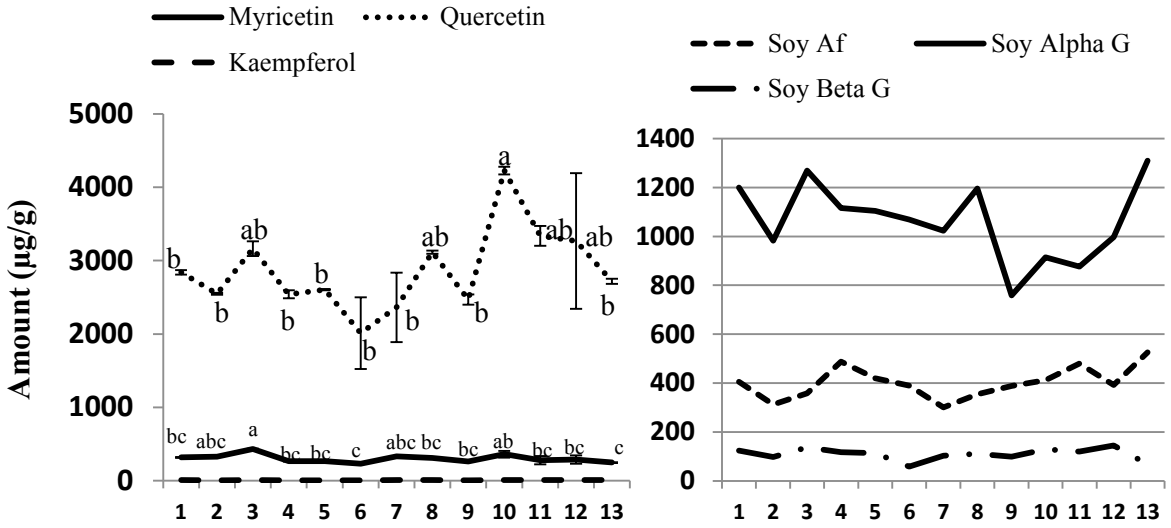

Figure 1. Amount of different bioactive components registered in Negro San Luis seed samples collected at 13 sites in the State of Durango, México. 2012. 1. Antonio Amaro; 2, 2 de Abril 1; 3. 2 de Abril 2; 4. Cuauhtémoc 1, 5. Cuauhtémoc 2; 6. Cuauhtémoc 3; 7. Ignacio Allende; 8. Pino Suárez, 9. La Purísima; 10. San José Nazareno; 11. Ignacio Allende 2; 12. Ignacio Allende 3; 13. Calixto Contreras. Letters different in same line represents significant differences DSH $\alpha=0.05$.

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CONTRIBUTION OF THE MESO AMERICAN BIOFORTIFIED BEAN *(PHASEOLUS VULGARIS* L.) TO FOOD SECURITY AND COMPETITIVENESS IN THE AGRONOMIC PRODUCTION CHAIN IN THE CARIBBEAN COAST OF COLOMBIA

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INTRODUCTION

The main purpose of the increase in use of nutritionally improved crops is to reduce nutritional deficits of the world´s population. Nutritional improvement of crops does not necessarily involve genetic transformation, but simply crosses between varieties using controlled polinization processes (Tofiño *et al*., 2012). The objective of the study was to strengthen the competitiveness of the agronomic production chain of bean by offering the traditional producer of the Caribbean coast of Colombia new varieties with enhanced agronomic, nutritional and agroindustrial potential. In order to comply with these objectives, an evaluation of the impact of auto consumption of biofortified beans compared to traditional varieties on the consumers' nutritional status is necessary. Likewise, the agroindustrial potential needs to be determined in order to define the socioeconomic viability of such intervention in the rural state of Cesar, Colombia.

MATERIALS AND METHODS

The study included several steps completed between February, 2010 and November, 2012 in four locations, 800-1300 masl, in Perijá and the Sierra Nevada of Santa Marta, Cesar, Colombia: 1. Evaluation by surveys, of consumption, production, and visual preference of common bean in 300 producers and their families, as well as the calculation of the added nutritional value of biofortified beans in the diet, following Muñoz *et al.* (2008). 2. Participative evaluation of the field performance, and the valuation of the mineral content using atomic absorption spectroscopy, of nine genotypes of biofortified beans. 3. Sensory analysis by 273 persons, including categorical valuation of consistency, taste and texture of four biofortified bean varieties and one local control prepared by the same recipe, and results analyzed using the chi square test.

RESULTS AND DISCUSSION.

95.6% of the producers reported to have consumed beans during the last week (2.76 ± 1.01 times and 4.1 ± 3.1 kg), with 40.7% of the beans originating from their own production. This corresponds to 110 g/p/d for a family with in average 5.92 members and a 24.6% and 16% additional supply of iron (Fe) and zinc (Zn), respectively, if the local variety of bean were to be exchanged for a biofortified genotype, due to its enhanced content of Fe and Zn (Table 1). According to the surveys, two genotypes of each color (red mottled: SMC14, SMC5; cream: SMC7, SMB17; black: SMN18, DOR 390; and red: RCB591, SCR3) were visually acceptable (SMN18, DOR 390, RCB591 and SCR3 were preferred by 7, 15, 20 and 30% of the persons participating in the survey, respectively). Genotype SMN18 was determined, in comparison to the local control variety, to have the best agronomic characteristics, as well as highest accumulation of Fe and Zn (Table 2). The high average concentration of crude protein in Valledupar (25.6%) and mineral nutrients in La Paz (15.9 mg/kg Mn), superior to values reported in other Mesoamerican beans, show that its nutritional value does not only rise from its Fe and Zn content and supports its po-

tential for transformation (Torga *et al*., 2011). Additionally, according to the categorical sensory evaluation, the biofortified varieties showed superior results in texture and consistency ($p<0.05$) compared to the local control variety.

Table 1. Potential contribution of biofortified bean in iron (Fe) and zinc (Zn) content of the diet of the families participating in the survey

Crop	Production for consumption	Nutrients added to the crop through biofortification		Added nutritional value through biofortification			
	(g/p/d)*	**Fe (µg/g)**	**Zn (µg/g)**	**Fe (µg/p/d)**	**Zn (µg/p/d)**	**RDA Fe (%)†**	**RDA Zn (%)†**
Bean	110	40≠	16≠	4400	1760	24.6	16

*Production for family consumption (kg/semester) multiplied by the number of semesters in a year (2) and divided by the average number of persons per family (5.92), the number of days in a year (365) and grams in one kilogram (1000).
≠ The additional nutritional values of the biofortified bean compared to the local control variety were obtained in the second semester of 2012 † Recommended Dietary Allowance (RDA) of the USA for adults: 18000 µg/d Fe and 11000 µg/d

Table 2. Agronomic and nutritional characteristics of biofortified genotypes in Cesar

Genotype identification CIAT	**1000 seed weight**	**Mun-sell scale colour**	**Yield**	**Nutritional content of crude, biofortified bean seeds**									
			2010	**Fe**	**Zn**	**Cu**	**Mn**	**Crude protein**	**Fe**	**Zn**	**Cu**	**Mn**	**Crude protein**
				Valledupar					**La Paz**				
			kg/ha	**mg/kg**				**%**	**mg/kg**				%
SCR3	341	10	1526	74.4	25.1	6	14.7	23.9	61	21	5.3	15	21
SMR 4	256	5	1296	98.7	27.5	6	16.7	26	107	34	10	14	24.7
SMR 39	346	4	962	106.1	36.7	4.7	17.3	27.2	117	26	8.7	17.3	21.6
SMR43	308	10	946	94.9	34.8	6.7	14.7	24.3	87	26	13.3	18.7	19
RCB 591	336	11	974	86.08	30	7.3	17.3	24.1	76	21	4.7	12.7	23.2
DOR 390	181	100	1974	81.4	29.9	5.3	17.3	24	74	19	5.3	14.7	22.1
SMN 18	**245**	**100**	**1390**	**109.3**	**37.1**	**10**	**16.7**	**28.3**	**102**	**27**	**8.7**	**16**	**24.4**
SMB 17	195	849	747.7	115	8.7	15.3	25.1	26	116	21.9	16	16	11
SMC 14	198	1043	678.8	120	7.3	16.7	23.6	27	121	21	18.7	18.7	15
Control	141	700	500	40	4	14	20.5	20.4	60	18	12	12	4.7

CONCLUSIONS

The extension of the use of biofortified beans would contribute to the improvement of the nutritional status of the producers given the significant rate of auto consumption registered in the region. good sensory characteristics of the grain and the high micronutrient content that maintains an excellent nutritional value also after normal losses in the transformation process.

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EVALUATION OF BLACK SEEDED CULTIVARS FOR YIELD AND BIOACTIVE COMPONENTS IN NUEVO LEÓN, MÉXICO

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INTRODUCTION: Grains of common bean (*Phaseolus vulgaris*) black seeded cultivars are needed as a raw material for food and pharmaceutical industries. Several black seeded cultivars have been developed by INIFAP´s breeding program for the Tropics and the Highlands of Mexico (Rosales *et al*., 2004). Cultivars (such as Negro San Luis) and bioactive compounds (myricetin, quercetin, saponins) have been found mainly in black commercial class (Guajardo *et al*., 2012). Common bean is planted in spring and summer in the State of Nuevo León under irrigation and rainfed conditions. Black seeded cultivars are considered as an option to increase yields, elaboration of processed foods and to obtain bioactive extracts. For black commercial class low prices (<5.0 MXN pesos per kilogram) and trading problems are observed during high production years. Flavonoids, saponins and other bioactive components (Guajardo *et al*., 2012) extracted from black seeded cultivars are actually used in pharmaceutical industry. In Northern México, selection for adapted cultivars with enhanced seed yield and high level for bioactive compounds need to be done in order to supply pharmaceutical and food industry. The objective was to evaluate seed yield and levels for bioactive compounds in eight cultivars planted in Hualahuises, N. L.

MATERIAL AND METHODS: In 2012, eight common bean cultivars were planted under irrigation at spring (March 13th) and summer (August 31th). A randomized complete block design with three replicates was used and experimental plot consisted in a 20 m in length double row beds, 1.20 m apart and 20 cm spacing between plant lines. Organic inoculants such as *Rhizobium*, *Glomus* and *Mycorrhizae* were applied in the seeds. Under field conditions, data were recorded for yield and 100 seeds weight. Analysis of variance was performed using a randomized complete block design and mean comparison was obtained based on the honest significant difference (HSD, $\alpha = 0.05$). In the laboratory and after homogenization of the flour obtained from milled seed sub-samples (Krups GX4100, DF, Mexico), 2.5 g were accurately weighed and extracted with 15 mL of 80 % aqueous methanol (DEQ Monterrey, México). Extraction was carried out in a Vortemp (Vortemp 1550, Labnet International, Inc. Edison, NJ) for 30 min at 2.87 g and 25 °C using eight 50 mL centrifuge tubes. Then, the resulting extract was filtered using a No. 1 Whatman filter paper and the solids were rinsed with 80 % methanol to adjust to a 15 mL final volume. Saponins and flavonoids were quantified by triplicate using an HPLC–DAD–ELSD (Agilent Technologies, Santa Clara, CA) system. Separation was performed in a Zorbax SB-Aq 4.6 mm ID x 150 mm, 3.5 µm reverse column (Agilent Technologies, Santa Clara, CA) with a flow of 0.5 mL min^{-1}. Elution was conducted with (A) HPLC-grade water adjusted to pH 2 with trifluoroacetic acid (Sigma, St. Louis, MO) and (B) HPLC-grade acetonitrile (100 %). Separation was achieved with 20 % B for the first 6 min, increasing the B concentration to 50 % at 12 min and to 100 % at 30 min. B solvent concentration was held for the next 10 min. Peaks in each chromatograms were integrated by the HP-Agilent Software (Chemstation 1990–2003). Peak identification of flavonoids was based on retention time and UV spectra. To confirm the detection of the DDMP-conjugated saponins their absorption maximum at 295 nm was obtained (Guajardo *et al*., 2012).

RESULTS AND DISCUSSION: Except for Sahuatoba (484 kg ha^{-1}) in the spring season, similar seed yield was observed for all the evaluated cultivars at two planting seasons (Table 1). In the spring seed yield fluctuations were observed between 1,544 kg ha^{-1} in Tacaná to 484 kg ha^{-1} in Sahuatoba. In the summer season higher yields were observed for FrijoZac N101 (1,573 kg ha^{-1}) and Pacífico (1,500 kg ha^{-1}), but resulted statistically similar to Tacaná (1,042 kg ha^{-1}). Reduction for average 100 seed weight was observed in the spring season (17 g/100 seeds) compared to summer (29 g/100 seeds). In despite of variation observed for levels of bioactive compounds among replications and cultivars, only statistically significant ($p<0.05$) differences were detected for myricetin content. Among cultivars highest values were observed for soyasaponin Af and the lowest for soyasaponin V. FrijoZac N101 (746 µg g^{-1}) and Pacífico (633 µg g^{-1}) showed highest values for soyasaponin Af. Soyasaponin αg registered values between 444 µg g^{-1} (Pacífico) and 328 µg g^{-1} (Tacaná); soysaponin βg 195 µg g^{-1} (Pacífico) and 137 µg g^{-1} (Tacaná) and Soysaponin V 33 µg g^{-1} (Sahuatoba) to 14 µg g^{-1} (Nayarit). Negro Sahuatoba showed the highest values for myricetin (175 µg g^{-1}) and could be considered as an option to extract this bioactive compound when is planted during summer season in Nuevo León, and similar environments where this cultivar is adapted. Selection for the best combination for seed yield and levels for specific bioactive compounds need to be obtained among common bean black seeded cultivars in order to increase productivity and to offer low cost-healthy products for poor people.

Table 1. Traits registered for eight common bean cultivars at two planting seasons. Hualahuises, N. L. Méx. 2012.

Cultivar	Yield (kg ha^{-1})		100 seeds Wt (g)	
	spring	summer	spring	summer
Nayarit	1,351[a]	1,417	14[b]	23[d]
Tacaná	1,544[a]	1,042	16[ab]	25[cd]
Pacífico	1,539[a]	1,500	16[ab]	24[d]
Sahuatoba	484[b]	1,396	14[b]	28[c]
Altiplano	1,121[ab]	1,438	21[a]	33[b]
FrijoZac	1,117[ab]	1,573	21[a]	35[b]
San Luis	--	1,188	--	43[a]
FNP_{NL}	1,263[a]	1,229	14[b]	22[d]
Average	1,203	1,348	17	29
C. V. (%)	19	37	11	5

[a-b]Letters in the same column show significant differences among cultivars according to HSD test ($\alpha = 0.05$).

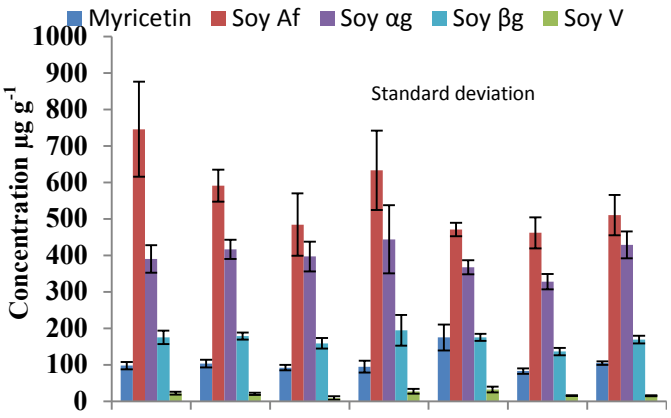

Figure 1. Values observed for different bioactive compounds in seven common bean cultivars

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STORAGE PROTEINS OF COMMON BEAN IDENTIFIED WITH 2D-PAGE

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INTRODUCTION: The common bean is a significant source of protein, complex carbohydrates, fiber, and minerals. Traditionally, beans are considered the "poor man's meat" because they provide a high amount of protein at a lower cost than animal protein to millions of people. Seeds of most dry beans contain 15 to 25% protein on a dry weight basis. This protein is rich in lysine but low in the sulfur containing amino acids cysteine and methionine. The majority of common bean proteins are the salt-soluble globulins and the water-soluble albumins. In this study, we used proteomic technologies involving the protein separation technique called 2D-PAGE. With this technique, proteins are separated by charge (isoelectric point) in the first dimension and by molecular mass in the second dimension. The separated proteins were then identified by mass spectrometry (MS) analysis. In addition, bioinformatics tools were used to characterize and identify common bean storage proteins.

MATERIALS AND METHODS: Seeds of common bean G 12910 were produced by growing plants under greenhouse conditions. The seeds were mechanically grounded prior to protein analyses. We used modified Trichloroacetic acid (TCA)/acetone precipitation/Urea solubilization extraction buffer to extract seed proteins (1). Proteins were separated by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and characterized using mass spectrometry and further identified by searching the data with different databases.

RESULTS AND DISCUSSION: Common bean seeds contain different types and proportions of storage proteins namely albumin (12-30%), globulin (54-79%), prolamin (2-4%) and glutelin (20-30%). Phaseolin, the major globulin fraction in the seed, is deficient in methionine, cysteine and tryptophan and therefore a principle target for modification to obtain value added common bean. The phaseolin protein has been used to differentiate wild and cultivated common beans belonging to the Mesoamerican gene pool from common beans of the Andean gene pool (2). We found multiple spots of phaseolin in our 2D-PAGE gels of common bean seed proteins (Fig. 1). Our results showed that 29 protein spots (#1-29) were identified as alpha-phaseolin. In addition, two spots (30, 31) were identified as beta-phaseolin and 28 spots (#32-59) were identified as phaseolin. Subunit patterns of the purified phaseolins were investigated using SDS-PAGE and it was found that these subunits varied from 2-6 bands with specific MWs ranging from 54.7-41.1 kDa.Legumin is another storage protein of the globulin fraction and was first described in several species including *Phaseolus vulgaris*, *Pisum sativum*, and *Vicia faba*. Legumin, a hexameric 11S globulin, consists of the acidic alpha subunit and the basic beta subunit and linked together by a disulfide bond. We found 21 spots (# 60-80) protein spots that were identified as legumin by our 2D-PAGE analysis. In addition, we identified three protein spots as albumin-2 (# 81-83).

In summary, it is significant that in this study of storage protein of common bean, we identified several globulin proteins (alpha-Phaseolin, phaseolin, beta phaseolin, and legumin) and one albumin protein (albumin-2).

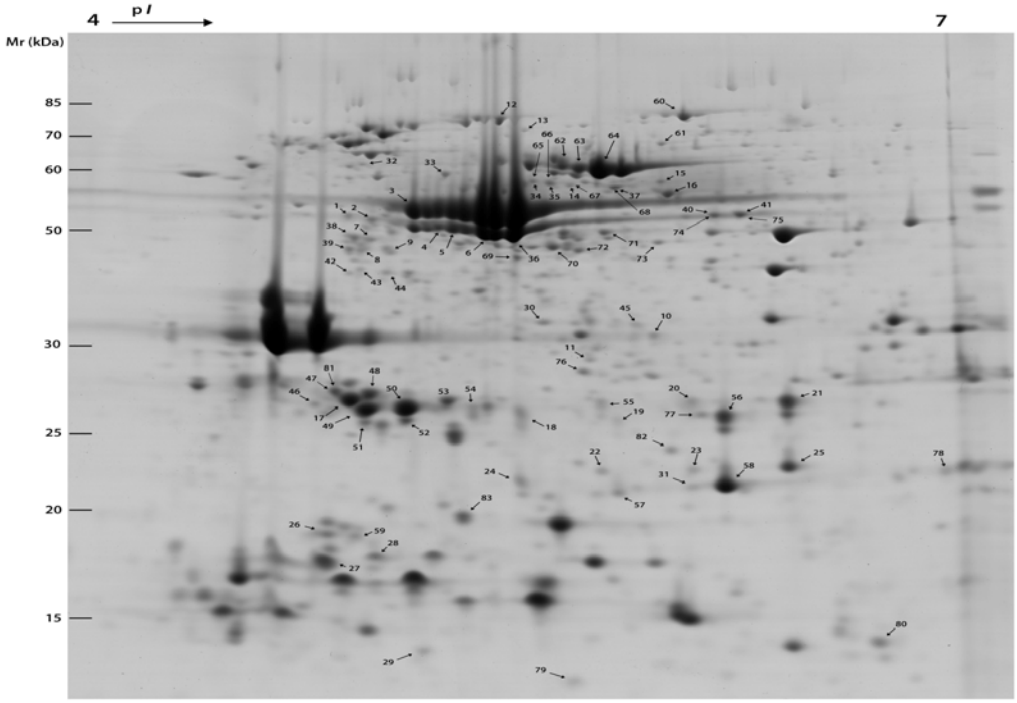

Figure 1: 2D-PAGE analysis of common bean storage proteins

Table 1: identified common bean storage proteins

ID[1]	Storage Protein [species]	Calc pI	Calc Mr (Da)	MS Score	GI number
1 - 29	Alpha-phaseolin [*Phaseolus vulgaris*]	5.25 - 5.42	48533 - 49241	63 - 1320	295832, 130169
30 - 31	Beta-phaseolin [*Phaseolus vulgaris*]	5.29	27536	99 - 115	130170
32 - 59	Phaseolin [*Phaseolus vulgaris*]	5.35 - 5.42	47525 - 48448	90 - 1355	403594, 403596
60 - 80	Legumin [*Phaseolus vulgaris*]	5.64	69081	114 - 847	312982406
81 - 83	Albumin-2 [*Phaseolus vulgaris*]	6.90	25604	86 - 346	312982408

[1]ID, spot number referred to in Fig. 1; calc pI/Mr, theoretical values for isoelectric point and molecular weight; MS Score, MOWSE Score

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NEW ALTERNATIVE FOR ASSESSING COOKING TIME OF COMMON BEAN PROGENIES

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INTRODUCTION: For a new common bean cultivar to be adopted by farmers and consumers, in addition to high yield, it must combine good culinary properties. Among them, cooking time is of greatest importance. In research, great stress has been placed on the identification of lines with shorter cooking time (Torga et al., 2011). The Mattson method was used in all these studies for characterization of this characteristic (Proctor & Watts, 1987). However, as there are hundreds of progenies to be evaluated in a common bean breeding programs, it is necessary to use a process for assessment of cooking time which is quicker and allows assessment of a large number of progenies. In light of the above, the purpose in the present study was to propose a new screening strategy for common bean progenies in relation to cooking time.

MATERIALS AND METHODS: The experiments were carried out at the Biology Department of the Universidade Federal de Lavras – UFLA (Federal University of Lavras), Lavras, MG, Brazil. Ten lines were evaluated, all of carioca type beans (beige with brown stripes). All these lines were collected in May 2012 from the same location. Before implementation of the tests, seeds from each line were classified for bean size, seeking to make each sample as uniform as possible. Cooking assessment was performed through two methodologies:
Mattson Method: The conventional method described by Proctor & Watts (1987).
Pressure Cooker Method: Each 100 grain sample was placed in a voile bag (Figure 1). These bags were placed in a pressure cooker which was kept heated for 10 minutes after the point at which the water began to boil (pressure cooker releasing steam). Next, the voile bags containing the samples from each line were incubated at 1-2 C^o for 30 minutes. After that, cooking was verified using the JAB-77 minor type equipment, just to check if the beans were cooked or not. This procedure was performed ten times. Thus, the percentage of grains cooked from each line was estimated.

RESULTS: To apply the new common bean cooking assessment methodology, some initial steps were carried out. One of them was to check what the minimum cooking time would be to reveal a difference among the lines tested. Ten minutes proved to be the optimal time that would allow best screening of the lines. As the beans in the voile bag are hot, they are removed from the pressure cooker and placed in the refrigerator for 30 minutes. It is recommended that the voile bags with the bean samples all be placed in contact with the bottom of the pot to avoid a possible effect from position in the container.
The experimental accuracy of the two processes was very similar, observed by the accuracy estimate (Table 1). In the Mattson method, it was not possible to identify groups of inbred lines, whereas in the pressure cooker method, the lines were classified in four groups, according to the percentage of beans perforated after ten minutes in the pressure cooker. The most quickly cooked line was CNFC11965 and the worst was EMB4.

This new methodology has some advantages, namely: i) It is possible to assess a large number of progenies simultaneously. If necessary, more than one pressure cooker may be used; ii) The procedure is easier and quicker than that of Mattson. In addition, it is not necessary to soak the beans in water beforehand; iii) The methodology establishes a cut-off or truncation point; above this time the line/progeny is considered to be worse for cooking. This is exactly the information breeders need for and selection of progenies. This time may be previously determined by the researcher; iv) What is done is exactly what homemakers do on a daily basis in relation to cooking beans. Therefore the results reflect the cooking procedure better than even the Mattson methodology.

Table 1. Mean percentage of beans cooked by the pressure cooker method and mean cooking time by the Mattson method of common bean lines. Lavras, Brazil. 2012.

Cultivar	Method	
	Pressure cooker	Mattson
CNFC11965	100[1]a	25.7[2]a
BRSMG MADREPÉROLA	76.7b	33.6a
P18163	73.3b	27.3a
RCII219	56.7c	30.6a
EMB9	53.3c	28.9a
BRSMG TALISMA	50.0c	24.7a
CNFC10432	46.7c	28.2a
BRSMG MAJESTOSO	43.3c	27.8a
VC21	26.7d	30.3a
EMB4	23.3d	29.8a
Mean	*55*[1]	*28.7*[2]
Accuracy	*96.1*	*85.3*

[1,2]: Percentage of beans perforated after 10 minutes of cooking in a pressure cooker and given in minutes respectively. Mean values followed by the same letter in the column do not differ by the Scott Knott test at 5% probability.

Figure 1. Steps in assessment of cooking time by the pressure cooker method.

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REVERSIBILITY OF SEED HARDNESS IN DRY BEAN GENOTYPES

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Cooking time is one of the parameters that define the commercial quality of bean genotypes. Cooking tests are essential in the selection process of the bean program at the Campo Experimental Valle de Mexico. More than 85% of beans are consumed through homemade preparations, so the bean cooking time is associated with time and fuel employed by a housewife.

Two phenomena associated with the hardness of the bean have been studied. *Hard-shell*, is associated with a limited water absorption capacity during soaking and *hard-to-cook* which is associated with hardness of the cotyledon. Both cause long-term cooking. Seed coat permeability, seed hardness and water absorption are affected by environmental factors and genetic*environment interactions (G*E) are usually present (Kigel, 1999).
In the high valleys of the Central Plateau of Mexico 95% of the bean is grown under rainfed conditions and sometimes hail occurs, affecting the plant's structure. Also due to climate change, the rainy period which is frost free has been reduced, so it is common that bean crop is affected by early frosts

It is necessary to adapt to the conditions imposed by climate change and for this it is required to determine the effect of early frosts on grain quality, and also try to obtain appropriate genotypes. In this context the objectives were a) Determine whether the hardening caused by frost in the final phase of bean development can be reversed, b) identifying outstanding genotypes that show greater adaptability to climate change

MATERIALS AND METHODS: 21 Recombinant Inbred Lines (RILs) of sulfur-yellow beans, grown in 2010 on experimental plots established in Chapingo and St. Lucia, locations of Texcoco, in the State of México were evaluated. During the growing season there were two hail-rainfalls at Chapingo and in both locations early frost occurred. When early frost happened, bean crop was about two weeks before its physiological maturity. Cooking time and water absorption capacity of the 21 RILs of both sites were evaluated. Two sets of St. Lucia's samples were stored, one set at 5 ° C and another at room temperature, both for 62 days. In periods of 35, 42 and 53 days some RILs were taken and their cooking time was determined.

RESULTS: It was observed that from the RILs produced in Chapingo, 20 had low capacity to absorb water during soaking, and all of them (21 in total) showed prolonged cooking times (134-166 min). In contrast, four of the RILs grown at St Lucia, exhibited water absorption capacity superior to 100% of its weight and the other 17 below 75%, that means occurence of hard-shell. There were highly significant differences in the cooking time among genotypes. In RILs with high water uptake cooking time was inferior to 100 minutes, whereas in the others it was from 120 to 172 minutes. Water uptake and cooking time were inversely proportional (r = 0. -63 **). The RILs taken at 35, 42 and 53 days showed that the cooking time decreased progressively over time (data not shown), particularly those RILs that when freshly harvested showed longer

cooking time. After 62 days in cold storage cooking time decrease between 18 and 51% (Fig. 1). According to Del Valle and Stanley, (1995) this is a reversible hardening which could be due to the pectin-phytate mechanism. To determine whether this decrease in cooking time was due to the low temperature, cooking time of RILs stored at room temperature (RT) (approx 23 ° C) was also mesasured. However no significant differences were detected. Also, no significant differences were in the water absorption capacity between the freshly harvested RILs and after being stored for 62 days either at room temperature or refrigerated. In the stored RILs, association between cooking time and water absorption capacity (r = -0.83 **) was higher than in the freshly harvested. The hardening (Hard-to-cook phenomenom) of RILs apparently caused by frost, was partially reversed by 62 days of storage, either chilled or at room temperature. Ten RILs which exhibited cooking times below 100 minutes were identified. The hard-shell in the RILs remained constant.

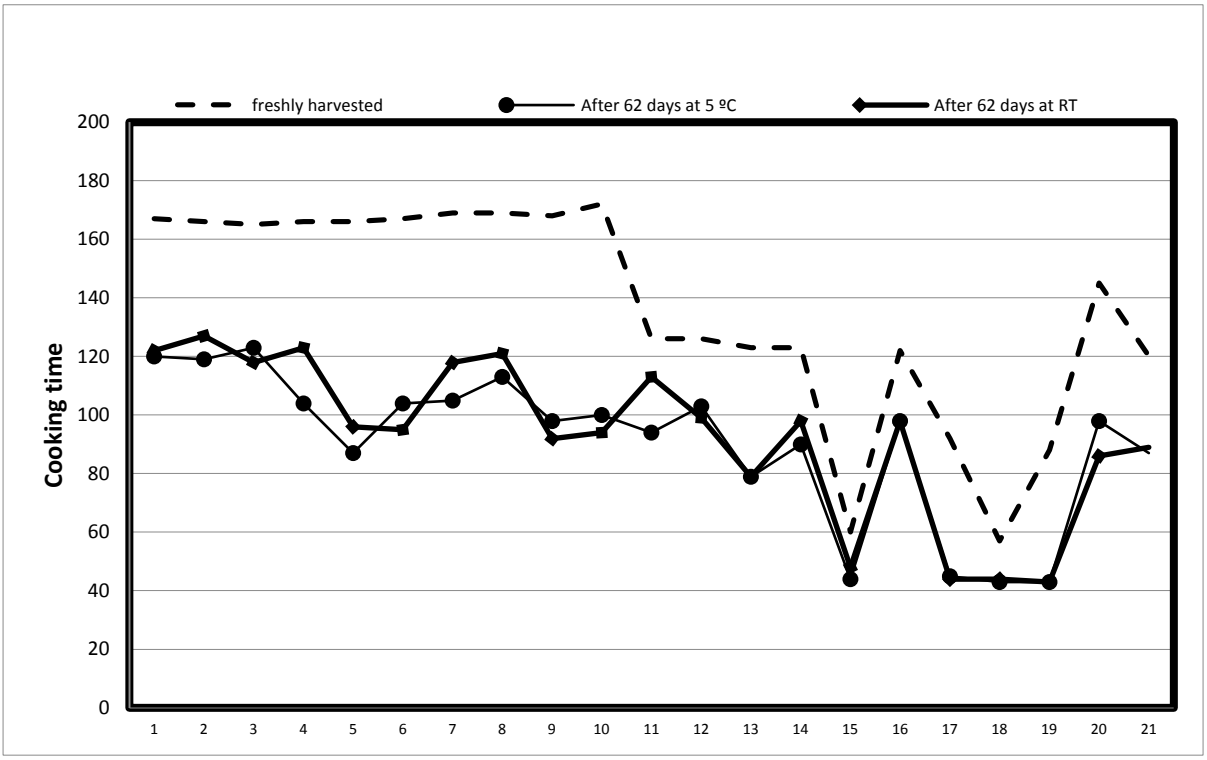

Figure 1. Cooking time of 21 RILS of sulfur-yellow dry beans stored for 62 days either at 5ºC or at room temperature (RT)

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ASSESSING PASTING PROPERTIES OF COMPOSITE WHEAT AND MAIZE FLOURS ENRICHED WITH COMMON BEAN FLOUR OR STARCH

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INTRODUCTION: In Mexico, the annual *per capita* consumption of maize (*Zea mays*) and wheat (*Triticum aestivum*) is 127 kg (González *et al*., 2011; Grajales *et al*., 2012) and 52 kg (Agrosíntesis, 2011), respectively. Maize and wheat grains are mainly used in flour production which represents an important ingredient to elaborate homemade tortillas and other traditional food items (Anton *et al*., 2008; Fajardo, 2008; Grajales *et al*., 2012). Common bean (*Phaseolus vulgaris*) need to be considered as an important input for the food and pharmaceutical industry in order to approach its nutritious and nutraceutical profiles. Carbohydrates, starch, protein and minerals are components of legume seeds that have the potential to be added for the biofortication of flours used for human consumption (Su-Qun and Ling-Yu, 2010). Pinto Saltillo cultivar is produced in large areas of the Sates of Durango, Chihuahua and Zacatecas, where this variety became popular due to improved agronomic performance, higher grain prices and longer shelf life. Bayo Victoria is another variety adapted in Durango, which usually sells at discount prices due to higher cooking time (> 150 min). Functional properties found in common bean resistant starch have been used to produce processed foods promoting human health. The objective was to evaluate the pasting properties of composite wheat and maize flours enriched with two common bean whole grain flours and their isolated starches.

MATERIALS AND METHODS: Ten formulations including commercial flours and 10 % of different components (flour and starch) of two common bean cultivars were evaluated (Table 1). Common bean flour was obtained milling grains in a hammer mill (Thomas-Wiley Miller Lab, Model 4) with 1 mm sieve and starch was extracted using a modified process derived from previous reports (Otto *et al*., 1997; Villarreal, 2010). Wheat flour tortillas were prepared by mixing the composite wheat flours with salt, water and baking powder. Likewise corn tortillas were prepared by mixing the resulting composite flours with water to produce dough (masa) that was transformed into tortillas by experienced housewives. Flour viscosity was evaluated using the RVA 4 device, considering 14 % as standard moisture content. Maximum weight per sample was 28.5 g, obtained adjusting the weight of the flour (3.25-3.42 g) and distilled water (25.25-25.08 g), according to flour initial moisture content. A 13 min standard cycle was used including 50 °C as starting temperature for 1 minute, then the temperature of the slurry was increased to 95 °C for 4 min and maintained for three additional minutes (heat holding time) and then the samples were cooled to 50 °C during a 5 min time period. Data was registered for viscosity peak, holding strength, breakdown, final viscosity, setback, peak time and pasting temperature.

RESULTS AND DISCUSSION: Significant differences ($p<0.01$) were observed among formulations obtained with wheat flour enriched with common bean flour or starch, for holding

strength, final viscosity, setback and pasting temperature. Wheat Commercial Flour (WCF) enrichment with common bean flour caused significant modifications for some rheological traits (Table 1), and in contrast no significant modifications were observed in WCF by the addition of common bean starch. Compared to the control treatment (WCF) significant reduction for holding strength, final viscosity, setback and pasting temperature was observed by the whole common bean flour addition. Significant differences ($p<0.05$) were observed among nixtamalized corn formulations for peak viscosity, holding strength, final viscosity, setback and peak time. Corn commercial flour (CCF) + 10 % Bayo Victoria starch registered highest values for peak viscosity (1,342 cP), holding strength (1,215 cP), final viscosity (2,364 cP), setback (1,149 cP) and the lowest value for peak time (5.5 min). Similar values were observed for corn commercial flour (CCF) compared to both formulations including 10 % of common bean whole flour (Pinto Saltillo and Bayo Victoria), for peak viscosity, holding strength, breakdown, and pasting temperature. Differential response was observed in WCF and CCF for the addition of common bean flour or starch. Similitude in most of the rheological traits was observed in WCF adding starch extracted from common bean grains. Difficulties were observed selecting the better formulation in CCF using rheological traits evaluated by RVA. According on housewives criteria, similar results were observed in all the formulations. Findings obtained herein support possibilities of using 10 % of common bean starch as partial substitute in wheat flour, in order to increment resistant starch and reducing glycemic load.

Table 1. Rapid Visco Analyzer (RVA) test Results for wheat and maize flour samples using 13 min temperature profile in RVA-3D.

Formulation	PV (cP)	HS (cP)	BD (cP)	FV (cP)	SB (cP)	PT (min)	PTP (°C)
Wheat Commercial Flour (WCF)	2,563^{a}	1,469^{ab}	1,095^{a}	3,063^{ab}	1,595^{ab}	5.8^{a}	57^{ab}
WCF+ Pinto Saltillo Flour	2,348^{a}	1,379^{b}	969^{a}	2,836^{b}	1,457^{b}	5.6^{a}	56^{b}
WCF + Bayo Victoria Flour	2,394^{a}	1,390^{b}	1,004^{a}	2,871^{b}	1,481^{b}	5.7^{a}	56^{b}
WCF+ Pinto Saltillo Starch	2,700^{a}	1,572^{a}	1,128^{a}	3,284^{a}	1,712^{a}	5.7^{a}	57^{ab}
WCF + Bayo Victoria Starh	2,688^{a}	1,594^{a}	1,094^{a}	3,332^{a}	1,738^{a}	5.7^{a}	60^{a}
Corn Commercial Flour (CCF)	1,039^{b}	976^{bc}	64^{a}	1,894^{c}	918^{c}	6.0^{b}	71^{a}
CCF+ Pinto Saltillo Flour	932^{b}	877^{c}	55^{a}	1,622^{d}	745^{d}	6.9^{a}	72^{a}
CCF + Bayo Victoria Flour	944^{b}	873^{c}	71^{a}	1,609^{d}	734^{d}	6.6^{a}	73^{a}
CCF+ Pinto Saltillo Starch	1,244^{a}	1,139^{ab}	106^{a}	2,196^{b}	1,057^{b}	5.7^{bc}	71^{a}
CCF + Bayo Victoria Starch	1,342^{a}	1,215^{a}	127^{a}	2,364^{a}	1,149^{a}	5.5^{c}	70^{a}

PV= peak viscosity; HS= holding strength; BD= breakdown; FV= final viscosity; SB= setback; PT= pasting time; PTP= pasting temperature. $^{a-c}$Differences among cultivars and treatments. Letters in the same column show significant differences among formulations according to HSD Tukey test ($\alpha = 0.05$).

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RUDÁ × AND 277 RILS: A POTENTIAL NEW CORE MAPPING POPULATION FOR COMMON BEAN

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The main population used for genetic mapping in common bean (*Phaseolus vulgaris*) is the BAT 93 × Jalo EEP 558 RIL (Recombinant Inbred Lines) population, reported by Nodari *et al.* (1993) and Freyre *et al.* (1998), which is currently composed of 75 lines. BAT 93 and Jalo EEP 558 belong to the Mesoamerican and Andean gene pools, respectively. These parents contrast for many important traits, such as disease and insect reactions, grain nutritional quality and other morphological and agronomical traits. Another important common bean RIL mapping population that has been used by the bean research community was developed by CIAT (Cali, Colombia) from crosses between DOR 346 (Mesoamerican) and G 19833 (Andean). This population is currently composed of 87 lines (Blair *et al.*, 2003; Galeano *et al.*, 2009). Several other bean mapping populations have also been developed and used by different groups. However, a serious limitation of most of these populations is their reduced size, which drastically affects the accuracy of the estimates of recombination rates and consequently the precision of the resulting genetic maps.

According to Silva *et al.* (2007), the minimum population size and respective genome saturation level to develop reliable genetic maps using a RIL mapping population should be 200 lines/5.0 cM, 300 lines/10.0 cM or 500 lines/20 cM. For this reason, a core mapping population with appropriated size is still lacking for common bean. In addition, with the ongoing *P. vulgaris* genome sequencing projects, a large number of molecular markers are being developed and will be available soon for the bean research community. In this sense, the fine-mapping of candidate genes and QTLs will become possible and highly demanded.

In an attempt to develop a potential new core mapping population for common bean, the BIOAGRO/UFV and Embrapa bean research groups developed a RIL population with 500 lines from crosses between Rudá (Mesoamerican) and AND 277 (Andean). These parents present considerable phenotypic contrast for many morphological and agronomical traits, in addition to disease reactions (Table 1). The genetic variability among them has also been checked at the molecular level. The estimated genetic distance based on a set of 126 SSR markers discovered by Embrapa group, most of them reported by Grisi *et al.* (2007), was 78.6%, and 71.3% using 677 SNP markers reported by Souza *et al.* (2012). Rudá is a 'carioca' seeded cultivar (beige background with brown stripes) developed by CIAT and released in Brazil by Embrapa. It derived from crosses between the cultivars Carioca and Rio Tibagi. Although it is a high yielding cultivar, Rudá is susceptible to the main bean diseases occurring in Brazil. AND 277 is a 'manteigão' seeded line (cream background with light red stripes) also developed by CIAT from multiple crosses [(Cargabello × (Pompadour Checa × Linea 17) × (Linea 17 × Red Cloud))]. It is resistant to different pathotypes of the angular leaf spot (ALS), anthracnose and rust pathogens (Table 1), harboring the ALS and anthracnose resistance genes *Phg-1* and *Co-1*^{4}, respectively.

Crosses and generation advancement from F_2 to F_9 generations, using the SSD (Single Seed Descent) method, were performed under greenhouse conditions. To identify the true hybrids, all F_1 plants and F_2 seeds were analyzed morphologically. Rudá × AND 277 RILs have been grown in the field for seed multiplication and phenotyping for important morphological and agronomical traits. In addition, these RILs are being genotyped at Embrapa using SSR and SNP markers, and also GBS. Seeds of the Rudá × AND 277 RIL mapping population should be available soon upon request. We expect that a cooperative use of these RILs as a reference mapping population should help the development of a reliable, integrated and saturated core genetic map for common bean.

Table 1. Reactions of the common bean lines Rudá and AND 277 to some of the main bean diseases.

Disease	Pathogen	Pathotype	Disease Reaction[a]	
			Rudá	AND 277
Angular leaf spot	*Pseudocercospora griseola*	7-15[b] ; 15-7[b]; 23-23[b] ; 31-7[b] 47-39[b] ; 63-6[b] 63-7[b] : 63-23[b] 63-31[b] : 63-47[b] 63-63[b]	S	R
Anthracnose	*Colletotrichum lindemuthianum*	7[c] ; 55[c] ; 87[c]	S	S
		8[c] ; 9[c]; 64[c] ; 65 72[c] ; 73 ; 77 ; 81; 453[c]; 1609; 2047[c]	S	R
		71[c] ; 89[c]	R	R
Rust	*Uromyces appendiculatus*	21-3; 53-19	S	R
		29-3	S	S
White mold	*Sclerotinia sclerotiorum*	SsEpamig01	S	S

[a]Resistant (R) and susceptible (S) reaction. [b]Disease reaction reported by Balbi *et al.* (2009) and [c] by Arruda (2009).

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THE CULTURE COLLECTION OF PHYTOPATHOGENIC MICROORGANISMS: AN IMPORTANT SOURCE OF INFORMATION TO COMMON BEAN RESEARCH

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The culture collection of common bean pathogenic microorganisms of Embrapa Rice and Beans was created in the 1980's aiming to estimate the variability of the major pathogens and thus guide the achievement of resistant cultivars. Currently the collection has more than 4.000 isolates of bean pathogens, being the most relevant species: *Colletotrichum lindemuthianum, Pseudocercospora griseola, Fusarium oxysporum f. sp. phaseoli, Sclerotinia sclerotiorum, Uromyes appendiculatus, Curtobacterium flaccumfaciens* pv. flaccumfaciens and *Xanthomonas axonopodis* pv. phaseoli. With the creation of the Embrapa Microbial Genetic Resources Network in 2010, evidenced the importance of the collection in the context of the Brazilian genetic heritage and with the financial support made possible the cataloging, maintenance and monitoring more appropriate for the collection. The challenge now is the implementation of the eletronic database and a search system, available to internal and external audiences, with the most relevant information to users, including data obtained with the use of molecular markers. Thus, in addition to facilitating access to information contained in the collection to the public internal company also will facilitate access to information by other external research groups, contributing more strongly to the development of research related to pathosystems. The objective of this study was to group the various data relating to the common bean pathogenic microorganisms from Embrapa Rice and Beans culture collection, aiming to emphasize its complexity and importance to the development of disease-resistant plants.
Samples from several common beans producing regions of Brazil were received over 30 years (1981-2011) by the Laboratory of Plant Pathology at Embrapa Rice and Beans. The data collection of each material was carefully recorded in the minute book containing collector name, collection site, collection date, and georeferenced cultivar information. The plant material was evaluated on the types of symptoms, disinfection with 70% ethanol and diluted sodium hypochlorite was done. The isolation of pathogens was performed differently for bacteria and fungi. For the isolation of bacteria the sample was macerated and the formed suspension was inoculated in Petri dishes containing culture medium potato dextrose agar (PDA) or nutrient agar, depending on the type of disease. For the isolation of fungi it was used the technique for microorganism monosporic and inoculation in Petri dish with BDA medium. Both bacteria and fungi strains were incubated at specific temperatures for each pathogen. After morphological, biochemical and molecular characterization, the identified isolates were subjected to long-term preservation in three different methods: cryopreservation, Castellani (in water) and filter paper. Besides, isolates were also characterized according to their pathogenicity by using a differential cultivar series according to each pathogen (CIAT, 1990; Rava & Sartorato, 1994, Pastor-Corrales & Abawi, 1987) or by specific molecular detection (Tegli et al. 2002, Alves-Santos et al., 2002).

The bean pathogens collection grew uninterrupted since 1981 due to the constant sampling of plant material with symptoms of disease, obtained from many common bean production regions in Brazil. Along the thirty years it has been obtained over 3500 isolates from seven different species of pathogens that cause diseases in common bean plants (Figure 1). All pathogenic microorganisms were evaluated morphologically to observe the spores shape and size (i.e. conidia of C. *lindemuthianum*) and the cell wall structure (i.e. gram positive bacteria of the specie C. *flaccumfaciens* pv. flaccumfaciens) (Figure 2 A and B). Some of them were also evaluated using molecular marker, resulting on the clustering of the isolates (Figure 2C). Molecular and

pathogenicity data indicated a high degree of variability among the isolates of the same species and the predominance of certain pathotypes according to their origin regions, showing the need for sampling and continuous isolation over the years. The isolates are used mainly for the selection of disease resistant genotypes, pathogen-host interaction studies and on the characterization of physiological races. The on line query and request of common bean pathogenic isolates can be found at the National Research Center of Rice and Beans website http://www.cnpaf.embrapa.br/transferencia/informacoestecnicas/colecao_fitopatogenos/index.php

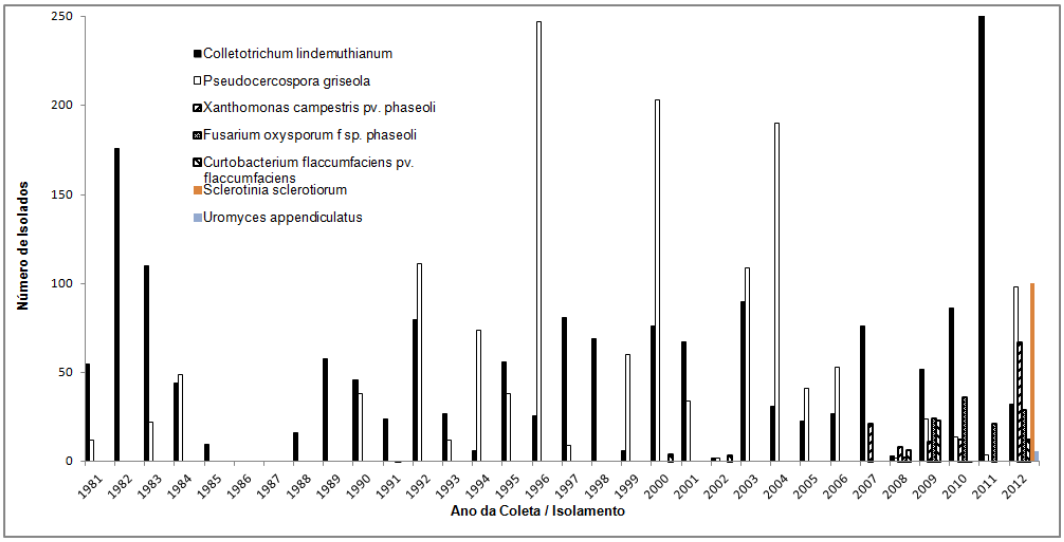

Figure 1. Number of different pathogenic species obtained between the years 1981 and 2012.

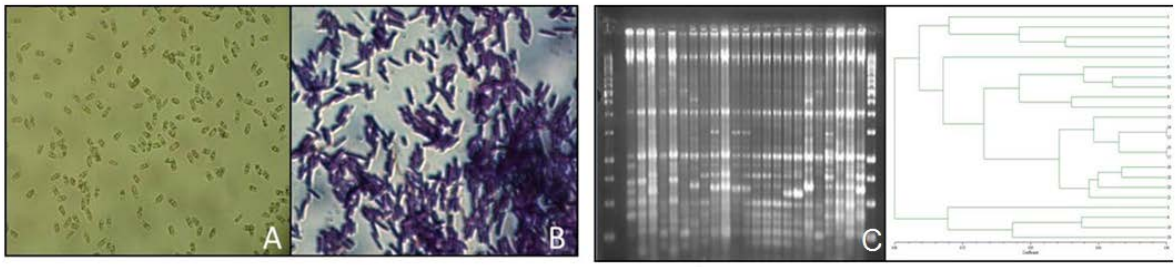

Figure 2. Morphological characterization: A) conidia of C. *lindemuthianum,* B) gram positive bacteria of the species C. *flaccumfaciens* pv. flaccumfaciens. C) Molecular characterization of different isolates of *C. flaccumfaciens* pv. flaccumfaciens.

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PROTEOMIC ANALYSIS OF DEFENSE-RELATED PROTEINS IN COMMON BEAN

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INTRODUCTION

Common bean is one of the world's most important edible legumes for direct human consumption. A myriad of diseases of common bean, especially those caused by fungal, bacterial, and viral pathogens often cause significant yield losses in common bean worldwide. These losses range from 10% for some root rots to 70% for angular leaf spot and 100% for anthracnose. Knowledge of defense-related proteins is an important area of research to find disease-management solutions, via modification of proteins, especially but not only for diseases for which there are not available resistance sources in common bean. Plants have developed different defense mechanisms by synthesis of specific proteins to protect and maintain the cell functions against biotic and abiotic stresses. Proteomic analysis has been effectively used to identify protein profiles of plant, animals and pathogens. The objective of our research was to conduct studies using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) for protein separation and mass spectrometry (MS) for protein characterization in common bean for the discovery of defense-related proteins in common bean.

MATERIALS AND METHODS

Seeds of common bean G 12910 were produced by growing plants under greenhouse conditions. The common bean proteins were extracted using a modified Trichloroacetic acid (TCA)/acetone precipitation/Urea solubilization extraction buffer. Proteins were separated by 2D-PAGE and analyzed by mass spectrometry and database searches (1).

RESULTS AND DISCUSSION

Proteomic approach was used to study the distribution of some of the defense and stress proteins in common bean seeds. Lectins defense-related carbohydrate-binding glycoproteins were identified in over 70 different legume species. They were responsible for innate immunity and defense mechanisms, as well as interaction with symbionts. We have identified twenty five lectin proteins and four alpha-amylase inhibitors using 2D-PAGE and MS. In this study we also have found another protein called Bowman-Birk inhibitor, which is a double headed trypsin inhibitor that consists of cysteine-rich protease inhibitors. They are widely distributed in common bean and mainly involved in plant protection from pests and pathogens. These proteins appeared as 2 spots. We also found one protein spot of superoxide dismutase. This protein increases dramatically in response to biotic and abiotic stresses, and also increases due to salinity. We also found eight spots of late embryogenesis abundant protein (LEA) and four spots of heat shock proteins (HSPs). LEAs and HSPs are involved in several protective functions. In summary, in this study, we are reporting the distribution of defense and stress proteins in common bean seeds using proteomic approach. This information is very important to scientists wishing to develop disease resistant varieties.

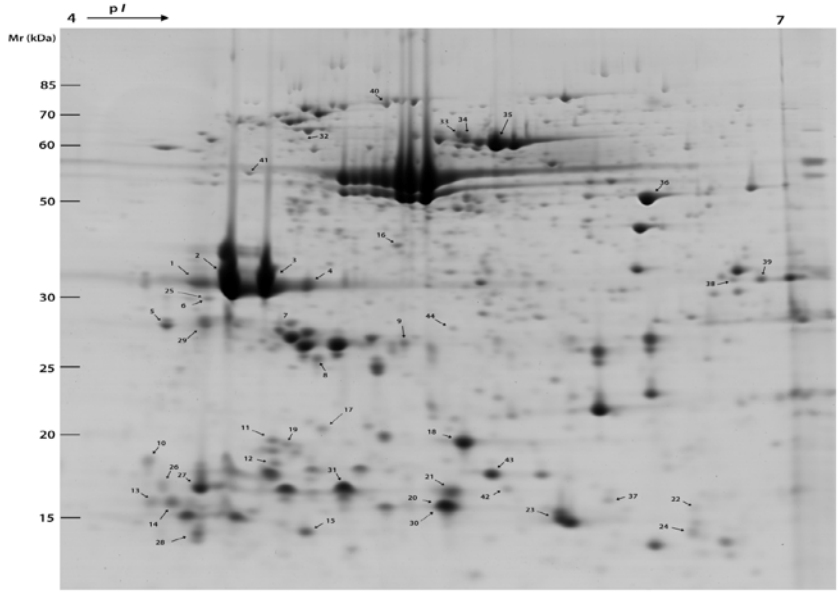

Figure 1: 2D-PAGE analysis of common bean defense/stress proteins

Table 1: identified common bean defense/stress proteins

ID	Defense / Stress Protein	Calc pI	CalcMr (Da)	MS Score	GI number / UniProt ID
1 - 25	Lectin	4.95 - 5.80	29- 31	73 - 898	19773406, 6822274, 19744132, 19577338, 501100
26 - 29	Alpha-amylase inhibitor	4.70 - 4.95	15 - 29	117 - 664	6456428, 1911780
30 - 31	Bowman-Birk inhibitor	6.38	12276	81 - 121	21304454
32 - 39	LEA protein	5.55 - 6.10	12- 51	65 - 482	75708857, 18499, Q39873, 6358640
40 - 43	HSP	5.28 - 6.36	17311 - 71894	107 - 237	357480003, 75279028, 507209
44	Superoxide dismutase	5.60	27881	93	351721352

[1]ID, spot number referred to in Fig. 1; calc pI, theoretical values for isoelectric point; calc Mr, theoretical values for molecular weight; MS Score, MOWSE Score

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EFFECT OF RECURRENT SELECTION FOR RESISTANCE TO ANGULAR LEAF SPOT ON SSR ALLELE FREQUENCY OF COMMON BEAN

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INTRODUCTION

Among the factors that most have limited the achievement of high grain yield in common bean, we highlight the occurrence of diseases, like angular leaf spot (*Pseudocercospora griseola*). As resistance to this disease has been attributed to one, two, three or more independent genes, an alternative to accumulate several resistant alleles is recurrent selection (RAMALHO et al., 2001; CARGNIN, 2007), a cyclical and dynamic system that aims to gradually increase the frequency of favorable alleles for a quantitative trait. With the advent of molecular markers, there is the possibility of checking whether the allele frequencies of microsatellite (SSR) loci were changed in the populations of the different cycles of recurrent selection. This is expected because the natural selection changes the allele frequency of most SSR loci in segregant populations (RODRIGUES e SANTOS, 2006). Likewise, it is possible to identify the alleles that might be associated with the resistance to angular leaf spot, and thus, they can be useful in future selections. Then, this study was done with the objective of determining if recurrent selection for resistance to angular leaf spot affects the frequency of microsatellite alleles specifically, to identify those who can assist in the selection.

MATERIAL AND METHODS

The recurrent selection program was set up for obtaining resistant lines to angular leaf spot with carioca grain type (ARANTES et al. 2010). The base population was obtained crossing two groups of parents using a diallel design: seven lines with carioca grain type (Carioca MG, CI-140, CI-128, ANPAT 8.12, IAPAR 81, ESAL 693 and Pérola) and ten sources of resistance to angular leaf spot (P. *griseola*) (AN 512561, AND 277, Ouro Negro, Compuesto Negro Chimaltenango, CAL 143, MAR 2, MAR 1, G 5686, MA 4137 and Jalo), including various types of grains. The entire procedure used for the selection of the 35 progenies (five progenies selected in seven recurrent selection cycles), is described in Arantes et al (2010).

The DNA extraction of the 35 progenies and of the 17 parents, as well as the amplification reactions occurred in accordance with the procedures used by Pereira et al. (2007). First, we verified the existence of polymorphism between the parents, with approximately 400 pairs of random SSR primers of *P. vulgaris*, whose sequences are available in www.css.msu.edu/bic . Subsequently, the 37 polymorphic SSR primers in the parents were used in the progenies of each cycle.

The identification of the amplified DNA fragments by microsatellite markers was done by performing a visual analysis, generating an array of 0, 1 and 2 that corresponds to the homozygous $A_i^1A_i^1$, the homozygous $A_i^2A_i^2$ and the heterozygous $A_i^1A_i^2$, respectively. A_i^1 and A_i^2 corresponds to alleles 1 and 2 of the i-th microsatellite marker. The genotypic proportions of

the parental lines and of the progenies were compared through the statistical chi-square (χ^2) test, that allows checking if the frequencies deviate from those expected or not, considering the expected genotypic frequencies those observed in the parents. This test was performed per locus and for each cycle, allowing a better comparison of the same locus in different cycles.

RESULTS AND DISCUSSION

Among the 37 polymorphic loci used, 28 (75.6%) suffered selection effect ($P \leq 0.05$) in at least one of the seven selection cycles, and 15 (40.5%) had genotypic frequencies affected in four or more cycles. The genotypic frequency changed in most SSR loci, and it implies that populations have suffered the effect of mechanisms that alter the allelic and genotypic frequencies, especially due to phenotypic selection for resistance to angular leaf spot.

Some of the loci influenced by selection in this study were also targets of selection in the study of Rodrigues and Santos (2006). Among them, there are BM154, BM210 and K03289. In locus BM154, all cycles were affected by selection. This marker is linked to quantitative trait loci (QTLs) that are involved in reducing the occurrence of angular leaf spot and it could have been retained as a result of recurrent selection.

The goal of a recurrent selection program is to increase gradually the frequency of favorable alleles during the selection cycles. Thus, the expected behavior of a particular allele subjected to this type of selection is that, in the most advanced cycles, its frequency is increased. However, we observed in the study quite high frequencies of some alleles since the first cycle and some even fixed. In the loci GATS11, ATA32, ATA27, PVct002, X6000, BM157, BM164 and BM175, it was observed allele fixation since the first selection cycle. Fixation always occurred in the alleles that were more frequent in the parents. As noted by Arantes et al. (2010), the progenies had low grades of severity of angular leaf spot in the early selection cycles, indicating that the population already had a high level of resistance to this disease. This may explain the high initial allele frequencies of some loci. However, it is important to note some loci for which allele frequencies in parents had greater changes in progenies like ATA7, BM202, ATA27 and PVct002. Therefore, there is a good chance that the alleles that increased their frequency might be close to major QTLs for resistance to angular leaf spot, in the conditions that have been carried out the selection.

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INNATE IMMUNITY-RESPONSIVE GENES IN A MAJOR QTL CONTROLING ANGULAR LEAF SPOT RESISTANCE

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INTRODUCTION

Angular leaf spot (ALS) causes great yield losses in common bean (*Phaseolus vulgaris* L.). This disease is caused by the hemibiotrophic fungus *Pseudocercospora griseola* (Sacc.) Crous & Braun. As the most effective approach to control this disease is to use resistant cultivars, understand the mechanisms controlling the host-pathogen interaction and the development of gene-specific molecular markers is important to plant breeding efforts. In this study, we determined whether putative genes located in a major ALS resistance QTL (ALS10.1) on the linkage group B10 [1] were responsive to flg22; a hallmark elicitor of plant innate immune response [2]. Available markers for these genes [3] were used in semi-quantitative RT-PCR (Reverse Transcriptase – Polymerase Chain Reaction) to access their expression levels. Indeed, we observed differential expression of two putative genes reinforcing the importance of the ALS10.1 QTL in bean defense responses. These results will facilitate further studies in cloning ALS resistance genes and the development of tools for marker-assisted breeding.

MATERIAL AND METHODS

The markers used in the BAC library screening for studying the QTL ALS10.1 [3] were aligned to gene sequences available in the NCBI non-redundant and EST databases using the tBLASTX ($\geq$50% identity and E-values $\leq 1 \times 10^{-5}$). Primers for the markers assigned to gene coding regions were used in RT-PCR. Leaves of the bean line G2333 were immersed in a solution of 10 mM flg22 or water (control) for 30 minutes, and then frozen in liquid nitrogen for total RNA extraction using the Plant RNeasy kit (Qiagen ®) according to the manufacturer's instructions. RT-PCR was carried out with gene-specific primers in a two-step reaction using the Takara ® RNA PCR kit according to the manufacturer's instructions. The *PvACT2* gene (GI: 165882002) was used to normalize the RNA input in the amplification reactions.

RESULTS AND DISCUSSION

Previously, we have characterized the genome structure of the major QTL for ALS resistance ALS10.1 [3]. In the present study, we found five markers as part of putative open reading frames (Table 1), which were chosen to assess gene expression. Two genes were found to be differentially expressed and three showed no response to flg22 treatment (Fig. 1).

Pathogen-associated molecular patterns (PAMPs) are typically essential components of whole classes of pathogens, such as fungal chitin, bacterial flagellin and its derived peptide flg22. Stimulation with PAMPs leads to PAMP-triggered immunity (PTI) in plants [2, 4]. The bean N-like protein containing the RGA07 marker was down-regulated in PTI response. It is a TIR-NBS-LRR protein, which provides a downstream signaling usually after recognition of pathogen virulence molecules [4]. This molecules leads to a second class of pathogen perception

called effector-triggered immunity (ETI) [4]. Therefore, this bean TIR-NBS-LRR protein could be involved in ETI, being down-regulated during PTI [5]. The putative ribonucleoprotein (PvM22 marker) was up-regulated during the bean PTI response. This kind of protein plays a role in many metabolic activities of plants, mainly through the genes involved in translational control [6]. Although the participation of ribonucleoproteins in stress responses was already established [6], its direct link with plant immunity remains elusive.

The three genes that were not responsive to flg22 treatment encoded for a ribosomal 40S protein (PvM13), an unknown protein (PvM127), and a TIR-NBS-LRR protein (P09O19). Ribosomal proteins act in protein synthesis and do not show a direct involvement in plant immune response. Although the P09O19 was assigned to a TIR-NBS-LRR gene that belongs to defense-associated gene families [4], it showed no differentially expression in response to flg22 indicating that gene-specific response may exist. The discovery of PAMP-responsive genes in the ALS10.1 region highlights its role in bean immune response.

Table 1 Markers assigned to gene coding regions of the ALS10.1 QTL.

Marker	Gene Annotation	E-value	Species
RAG07	TMV resistance protein N-like	4×10^{-86}	*Glycine max*
P09O19	TIR-NBS-LRR	6×10^{-20}	*Glycine max*
PvM22	Ribonucleoprotein	9×10^{-65}	*Glycine max*
PvM127	unknown protein	3×10^{-09}	*Arabidopsis thaliana*
PvM13	Ribosomal protein 40S	1×10^{-93}	*Glycine max*

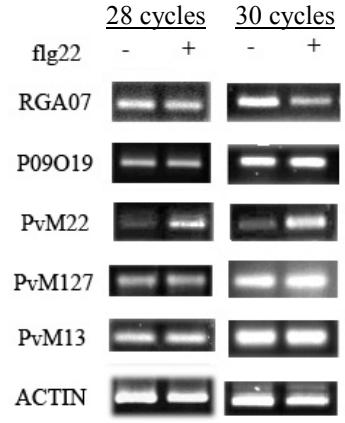

Fig 1. Genes in the QTL ALS10.1 region may play a role in bean innate immunity. RT-PCR was performed with total RNA extracted from bean leafs treated with flg22 (10 μM) or water (control) for 30 minutes. Left column shows amplicons after 28 PCR cycles whereas the second column shows the same amplicons after 30 PCR cycles. The experiment was performed in three biological replicates and the bean actin gene (*PvACT2*) was used as loading control.

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EVALUATION OF RESISTANCE IN COMMON BEAN GENOTYPES TO THE CAUSAL AGENT OF ANGULAR LEAF SPOT

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INTRODUCTION

Angular leaf spot disease of common bean (*Phaseolus vulgaris*), caused by *Pseudocercospora griseola,* is one of the most important disease of this crop in Brazil. Losses due to this disease can be as high as 80% depending on the cultivar genetic background, environment conditions and the pathogenicity of its causal agent (Singh et al., 2010). Many control strategies, including chemical applications, cultural practices and genetic resistance can be used for the management of disease. Genetic resistance is the most appropriate method to the pathogen control. The aim of this study was to determine the reaction of 28 bean genotypes to four strains belonging to 63-63 race of *P. griseola* under greenhouse conditions.

MATERIALS AND METHODS

Twelve seeds of each cultivar were sown in pots and tested with four strains. Plants were inoculated at the V3 development stage. To obtain spores the strains were cultured on bean leaf dextrose-agar medium and incubated for 14 days at 24 ± 2°C. Spore suspensions were obtained on sterile water and concentration adjusted to 2 x 10^4 conidia ml-1. Plants were inoculated and pots were maintained in a moist chamber at 22 °C and 12 h of photoperiod for 48 hours. Then, pots were transferred to the greenhouse, for more seven days when evaluations were performed. The scale from 1 to 9 developed by Schoonhoven and Pastor-Corrales (1987) was used to evaluate plant symptoms. The average scores were calculated and scores lower 3, 3,1 to 6 and upper 6 were considered as resistant, moderately resistant and susceptible, respectively.

RESULTS AND DISCUSSION

From the 28 genotypes tested, only 9 (Table 1) showed resistance to one or more strains of *P. griseola*. Most genotypes were resistant to only one strain. Most cultivars showed moderately resistance to strains of *P. griseola* used. It was observed variability among strains of 63-63 race. Therefore, the results show high aggressiveness of the strains analyzed. These results confirm the observation that most cultivars used in Brazil are susceptible or show resistance to only few *P. griseola* races, due to the wide pathogenic variability of the fungus (Sartorato, 2002; Garcia et al 2006). New sources of resistance need to be included on current common bean breeding programs to obtain cultivars resistant to more strains of *P. griseola*.

Table 1. Reaction of 28 common bean genotypes to four *Pseudocercospora griseola* strains belonging to race 63-63.

Cultivars	Psg-1	Psg-2	Psg-3	Psg-4
MAI-8-9	MR	MR	MR	MR
MAI 6.10	MR	R	MR	MR
MAII-10	MR	MR	MR	MR
MAII-8	MR	S	MR	MR
MAIII-16.155	MR	MR	R	MR
MAIII-16.159	R	MR	R	MR
MAIV-15.204	MR	MR	MR	MR
MAIV-15.203	MR	MR	MR	MR
MAV-3.36	MR	MR	MR	MR
MAV-7.85	MR	MR	MR	MR
MAVI-24	MR	MR	MR	R
MAVI-21	MR	MR	MR	MR
MAVII-34	MR	MR	R	MR
MAVII-92	MR	MR	MR	MR
MAVIII-78	MR	MR	MR	MR
MAVIII- 94	MR	MR	MR	MR
Ouro Negro	R	MR	MR	S
M-20	MR	MR	MR	MR
Jalo	MR	S	MR	S
BRSMG-Madrepérola	MR	S	S	S
Rosinha	MR	S	S	MR
BRSMG-Majestoso	R	MR	MR	MR
Carioca MG	MR	MR	S	S
Pérola	MR	S	MR	MR
Cornell 49242	R	MR	MR	MR
AND-277	R	MR	MR	MR
MAII-16	MR	MR	MR	MR
BRS- Horizonte	MR	MR	MR	MR

R = resistant; MR = moderately resistant; S = susceptible.

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REACTION OF COMMON BEAN GENOTYPES TO FIVE RACES OF *COLLETOTRICHUM LINDEMUTHIANUM* IN BRAZIL

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INTRODUCTION: Anthracnose of common bean (*Phaseolus vulgaris*), caused by *Colletotrichum lindemuthianum*, is a cosmopolitan disease. Favorable conditions for disease development, such as moderate temperature (15-20ºC) and high humidity, susceptible cultivars, and infections in early stages of plants can dramatically reduce yield (Paula Júnior & Zambolim, 2006). The high variability of *C. lindemuthianum* hampers the development of cultivars with durable resistance. Thus, the search for new sources of resistance, mainly among adapted genotypes, is a routine in bean breeding programs. The aim of this study was characterizing the reaction of genotypes of common bean from the germplasm of the Universidade Federal de Viçosa to the main races of *C. lindemuthianum* identified in the State of Minas Gerais, Brazil.

MATERIAL AND METHODS: The reaction of 283 genotypes of common bean to the races 65, 73, 81, 87, and 89 of *C. lindemuthianum* was evaluated. The genotypes Rudá (susceptible to all races) and Rudá-R (resistant to all races) were used as controls. Rudá-R has the resistance genes *Co*-4, *Co*-6 and *Co*-10 (Costa et al., 2010). The inoculum was produced in flasks containing sterile bean pods partially immersed in BDA (Pio-Ribeiro & Clark, 1975). Flasks were incubated at 23°C in darkness for 8-10 days. The inoculum concentration was adjusted for 1.2×10^6 conidia mL^{-1}. For each race, eight seedlings of each genotype were inoculated ten days after emergence. A suspension of each race was applied in both sides of the primary leaves with a DeVilbiss atomizer activated by an electric compressor. The plants were incubated for seven days in a mist chamber (20 ± 1°C and relative humidity > 95%) using a 12-hour photoperiod. After this period, plants were scored as resistant (R, scores 1-3) or susceptible (S, scores 4-9) (Pastor-Corrales, 1992). The results were analyzed considering the index of virulence (IV) of each race and the index of resistance (IR) of each genotype, in which: IV = number of susceptible genotypes x 100/total of genotypes, and IR = number of resistant genotypes x 100/total of races.

RESULTS AND DISCUSSION: The lowest and the highest IV scores were obtained with the races 87 (41.7%) and 81 (68.2%). Intermediate IV scores were obtained for the races 73 (52.3%), 89 (62.9%), and 65 (65.7%). These races are among the most frequent races found in Minas Gerais (Damasceno e Silva et al., 2007) and the most widely distributed races in Brazil (Rava et al., 1994). Twenty-three groups of genotypes with the same reaction (S or R) to the five races were obtained. The reaction combinations more frequent to the five races were: $S^{65}S^{73}S^{81}S^{87}S^{89}$ (83 genotypes), $R^{65}R^{73}R^{81}R^{87}R^{89}$ (44), $S^{65}S^{73}S^{81}R^{87}S^{89}$ (39), $S^{65}R^{73}R^{81}R^{87}R^{89}$ (17), $R^{65}R^{73}S^{81}S^{87}S^{89}$ (14), $S^{65}R^{73}S^{81}R^{87}R^{89}$ (13), and $S^{65}R^{73}S^{81}S^{87}S^{89}$ (10). Genotypes from different groups should be hybridized in order to increase the resistance to these races of *C. lindemuthianum*. One hundred and sixteen genotypes (41%) were resistant to at least three races

(Table 1), with IR ≥ 60%. Forty-four genotypes were resistant to all races and are promising sources of resistance to anthracnose. Among these genotypes there are some commercial cultivars, such as Ouro Vermelho, BRS Valente, Carnaval MG, BRS Grafite and BRSMG Tesouro.

Table 1. Common bean genotypes with resistance to at least three races of *Colletotrichum lindemuthianum*.

Reaction	Genotypes
$R^{65}R^{73}R^{81}R^{87}R^{89}$	1828 S 313 Venezuela, 1841 6 G, 1843 55 G, 1845 77 G, 1849 Floresta 13041, 1861 Sacavem 486, 63 F, AB136, AN 910902, AN 910970, AN 911021, AN 911120, AN 9122526, AN 9122551, BP 9116316, BRS Expedito, BRS Grafite, Carnaval MG, CB 733782, CB 734681, CNFJ 10301, CNFR 10245, Enxofre, Fe 732116, FT 85-113, G 2333, IAC Bico de Ouro, Kaboon, LM 96108804, Meia Noite, Ouro Vermelho, RAB 94, Serrano, TB 9401, TU, IAC UNA, Vagem Amarela, BRS Valente, Vermelho, Vermelho 2157, Vi 10-2-1, BRSMG Tesouro, W 22-50, Rudá-R (control)
$S^{65}R^{73}R^{81}R^{87}R^{89}$	2970149, 96 F, AN 9021334, BAT 1616, BRS Vereda, CF 880152, LM 9220225, OP-210P, OP-320P, Ouro Negro, P.16 Trujillo 4, SC 9029935, Vi 13100P, Vi 5500P, Vi 7800P, VP 1, Xodó
$R^{65}S^{73}R^{81}R^{87}R^{89}$	1831 S 353 Venezuela, BAT 304, Iraí, Roxo 90
$R^{65}R^{73}S^{81}R^{87}R^{89}$	3272, 1862 Sacavem 538, 1864 Sacavem 860, 1868 Sacavem 1061, FT 83-120, LM 95103904, MA 733322
$R^{65}R^{73}R^{81}S^{87}R^{89}$	A 525, DRK 18, Manteigão Preto, Pintado (Bolinha), Preto 60 Dias, Vermelho1
$R^{65}R^{73}R^{81}R^{87}S^{89}$	1836 S 464 Venezuela, VC19X3, Roxinho 1
$S^{65}S^{73}R^{81}R^{87}R^{89}$	1833 S 375 Venezuela, CB 733823, CF 880150
$S^{65}R^{73}S^{81}R^{87}R^{89}$	41 F, FEB 163, LM 95103786, OP-390P, PR 9115802, Roxinho 2, SX 2232-2, Vi 5700P, VP 4, VP 3, VP 5, VP 6, W22-34
$S^{65}R^{73}R^{81}R^{87}S^{89}$	Barriga Verde, BRS Supremo
$R^{65}S^{73}S^{81}R^{87}R^{89}$	1829 S 349 Venezuela, Capichaba Precoce, FE 821698
$R^{65}S^{73}R^{81}R^{87}S^{89}$	Compl. Negro Chimaltenango, FE 732614, Field grown 49-242, IAPAR 44, IAPAR 20, P. White 6301, POT 51, RAI 295, Novirex
$R^{65}R^{73}S^{81}S^{87}R^{89}$	BRS Campeiro, Tico-tico
$R^{65}R^{73}S^{81}R^{87}S^{89}$	84 VAN 166, AN 910390, AN 512568

Genotypes were inoculated with races 65, 73, 81, 87, and 89 of *C. lindemuthianum*; R – resistant; S – susceptible.

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INVESTIGATION OF SOURCES OF RESISTANCE TO ANTRACNOSE DISEASE IN A *PHASEOLUS VULGARIS* GERMPLASM COLLECTION IN BRAZIL

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INTRODUCTION

Anthracnose disease causes significant economic losses in Brazil and worldwide. Common bean breeding programs all over the country have been making efforts to provide new cultivars that acquired improvements to many traits. Therefore, every year, a great number of accessions are deposited at the Germplasm Colletions. The selection to diseases resistance depends of the natural occurrence of pathogens in the field, which relies on environmental conditions. Thus, greenhouse evaluations of these accesses can provide information of potential sources of resistance to introduce on current breeding programs. Studies have related high pathogenic variability in Brazil and the most frequent of races 65 and 81 (Pinto et al. 2012, Ishikawa et al. 2011, Pereira, et al. 2010). Our aim is to evaluate the reaction common bean accesses of the Germplasm Collection from Universidade Federal de Lavras, Brazil, to two races of *C. lindemuthianum* and obtain useful information to breeding programs.

MATERIAL AND METHODS

Five hundred common bean cultivars maintained at the common bean Germplasm Collection from the Universidade Federal de Lavras (UFLA) – Minas Gerais, Brazil were evaluated for their reaction to two *C. lindemuthianum* strains. The strains were provided by the *C. lindemuthianum* culture collection from the Department of Biology, UFLA.

Fungal colonies were maintained in M3S medium (Tu, 1985). To obtain spores, strains were inoculated on sterile bean pods and incubated at 22ºC for 10-15 days in the dark. Nine seeds of each cultivar were sown in a polystyrene tray of 162 cells with Multiplant® substrate. Two replicate trays were used totalizing 18 seeds of each cultivar. Spore suspensions were obtained on sterile water and concentration adjusted to 1.2×10^6 conidia/ml. When seedlings had fully expanded primary leaves the spore suspension was sprayed and trays were maintained in moist chamber at 22 °C and 12 h of photoperiod for 72 hours. Then, the trays were transferred to greenhouse for more seven days when evaluations were performed.

The scale from 1 to 9 developed by Schoonhoven and Pastor-Corrales (1987) was used to evaluate plant symptoms. The average scores were calculated and scores below 3 were considered as resistant, whereas plants scoring more than 3 were susceptible.

RESULTS AND DISCUSSION

The table 1 shows that most of the cultivars analyzed are susceptible to race 65 and resistant to race 81. It was possible to identify 124 cultivars resistant to both races. The germplasm collection maintains cultivars and inbreed lines from both Andean and Mesoamerican gene pool, therefore, existing wide genetic variability for many traits. The reaction of these cultivars to anthracnose disease provide useful information mainly because the resistance to these two races is being associated with other commercially favorable traits such as grain yield, grain color and

upright plant architecture, in order to identify potential sources or resistance to include in current breeding programs. The lines MA4137 and MAI-8.9 were developed by recurrent selection breeding program to angular leaf spot resistance and, furthermore, have presented resistance to both races of *C. lindemuthianum* evaluated. The line CI128 is resistant to both races tested and belongs to recurrent selection breeding program to grain yield and quality. Other cultivars such as ESAL547 and Vermelho 2157 were identified as resistant to both races and can be used as resistance sources.

Table 1. Reaction of 500 common bean lines to the races 65 and 81 of *C. lindemuthianum*.

Reaction/Strains	R65	R81
Resistant	188 (37.6%)	278 (55.6%)
Susceptible	312 (62.4%)	222 (44.4%)
Total	500	500

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IDENTIFICATION OF *COLLETOTRICHUM LINDEMUTHIANUM* ANTHRACNOSE RESISTANCE SOURCES IN CULTIVARS/LINES OF COMMON BEAN IN PARANÁ, BRAZIL

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INTRODUCTION

Anthracnose is one of the most important disease of common bean, its causal agent is the fungus *Colletotrichum lindemuthianum*. The use of resistant cultivars is the most commonly adopted strategy in order to control this pathogen (Pastor-Corrales et al. 1995). Nowadays, researches have confirmed the presence of 19 genes and four allelic series in the common bean genome, which confer resistance to *C. lindemuthianum* (Gonçalves-Vidigal et al. 2012). The integration of advances in biotechnology and molecular marker application with conventional plant breeding practices are important to obtain disease resistant cultivars. Thus, in order to find new sources of resistance to anthracnose, the present work had as objective to evaluate anthracnose genetic resistance of VCUs (Value for Cultivation and Use) elite lines on field and in laboratorial conditions, using inoculations and molecular analysis.

MATERIAL AND METHODS

The experiments were conducted in the period of 2010/2011 at Maringá and Umuarama, northwest region of Paraná state, Brazil. The treatments consisted of 11 elite lines and five commercial cultivars (control) assessed on field. The experimental design was randomized complete blocks with four replications. The evaluations of reaction to *C. lindemuthianum* were made on field and at Laboratório de Melhoramento do Feijoeiro Comum e Biologia Molecular of Núcleo de Pesquisa Aplicada a Agricultura (Nupagri-UEM). Ten seedlings of each cultivar were spray-inoculated with 2.0 ml of the spore suspension of each race of *C. lindemuthianum* (Table 1), using a De Vilbiss number 15 atomizer powered by an electric compressor. The plants were treated as replicates for the experiments. After inoculation, plants were maintained at >95% relative humidity for 2 days at 21-23^{o}C and 16-h day length (light intensity of 300 micromoles m^{-2} s^{-1} at 1 m height) in a reach-in controlled environment chamber. Plants were removed from the growth chamber and transferred to the greenhouse (16-h day length at 25^{o}C) until evaluation. Symptom visual evaluation was done 10 days after inoculation, using a scale from 1 to 9 (Pastor-Corrales, 1991). Plants scoring from 1 to 3 were considered resistant, whereas 4 to 9 were susceptible.

Molecular analysis was conducted with $SAS13^{950}$, once this marker was found closely linked to *Co-4*^{2}, a gene that confers resistance to 97% of the already identified *C. lindemuthianum* races (Young et al. 1998). The amplification cycles were conducted according to Williams et al. (1990).

RESULTS AND DISCUSSION

The evaluation of VCUs elite lines disease reaction on field conditions, in both Maringá and Umuarama, were as following: Guará, IPR Uirapuru, BRS MG Realce, CNFP 10104, CHC 01-175, CHP 98-66-20, TB 02-24, TB 02-07, SM 1107, LP 08-90, LP 07-80, UEM G1, UEM P1 and Tangará were resistant; meanwhile, the susceptible specimens were: Pérola and SM 1810. In

laboratorial assessment, the same cultivars had similar response, when inoculated with race 65 of *C. lindemuthianum*. Laboratorial results from inoculated plants which showed to be resistant to race 2047 of *C. lindemuthianum* were: CNFP 10104, TB 02-24, TB 02-07, SM 1810, SM 1107, LP 07-80, UEM G1, UEM P1 and Tangará; while Pérola, Guará, IPR Uirapuru, BRS MG Realce, CHC 01-175, CHP 98-66-20 and LP 08-90 were susceptible. It was observed that elite lines such as: CNFP 10104, TB 02-24, TB 02-07, SM 1107, LP 07-80, UEM G1, UEM P1 and the cultivar Tangará were resistant to both races, when inoculated. This evidence characterizes these plants as source of resistance to both races 65 and 2047 of *C. lindemuthianum*. In addition, during molecular analysis, these cultivars revealed the presence of the molecular marker $SAS13^{950}$. Meanwhile, the susceptible plants Pérola, Guará, IPR Uirapuru, BRS MG Realce, and the line CHP 98-66-20 did not present the $SAS13^{950}$ marker. The lines that showed resistance to race 2047 *C. lindemuthianum* can be traced through assisted selection, with the use of SAS13 molecular marker, once it presented 88.8% of efficiency on identification of resistant genes in cultivars. In conclusion, obtained results evidenced that CNFP 10104, TB 02-24, TB 02-07, SM 1107, LP 07-80, UEM G1, UEM P1 and Tangará cultivar can be considered as important anthracnose resistance source for future common bean breeding programs.

Table 1. Reaction of lines/cultivars of *Phaseolus vulgaris* L. to *Colletotrichum lindemuthianum* and molecular analysis

Entry	Reaction (in the field) [a, b]	Race 65	Race 2047	Molecular marker SAS13[c]
Pérola	R	5 (S)	4 (S)	-
Guará	R	1 (R)	6 (S)	-
IPR Uirapuru	R	1 (R)	5 (S)	-
BRS MG Realce	R	1 (R)	5 (S)	-
CNFP 10104	R	1 (R)	1 (R)	+
CHC 01-175	R	1 (R)	6 (S)	+
CHP 98-66-20	R	1 (R)	6 (S)	-
TB 02-24	R	1 (R)	3 (R)	+
TB 02-07	R	1 (R)	1 (R)	+
SM 1810	S	8 (S)	4 (R)	-
SM 1107	R	1 (R)	3 (R)	+
LP 08-90	R	1 (R)	5 (S)	+
LP 07-80	R	1 (R)	3 (R)	+
UEM G1	R	1 (R)	3 (R)	+
UEM P1	R	1 (R)	1 (R)	+
Tangará	R	1 (R)	1 (R)	+

R[a] = Resistant; S[b] = Susceptible; [c]Marker present (+); absent (-)

ACKNOWLEDGEMENTS

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INHERITANCE OF RESISTANCE TO RACE 73 OF ANTHRACNOSE IN THE NAVY BEAN LINE ACUG 10-1

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INTRODUCTION: Anthracnose of common bean *(Phaseolus vulgaris* L.), caused by the fungus *Colletotrichum lindemuthianum*, is a seed-borne disease of dry beans that can cause yield losses as high as 100% (Schwartz *et al*., 2005). Most commercial navy bean varieties grown in central and western Canada are susceptible to anthracnose race 73, the prevalent race in Ontario since 2003.

MATERIAL AND METHODS: 126 $F_{4:6}$ recombinant inbred lines (RILs) of ACUG 10-1 x H4784A-29844, the two parental lines, the 12-member anthracnose differential series (Dongfang *et al*., 2008), and 4 check cultivars were evaluated for resistance to anthracnose at two field sites: the Elora and Huron Research Stations (University of Guelph), in 2012. Each site was planted as hill plots in a 12 × 12 unbalanced square lattice design with two replications. Plots were inoculated by spreader rows, from seed infected with anthracnose race 73 collected at Elora in 2011, planted as borders and as every third row within the experiment. Anthracnose severity was rated from 0 to 10 based on a visual estimate of percent of pods showing symptoms, with a rating of 5 indicating that 50% of pods showed anthracnose lesions. Ratings were begun when the susceptible check had a rating of 6, and repeated 3 times every 7 days until maturity. The Area Under the Disease Progress Curve (AUDPC) was calculated based on the 4 ratings. DNA was extracted from young leaves harvested from each RIL. RILs were genotyped with a panel of 786 SNP markers developed at NRC Saskatoon and the University of Saskatchewan, from which 86 were found to be polymorphic between the parental lines and segregating in the population. A genetic linkage map was generated using JoinMap 4 (Van Ooijen, 2006) and compared with the physical map using MapChart 2.2 (Voorrips, 2002).

RESULTS: Anthracnose severity at the Elora Research Station was low, due to an unusually dry season. At the Huron Research Station, anthracnose severity ratings of susceptible lines reached 10, although that of Michelite (*Co-11*) was lower than expected for race 73. The frequency distribution of AUDPC followed a bi-modal distribution with a high frequency of lines in the two ends of the disease scale (Fig 1). Linkage maps were constructed using the polymorphic markers and single marker QTL analysis was performed to identify genomic regions associated with resistance to anthracnose in ACUG10-1 (Table 1). Six SNP markers in a 1.9 cM region were significantly associated with anthracnose AUDPC accounting for up to 54 % and 85 % of variation at the Elora and Huron Research Stations, respectively (Table 1).

DISCUSSION AND CONCLUSIONS: The significant markers are likely located on chromosome 1, due to a close linkage in another one of our mapping populations (Xie *et al*, unpublished) of these six SNP markers with the SSR BMd10, which has been mapped to Pv01 (Blair *et al*, 2003). A BLAST search of the sequences of the markers in the QTL region was performed on Pv0.9 (US Department of Energy Joint Genome Institute); the QTL region was

found to fall within a 1 Mbp region on the pseudomolecule that corresponds to Pv01. Of the previously identified anthracnose resistance genes, only *Co-1*, from the Andean gene-pool, has been reported on chromosome 1. It is therefore likely that resistance to anthracnose race 73 in ACUG 10-1 is conditioned by one of the allelic forms of *Co-1*. Lines with *Co-1*, even though resistant to race 73 of anthracnose, are susceptible to other North American anthracnose races (23 and 105) and therefore are not expected to provide durable resistance.

Table1. Summary of the results of single marker QTL analysis for anthracnose responses of the RILs in anthracnose nurseries at the Huron and Elora Research Stations in 2012.

			Huron				Elora			
Chr	cM	SNP Marker	*P-value*	R^2_p	Additive effect	Dominance effect	*P-value*	R^2_p	Additive effect	Dominance effect
1	81.5	Pv09sc00008p1341927	< 0.0001	0.74	105	81	< 0.0001	0.48	23	23
1	81.5	Pv09sc00008p1384743	< 0.0001	0.71	106	80	< 0.0001	0.48	23	23
1	81.5	Pv09sc00008p1393869	< 0.0001	0.74	106	81	< 0.0001	0.48	23	23
1	81.5	Pv09sc00008p1467830	< 0.0001	0.72	106	81	< 0.0001	0.48	23	23
1	83.4	Pv09sc00008p1574781	< 0.0001	0.85	113	88	< 0.0001	0.54	25	25
1	83.4	Pv09sc00008p1922017	< 0.0001	0.84	112	88	< 0.0001	0.54	25	25

R^2_p: the proportion of phenotypic variance accounted for by the single marker linear model; Additive effects were estimated as half of the difference between the two homozygous groups at each marker locus. Dominance effects were estimated as the deviation of heterozygotes from the mid-value between the two homozygous groups.

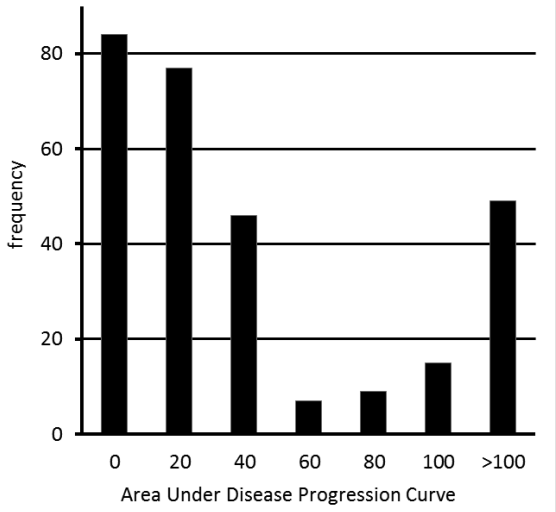

Fig 1. Frequency distribution of AUDPC for the RIL population in 2012 at the Huron Research Station

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CHARACTERIZATION OF THE ANTHRACNOSE RESISTANCE GENE IN THE MESOAMERICAN COMMON BEAN CULTIVAR CRIOULO 159

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INTRODUCTION

Anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara is among the most widespread diseases of the common bean in the tropics. This disease is widespread in Brazil and Eastern and Southern Africa, causing high crop losses of up to 100% (Pastor-Corrales and Tu 1989). The use of resistant cultivars is the most efficient method to control common bean anthracnose, and it is important a continuing search for new resistance sources. Currently, the resistance to *C. lindemuthianum* is conditioned by 19 anthracnose resistance loci identified by the Co symbol (Kelly and Vallejo 2004, Gonçalves-Vidigal et al. 2012). Previous studies carried out at Nupagri-UEM, had shown that Crioulo 159 genotype, a landrace collected in small farms in Santa Catarina State, is resistant to 2, 64, 73, and 2047 races of *C. lindemuthianum*. The present work aimed to characterize genetic resistance of Mesoamerican cultivar Crioulo 159 to races 2, 64, 73, and 2047 of *C. lindemuthianum* through resistance inheritance study and allelism tests.

MATERIAL AND METHODS

The Mesoamerican common bean cultivar Crioulo 159 was crossed with Michelite, Michigan Dark Red Kidney (MDRK), Cornell 49-242, Mexico 222, TO, TU, AB 136, G 2333, SEL 1308, AND 277, H1 Line, BAT 93, Ouro Negro, Jalo Vermelho (JV), Jalo Listras Pretas (JLP), Pitanga, Corinthiano in order to obtain F_2 populations. Parents, F_1 and F_2 of each cross, were spray-inoculated with standardized spore concentration (1.2×10^6 spores mL^{-1}), of each race of *C. lindemuthianum*, using a De Vilbiss number 15 atomizer powered by an electric compressor. After inoculation, plants were maintained at high relative humidity (>95%) for 48 h at 21-23^{o}C. The inheritance test was conducted in F_2 population from cross between Crioulo 159 × Cornell 49-242 cultivars, inoculated with 2047 race. Allelism tests were carried out in the F_2 from the crosses (R × R) where both cultivars resistance reaction to 2, 64, 73 and 2047 races, in order to evaluate the independence of the gene presented in Crioulo 159 cultivar from the other previously characterized. Symptom visual evaluation was done 10 days after inoculation, using a scale from 1 to 9 (Pastor-Corrales, 1991). Plants scoring from 1 to 3 were considered resistant, whereas 4 to 9 were susceptible. Genetic analyses of F_2 population were done by using Chi-Square test (χ^2).

RESULTS AND DISCUSSION

The inheritance study demonstrated a 3R:1S ratio in F_2 population from the cross between Crioulo 159 × Cornell 49-242 cultivars, inoculated with 2047 race. This fact indicates the presence of one resistant dominant gene in Mesoamerican cultivar Crioulo 159. Allelism tests in the crosses involving Crioulo 159 with Michelite, Michigan Dark Red Kidney (MDRK), Cornell 49-242, TO, TU, AB 136, G 2333, Jalo Listras Pretas (JLP), Jalo Vermelho, BAT 93, Ouro Negro, AND 277, H1 Line, Pitanga, Corinthiano and SEL 1308 cultivars fitted a 15R:1S ratio, indicating the action of two dominant genes, one of them present in Crioulo 159 cultivar and the other in each one of the tested cultivars. On the other hand, a segregation ratio of 63R:1S was

observed in the cross of Crioulo 159/Mexico 222, which indicates the action of three dominant genes that confer resistance to *C. lindemuthianum*. One of these genes is present in Crioulo 159 and the other two are present in Mexico 222 (Table 1).

Table 1. Allelism tests in F_2 populations from R x R crosses inoculated with races 2, 64, 73 and 2047 of *Colletotrichum lindemuthianum*

Cross	Race	Resistance Gene	Observed Plants		Expected Ratio	χ^2	P-Value
			R[a]	S[b]	R:S		
Crioulo 159 × Cornell 49-242	2	*Co-2*	94	6	15:1	0.011	0.92
Crioulo 159 × Mexico 222	2	*Co-3*	108	2	63:1	0.047	0.83
Crioulo 159 × H1 line	2	*Co-7*	95	5	15 : 1	0.266	0.61
Crioulo 159 × Michelite	2	*Co-11*	93	7	15 : 1	0.096	0.76
Crioulo 159 × Ouro Negro	64	*Co-10*	93	7	15 : 1	0.096	0.76
Crioulo 159 × Jalo Vermelho	64	*Co-12*	93	7	15 : 1	0.096	0.76
Crioulo 159 × JLP	64	*Co-13*	94	6	15 : 1	0.011	0.92
Crioulo 159 × MDRK	73	*Co-1*	94	6	15 : 1	0.011	0.92
Crioulo 159 × TO	73	*Co-4*	94	6	15 : 1	0.011	0.92
Crioulo 159 × SEL 1308	73	*Co-4*^{2}	94	6	15 : 1	0.011	0.92
Crioulo 159 × TU	73	*Co-5*	95	5	15 : 1	0.266	0.61
Crioulo 159 × AB 136	73	*Co-6*	92	8	15 : 1	0.522	0.47
Crioulo 159 × BAT 93	73	*Co-9*	92	8	15 : 1	0.522	0.47
Crioulo 159 × AND 277	2047	*Co-1*^{4}	93	7	15 : 1	0.096	0.76
Crioulo 159 × G 2333	2047	*Co-4*^{2}	269	21	15 : 1	0.486	0.49
Crioulo 159 × Pitanga	2047	*Co-14*	94	6	15 : 1	0.011	0.92
Crioulo 159 × Corinthiano	2047	*Co-15*	88	8	15 : 1	0.711	0.40

R[a] = Resistant; S[b] = Susceptible; JLP = Jalo Listras Pretas; MDRK = Michigan Dark Red Kidney.

CONCLUSION: It is concluded that Crioulo 159 cultivar has a dominant resistant gene through inheritance and allelism tests and this gene is independent from those genes previously characterized. Thus, the authors propose the symbol *Co-16* to named the referred gene present in Crioulo 159 cultivar. The identification of cultivar Crioulo 159 as a new source of resistance to race the 2047 of *C. lindemuthianum* is very important for common bean breeding programs due to the presence of a new dominant Mesoamerican resistant gene to race with high virulence.

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MOLECULAR MAPPING OF THE ANTHRACNOSE RESISTANCE GENE *CO-15* IN THE COMMON BEAN CULTIVAR, CORINTHIANO

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INTRODUCTION

Anthracnose, caused by the fungus *Colletotrichum lindemuthianum* is one of the most important diseases affecting beans that reduces crop yields and depreciates the quality of the seed product (Pastor-Corrales and Tu 1989). One of the most efficient and economic alternatives to control this disease is the use of resistant cultivars (Pastor-Corrales et al. 1995). Therefore, the characterization of new germplasm sources and genes involved in the process of resistance has been studied repeatedly. Previous studies conducted by Gonçalves et al. (2010) revealed that Andean genotype Corinthiano carries the *Co-15* gene, which conditions resistant to races 2, 8, 23, 64, 65, 89, 73 and 2047 of *C. lindemuthianum*. Thus, the objective of this study was to identify molecular markers linked to *Co-15* resistance gene present in the Corinthiano cultivar.

MATERIALS AND METHODS

The evaluations were conducted in laboratories and greenhouses at the at the Núcleo de Pesquisas Aplicadas à Agricultura (Nupagri), Universidade Estadual de Maringa (UEM), Brazil. The molecular analysis were carried out at the Department of Plant, Soil and Microbial Sciences at Michigan State University (MSU), USA, in the period between November 2011 and September 2012. The Corinthiano cultivar, resistant to race 2047 of *C. lindemuthianum*, was crossed with Cornell 49-242 (susceptible parent) in order to obtain the F_1 and the F_2 seeds. The parents, F_1 generation and 86 F_2 plants were spray-inoculated with standardized spore concentration (1.2×10^6 spores mL^{-1}) of 2047 race, using a De Vilbiss number 15 atomizer powered by an electric compressor. After inoculation, plants were maintained at high relative humidity (>95%) for 48 h at 21-23^{o}C. The disease reaction was performed according to a scale from 1 to 9, where 1-3 considered resistant plants, 4-9 considered susceptible plants (Pastor-Corrales et al. 1995). After the evaluation, each resistant F_2 plant was transplanted to obtain the $F_{2:3}$ families. Additionally, this test was performed with the data for the 69 $F_{2:3}$ families from Corinthiano × Cornell 49-242 cross according to a segregation hypothesis of 1:2:1 (RR : Rr : rr).

These families were analyzed to construct two homozygous contrasting resistant and susceptible DNA bulks based on virulence data obtained (Michelmore et al. 1991). Equal volumes of DNA from five homozygous resistant and five susceptible F_2 plants were used. A total of 350 molecular markers were tested with contrasting amplification patterns in parental materials and in the resistant vs. susceptible bulks and individuals from the bulks. A goodness-of-fit test for a 1:1 segregation ratio was performed for the segregation of the g2685 marker in the Bat/Jalo bean consensus mapping population.

Linkage analyses were performed using the computer software Mapmaker/EXP 3.0 (Lincoln and Lander 1993). Linkage group nomenclature follows Pedrosa-Harand et al. (2008) and the map was drawn using the computer software MapChart (Voorrips 2002).

RESULTS AND DISCUSSION

A segregation of 69 resistant and 17 susceptible individuals in the F_2 population fitted to the expected 3R:1S ratio (p = 0.4959). This segregation data confirmed the monogenic resistance in the Corinthiano cultivar to race 2047 of *C. lindemuthianum* carried out by Gonçalves et al. (2010). These 69 $F_{2:3}$ families inoculated with race 2047 segregated in classes as follows: 23 RR : 45 Rr : 18 rr (p = 0.68). The linkage analysis revealed that the $g2685^{150}$ marker was linked to the *Co-15* gene at a distance of 5.6 cM on Pv04 (Figure 1), when tested in the F_2 individuals from the Corinthiano × Cornell 49-242 cross. The molecular marker $g2685^{150}$ previously mapped to chromosome Pv04 (McConnell et al. 2010) was tested in the BAT 93/Jalo EEP 558 (BJ) RI population, resulted in segregation of 27(+):34(-) (χ^2 = 0.80; p =0.37) for a good fit to a 1:1 ratio. Corinthiano has been shown to be an important source of resistance to anthracnose, and the fact that *Co-15* gene is an Andean gene reinforces its use in common bean breeding programs where there is a lack of effective Andean resistance genes for anthracnose.

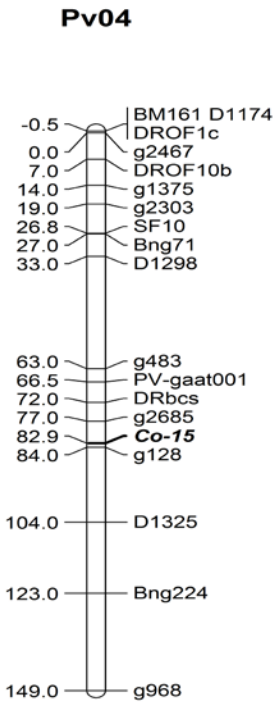

Figure 1. Genetic distance and location of the *Co-15* locus for resistance to common bean anthracnose, and molecular marker $g2685^{150}$ on chromosome Pv04 of *Phaseolus vulgaris* L., using the population from the Corinthiano × Cornell 49-242 cross. The map was drawn with MapChart (Voorrips 2002).

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USE OF MUTI SITE SCREENING TO IDENTIFY AND VERIFY PARTIAL RESISTANCE TO WHITE MOLD IN COMMON BEAN IN 2012

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Data also from H. Schwartz (CO), S. Singh (ID), J. Kelly (MI), M. Wunch (ND), P. Griffiths (NY), J. Myers (OR), P. Miklas (WA), K. Kmiecik (WI), and John Theuws (BEL)

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/pathologists from across the USA with broad and/or specific partial resistance to WM.

Breeders sent seed of 27 bean lines with putative sources of resistance to our laboratory where the seeds were divided in equal amounts for field (400g/line) and/or greenhouse (25 seeds/ line) tests and then sent to nine locations to be evaluated by standardized greenhouse and/or field screening methods. Three bean lines were included in both tests as controls: partially resistant G122, Bunsi with field avoidance and susceptible GN Beryl.

The field tests consisted of two rows of each of the 12 entries and one row of a local semi vine WM susceptible genotype, resulting in a three-row plot 4.6 m (15 ft) long replicated three times in a randomized complete block design. There were six field tests conducted in six locations. The field nurseries were all evaluated using a CIAT 1 to 9 scale (1 = no visible symptoms to 9 = death) (Van Schoonhoven et al., 1987). Nebraska, North Dakota and Wisconsin did not have results due to weather. These problems that resulted in no data were disappointing, but demonstrate the importance of testing in multiple locations. In the field tests, all 9 lines were significantly more resistant than Beryl (Table 1). The field results of the three fields that reported disease support 2 bean lines, A195 and VRW32, having resistance similar to G122 (the resistant check). The remaining 7 lines were rated from 2.9 to 5.3.

The greenhouse trials tested 27 entries, plus 3 controls, using the straw test to inoculate 21- to 28-day-old plants. The plants were innoculated 2.5 cm above the fourth node with a plug of PDA media containing young *S. sclerotiorum* mycelia pressed into a 2.5 cm clear drinking straw sealed at one end and fitted over the cut internode. The infected plants were evaluated 8 days later using the modified Petzoldt and Dickson scale (Teran et al, 2006). The greenhouse results (Table 2) support 11 bean lines with resistance similar to G122. There were four lines that showed intermediate resistance in the field and susceptible in the greenhouse test. These lines exhibited escape or avoidance mechanisms. A navy, small red and pinto exhibited resistance in the field and greenhouse. Pinto, small red, great northern and cranberry seed classes showed resistance in the field and in the greenhouse. Progress in incorporating WM resistance into dry bean lines with commercial potential validates use of multisite screening and National Sclerotinia Initiative support over the last 10 years.

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

ENTRY	SEED CLASS	COLLABORATOR	MI	OR	WA	Mean	t Grouping
BERYL	G. NORTHERN	Susceptible Check	9.0	9.0	7.5	8.5	A
EXRICO	NAVY	Intermediate Check	6.7	4.5	5.3	5.5	B
ND060514	NAVY	J. Osorno - ND	4.8	5.0	6.0	5.3	B C
ND080547	SMALL RED	J. Osorno - ND	5.2	5.2	5.0	5.1	B C D
USPTWM12	PINTO	P. Miklas- WA	4.4	5.5	5.0	5.0	B C D E
S08418	PINK	J. Kelly - MI	3.7	4.8	5.3	4.6	B C D E
Z0726974	PINTO	P. Miklas- WA	3.7	4.0	4.0	3.9	C D E F
P07863	PINTO	J. Kelly - MI	2.2	4.8	4.3	3.8	D E F
PS02028A3B2	G. NORTHERN	P. Miklas- WA	4.4	2.7	3.5	3.5	E F G
VRW32	SM GREY-BROWN	S. Singh- ID	3.0	2.7	3.0	2.9	F G H
G122	CRAN	Resistant Check	3.7	1.2	2.0	2.3	G H
A195	LG CREAM	S. Singh- ID	1.9	1.0	3.0	2.0	H

*CIAT Scale: 1 = no disease, 9 = plants dead **Alpha = 0.05, LSD = 1.44
ND, NE and WI had no data from field due to weather

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

ENTRY	SEED CLASS	COLLABORATOR	CO	BEL	MI	NE	NY	OR	WI	WA	ID	Mean	t Grouping
BERYL	G. NORTHERN	Susceptible Check	7.8	6.3	7.7	9.0	6.3	8.5	7.6	5.3	6.7	7.2	A
PS06-026-1-4-5-B3	PINTO	P. Miklas- WA	3.1	2.5	8.1	9.0	8.0	9.0	7.9	7.8	8.7	7.1	A
NE1-11-15	G. NORTHERN	C. Urrea- NE	6.0	7.0	6.3	7.8	7.6	9.0	5.0	6.9	6.0	6.8	A B
P07863 (El Dor.)	PINTO	J. Kelly - MI	5.1	3.0	6.7	9.0	6.2	9.0	8.0	5.3	8.0	6.7	A B C
S08418 (Rosetta)	PINK	J. Kelly - MI	5.5	4.8	7.4	9.0	3.7	9.0	6.2	5.8	7.6	6.6	A B C D
PS08-040B-2-B2	PINTO	P. Miklas- WA	4.3	4.3	7.7	8.8	7.4	8.8	6.7	5.4	5.4	6.5	A B C D E
PS08-040B-6-B2	PINTO	P. Miklas- WA	4.0	3.0	6.8	8.2	7.1	9.0	7.7	6.8	5.7	6.5	A B C D E
PS08-039A-4-B2	PINTO	P. Miklas- WA	4.3	4.5	6.1	9.0	5.5	8.8	5.8	5.5	6.2	6.2	A B C D E F
PS06-026-1-6-1-B2	PINTO	P. Miklas- WA	3.0	3.0	6.8	9.0	4.1	8.5	5.8	6.9	8.5	6.2	A B C D E F
PS03-038-4-B3-B6	G. NORTHERN	P. Miklas- WA	2.4	5.7	5.2	9.0	6.3	8.4	5.9	6.8	5.8	6.2	A B C D E F
Z0726-9-74	PINTO	P. Miklas- WA	4.5	4.0	6.0	9.0	5.4	8.9	5.0	4.8	7.3	6.1	A B C D E F
EXRICO (BUNSI)	NAVY	Intermediate Check	3.4	7.0	5.1	9.0	4.5	9.0	5.5	3.4	7.7	6.1	A B C D E F
PS02-028A-6-B2	G. NORTHERN	P. Miklas- WA	3.8	5.3	6.2	8.6	5.1	6.7	5.5	6.5	6.0	6.0	A B C D E F
VRW 32	SM GREYISH-BROWN	S. Singh- ID	3.4	5.3	5.3	9.0	5.5	8.1	5.2	5.3	5.0	5.8	A B C D E F G
PS02-028A-3-B2	G. NORTHERN	P. Miklas- WA	4.7	4.3	4.7	8.0	6.4	7.3	5.2	5.3	5.9	5.8	A B C D E F G
PS08-039A-5-B2	PINTO	P. Miklas- WA	2.7	3.8	7.3	6.2	6.4	8.8	5.7	4.6	6.2	5.7	A B C D E F G
NE2-11-13	PINTO	C. Urrea- NE	3.8	3.0	5.8	7.8	5.6	6.8	6.1	5.2	7.4	5.7	A B C D E F G
NE14-11-8	SMALL RED	C. Urrea- NE	5.4	3.0	6.4	6.8	4.5	7.9	3.9	5.6	5.6	5.5	B C D E F G H
COWM 16099	PINTO	H. Schwartz- CO	4.3	5.0	5.4	8.5	5.0	5.2	4.7	4.5	6.3	5.4	B C D E F G H
ND060514	NAVY	J. Osorno - ND	4.0	4.7	4.8	7.8	5.3	6.3	4.5	4.8	4.7	5.2	C D E F G H I
PS08-021C-4-B2	PINTO	P. Miklas- WA	2.8	2.8	5.0	7.8	4.6	6.8	4.7	4.3	6.8	5.1	D E F G H I
ND080547	SMALL RED	J. Osorno - ND	5.6	2.3	4.7	5.0	5.1	8.2	4.2	3.9	5.8	5.0	E F G H I
COWM 16079	PINTO	H. Schwartz- CO	5.3	2.3	5.8	3.6	5.5	6.9	4.1	4.3	5.7	4.8	F G H I
PS08-031A-11-B2	G. NORTHERN	P. Miklas- WA	4.4	2.0	4.9	6.8	5.2	4.1	5.3	4.4	6.2	4.8	F G H I
COWM 16100	PINTO	H. Schwartz- CO	1.5	3.7	7.5	6.0	4.7	8.0	3.9	3.8	4.0	4.8	F G H I
PS08-031A-8-B2	G. NORTHERN	P. Miklas- WA	4.4	5.3	5.1	7.0	4.0	6.3	4.8	.	4.8	4.6	F G H I
USPT-WM-12	PINTO	P. Miklas- WA	3.5	2.5	3.8	5.6	3.6	5.8	5.3	4.8	4.3	4.4	G H I
NE10-11-16	CRAN	C. Urrea- NE	3.0	1.5	3.9	4.0	2.6	5.7	5.0	5.0	5.0	4.0	H I
G122	CRAN	Resistant Check	4.9	1.0	4.4	4.8	4.0	5.6	1.6	4.7	4.3	3.9	H I
A195	LG CREAM	S. Singh- ID	5.0	2.0	3.9	3.3	2.6	4.3	5.0	4.5	4.2	3.9	I

*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 = 1.6

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PHYSIOLOGICAL RESISTANCE TO *SCLEROTINIA SCLEROTIORUM* IN COMMON BEAN LINES IN THE STATE OF MINAS GERAIS, BRAZIL

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INTRODUCTION

White mold caused by *Sclerotinia sclerotiorum* is the most serious disease of common bean (*Phaseolus vulgaris*) in the irrigated areas of the State of Minas Gerais (MG), Brazil. The moderate temperatures (15-22°C) during the fall-winter season favor the development and spreading of the disease. Commercial cultivars are generally susceptible. However, many lines of the MG common bean breeding program (Federal Universities of Lavras and Viçosa, Epamig and Embrapa Rice and Beans) have consistently shown lower symptoms in field compared to the commercial cultivars. The development of bean cultivars combining physiological resistance and architectural avoidance to white mold could be a strategy to reduce disease and improve yield. The purpose of this work was to identify physiologically resistant genotypes to *S. sclerotiorum* among advanced lines and cultivars of the MG bean breeding program.

MATERIALS AND METHODS

Fifteen bean genotypes with partial field resistance to white mold were tested in two greenhouse experiments. Four cultivars (BRSMG Majestoso, Ouro Negro, Ouro Vermelho, and Pérola) were used as control as well as the line A 195, which is known for its resistance (Singh et al., 2007). Plants were grown in 3.0 L-pots. Each plot was a pot with three plants. Treatments were replicated four times in a randomized block design. In the first experiment, plants were inoculated with an isolate of *S. sclerotiorum* from Oratórios, Zona da Mata of MG; in the second, with an isolate from Unaí, Northeast of MG. Inoculation was done according to Petzoldt & Dickson (1996). Length of lesions was measured and plants were rated for disease severity at seven days after inoculation, using the 1-9 scale of Terán et al. (2006), in which 1 represents plants with no symptoms and 9, stem/branch invasion of the third internode > 1 inch leading to plant death.

RESULTS AND DISCUSSION

The bean genotypes were separated into three (isolate of *S. sclerotiorum* from Oratórios) or two (isolate from Unaí) groups based on disease severity and in two groups based on lesion length (Table 1). Sources of physiological resistance to white mold are present in the genotypes. Severity and lesion size of BRSMG Majestoso, CAL 96, Ouro Branco, Ouro Negro, Ouro Vermelho, and VP-21 were similar to those of A 195. BRSMG Majestoso, Ouro Negro, and Ouro Vermelho have type III growth habit, which favor the white mold development in the field, as observed by Vieira et al. (2012). On the other hand, CAL 96, Ouro Branco, VP-21, and A 195, have type I growth habit, which might contribute to reduce the disease in the field. Our results indicate that some genotypes of the MG common bean breeding program have both physiological resistance to white mold and architectural characteristics that could be combined in order to reduce the disease in field.

Table 1 – Effects of common bean genotypes on white mold symptoms assessed seven days after inoculation of plants with two isolates of *Sclerotinia sclerotiorum*

Genotype	Plant type	Isolate from Oratórios		Isolate from Unaí	
		Severity	Lesion length	Severity	Lesion length
RP-1	II	8.2 a	7.7 a	7.1 a	5.9 b
CNFC 10720	II	7.6 a	8.3 a	8.0 a	7.4 a
BRS Estilo	II	7.5 a	9.3 a	7.2 a	6.7 a
CNFP 11980	II	7.2 a	9.2 a	7.2 a	7.0 a
CNFC 10432	II	7.2 a	7.7 a	6.6 a	5.8 b
CNFC 11965	II	7.1 a	7.6 a	6.9 a	6.9 a
BRS Vereda	III	6.9 a	7.1 a	6.5 a	5.0 b
CNFC 10722	II	6.3 a	6.2 b	6.5 a	5.0 b
VC 17	III	6.2 b	7.9 a	6.9 a	7.4 a
CNFP 10798	II	6.1 b	6.7 a	6.6 a	6.8 a
CNFP 11990	II	5.4 b	6.7 a	7. 1 a	7.2 a
BRS Executive	III	5.2 c	8.6 a	6.8 a	9.3 a
VP 21	II	4.9 c	5.2 b	6.5 a	6.2 b
Ouro Branco	I	4.7 c	5.3 b	4.7 b	4.9 b
Cal 96	I	4.2 c	4.0 b	4.7 b	4.8 b
BRSMG Majestoso	III	5.7 b	6.8 a	5.5 b	5.6 b
Ouro Negro	III	5.2 c	5.9 b	5.2 b	5.7 b
Ouro Vermelho	III	4.6 c	4.5 b	5.5 b	5.4 b
Pérola	III	6.0 b	7.4 a	6.8 a	7.6 a
A 195	I	4.5 c	4.3 b	4.9 b	5.6 b

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

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IDENTIFICATION AND CHARACTERIZATION OF NEW SOURCES OF RESISTANCE TO WHITE MOLD IN DRY BEANS

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INTRODUCTION

White mold is caused by the necrotrophic fungus *Sclerotinia sclerotiorum* Lib.de Bary is a major constraint to dry bean (*Phaseolus vulgaris* L.). This fungus is ranked as the main disease problem causing severe yield losses in North Dakota and Minnesota, which are ranked as top producers in the US. The use genetic resistance is one of the most effective and cost- efficient methods to control the disease.

Two breeding lines ND060514 (navy) and ND080547 (small red) from the North Dakota State University dry bean breeding program were identified as potential sources of resistance to white mold. These lines are at advanced breeding stages and could be released in the near future given its competitive yield and good agronomic performance.

The objectives of this project were to study the potential source of resistance to white mold in these lines, by screening them at the molecular level with some known molecular markers, associated with resistance to white mold in common bean and to evaluate if the gene(s) are different and unique when compared with sources previously reported.

MATERIALS AND METHODS

DNA was extracted from both resistant lines along with some other resistant and susceptible genotypes, from leaf tissue by using CTAB method. Screening of these lines at the molecular level was done with 17 RAPD (Random Amplification of polymorphism DNA), 2 SRAP (Sequence-related amplified polymorphism), 6 SCAR (Sequenced Characterized Amplified Region) and 7 SSR (Simple Sequence Repeats) markers, linked to loci for resistance. To amplify these markers, PCR was performed following the protocol by Soule et al. (2011).

RESULTS AND DISCUSSION

Preliminary results showed that at least 9 QTL's (WM2.2, WM4.2, WM5.3, WM5.4, WM6.1, WM8. 2, WM 8.3, WM8.4 and WM9.1) could be positively associated with resistance in breeding lines ND060514 and ND080547. Out of total 17 makers, 11 markers (5 RAPD, 3 SCAR, and 3 SSR) showed polymorphism when screened. In addition, pedigrees of these two lines also suggest that the resistance found in ND060514 may come from ICA-Bunsi while resistance in ND080547 is still not clear.

The present study confirmed the levels of resistance observed in these breeding lines. Nonetheless, these lines and tagged QTL's could be useful in breeding programs and for understanding the source of resistance in the lines and selection of beneficial alleles at these loci for improvement of resistance. Since resistance in breeding line ND080547 remains unclear based on the pedigree and marker information, development of mapping populations is underway

Table 1. Screening of two lines (ND060514 and ND080547) along with resistant and susceptible checks, with 11 molecular markers linked to loci for resistance to white mold.

QTL	Pop.	Primer	A55	G122	Bunsi	Newport	Huron	Aztec	CO72548	Va19	I9365-31	Vax 3	ND060514	ND080547	115M	Vax5	Othello	Xan 159	USWK CBB-17	NY 6020-4	Benton
WM8.4	BR	L04.1	+	0	+*	0	+	+	0	0	0	+	+	+	0	+	0	+	0	0	+
WM2.2	HN/BN	BC20.1800	+	0	+*	-	-	-	0	0	0	-	0	-	0	-	0	-	0	0	-
WM8.2	PX	AH05.1	0	0	+	0	+	+	0	0	0	+	+	0	0	+	0	+	0	0	+
WM5.1	PX	D05.1100	0	0	+	0	+	-	0	0	0	+	+	0	0	-	0	+	+	-	-
WM8.3	BV	SF13R10.410	0	+	0	0	0	0	0	+*	+	0	0	0	0	0	0	0	+	0	+
WM2.2	BV	SMe1Em5.110	-	+	-	-	-	+	+	+*	+	-	-	-	+	-	+	+	+	-	+
WM2.2	BV	SF6Em3.220	+	+	+	+	+	+	+	-	+	+	+	+	_-	-	+	+	+	+	+*
WM9.1	GC	BM154	+	-	+	+	-	-	-	-	-	+	+	+	+	+	-	-	-	0	-
WM5.4	R31	H19.725	+	+	-	-	-	-	-	+	+*	-	-	-	0	-	-	+	0	0	+
WM5.3	R31	BM138	+	+	+	+	+	+	+	+	+*	+	+	+	+	-	+	+	+	+	+
WM4.2	R31	BMd-15	-	+	+	+	+	-	-	-	+*	-	-	-	-	-	-	+	+	+	+

- A plus sign ("+" / dark color) indicates presence of the marker and minus sign ("–"/ light grey color) indicates absence. A "0" Indicates no band.
- A "*" sign indicates the parental alleles.
- The population in which QTL have been identified are abbreviated PX = PC-50/XAN-159, BN = Bunsi/Newport and HN = Huron/Newport, BR = Bunsi/Raven, GC = G122/CO72548, and BV = Benton/VA19 and R31 = Raven/I9365-31.

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MYCELIAL COMPATIBILITY GROUPING AND AGGRESSIVENESS OF *SCLEROTINIA SCLEROTIORUM* ISOLATES FROM FOUR STATES OF BRAZIL

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INTRODUCTION

Greenhouse resistance screening tests have been conducted in Brazil to evaluate the physiological resistance of common bean genotypes to white mold (WM). Inoculations made with one or few isolates of *Sclerotinia sclerotiorum* may not represent the existing pathogenic variability in field populations. Therefore, it is necessary to study the variability of the pathogen population, mainly regarding to genotypic and pathogenic (aggressiveness) characteristics. The purpose of this work was to characterize the vegetative compatibility and aggressiveness of isolates of *S. sclerotiorum* collected in four Brazilian states.

MATERIALS AND METHODS

Twenty isolates of *S. sclerotiorum* collected from common bean fields in Minas Gerais, São Paulo, Espírito Santo and Paraná were tested. Vegetative compatibility was evaluated according to Schafer & Kohn (2006). The isolates of *S. sclerotiorum* were paired in Petri plates (60 x 15 mm) containing BDA added with 75 μL L^{-1} of McCormick's red food coloring. Disks (5-mm-diameter) from colonies grown on BDA for two days at 23ºC were distributed in a Petri plate, with a total of four pairings per plate. The plates were incubated at 23°C in the darkness. Vegetative compatibility was checked visually after three and six days of incubation. Each pairing was replicated at least twice. When the results were inconsistent, the pairing was replicated twice again. Isolates were paired in all-pairwise combinations. Compatible isolates were distinguished by the fusion of mycelia, without an accumulation of red dye in the fusion zone. The straw test (Petzoldt & Dickson, 1996) was used to characterize the isolates according to their aggressiveness. Two experiments were carried out in greenhouse with the cultivar Pérola (susceptible to WM) and the line A 195 (partially resistant). Plants were grown in 3.0 L-pots. Each plot was a pot with three plants. Treatments were replicated four times in a randomized block design. The WM reaction was scored at seven days after the inoculation using the 1-9 scale of Terán et al. (2006), in which 1 represents plants with no symptoms and 9, stem/branch invasion of the third internode > 1 inch leading to plant death.

RESULTS AND DISCUSSION

All 20 self-pairings were compatible and nine mycelial compatibility groups (MCGs) were identified (Table 1). All isolates from Zona da Mata, one from São Paulo and one from Espírito Santo were assigned to MCG1. Four MCGs were composed of a single isolate and two MCGs were composed of isolates collected in different regions. The mean severity score was 3.9 for A 195 and 6.5 for Pérola (Table 1), which confirms the potential of A 195 as a source of physiological resistance to WM. The isolates collected in the Northwest region of Minas Gerais and the isolates 23 and 217 were the most aggressive. Studies have been conducted with more

isolates and including other regions in order to characterize the population of *S. sclerotiorum* in Brazil.

Table 1 – Mean severity scores of white mold on common bean plants at seven days after inoculation and mycelial compatibility groups (MCG) of 20 *Sclerotinia sclerotiorum* isolates.

Isolate	State (region)	Severity (Pérola)	Severity (A195)	MCG
2	Minas Gerais (Zona da Mata)	5.7 b	2.4 b	1
8	Minas Gerais (Zona da Mata)	6.2 b	3.3 b	1
19	Minas Gerais (Zona da Mata)	6.6 b	3.7 b	1
23	Minas Gerais (Zona da Mata)	7.0 a	4.2 a	1
51	Minas Gerais (Northwest)	7.8 a	4.1 a	3
56	Minas Gerais (Northwest)	7.0 a	4.5 a	3
70	Minas Gerais (Northwest)	6.7 a	4.2 a	2
79	Minas Gerais (Northwest)	7.3 a	4.4 a	2
136	São Paulo	5.8 b	4.4 a	4
138	São Paulo	6.1 b	3.4 b	8
141	São Paulo	6.6 b	4.2 a	1
217	São Paulo	7.7 a	5.0 a	4
165	Espírito Santo	6.3 b	4.6 a	2
166	Espírito Santo	6.6 b	3.2 b	2
173	Espírito Santo	6.1 b	3.9 a	6
174	Espírito Santo	6.6 b	4.4 a	1
193	Paraná	5.7 b	3.4 b	7
196	Paraná	6.1 b	3.5 b	5
203	Paraná	6.1 b	4.2 a	9
204	Paraná	5.4 b	4.6 a	5
	Mean	6.5	3.9	

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

ACKNOWLEDGMENTS

Capes, CNPq and Fapemig for financial support.

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IDENTIFYING SOURCES OF BACTERIAL BROWN SPOT IN COMMON BEANS

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INTRODUCTION

Bacterial brown spot caused by *Pseudomonas syringae* pv. *syringae* was first observed from western Nebraska dry bean fields on a limited basis throughout the late 1960s. Epidemics still occur sporadically, but the pathogen's presence has been increasing in incidence and damage during the past 20 years in the Central High Plains. When the disease occurs today, it can be very damaging due to the lack of resistance in modern commercial varieties. Due to a high incidence of brown spot in Nebraska production during 2009 (almost 15% of the samples from a survey), there is a renewed interest and need for developing new varieties with resistance to brown spot. Bacterial brown spot is subject to phytosanitary regulations for some countries that affects the seed movement between countries. The main goal of this project is to identify sources of bacterial brown resistance in both US and CIAT's (International Center for Tropical Agriculture) dry bean core collections and to transfer that resistance into Nebraska elite dry bean lines.

MATERIALS AND METHODS

A protocol to screen bacterial brown spot was developed. Six bacterial brown spot isolates (GN2, 82 JL, 9907, PS 10, PSM 5, and PS 18) were screened in concentrations of 1.5 and 3.0 x 10^8 cfu ml^{-1} using Neb. Sel.1 # 27 and Orion as resistant and susceptible checks, respectively. One leaflet of the first expanded trifoliolate leaf of each plant was inoculated using the multiple needle method (Andrus, 1948 and Zapata et al., 1985). Two plants per accessions were planted and inoculated. The plants were evaluated 7 and 14 days after inoculation using a 1-9 scale, where 1= immune and 9= highly susceptible (CIAT, 1987). Reactions from 1 to 4 were considered resistant and from 5 to 9 were susceptible (Figure 1). This experiment was replicated twice.

The isolate PSM 5 was utilized in a concentration of 1.5 x 10^8 cfu ml^{-1} caused infection enabling us to differentiate between the resistant and the susceptible parents. This isolate was selected to screen both US and CIAT's Core Collections. The entire US Dry Bean Core Collection (424 accessions) was screened with the PSM 5 Bacterial Brown Spot isolate. About 1,353 accessions from CIAT's Core Collection were screened with the PSM 5 Bacterial Brown Spot isolate.

Koch's postulates were verified by re-isolation of the pathogen from symptomatic plants.
A total of (178) accessions showing resistance (disease ratings from 1 to 3) from both core collections are being inoculated in 2013 with the PSM 5 isolate, and the resistant accessions will be inoculated to the other five bacterial brown isolates GN2, 82 JL, 9907, PS 10, and PS 18 in a concentration of 1.5 x 10^8 cfu ml^{-1}.

RESULTS AND CONCLUSION

A protocol for screening bacterial brown spot was developed. Both U.S. and CIAT's dry bean core collections were screened to the PSM 5 isolate. From the U.S. Core Collection, 11.8% (50), 16% (68), and 72.2% (306) showed resistance, intermediate, and susceptible reaction to the bacterial brown isolate PSM 5, respectively. From CIAT Core Collection, 9.5% (128), 20.5% (277), and 70.0% (948) showed resistance, intermediate, and susceptible reaction to the bacterial brown isolate PSM 5, respectively.

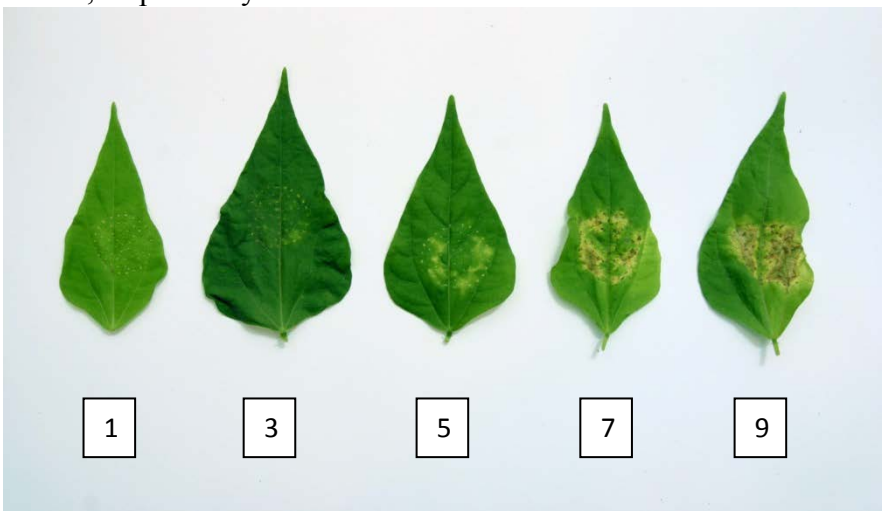

Figure 1. Reactions from 1to 3 were considered resistant, from 4 to 6 intermediate, and from 5 to 9 were susceptible.

ACKNOWLEDGEMENTS

Financial support of the Nebraska Department of Agriculture is highly appreciated.

ENDORNAVIRUSES RECURRENTLY DETECTED ON MESOAMERICAN BUT NOT IN ANDEAN BEAN CULTIVARS

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INTRODUCTION

Endornaviruses are double-stranded RNA (dsRNA) viruses of the family *Endornaviridae* that range from 10 to 17 kbp; infect plants, fungi, and oomycetes. They are transmitted only via gametes and do not cause apparent symptoms. Endoranviruses infect economically important crops, such as barley, pepper, common bean, rice, and some plant pathogenic fungi. Recently, we discovered that Black Turtle Soup and other common bean cultivars were infected by two distinct endornaviruses which we have been designated as *Phaseolus vulgaris* endornavirus 1 (PvEV-1) with a genome of 14 kbp and *Phaseolus vulgaris* endornavirus 2 (PvEV-2) with a genome of 15 kbp (1). The main objective of this investigation was to determine the occurrence of PvEV-1 and PvEV-2 in common bean genotypes of Mesoamerican and Andean origin.

MATERIALS AND METHODS

In this study we analyzed of 113 common bean genotypes that were grown in a greenhouse in Baton Rouge, Louisiana State University. Three grams of foliar tissues from 2- to 3-week-old plants were collected, and used for dsRNA extractions. DsRNA was extracted by phenol and CF-11 chromatography as described by Valverde et al (2). Aliquots of dsRNA samples were electrophoresed in 0.75 % agarose gels for 16 h at 30 V. Presence of dsRNAs was determined by ethidium bromide staining and visualization under UV light. Other dsRNA aliquots were used in reverse transcription-PCR (RT-PCR). A duplex, single tube RT-PCR for the simultaneous and discriminatory detection of both PvEV-1 and PvEV-2 developed in previous research (1) was used to ascertain the nature of dsRNA bands observed in gels after electrophoresis.

RESULTS

Our results indicated that 75 of 113 common bean cultivars tested contained Endornaviruses or putative endornaviruses. See Table 1 showing the results on selected cultivars. Endornaviruses were almost universally present in common bean cultivars of Mesoamerican origin. In contrast, most cultivars of Andean origin were virtually free of these endornaviruses. All detected 14 kbp dsRNAs in all 68 common bean genotypes (66 Mesoamerican and 2 Andean) were confirmed to be the genome of PvEV-1 by RT-PCR. However, not all tested common beans cultivars with a 15 kbp dsRNA yielded the expected RT-PCR amplicon. Some of them tested negative and in some cases the presence of PvEV-2 could not be confirmed. Endornaviruses are transmitted at high percentages through both gametes and their source in some crop cultivars have been infected cultivars used during the breeding process. Thus, the exceptions we found here are most

likely the product of introgressions of gene pools by current plant breeding activities. At present, we do not know the effect that these viruses have in common bean.

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Table 1. Common bean cultivars lines tested for PvEV-1 and PvEV-2 by gel electrophoresis.

Common Bean Cultivar	Market Class	Electrophoresis		RT-PCR	
Mesoamerican		14 kbp[a]	15 kbp	PvEV-1	PvEV-2
Black Turtle Soup	Black	+[b]	+	+	+
T-39	Black	+	+	+	NT[c]
Loreto	Black	+	+	+	-[d]
Big Horn	Great Northern	+	+	+	+
Matterhorn	Great Northern	+	+	+	+
UC Pink 9634	Pink	+	+	+	-
Roza	Pink	+	+	+	-
Jackpot	Pinto	-	-	-	-
Santa Fe	Pinto	+	+	+	+
Windbreaker	Pinto	+	+	+	+
Vista	Navy	+	+	+	+
Seahawk	Navy	+	+	+	+
Merlot	Small red	+	+	+	NT
R930365	Small red	+	+	+	-
Andean					
CELRK	Light Red Kidney	-	-	NT	NT
Mogul	Light Red Kidney	-	-	NT	NT
CPC 00247	White Kidney	-	-	-	-
Beluga	White Kidney	-	-	-	-
Lassen	White Kidney	-	-	-	-
Montcalm	Dark Red Kidney	-	-	NT	NT
Redhawk	Dark Red Kidney	-	-	NT	NT
BD 1003	Cranberry	-	-	-	NT
Capri	Cranberry	-	-	-	-
Blue Lake	Snap Bean	-	-	-	NT
Black Valentine	Snap Bean	-	-	-	-
Cherokee Wax	Snap Bean	-	-	-	-
Top Crop	Snap Bean	-	-	NT	NT
Wintergreen	Snap Bean	-	-	NT	NT

[a] dsRNA size, [b] Positive, [c] Not tested, [d] Negative

PATHOGENIC AND MOLECULAR CHARACTERIZATION OF *FUSARIUM SOLANI* F. SP. *PHASEOLI* USING MULTILOCUS SEQUENCE ANALYSIS

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INTRODUCTION: *Fusarium solani* f. sp. *phaseoli* (syn. *F. phaseoli* T. Aoki & O'Donnell, *F.cuneirostrum* O'Donnell & T. Aoki) is a soilborne fungal pathogen that causes Fusarium root rot, one of the most economically important and widespread diseases of dry bean (*Phaseolus vulgaris* L.) (Abawi and Pastor-Corrales, 1990). The genetics of Fusarium root rot resistance has been found to be quantitative (Roman-Aviles and Kelly, 2005). However, disease resistance is also dependent on the biology (e.g., sexual reproduction) and population genetic structure of the pathogen (McDonald and Linde, 2002). In contrast to *F. oxysporum* f. sp. *phaseoli*, which strictly reproduces asexually, some members of the *F. solani* species complex have known sexual stages and molecular phylogenetic analysis indicate that *F. solani* isolates from dry bean share the same sexual form (*Nectria haematococca*) of the pea pathogen *F. solani* f. sp. *pisi* (O'Donnell and Gray, 1995). The objectives of this study were to 1) characterize the intra-specific genetic diversity using multilocus sequence analysis, 2) determine the presence of mating-type alleles, and 3) assess the virulence of *F. solani* isolates from dry bean breeding nurseries in Washington State, USA.

MATERIALS AND METHODS: Several dry bean cultivars, randomly distributed in the field, showing typical symptoms of Fusarium root rot were collected during the Summer of 2012 from two different bean breeding nurseries in Washington State University at the Roza (cvs. OAC-Inferno, Red Hawk, SDIP, and Stampede) and Othello (cvs. Badillo, Buster, Red Hawk, and UCD-0801) field units. In addition, a symptomatic volunteer dry bean was also collected at the USDA-ARS Paterson field unit. All fields are separated from each other by >50 km. A total of nine single-spored isolates were obtained from collected plants using *Fusarium* semi-selective medium (PCNB-PDA). Morphological identification was carried out on isolates grown on PDA plates and following The *Fusarium* Laboratory Manual (Leslie et al., 2006). The nuclear ribosomal internal transcribed spacer (ITS) region, the entire nuclear ribosomal intergenic spacer (IGS), a portion of the translation elongation factor 1-α (*EF-1a*), and portions of the two idiomorphic alleles (*MAT-1, MAT-2*) of the mating type locus were amplified by PCR and sequenced using previously reported primers (Kerenyi et al., 2004; Aoki et al., 2005 and references therein). A phylogenetic tree for each sequence (except for IGS region and *MAT* alleles) was reconstructed by maximum-parsimony using MEGA 5.1 software. In addition, sequences from isolates previously identified as *F. solani* f. sp. *phaseoli* and/or isolated from dry bean or soybean were retrieved from the NCBI database and included in the phylogenetic analysis. *F. oxysporum* f. sp. *phaseoli,* and *F. lateritium* (previously reported to cause similar symptoms of Fusarium root rot in Mexico), were used as outgroup references. Virulence tests for all isolates were done on the moderately susceptible commercial cultivar Othello adapting a seed soak inoculation method in the greenhouse (Porter and Coffman, 2011). For this study, disease severity was scored using a scale from 1 to 9, where 1= no visible symptoms and 9= complete necrosis of root system and advanced necrosis on the underground hypocotyl.

RESULTS AND DISCUSSION: All fungal strains isolated from diseased roots of dry bean were identified as *F. solani* based on morphological characters and multilocus (ITS, *EF-1a*, and IGS) sequence analysis. Both mating types (*MAT-1, MAT-2*) were present in all isolates and sampling locations (Fig.1). In the phylogenetic analysis, *N. haematococca* groups together with our isolates providing another line of evidence that these isolates belong to the species *F. solani,* and that they also share the same sexual reproductive stage. The phylogenetic analysis using the *EF-1a* and ITS sequences exhibited a similar topology (only the *EF-1a* is shown) and demonstrated that our isolates form a distinct group, which can be divided into two sub-groups characterized by geographic location (Fig. 1). Additionally, our isolates are genetically different from others reported to be isolated from dry bean, indicating that *F. solani* isolates causing disease on dry bean are genetically more diverse than previously thought. The analysis also suggest that gene flow exist among fields as isolates from Paterson (Volunteer bean) and Othello (Buster) fields group within the sub-group which includes isolates from the Roza field. Finally, fungal isolates showed a range of virulence to the cultivar Othello, with the most virulent isolates (from the Roza field) producing typical symptoms of Fusarium root rot disease (Fig. 1). All isolates were recovered from surface-desinfected root tissues. In summary, the high level of genetic variability and the presence of both mating types suggest that this fungal population has potential for sexual recombination in the field, likely contributing to the observed range in virulence.

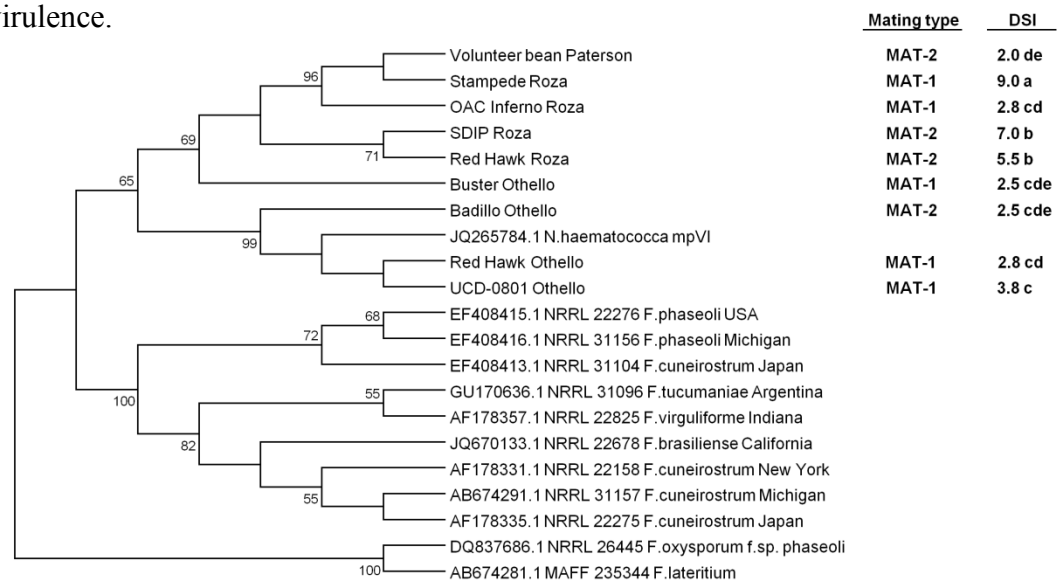

Fig. 1. A consensus bootstrap (500 replicates) maximum-parsimony tree inferred from *EF-1a* sequence, distribution of mating types (*MAT-1, MAT-2*), and disease severity index (DSI; scale 1 to 9) of *F. solani* isolates. The DSI is the mean of four replicates (one plant per replicate) in a single trial, separated by Tukey's test (α=5%).

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GENETIC VARIABILITY OF COMMON BEAN ROOT SYSTEM MORPHOLOGY

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INTRODUCTION: The root system has been poorly studied over the last 150 years in spite of there being a large range of genetic variation in bean root morphology and anatomy that is unused in modern plant breeding. The objective of this study was to assess the genetic variation in root morphology in common bean, given that there may be evidence that breeders have unconsciously selected plants with smaller root systems that may limit drought tolerance and seed yield under normal irrigation or rainfed conditions.

MATERIALS AND METHODS: Seeds of a set of 18 common bean parents of different RIL populations were germinated in Petri dishes on September 07, 2012. Seven days later, seedlings with similar growth were transplanted in polyethylene tubing bags sleeved into polyvinyl chloride (PVC) tubes, 80 cm long and 10 cm in diameter. Two drainage holes made at the bottom of each bag were covered with filter paper before being filled with 8.5 kg of dry silica sand #30 with 24% field capacity (w/w). The set of 18 common bean genotypes included the parental components of 12 different RIL's; populations that consist of crosses between parental genotypes with similar or contrasting plant growth habit, namely determinate (I) or indeterminate (II or III) type of architecture (Table 1). A randomized complete block design with four replicates was used. After transplanting the bean seedlings, plants were grown for 49 days under well water conditions in a cooled greenhouse at the University of California Riverside. Most parental bean genotypes reached the phenological stage of flowering and pod development before they were harvested. Shoot biomass, including developing pods, was harvested from the sand surface, dried in a forced air oven, then weighed. The root system was washed without damage, basal lateral roots were counted and longest root measured (root length) , the root system was separated into two parts; shallow roots included all roots less than 30 cm and deep roots consisted of all roots greater 30 cm. Roots were dried and weighed. Root biomass was calculated as the sum of the two parts. Data were subjected to ANOVA for each of trait.

Table 1. Common bean parental genotypes evaluated for their root system morphology under greenhouse conditions in 2012 at the University of California Riverside, USA.

Genotype Name	Growth habit		Genotype name	Growth habit		Genotype name	Growth habit
Othello	III		G-19833	III		OSU-5446	I
VAX-3	II		I9365-25	III		RR-6950	III
G-122	I		Montrose	III		Raven	II
A-55	II		BelNebRR-1	III		I9365-31	III
Montcalm	I		Taylor Hort	I		Bunsi	III
DOR-364	II		Cardinal	I		Benton	I

RESULTS: Significant differences were found among the genotypes for all root traits measured or calculated as well as for shoot biomass. In general, determinate growth habit type I architecture parental lines had a tendency to develop higher number of basal lateral roots (12.5) than indeterminate type II (8.2) or type III (9.9). The average root length among individual genotypes varied from 53.3 to 82.7 cm (Table 2); however, when the three growth-habit type of architecture was compared, they were closest to the grand mean (73.6 cm). For some genotypes the longest root may have been restricted by the depth of the 80 cm tubes. Type III and II genotypes were similar but the latter had 24.2% higher shallow root weight than type I (2.5 g). In contrast, type III genotypes developed almost three-fold higher deep-root weight than type II (1.2g) and 30% more than type I (2.3 g). Root biomass calculated as the sum the two parts was similar for the tree growth habit types of architecture with an average of 5.1 g. Percent root weight was similar for both, type II (30.1) and III (25.5), but higher than type I (20.1). Shoot biomass was similar between type I (18.4 g) and III (17.9 g) but 36.8 - 38.6% higher that type II.

Table 2. Root and shoot traits of common bean parental genotypes grouped according to growth habit and grown in PVC tubes under greenhouse conditions.

Plant traits	Parental genotypes grouped by growth habit				
Root and shoot traits	Type I(6)	Type II(4)	Type III(8)	Average (18)	LSD 0.05
Basal lateral roots	12.5	8.2	9.9	9.9	2.96
Root length (cm)	76.3	70.7	79.8	73.6	10.95
Shallow root wt (g)	2.5	3.3	2.7	2.7	0.65
Deep root wt (g)	2.3	1.2	3.3	2.4	1.22
Total root wt (g)	4.8	4.6	6.1	5.1	1.51
% Root wt	20.1	30.1	25.5	24.3	6.33
Shoot dry wt (g)	18.4	11.3	17.9	15.7	4.50
Plant dry wt (g)	23.2	15.9	24.0	20.8	5.64
Days to flowering	46.8	54.8	50.0	50.4	3.14

Numbers in parenthesis indicate number of parental lines included in the group

CONCLUSIONS: These preliminary results indicated that genetic variation exists among common bean parental genotypes evaluated for root morphology traits. A second study might be to screen the RIL's derived from a parental pair with contrasting root morphology differences to act as potential candidates for QTL analysis of root traits in the following crosses: I9365-25 / Montrose, G-122 / Montcalm, OSU -5446 / RR-6950 and Othello / VAX-3.

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SURVEY OF ANDEAN GERMPLASM FOR TERMINAL DROUGHT RESPONSE

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Plant breeding efforts toward drought resistance in common bean have generated a large amount of data that has contributed to our understanding of the impact of drought on dry bean (for review: Beebe et al, 2010). Although QTL for drought tolerance have been identified significant QTL X environment interactions indicate that further characterization of drought response is necessary. There has been some success for enhancing drought resistance in Middle American (MA) germplasm but less in Andean types. In this study we selected a subgroup from the Andean Diversity Panel (ADP) comprised of North American (NA) and African materials with vine and bush growth habits and tested them for response to terminal drought stress with the goal of identifying adapted drought tolerant lines for use in breeding populations and inheritance studies.

MATERIALS AND METHODS

A total of 143 Andean lines plus 1 MA check were planted in a lattice split-plot design with two replications and two treatments: drought stress (DS) and non-stress (NS). Both treatments were furrow irrigated on a regular watering schedule until flowering. After flowering, only the NS treatment received water, simulating terminal drought stress for the restricted water treatment. Soil water content was collected using a neutron probe (data not shown). Yield, seed weight, flowering date, maturity, plant height, plant stand (emergence and early vigor), SPAD reading, and pod wall to seed ratio data were collected and analyzed using SAS.

RESULTS AND DISCUSSION

The overall mean for drought stress was 2300 kg ha^{-1} compared to 4000 kg ha^{-1} for non-stress indicating moderately severe drought intensity index of 0.40. Excluding the MA check PT7-2 (5730 kg ha^{-1}), 15 of the highest yielding 20 lines were from Africa. Conversely, only eight of the lowest yielding 20 lines were from Africa. Clearly, as a group, the African materials exhibited greater tolerance to terminal drought stress than the NA lines. Overall indeterminate lines performed better than determinate bush lines as expected (data not shown). The pod wall ratio did not differ between treatments, but it was negatively correlated with geometric mean for yield (r^2 = -0.46) (Fig. 2). These preliminary data suggest potential germplasm for use in breeding for enhanced drought tolerance in the Andean bean market classes.

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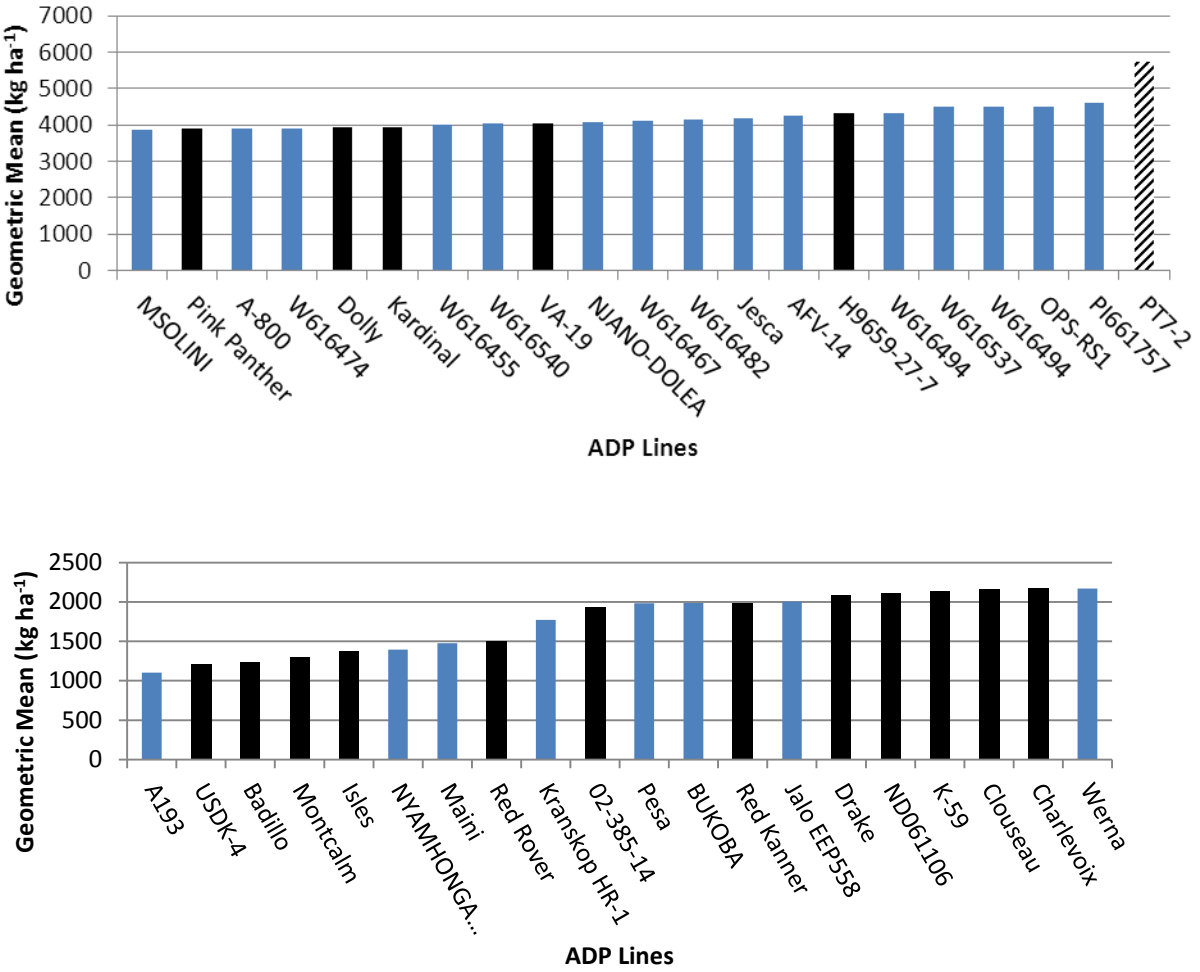

Figure 1. Geometric mean (kg ha^{-1}) of the 20 highest yielding ADP lines (+MA check) (top figure) and 20 lowest yielding ADP lines (kg ha^{-1}) under terminal drought stress, Othello, WA, 2012. Dark bars indicate North American lines.

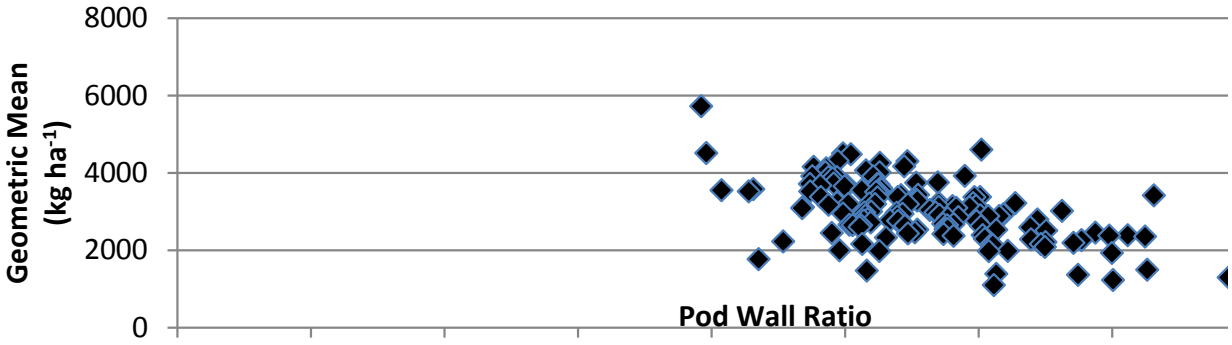

Fig. 2. Scatterplot for correlation between geometric mean and pod wall ratio (r^2 = -0.46**).

COMPARISON OF SNAP BEAN GENOTYPES FOR YIELD POTENTIAL UNDER DROUGHT CONDITIONS

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INTRODUCTION: Drought is considered to be yet most limitation on bean yields after soil fertility, affecting more than 60% of production areas worldwide. Agronomic solutions are often not practical, capital intensive and may be of difficult adoption by farmers. A genetic solution to drought is therefore attractive but difficult to attain. Genotypic differences for drought resistance have been reported for common bean (Abebe *et al* 1998, Acosta-Gallegos *et al* 1997). The most effective selection criterion, among various morphological, physiological, phenological, yield, and yield related traits, for identifying drought resistant genotypes is seed yield of DS (drought stress) and NS (nonstress) environments (Abebe *et al* 1998, Ramirez-V. & Kelly 1998, White *et al* 1994). The objective of this study was to compare pod yield of two snap bean cultivars under drought stressed and nonstressed environments.

MATERIAL AND METHODS: The study was carried out at MVCRI- Plovdiv, under field conditions from April to June 2010 and 2011 with two previously developed snap bean cultivars - Tangra and Zaria. Plots were grown in DS (applying half – reduced irrigation) and NS (applying supplementary irrigation), consisted of two rows in two replications, both adjacent to each other in a similar design and plot size. Plots were kept free from weeds, diseases, and insect pests by means of a combination of preventive chemicals and hand labor. In addition to pod yield (g plant $^{-1}$) (SY), data were also recorded for number of days to flowering (DF) and geometric means (GM) were determined as GM = (NS X DS). Percent reduction (PR) due to drought stress in relation to cultivars of NS environment was also determined for pod yield as PR= 100*(1 - DS/NS). Drought susceptibility index for pod yield for each genotype was calculated as follows: DSI = (1 - Yds/Yns)/(1-(Ymds-Ymns), where Yds and Yns are mean yields of a given genotype and Ymds and Ymns are means across all genotypes in DS and NS environments, respectively (Fischer & Maurer 1978). Simple correlation coefficients among different traits were also determined. All data were analyzed by a SPSS 12.0 statistical package for Windows.

RESULTS AND DISCUSSION: By taking advantage of relatively reduced rainfall during the growing season in the past decades in Bulgaria (Cholakov 2002), and by applying half – reduced irrigation on the half of the plants, a moderate level of terminal drought stress can be simulated (Table 1). There was observed moderate levels for DII for both cropping seasons of the experiment (≥ 0.3). There was recorded that drought stress, on the average, reduced green bean yield by 31.16 %. Pod yield of Tangra in DS was significantly lower than its counterpart in NS environment for both years and did not differ significantly in Zaria (Table 2). The largest reduction in pod yield due to drought stress was recorded in 2011 for Tangra (39.7). In contrast, Zaria showed comparatively less yield reduction in both cropping season (21.6% and 24.1%, respectively). The DSI was comparatively low (0.71-0.78) for Zaria, and high DSI (≥1.28) was observed for Tangra. Both early and late maturity may provide bean crop with means of escaping drought. For Bulgaria climatic zone earliness is advantageous for beans, because soil moisture is

Table 1. Cumulative rainfall (l/m^2) for month, growing season and year, & drought intensity index-DII

Cropping season	April	May	June	July	Growing season	Annual	DII
2010	38	19	60	120	237	629	0.30
2011	19	41	15	41	116	447	0.31
Rainfall normals	45	65	63	49	222	539	

adequate early in the season. The flowering stage is the most sensitive to water stress. The results showed that drought stress accelerated flowering of Tangra and did not affect significantly Zaria. Correlation between yield and days to flowering showed clear advantage for earliness in both cultivars. Thus, breeding for early flowering in a practical limit is promising approach for drought escape in determinate snap beans in Bulgaria considering that earliness leads to loss of productivity.

Table 2. Mean yield and days to flowering for two snap bean genotypes evaluated in drought-stressed and nonstressed environments at two cropping seasons in 2010 and 2011

Genotype		Pod yield			Days to flowering					Correlation of pod yield X Days to flowering for total cropping period		
		g plant $^{-1}$			d		d					
	Year	NS	DS	GM	PR	DSI	NS	DS	GM	NS	DS	GM
Zaria	2010	106.7	83.6 ns	94.5	21.6	0.71	32	32	32			
	2011	160.2	121.5 ns	139.5	24.1	0.78	40	37	38	- 0.69	- 0.65	- 0.64
Tangra	2010	107.9	65.7 ++	84.2	39.0	1.29	29	30	29	+	+	+
	2011	130.2	78.4+++	101.1	39.7	1.28	45	41	43			

NS = nonstressed, DS = drought-stressed, PR = percent reduction in the DS in relation to the NS environment, GM (geometric mean) = (NS X ND), and DSI (drought susceptibility index) = (1- Yds/Yns)/DII, where Yds and Yns are mean yields of a given genotype in DS and NS environments, respectively.
ns - Paired values of NS and DS environments for each genotypes not differ significantly
+';'++'; '+++' - Paired values of NS and DS environments for each genotypes differ at P=0.5; P =0.01 and P=0.001, respectively

CONCLUSION: It was concluded that Zaria genotype was less affected by drought stress than Tangra. Greater tolerance in Zaria probably depends essentially on genetic variability among the parental sources, and optimum recombinogenesis of genes in the new genotype. Use of drought resistant germplasm from Zaria should therefore be maximized in cultivar development programs conducted at Maritsa VCRI, aimed at reducing production costs and maximizing water-use efficiency. Further search and use of new sources of drought resistance in multiple-parent interracial and intergene pool populations should be extend. If full season, drought tolerant cultivars are available, these could offer both protection against drought and better yield potential in varying climatic conditions.

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WATER RELATIONS IN COMMON BEAN DURING SHORT AND MODERATE WATER DEFICIENCY

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INTRODUCTION

Water deficit is an abiotic factor that affects the agricultural production with greater frequency and intensity, influencing aspects related to plant development such as decrease in photosynthesis rate, reduction in leaf area (Fontana et al., 1992), and closing stomatal (Santos and Carlesso, 1998).

As in other plants, performance of *Phaseolus vulgaris* also is affected by water deficiency, in which can provoke lower growth and development, with progressive reduction in leaf dry matter (Costa et al., 2011) and consequent repercussion on production parameters as number of grains and pods per plant.

Based in this overview, this study has aim to investigate influences of short and moderate water deficit on water relations of *Phaseolus vulgaris* plants, measuring leaf water potential, stomatal conductance and transpiration rate.

MATERIALS AND METHODS

Study was conducted in greenhouse located in Laboratório de Fisiologia Vegetal Avançada (LFVA), Universidade Federal Rural da Amazônia (UFRA). Plants remained in greenhouse environment under natural conditions with air temperature minimum and maximum of 26.9 and 35.5°C, respectively. Air relative humidity oscillated between 62 and 91%. The photoperiod medium was of 12 h of light.

In plant material were used seeds of IAPAR 81, and as substrate Plantmax®, which was placed in pot with 3L of capacity. Experimental design employed was entirely randomized with 2 water conditions (water deficit and control) combined with 3 evaluation points (0, 2 and 6 days), totalizing 6 treatments. It was composed 5 replicates and 30 experimental units, being each experimental unit was constituted by 1 plant pot^{-1}.

In this study were evaluated leaf water potential, stomatal conductance, and transpiration rate, being these parameters evaluated in well expanded trifoliolate leaves 3rd located at the middle of the main branch during the stage V_4. Data were analyzed employing a variance analysis, and using Scott-Knott test at 5% level of probability. Standard errors were also calculated in all treatments evaluated.

RESULTS AND DISCUSSION

Plants exposed to water deficit presented reduction in leaf water potential (Figure 1A), being a significant change only during moderate water deficiency (6th day). The reduction in leaf water potential is due to minor absorption rate of water by plant via root, and water loss occasioned by gas exchanges through stomatal.

In stomatal conductance were observed significant modifications in short and moderate water deficit, resulting in decrease in this parameter (Figure 1B). Reduction in stomatal conductance can explain by reduction in water availability in substrate, which

will produce decrease in leaf water potential, resulting in closing stomatal. Results revealed that in conditions of limited water supplement in substrate there is increase in abscisico acid (ABA) concentration in xylem sap, and it will promote stomatal closing (Santos and Carlesso, 1998).

For transpiration rate the water stress promoted significant decrease in 2 and 6th day after implementation of water restriction (Figure 1C). Decrease in transpiration rate of *Phaseolus vulgaris* plants can be attributed to stomatal behavior, that under water deficit are keep partially closed, contributing to variation of transpiratory behavior of plant (Oliveira et al., 2005). Leite and Filho (2004) describe that reduction in transpiration works as important mechanism of tolerance to drought.

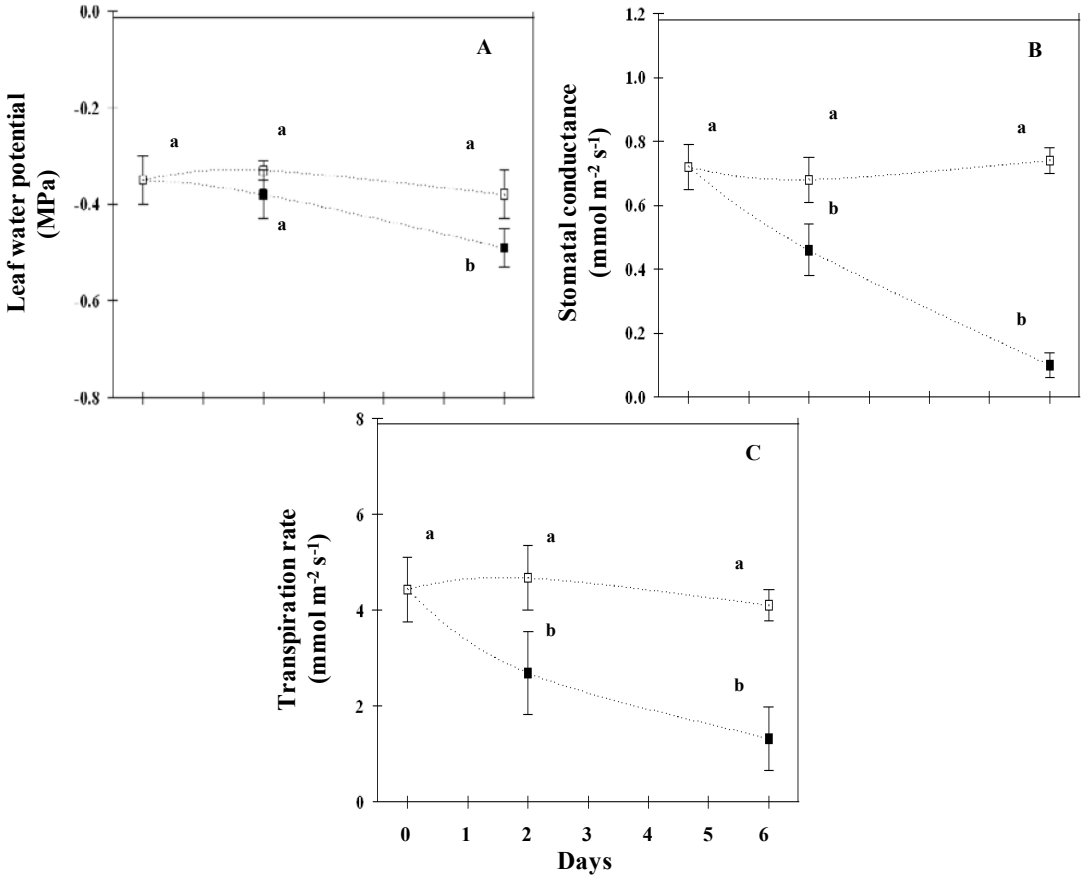

Fig. 1: (A) Leaf water potential, (B) stomatal conductance, and (C) transpiration rate in *Phaseolus vulgaris* plants exposed to short and moderate water deficiency. Same letters into period (day) do not show significant differences at Scott-Knott test ($P < 0.05$). Bars represent the mean standard error.

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PHOTOSYNTHESIS AND LEAF TEMPERATURE IN *PHASEOLUS VULGARIS* PLANTS EXPOSED TO SHORT AND MODERATE WATER DEFICIT

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INTRODUCTION

Common bean crop is considered one of the more important leguminous in world, and grain presents higher protein and carbohydrates amounts. This culture is economically favorable to Brazil due to adequate climatic conditions and large consumer market, placing this country as main producer and consumer worldwide (Broughton et al., 2003; Vieira, 2005).

Environment inadequate conditions produced by abiotic factors such as water, temperature, salt, and mineral elements can provoke reductions in growth and development in plants, disorders in physiological, biochemical, and molecular behaviors, besides yield losses in several crops (Tan et al., 1999; Leport et al., 1999; Oliveira Neto et al., 2009).

Aim of this study was to study impact promoted short and moderate water deficit on photosynthesis and leaf temperature of common bean plants.

MATERIALS AND METHODS

Study was conducted in greenhouse located in Laboratório de Fisiologia Vegetal Avançada (LFVA), Universidade Federal Rural da Amazônia (UFRA). Plants remained in greenhouse environment under natural conditions with air temperature minimum and maximum of 26.9 and 35.5°C, respectively. Air relative humidity oscillated between 62 and 91%. The photoperiod medium was of 12 h of light.

In plant material were used seeds of IAPAR 81, and as substrate Plantmax®, which was placed in pot with 3L of capacity. Experimental design employed was entirely randomized with 2 water conditions (water deficit and control) combined with 3 evaluation points (0, 2 and 6 days), totalizing 6 treatments. It was composed of 5 replicates and 30 experimental units, with each experimental unit constituted by 1 plant pot^{-1}.

In this study were evaluated photosynthesis rate, photosynthetic efficiency, and leaf temperature, being these parameters evaluated in well expanded trifoliolate leaves 3rd located at the middle of the main branch during the stage V_4. Data were analyzed employing a variance analysis, and using Scott-Knott test at 5% level of probability. Standard errors were also calculated in all treatments evaluated.

RESULTS AND DISCUSSION

Photosynthesis rate was significant affected during short and moderate water stress (2 and 6 days), provoking fall more in 6th day of water restriction (Figure 1A). Reduction in photosynthesis is connected to stomatal closing induced by lower water availability in substrate. In addition, this decrease will interfere directly in carbon metabolism with consequent minor expression of plant production potential. Results on reduction in

photosynthesis found in stressed plants are similar with reported by Santos et al. (2009) studying five common bean genotypes exposed to mild water deficit.

Results linked to photosynthetic efficiency reveal that plants exposed to water deficiency present reduction in this physiological parameter (Figure 1B), with significant interference only under moderate water deficiency.

For leaf temperature were observed significant increases during 2 and 6th after implementation of water deficit (Figure 1C). Results linked to higher leaf temperature are explained by the reduced water amount in tissue/leaf after water restriction, and this increase probably provokes negative consequences in gas exchanges and photosynthesis rate, in which are processes that work under adequate temperature range.

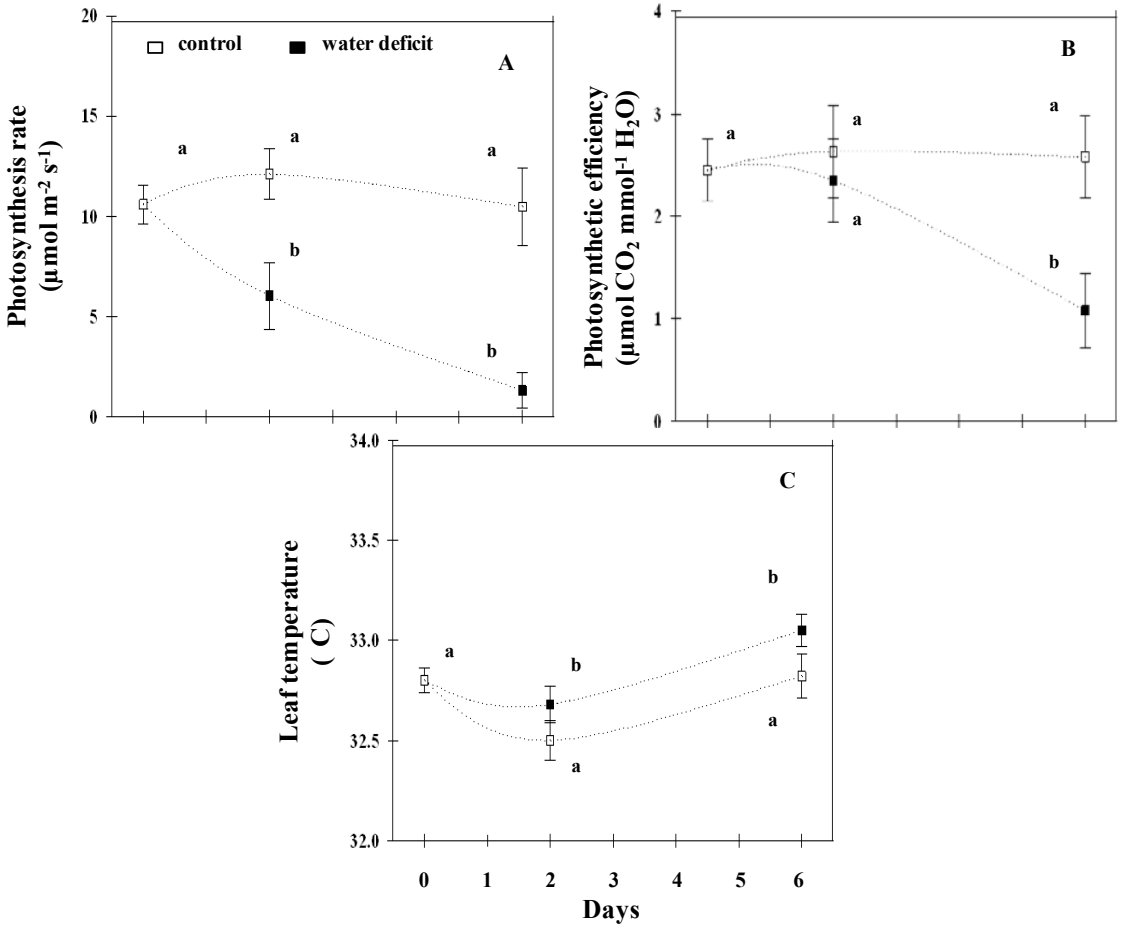

Fig. 1: (A) Photosynthesis rate, (B) photosynthetic efficiency, and (C) leaf temperature in *Phaseolus vulgaris* plants exposed to short and moderate water deficiency. Same letters into period (day) do not show significant differences at Scott-Knott test ($P < 0.05$). Bars represent the mean standard error.

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SCREENING THE ANDEAN DIVERSITY PANEL UNDER MULTIPLE STRESSES

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The dry bean Andean Diversity Panel (ADP) consisting of ~400 lines (landraces, advanced breeding lines, cultivars) from Africa and the Americas was developed by the USAID PulseCRSP and ARS-FtF projects for the genetic characterization of targeted traits and identification of breeding materials with the ultimate goal of developing improved Andean germplasm for BNF, disease resistance, nutrient content, cooking time, and tolerance to abiotic stresses. The panel is currently in the queue to be genotyped with 6000 SNPs from the BeanCAP project for Association Mapping the above traits.

Breeding for abiotic stress resistance has been ongoing for over 50 years at the Roza research unit located in Prosser, WA. Also known as the 'Purgatory Plot', the test field typifies soil compaction, drought (intermittent), low soil fertility, and moderately high root rot pressure. The plot has a short two year rotation with a small grain. We sought to assess the performance of a subset of ADP lines in the purgatory plot for identification of multiple stress resistant lines to be used as parents in breeding and genetic populations for inheritance studies.

MATERIALS AND METHODS

A total of 225 lines including 208 ADP accessions were planted in one 3-m row plots, with 3 replications using a lattice design (2012). Plants were watered normally using overhead irrigation to establish early plant growth. Just prior to flowering, water was applied at 10-d intervals to match a 50% rate of evapotranspiration and simulate intermittent drought conditions. Watchdog stations were used to record soil moisture (data not shown). Yield, seed weight, plant biomass, plant N, flowering date, and plant stand (emergence and early vigor) data were collected and analyzed using SAS.

RESULTS AND DISCUSSION

The Middle American (MA) checks were the highest yielding group (2928 kg ha^{-1}) and the non-nods were the lowest yielding (493 kg ha^{-1}) (Fig. 1). African bush and vine types include African cultivars and Tanzanian landraces. As a group, the African Type III vine (1417 kg ha-1) type out-yielded the adapted North American Type III vine (1102 kg ha^{-1}) and Type I bush (677 kg ha-1) materials. The highest yielding Andean line was a Tanzanian Type III landrace with large red seeds which had the third highest yield (3833 kg ha^{-1}) following two well adapted stress tolerant MA lines 'BR-88' (4936 kg ha^{-1}) and 'Roza' (4544 kg ha^{-1}) (Fig. 2). Excepting MA checks, 8 of the 10 highest yielding lines were Tanzanian landraces with indeterminate vine growth habit (Fig. 2). The trial will be repeated in 2013. The better performance of MA checks suggests that wide inter-gene pool crosses should be revisited as a means to introgress abiotic stress tolerance into Andean beans. Additionally, with more extensive and accurate phenotyping, there will be potential for identifying genes associated with abiotic stress tolerance through association mapping.

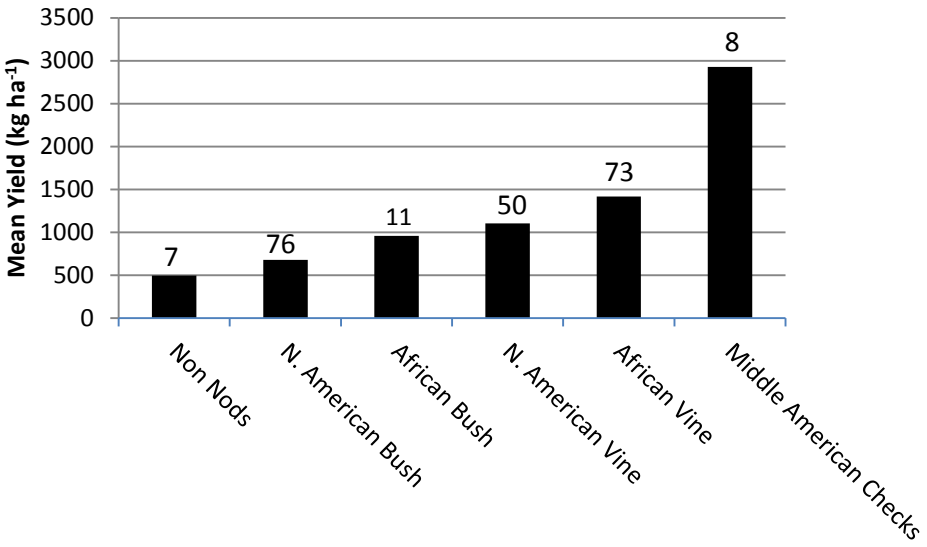

Figure 1. Mean yield (kg ha^{-1}) of subgroups by origination and growth habit, in the partial ADP multiple stress experiment, Prosser, WA, 2012. Number above bar represents the number of individuals in each group.

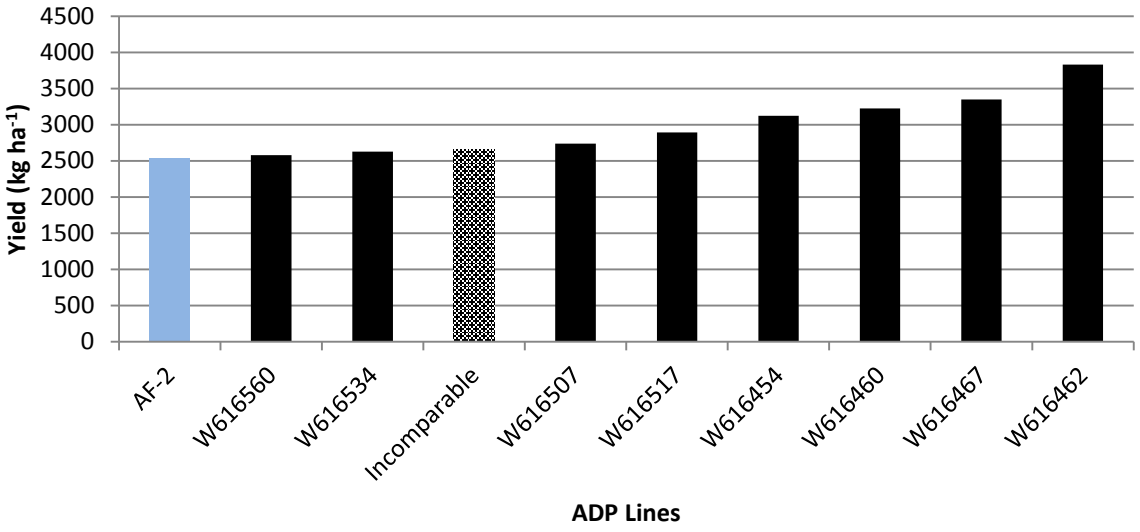

Figure 2. Ten highest yielding ADP lines under multiple stress, Prosser, WA, 2012. Dark bar = Tanzanian vine landraces; patterned bar = Tanzanian bush; light bar = African bush cultivar.

YIELD OF COMMON BEAN (*PHASEOLUS VULGARIS* L.) IN ACID SOILS

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INTRODUCTION

In Mexico (the State of Veracruz, Gulf of Mexico's coastal area), 20000 ha that is, one third of the area planted to beans in the fall-winter cycle, is located in acid soils. The crop grows rain-fed and with the soil residual moisture. The soils are acid with a pH below 5.0, with low content of organic matter. These soil conditions favor a low cation exchange and reduce the growth and yield of beans (Zetina-Lezama *et al*., 2005). To increase bean yield, a possible approach is the selection of bean varieties better adapted to low pHs. Therefore, the objective of the present work was to compare the yield of 13 varieties of beans grown in acid soil.

MATERIALS AND METHODS

The study was conducted in the vicinity of J. Rodríguez Clara, State of Veracruz (Mexico) (18º 00' N; 95º24' W), 140 m altitude; hot subhumid climate (AWo), and average temperature and rainfall of 24.5 ^{o}C and 1462 mm per year respectively (Soto y García, 1989). In the case of the experimental plot, the soil was a sandy loam with pH 4.6; the experimental plot was prepared for sowing as customary. Sowing was done at equivalent density of 250,000 plants/ha, on September 22, 2012 in humid soil. NPK at the rate 40-40-0 was applied ten days after seedling emergence. The experimental design was a complete randomized blocks with 13 treatments (varieties) and four replications. The experimental unit consisted of five rows, 5 m long and 0.8 m between rows**.** The plants developed during the fall-winter season without supplemental irrigation using partly the soil residual humidity. The harvest started on December 17. The bean varieties were**:** Negro (N) Veracruz, N Cotaxtla, N Michigan, Michoacán 138, Criollo San Andrés, Criollo Tesochoacán, Criollo Bola, Flor de Mayo (FM) Bajío, FM M38, FM Sol, FM Noura, FM MRC and Flor de Junio (FJ) Marcela. Weeds and insect pests were controlled with commercial products. At the crop maturity 3 m^2 area of the each experimental unit was harvested. The seed was separated and weighted, its moisture content determined and the yield (kg ha^{-1}) was calculated, adjusted to 14 percent moisture. The data were analized using SAS, 9.0 version (SAS Institute, 1999) and Tukey (DSH

RESULTS AND CONCLUSIONS

The assayed varieties responded differentially to the acid condition of the soil (Table 1). The varieties might by grouped as follows: (1) Highest yield; 2) Intermediate yield and 3) Lowest yield. The varieties N Veracruz, Criollo San Andrés, N Cotaxtla and FJ Marcela first group, exhibited the highest yield. The varieties FM M38, Michoacán 128, FM Sol, FM Bajío and Criollo Tesochoacán second group, exhibited an intermediate yield. N. Michigan, FM Noura, FM MRC and Criollo Bola, third group, exhibited the lowest yield.

Table 1. Seed yield of 13 varieties of bean (*Phaseolus vulgaris* L.) grown in acid soil. 2012.

Variety	Yield of grain (kg ha^{-1})
N. Veracruz	1153.58 a
Criollo San Andrés	1125.32 ab
N. Cotaxtla	1100.34 abc
F. J Marcela	1050.19 abc
FM M38	972.13 bcd
Michoacán 138	943.95 cd
FM Sol	872.43 de
FM Bajío	826.34 de
Criollo Tesechoacán	818.29 de
N. Michigan	763.25 ef
FM Noura	753.76 ef
FM MRC	749.67 ef
Criollo-bola	693.02 f
ANOVA	**
Tukey, 0.05	159.98

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ROOT TRAITS AND NODULATION OF RECOMBINANT INBRED BEAN LINES FROM A 'JAMAPA × CALIMA' POPULATION INOCULATED WITH TWO STRAINS OF RHIZOBIUM

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Bean cultivars of Andean (A) and Middle American (MA) origin often have contrasting above-ground traits. Less is known, however, of possible differences in root traits of beans from different gene pools. Recombinant inbred lines (RIL) derived from a cross between the Andean cultivar 'Calima' and the Middle American cultivar 'Jamapa' (Vallejos *et al.*, 2000) were evaluated in a laboratory trial for root traits and nodulation. The RILs, Jamapa and Calima were cultivated using a hydroponic method with Nitrogen free nutrient solution (Broughton and Dilworth, 1971) and evaluated in root growth pouches using procedures described by Somasegaran and Hoben (1994). Three pouches of each RIL were inoculated with *Rhizobium etli* strain CIAT 632 and three pouches were inoculated with *Rhizobium tropici* strain CIAT 899, using a concentration of 4.0×10^{10} CFU at four days after germination. *R. etli* is the predominant occupant of nodules in both centers of origin although *R. tropici* has been reported to be more tolerant to acid soils and high temperatures (Martínez-Romero, 2003). At 16 days after planting, the roots were evaluated for number of basal root crowns and roots, and for nodulation. The number of nodules were counted and nodule size was evaluated using a 1-3 scale, where 1 = small, 2 = medium and 3 = large nodule size. Means of root and nodulation traits were compared using Least Significant Differences ($P \leq 0.05$). Phenotypic correlations and narrow sense heritabilities (Knapp, 1985) were calculated for root and nodulation traits.

Significant differences were observed among RILs for root and nodulation traits. Although mean number of basal root crowns/plant and roots/plant were similar for the RILs, Jamapa and Calima, a few RILs produced significantly more basal root crowns and roots than the parents (Table 1). Phenotypic correlations between number of basal crown roots/plant and number of roots/plant were positive and highly significant. Narrow sense heritability estimates for number of basal crown roots/plant were > 0.6 and for number of roots were near 0.5 (Table 3). Calima (A) tended to produce more nodules than Jamapa (MA), especially when inoculated with the *R. tropici* strain CIAT 899 (Table 1). These results support the co-evolution of the Rhizobium and common bean in the centers of genetic diversity which was suggested by Aguilar et al. (2004). The RILs expressed a wide range in nodule number including lines that produced significantly more nodules than either parent or RILs that produced very few nodules when inoculated with a particular *Rhizobium* strain. It should be noted, however, that only a few RILs produced > 50 nodules/plant. The number of nodules was positively correlated with both number of basal root crowns and number of roots (Table 2). Narrow sense heritability estimates for nodule number/plant ranged from 0.40 to 0.59 (Table 3). Mean nodule size scores of the parents and the RILs were similar (Table 1). Phenotypic correlations of nodule score with number of basal root crowns and number of roots/plant were low or not significant (Table 2). Narrow sense heritability estimates for nodule score were 0.41 & 0.43. The phenotypic data from this RIL population will be used to identify QTLs for root and nodulation traits.

Table 1. Mean number of basal root crowns per plant, mean number of roots per plant, mean number of nodules per plant and mean nodule size score of Jamapa x Calima (JxC) RIL's inoculated with two strains of Rhizobium.

	Rhizobium tropici strain 899				*Rhizobium etli* strain 632			
	Crowns no.	Roots no.	Nodules no.	Nodule size score[1]	Crowns no.	Roots no.	Nodules no.	Nodule size score[1]
JxC RILs								
Range	2.0 - 6.0	7.7 - 19.0	1.7 - 127.7	0.7 – 3.0	2.3 – 6.0	5.3 - 16.3	6.5 – 123.6	1.0 – 3.0
Mean	3.4	11.1	36.9	1.6	3.8	11.3	48.0	1.9
LSD(0.05)	1.0	3.9	34.7	1.0	1.1	3.9	50.1	NS
CV(%)	17.5	21.7	58.3	38.3	18.3	21.5	64.5	33.7
Jamapa								
Mean	3.2	9.9	32.8	1.8	3.2	10.1	51.6	2.2
CV(%)	12.6	16.9	73.0	46.8	20.6	15.2	39.3	38.1
Calima								
Mean	3.6	11.7	67.8	1.8	3.7	11.7	79.4	2.2
CV(%)	27.9	15.8	60.2	42.9	20.2	22.4	46.8	25.8

[1] Rated on a scale from 1 to 3 where 1= small and 3 = large nodule size.

Table 2. Phenotypic correlations among root and nodule traits of Jamapa x Calima RILs Inoculated with Rhizobium strains 899 and 632.

	Rhizobium tropici strain 899		*Rhizobium etli* strain 632	
	Basal root crowns/plant no.	Number of roots/plant	Basal root crowns/plant no.	Number of roots/plant
Roots/plant no.	0.82**		0.81**	
Nodules/plant no.	0.45**	0.58**	0.28*	0.19*
Nodule score	0.17^{NS}	0.25*	0.27*	0.14^{NS}

Table 3. Narrow sense heritabilities for root and nodule traits of Jamapa x Calima RIL population inoculated with Rhizobium strains 899 and 632.

	Rhizobium tropici strain 899		*Rhizobium etli* strain 632	
	Narrow sense heritability	CI (1-α=90%)	Narrow sense heritability	CI (1-α=90%)
Number of basal root crowns/plant	0.72	0.59 to 0.80	0.64	0.48 to 0.74
Number of roots/plant	0.50	0.28 to 0.64	0.49	0.26 to 0.63
Number of nodules/plant	0.59	0.40 to 0.70	0.40	0.09 to 0.59
Nodule score	0.41	0.15 to 0.58	0.43	0.14 to 0.62

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BIOLOGICAL NITROGEN FIXATION ASSESSMENT IN COMMON BEAN GENOTYPES WITH DIFFERENT VEGETATIVE CYCLE

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INTRODUCTION

Nitrogen (N) is the nutrient absorbed in higher amounts by common bean (*Phaseolus vulgaris* L.) crops, playing an important role on the development and grain yield of most crops (MALAVOLTA, 1989). According to DÖBEREINER and DUQUE (1980) common bean is able to fix atmospheric N when in symbiosis with rhizobia inoculants through biological nitrogen fixation (BNF) process, representing the cheapest way for N obtaining by leguminous crops. However, for common bean crops the BNF is not able to supply all N requirements. This may occur in function of many factors related to the plant genotype and bacterial strains, besides environmental factors (GRAHAM, 1981; MOAWAD et al., 2004). A key strategy for the success of BNF in common bean crops is the development of research with focus in the interaction between both symbioses. This work aimed to evaluate nodulation parameters of common bean cultivars with different vegetative cycle duration in association with different rhizobia strains.

MATERIAL AND METHODS

The assessment of common bean nodulation was carried out under greenhouse condition of the Agronomy and Food Engineering School at Universidade Federal, Goiânia, Goiás, Brazil. The experiment was performed in 5 L pot filled with a sub superficialOxisol on a randomised block experimental design with three replications in a 6X5 factorial arrangement of treatments with six common bean genotypes (CNFC 15873, CNFC 15874, Carioca precoce, IPR Colibri, Pérola and BRS Estilo) and five nitrogen sources (3 rhizobia strains = SEMIA4080, SEMIA4088 and SEMIA4077, 1 nitrogen treatment = 90 kg N ha^{-1} and 1 control treatment). Plants were collected at V4 stage at the soil surface and shoot plants were placed to dry at 65 ºC until constant weight to determine the shoot dry weight (SDW). Roots were carefully taken from the pots, washed and nodules were collected and dried at 65 ºC until constant weight to determine the nodule dry weight (NDW). Values of SDW were divided by NDW to determine the nodule efficiency (NE). Data were submitted to a variance analysis and the means were compared by the Skott-Knott's test at 5% of significance.

RESULTS AND DISCUSSION

The analysis of variance had been shown differences among the cultivars of common bean for the evaluated parameters, besides interaction with the rhizobia strains (Table 1). Regarding NDW, BRS Estilo genotypes showed greater values, followed by Carioca precoce and IPR Colibri; however, rhizobia strains did not differ for NDW. The greater values of SDW were found for BRS Estilo and Carioca precoce genotypes. Among the rhizobia strains SEMIA 4077 and SEMIA 4080 provided equal SDW values as compared to the N treatment. Greater values of NE were found for CNFC 15873, CNFC 15874 and Pérola genotypes and also for these genotypes when inoculated with SEMIA 4080, which showed the greatest value among the rhizobia strains.

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Table 1. Nodule dry weight (NDW), Shoot dry weight (SDW) and Nodule efficiency (NE) of common bean cultivars under different sources of nitrogen.

Cultivars	SEMIA4080	SEMIA4088	SEMIA4077	NT	Control	Mean
	NDW (mg plant $^{-1}$)					
CNFC 15873	2.10 Ca	6.80 Ba	2.63 Ba	0.00 Aa	0.00 Aa	2.31 C
CNFC 15874	1.77 Cb	14.30 Aa	6.77 Ba	0.00 Ab	0.00 Ab	4.57 C
Carioca precoce	24.13 Ba	18.30 Aa	11.50 Bb	0.00 Ac	0.00 Ac	10.79 B
IPR Colibri	21.93 Ba	15.70 Aa	12.50 Ba	0.00 Ab	0.00 Ab	10,03 B
Pérola	1.90 Ca	3.43 Ba	4.63 Ba	0.00 Aa	0.00 Aa	1.99 C
BRS Estilo	43.83 Aa	22.20 Ab	30.76 Ab	0.00 Ac	0.00 Ac	19.36 A
Mean	15.94 a	13.46 a	11.47 a	0.00 b	0.00 b	
	SDW (g plant $^{-1}$)					
CNFC 15873	0.43 Ba	0.30 Aa	0.83 Aa	0.57 Aa	0.31 Ca	0.48 B
CNFC 15874	0.38 Ba	0.40 Aa	0.53 Ba	0.50 Aa	0.24 Ca	0.41 B
Carioca precoce	1.70 Aa	0.61 Aa	0.35 Bb	0.40 Ab	0.74 Cb	0.76 A
IPR Colibri	0.43 Bb	0.33 Ab	0.54 Bb	0.34 Ab	0.97 Ba	0.52 B
Pérola	0.44 Ba	0.28 Aa	0.38 Ba	0.20 Aa	0.55 Ca	0.37 B
BRS Estilo	0.58 Bb	0.46 Ab	0.96 Aa	0.30 Ab	1.45 Aa	0.75 A
Mean	0.66 a	0.40 b	0.60 a	0.70 a	0.38 b	
	NE (g mg^{-1})					
CNFC 15873	0.22 Aa	0.05 Ab	0.30 Aa	0.00 Ab	0.00 Ab	0.11 A
CNFC 15874	0.24 Aa	0.04 Ab	0.08 Bb	0.00 Ab	0.00 Ab	0.08 A
Carioca precoce	0.07 Ba	0.04 Aa	0.07 Ba	0.00 Aa	0.00 Aa	0.04 B
IPR Colibri	0.11 Ba	0.02 Aa	0.04 Ba	0.00 Aa	0.00 Aa	0.04 B
Pérola	0.21 Aa	0.08 Ab	0.09 Bb	0.00 Ac	0.00 Ac	0.08 A
BRS Estilo	0.01 Ba	0.03 Aa	0.03 Ba	0.00 Aa	0.00 Aa	0.01 B
Mean	0.15 a	0.04 c	0.10 b	0.00 d	0.00 d	

NT= nitrogen treatment (90 kg N ha^{-1})

Means within the same column followed by the same capital letter are not significantly different by Skott-Knott's test ($p<0.05$). Means in the same row followed by same lowercase letter are not significantly different by Skott-Knott's test ($p<0.05$).

ASSESSMENT OF MESOAMERICAN AND ANDEAN COMMON BEAN GENOTYPES FOR HIGH-NODULATION EFFICIENCY

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INTRODUCTION

Nitrogen is a key limiting nutrient for agriculture. The association among diazotrophic bacteria and legumes is an important technology to supply nitrogen to the plant without major environmental impacts, and encourages the development of sustainable agriculture. However, is widely known that biological nitrogen fixation (BNF) in common bean is a controversy issue triggered by low efficiency results caused by several factors (GRAHAM, 1981; 2000). Considering the importance of this crop as protein source in developing countries, alternatives that increase the BNF have strategic importance. Therefore, there is a growing interest by the common bean breeding programs for improved cultivars with high BNF efficiency. Thus, the screening of genotypes for nodulation response is an important part of pre-breeding of common bean.

MATERIAL AND METHODS

Aiming to evaluate the nodulation of 882 Mesoamerican and Andean genotype of common bean a greenhouse experiment was carried out at the National Rice and Beans Research Center of Embrapa, located in the county of Santo Antônio de Goiás, Goiás, Brazil. The experiment was performed on a randomized block design, in which the genotypes of common bean, obtained from the active bank of genotype of the Embrapa Rice and Beans, were evaluated under sterile conditions. Two seeds of each genotype were planted in 3 L pots filled with sterile sand and vermiculite (2:1). Seven days after emergence (DAE), plants were inoculated with a mixture of three strains of *Rhizibium tropici* (SEMIA 4077, SEMIA 4080; SEMIA 4088), on a final concentration of 10^9 C.F.U. mL^{-1}. Ouro Negro was also inoculated with the rhizobial mixture and used as a reference. Once a week, 200 mL per pot of a Norris' solution were added until harvest. Common bean plants were harvested 30 DAE and it were determined the relative number of nodules (NN), relative nodule weight (RNW) and total nodule dry weight (TNDW). These data were used to generate a Relative Nodulation Index (RNI) (FERREIRA et al., 2010). Data of nodulation were submitted to a variance analysis and the averages were compared by the Scott-Knott´s test at 5% of significance.

RESULTS AND DISCUSSION

In this first step, all genotypes were characterized compared to Ouro Negro, a commercial bean cultivar developed for high nitrogen fixation efficiency (HENSON et al., 1993). Among the 882 genotypes tested, 687 of them were able to show nodulation. The analysis of variance had been shown differences among genotypes of common bean for NN, RNW, TNDW and RNI (Fig. 1). About 21%, 32% and 54% of the genotypes showed greater NN, RNW and TNDW, respectively than the reference cultivar (Figs. 1A, B and C). Regarding IRN about 43% of the genotypes

presented greater values then the reference genotype, while about 30% showedsimilar values of RNI and other 30% showed lower values of RNI as compared to the reference genotype (Fig. 1D). The 43% with greatest RNI were divided into four classes with an extreme group comprising 0.44% of the genotypes (Fig. 1D). This extreme group is represented by three genotypes, which will take part on a field experiment as a promising source for high BNF efficiency.

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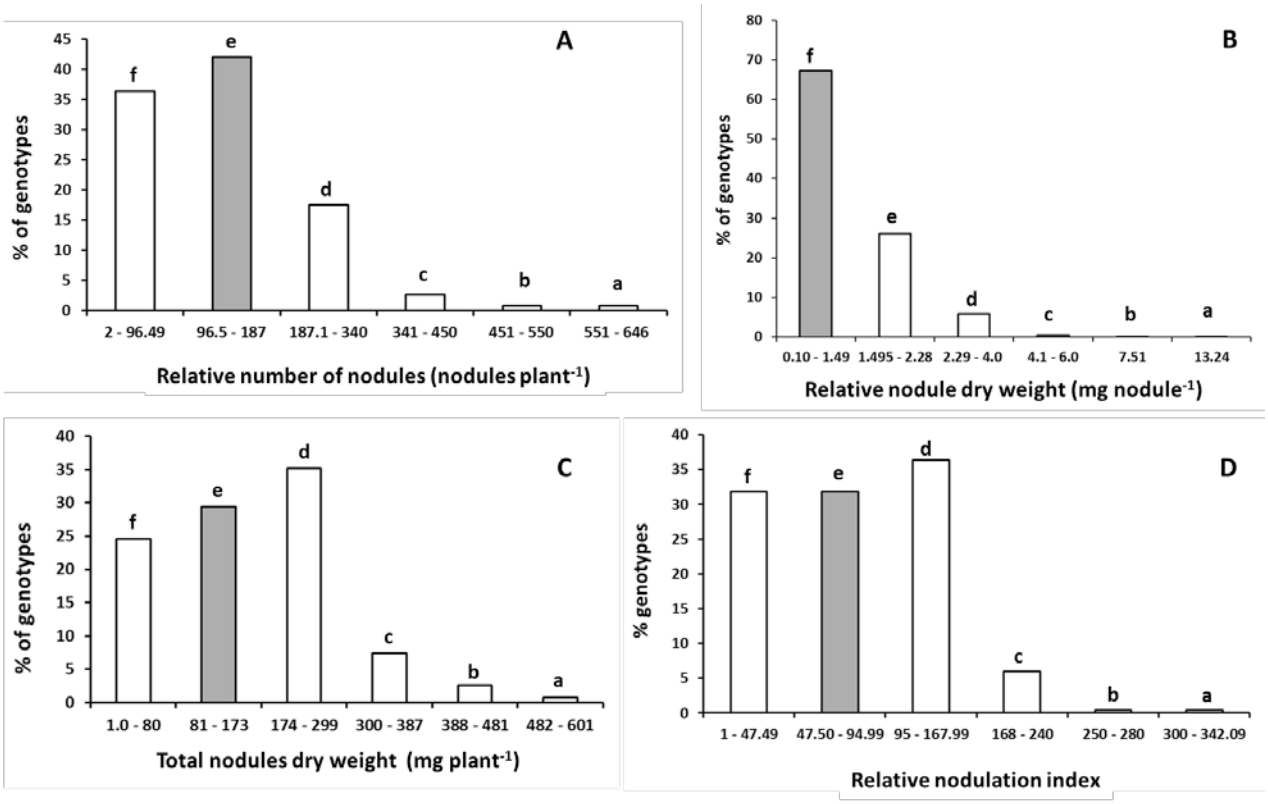

Figure 1 – Percent distribution of the genotypes of common bean according to the different classes: A) relative number of nodules; B) relative dry weight (mg nodule^{-1}); C) nodule dry weight (mg plant^{-1}); D) relative nodulation index. Hachured columns indicate the classes which comprise the reference cultivar (Ouro Negro).

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values varied from 16.93 to 121.96 mg per plant. The lowest values were found for BRS MG Realce, BRS Embaixador and BRS Radiante. In general, BRS Radiante an earlier maturation cultivar showed the worst nodulation performance. According to GRAHAM (1981) earlier maturation genotypes tend to show low efficiency in N fixation due to the short time available for BNF comprising the development and infection of root system to nodules senescence (RUSCHEL et al., 1982; RENNIE & KEMP, 1983). The best nodulation performance was observed for BRS Notável and BRS Marfim, which could be used as parent in common bean breeding programs aiming to obtain common bean cultivars showing high N-fixing efficiency.

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Table 1. Number of nodules (NN= nº plant^{-1}), Percentage of active nodules (%NA) and nodule dry weight (MSN= mg plant^{-1}) of different common bean genotypes.

Genotypes	**NN**	**%AN**	**NDW**
BRS Radiante	25.06 c	30.67 a	35.11 b
BRS MG Talismã	53.00 b	31.33 a	81.55 a
BRS Horizonte	68.77 b	37.33 a	97.87 a
BRS Notável	115.09 a	34.00 a	121.96 a
BRS Marfim	91.25 a	46.00 a	100.60 a
BRS Agreste	72.70 b	26.00 a	105.14 a
BRS Campeiro	63.51 b	37.33 a	104.13 a
BRS Embaixador	25.84 c	18.66 b	33.32 b
BRS MG Realce	12.53 c	12.66 b	16.93 b
CNFC 10429	44.45 b	14.00 b	64.52 b

Means within the same column followed by the same letter are not significantly different by Skott-Knott's test ($p<0.05$).

GROWTH AND ACCUMULATION OF N IN BEAN PLANT CULTIVRS INOCULATED WITH RHIZOBIUM STRAINS

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INTRODUCTION: Nitrogen (N) is the nutrient most extracted and exported by the bean plant which along with other legume plants is capable of utilizing the N fixed in is roots by bacteria as of the genus *Rhizobium*. Nevertheless, a number of biotic and abiotic factors can influence the symbiotic process. So, it was intended through this work to verify whether common bean plant cultivars respond equally to inoculation of the seeds with rhizobium, investigate the behavior of new cultivars submitted to inoculation and detect whether there is difference in the efficiency when inoculating the seeds with different rhizobium strains.

MATERIALS AND METHODS: Two field experiments (Patos de Minas and Uberaba, State of Minas Gerais, Brazil) in the winter crop of 2010. The statistical design was randomized blocks with three replications and factorial scheme 8x3, involving eight cultivars (União, Madrepérola, Supremo, Radiante, Bolinha, Ouro Negro, Ouro Vermelho and Majestoso) and three types of inoculation (seeds non-inoculated nor inoculated with the strains CIAT 899^T or UFLA 04-173). The inoculant was prepared with autoclave-sterilized peat, at the 3:1 proportion of peat:cultures in a semi-solid medium YMA at the log phase (SOARES et al., 2006). The resulting material was employed on the basis of 10 g per kg of seed. At flowering, 10 plants of each plot were collected for determination of the shoot dry matter (MSPA) and content (TNPA) and nitrogen accumulation in the shoot (ANPA). The N content (%) was determined by the semi-microkjedhal method according to Sarruge and Haag (1979) and the N accumulated was calculated by the product MSPA X TNPA divided by 100. The data were submitted to normality and homoscedasticity tests of variances and, whenever necessary, were transformed in $(x)^{0.5}$, except TNPA, transformed in arcsine $(x/100)^{0,5}$. Next, the data were submitted to the joint variance analysis. In the cases of treatment effect, the means clustering was done by the Scott-Knott test.

RESULTS AND DISCUSSION: The values of MSPA were affected by the three factors under study, but not by the interactions. The cultivars. Madrepérola, Ouro Negro, União and Ouro Vermelho, all of type III habit, presented results better than the others (Table 1), which is consistent, since the cultivars of the type III present larger branching and larger leaf area than the ones types I and II. Inoculation promoted larger MSPA (Table 1), result which stands out greater influence of the inoculated strains in making N available than the native rhizobia. Increased MSPA was found in Uberaba and took place likely by the presence of straw (no-tillage planting).

The cultivars presented differences as to the TNPA and to the ANPA, but this effect proved influenced by inoculation (Table 2). In terms of TNPA, the cultivars differed only when they were inoculated with the strain UFLA 04-173, situation in which the cultivars Madrepérola, Ouro Negro and União were outyielded by the others. In the absence of inoculation and in the presence of the train CIAT 899^T, the cultivars had identical behavior. Inside each cultivar, the inoculated treatments differed when they were applied to the cvs. Madrepérola (in which the strain CIAT 899^T provided greater TNPA) and in the cv. Bolinha (in which both the strains outyielded the non-inoculated treatment) (Table 2).

As the ANPA is concerned, the unfolding of the interaction CxI pointed out that when inoculating the seeds with the strain CIAT 899^T, the cvs. União, Radiante and Bolinha

outyielded the others. In the presence of strain UFLA 04-173, the cvs. Majestoso, Radiante and Bolinha outstood. All the cultivars presented the same behavior when non-inoculated.

Inoculation within cultivars demonstrated differences only within cv. Madrepérola, in which the UFLA 04-173 presented performance poorer than that of CIAT 899^T and of the native strains (Table 2). The results relative to both TNPA and ANPA point out, therefore, that the cv. Madrepérola seems to have more affinity with the strain CIAT 899^T than the native strains which present less specificity in symbiosis with the bean plant in relation to the selected strains and that there is differential behavior of the strains and cultivars.

Table 1. Average values of shoot dry matter (MSPA) and Nitrogen content (TNPA) and accumulation in the shoot (ANPA) in relation to cultivars, types of local inoculation[1]

Cultivars	MSPA	TNPA	ANPA
	(g/10pls)	(%)	(mg/10pls)
Madrepérola	40.46 a	3.14	710
Ouro Negro	41.53 a	3.40	850
União	42.81 a	3.02	970
Supremo	29.75 b	3.70	770
Radiante	35.07 b	3.66	1150
Bolinha	35.24 b	3.58	920
Ouro Vermelho	37.24 a	3.58	820
Majestoso	32.75 b	3.66	970
Inoculation			
Absent	32.95 b	3.44	890
CIAT 899^T	38.15 a	3.50	920
UFLA 04-173	39.48 a	3.46	860
Local			
Patos de Minas	30.44 b	3.36	920
Uberaba	43.28 a	3.58	860
Mean	36.86	3.47	890
CV(%)	15.56	11.18	11.27

[1]Means followed by the same letter belong to a same group according to the Scott-Knott test (p>0.05)

Table 2. Average values of Nitrogen content (TNPA) and Nitrogen accumulation (ANPA) in the shoot in relation to cultivars and inoculation. Average values of two locations[1]

Cultivars	TNPA (%)			ANPA (mg/10 plants)		
	Absent	CIAT 899^T	UFLA04-173	Absent	CIAT 899^T	UFLA04-173
Madrepérola	2.27 bA	3.53 aA	2.63 bB	770 aA	820 aB	550 bC
Ouro Negro	3.72 aA	3.38 aA	3.10 aB	930 aA	870 aB	750 aB
União	3.25 aA	3.10 aA	2.70 aB	1070 aA	990 aA	850 aB
Supremo	3.88 aA	3.20 aA	4.02 aA	830 aA	670 aB	810 aB
Radiante	3.27 aA	3.87 aA	3.85 aA	1020 aA	1270 aA	1160 aA
Bolinha	2.90 bA	3.93 aA	3.92 aA	860 aA	1030 aA	1050 aA
O. Vermelho	3.70 aA	3.47 aA	3.57 aA	830 aA	820 aB	790 aB
Majestoso	3.57 aA	3.55 aA	3.87 aA	800 aA	880 aB	930 aA

[1]Means with same capital letter in column and small in row are in the same group, Scott-Knott test (p>0.05).

CONCLUSIONS: The inoculation with the strains CIAT 899 and UFLA 04-173 furthers the growth of the bean plant. As to the N content and accumulation in the shoot, there is differential behavior of the strains of *Rhizobium* and bean plant cultivars. When the inoculation is done with the strain UFLA 04-173, the cultivars differ as to the N content and accumulation in the shoot. In the case of the cv. Madrepérola, the strain CIAT 899^T contributed toward increased N content and accumulation in the shoot.

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INOCULATION OF COMMON BEAN SEEDS WITH *RHIZOBIUM TROPICI*, ASSOCIATED WITH MINERAL N FERTILIZATION AND OTHER ADDITIVES

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INTRODUCTION

The low technology level employed by farmers and the cultivation of common bean in soils with low fertility, especially poor in nitrogen, constitutes the main factors affecting the Brazilian productivity of the bean crop. In this context, the proper management of nitrogen fertilizer is one of the main difficulties to the bean crop, further on the application of excessive doses of N will increase the production cost and can cause serious environmental damage. Furthermore, the use of insufficient quantities may limit the potential yield of bean (Santos et al., 2003). On the other hand, studies have shown that it is possible that the culture can benefit itself, under field conditions, by the process of biological nitrogen fixation which can achieve productivity levels above 2,500 kg ha^{-1} (Hungria et al., 2000).

The present study aimed to evaluate the effects of nitrogen fertilization, inoculation with *R. tropici* and some treatments of the bean seeds, whether or not associated with a foliar fertilization on nodulation, shoot dry weight and N total accumulation on shoot of bean plants.

MATERIAL AND METHODS

The experiment was carried out in Aquidauana, MS, Brazil (20 º 28'S, 55° 47'W, 147m), in the Universidade Estadual de Mato Grosso do Sul (UEMS) experimental field. Sowing of common bean, cultivar BRS Radiante, was done on 04/09/2012, using 00-20-20 fertilizer formulated at the dose of 250 kg ha^{-1}. Each plot consisted of five rows of 4.0 m, 0.45 m spaced. The experimental design was in randomized complete block with three replications. The following treatments were tested: T1 = control (no N and no inoculation), T2 = Inoculation with *R. tropici* (SEMIA 4080 + SEMIA 4077); T3 = inoculation + 60 kg ha^{-1} N (30 $kgha^{-1}$ at 15 and 40 days after emergence), T4 = 80 kg N ha^{-1} at sowing + 40 $kgha^{-1}$ at R7 stage; T5 = inoculation + 2 foliar applications with $CaNO_3$ (in V3 (2 kg ha^{-1}) and V6 (8 kg ha^{-1}); T6= inoculation + chemical treatment of seeds (Standak Top); T7 = seed chemical treatment of seeds (Standak Top) + N (60 $kgha^{-1}$, 50% to 15 + 50% at 40 days after emergence), T8 = Inoculation + 2 foliar applications with 1.2 kg ha^{-1} P_2O_5 (stages V3 and V6). The nodules were collected 30 days after emergence, following the methodology described by Cardoso et al. (2009). On the same occasion was collected the shoot plants for the evaluation of the nodulation, shoot dry weight and N content on shoot bean plants.

RESULTS AND DISCUSSION

The sowing fertilization with 80 kg ha^{-1} of mineral N (T2) and seed treatment with fungicide + insecticide application associated with 60 $kgha^{-1}$ from N (T7), promoted a significant reduction ($p < 0.05$) in nodulation (number and nodules shoot dry weight) of bean plants compared to the control treatment (Table 1). To the other treatments were not observed significant differences ($p < 0.05$).

Both as the shoot dry weight and shoot nitrogen content of the common bean plants were not affected significantly ($p < 0.05$) by any of the treatments tested (Table 1). However, it should be noted that the plants only inoculated with *R. tropici* showed values that did not differ from those obtained in other treatments with fertilizer and chemical treatment of seeds (Table 1), indicating that this practice could replace the N fertilization with a lower cost, contributing to the farmer net income.

Table 1. Nodulation, shoot dry weight and shoot nitrogen content at of the common bean plants inoculated with *R. tropici*, associated with nitrogen fertilization and other additives. Aquidauana, MS. Brazil. Harvest 2012.

Treatments	*Nodule (number pl^{-1})	*Nodule Dry weight (mg pl^{-1})	Shoot dry weight (g pl^{-1})	Shoot N content (g kg^{-1})
T1. Control	1.99 a	6.35 a	1.74 a	34.7 a
T2. 120 kg ha^{-1} de N	1.04 b	0.13 b	1.79 a	36.0 a
T3. Standard Inoculation (SI)	1.88 a	6.79 a	1.58 a	36.9 a
T4. SI + 60 kg ha^{-1} de N	1.90 a	4.77 a	1.75 a	35.3 a
T5. SI + $CaNO_3$	2.17 a	5.66 a	1.56 a	35.0 a
T6. SI + fungicide + insecticide	2.06 a	4.54 a	1.62 a	33.1 a
T7. Fungicide + insecticide in seed + 60 kg ha^{-1} de N	1.35 b	0.89 b	1.94 a	33.7 a
T8. SI + P foliar fertilization	2.23 a	6.83 a	2.64 a	37.4 a
CV (%)	38	46	35	12

* Transformed data $(x+1)^{1/2}$. Means followed by the same letter in the column do not differ by Scott-Knott test at 5% probability.

CONCLUSIONS

- The bean seeds treatment with fungicide + insecticide or fertilization with 80 kg ha^{-1} of mineral N at sowing promote significant reductions in common bean plants nodulation compared to the control treatment.
- The shoot dry weight and the shoot N content in common bean plants inoculated with *R. tropici* did not differ significantly from those plants fertilized with 80 kg ha^{-1} of N at sowing + 40 kg ha^{-1} of N at coverage fertilization.
- The *R. tropici* inoculation in common bean crop can replace the need for nitrogen fertilization, reduce production costs and increase the farmer net income.

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LEAF AREA AND BIOMASS OF SNAP BEAN IN RELATION TO SHADING AND NITROGEN

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INTRODUCTION: The solar radiation (SR) and nitrogen supply are decisive for the production of crops. However, some species require less intensity of radiation (ISR) to express higher growth and yield. The IR can be manipulated using meshes with different opening generating shading, which can also alter temperature, humidity, growth and performance of the plant. The shadow as well as reducing photoinhibition, reflect the SR infrared and reduces the emission of heat to the plant canopy, also prevents room temperature rises to the degree that partly avoided, the incidence of abiotic diseases, reducing susceptibility to attack by pests and diseases (Bustamante, 2001).The effect of shading on the growth of the dry bean has been reported by Escalante *et al.* (1980). On the other hand, fertilization with nitrogen (FN) produces a greater leaf area, intercepted radiation, growing and yield of crops (Escalante and Rodriguez, 2010). So, with the combination of these management practices, could be more appropriate conditions for some crops that require to express higher growth and yield with ISR lower than the incident. The objective of the study was to determine, the effect of shading and nitrogen on plant height, number of leaves, leaf area and dry matter distribution in snap bean.

MATERIALS AND METHODS: The study was established in a glasshouse of the Colegio de Postgraduados Campus Montecillo, México. Planting bush snap bean cv."Strike" was 10 October 2011 in 1 litre pots. 25 days after sowing (das) seedlings presented the first pair of leaves, well exposed and was thinning. During this time, watering with tap water was applied. Subsequently, in each pot with treatment of N is supplied with 100 ml of a solution of urea 212 mg l^{-1} daily. In total 11 irrigations were applied. The treatments were as follows: 1) shaded with black mesh and N (BSN +); 2) Shaded with black mesh without **N** (BSn0); 3) Shading with white mesh and N (WSN +); 4) Shading with white mesh without N (WSn0); 5) Without shading with N (S0N +); and 6) without shading without N (S0n0). The experimental design was factorial randomized with twenty replicates. The time to occurrence of phenological phases was recorded. To 25 and 50 das, ten plants were harvested by treatment to register, (HP) height, leaf area (LA) and dry matter (DM) per plant. The SR at noon, was measured with a sensor linear quantum, inside and out of glasshouse and under the shaded mesh. The temperature was also recorded. An analysis of variance (ANOVA) and a test of comparison of means between treatments (Tukey, 0.05) was applied .

RESULTS AND DISCUSSION: The SR inside glasshouse was 26% lower that on the glasshouse (957 Wm^{-2}) and under the shaded-mesh was 83% lower. The temperature was 32 °C and under the mesh of 30 ° C. The occurrence of phenological stages was similar among treatments. Emergency (V1) was 8 das, first pair of leaves (V2) to 13 das and first trifoliolate leaf (V3) 21 das and R5 stage or flowering at 50 das. To 25 das, the mean of the HP was 5 cm, the LN 2, LA 26 cm^2, stem DM 83 mg, root DM 127 mg, leaves DM 45 mg, and total DM or biomass (TB) of 255 mg per plant. To 50 das, HP, LN, LA and TB per plant showed significant changes by effect of shading (S), nitrogen (N) and interaction S*N (table 1).

Snap bean with shading in particular with white mesh (WS) presented greater HP, LN, LA, root DM, stem DM, leaves DM and TB. The root accumulate highest DM, followed of leaves and stem. Similar trends were found in the snap beans with FN. Escalante and Rodríguez (2010) for dry bean under field conditions reported similar results. In terms of the interaction of S * N, the highest values of the variables in study corresponded to the bean planted with shaded-mesh and FN, particularly with white mesh. These results indicate that under conditions of high temperature, with the use of shaded-mesh be achieved higher growth and consequently greater production of TB in SNAP bean cv.Strike. This could also be applicable for planting of beans combined with other crops, under non-limiting conditions of water and nutrients.

Table 1. Plant height (cm), leaves number, leaf area (cm^2), dry matter (g) and its distribution in snap bean (*P.vulgaris* l.) cv. Strike in relation to shading and nitrogen. Data per plant to 50 das.

Shading (S)	Nitrógen	High (cm)	LN	LA dm^2	RDM (g)	SDM(g)	LDM (g)	TB (g)
S0		8 b	4 c	1.7 b	0.60 b	0.26 b	0.51 c	1.5 b
BS		11 a	5 b	3.0 a	0.76 a	0.27 b	0.64 b	1.6 b
WS		10 ab	6 a	3.1 a	0.80a	0.36 a	0.76 a	1.9 a
	N+	10 a	5.7	3.0 a	0.72 a	0.34 a	0.81 a	1.9 a
	n0	9 a	5.0	2.2 b	0.73a	0.25 b	0.47b	1.4 b
Prob F.	N	**	**	**	*	*	**	*
	S	**	**	**	**	*	**	*
	S*N	**	**	**	**	*	*	*

S0 = unshaded (control); BS and WS = shading mesh black and white, respectively; NL = number of leaves; LA = leaf Area; RDM, SDM, LDM and TB indicate dry matter in root, stem, leaves and total accumulated per plant, respectively. N += with application of N; N0 = without application of N; S = shading. *, * indicates F > 0.05 and 0.01, respectively. In columns values with different letter indicates statistical difference (Tukey $\alpha = 0.05$).

CONCLUSIONS._With shading and nitrogen fertilization is achieved higher growth of cv. Strike snap bean and greater distribution of dry matter in the plant organs. The shading * nitrogen interaction affects the magnitude of the growth variables of snap bean.

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GROWTH ANALYSIS OF THE SNAP BEAN IN RELATION TO SHADING AND NITROGEN

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INTRODUCTION

Growth analysis has been a valuable tool in the quantitative analysis of plant and crop growth (Hunt *et al*., 2002). This knowledge is important to the timely agronomic management and achieve a maximum production of the crop. Despite that studies have demonstrated the usefulness for a better understanding of how environmental changes affect the dynamics of growth and generation of the crop yield , its use has not been generally extended. In Mexico, background on analysis of growth in dry bean (*Phaseolus vulgaris* L.) in relation to effects of shading and nitrogen fertilization have been presented by Escalante and Kohashi (1982); Escalante and Rodríguez (2010), respectively. The objective of the study was to determine in bush snap bean, under glasshouse conditions in temperate climate, the effect of artificial shading and the nitrogen supply on growth rate (GR) and canopy efficiency for dry matter production.

MATERIALS AND METHODS

The study was established in a glasshouse of the Colegio de Postgraduados Campus Montecillo, México. Planting bush snap bean cv."Strike" was 10 October 2011 in 1 litre pots. To 25 days after sowing (das) seedlings presented the first pair of leaves well exposed and was thinning. During this time, watering with tap water was applied. Subsequently, in each pot with treatment of N is supplied with 100 ml of a solution of urea 212 mg l^{-1} daily. In total 11 irrigations were applied. The treatments were as follows: 1) shaded with black mesh and N (BSN +); 2) Shaded with black mesh without **N** (BSn0); 3) Shading with white mesh and N (WSN +); 4) Shading with white mesh without N (WSn0); 5) Without shading with N (S0N +); and 6) without shading without N (S0n0). The experimental design was factorial randomized with twenty replicates. The time to occurrence of phenological phases was recorded. To 25 and 50 dds, ten plants were harvested by treatment to register, (HP) plant height ,leaf area (LA), total dry matter or biomass (BT) per plant to calculate the height growth rate (HGR, cm d^{-1}) specific leaf area cm^2 g^{-1},SLA), the absolute growth rate of leaf area (LGR, dm^{-2} d^{-1}), plant growth rate (PGR, g d^{-1}), leaf area duration (LAD, cm^{-2} d^{-1}), biomass duration (BD; g d^{-1}) and net assimilation rate (NAR, g dm^{-2} d^{-1}) in accordance with the criteria outlined in Escalante and Kohashi (1993). The SR at noon, was measured with a sensor linear quantum, inside and out of glasshouse and under the shaded mesh. The temperature was also recorded. An analysis of variance (ANOVA) and a test of comparison of means between treatments (Tukey, 0.05) was applied .

RESULTS AND DISCUSSION

The SR inside glasshouse was 26% lower that on the glasshouse (957 Wm^{-2}) and under the shaded-mesh was 83% lower. The temperature was 32 ° C and under the mesh of 30 ° C. The occurrence of phenological stages was similar among treatments. Emergency (V1) was 8 das, first pair of leaves (V2) to 13 das and first trifoliolate leaf (V3) 21 das and R5 stage or flowering at 50 das. The ANOVA showed significant differences by effect of the shading (S), nitrogen (N) and the interaction of S * N. The snap beans grown under shaded particularly with white mesh, presented the highest GR in relation to control. Similar trends have been reported for dry bean by Escalante *et al*. (1982). Likewise, with N plants showed a greater HGR, PGR, LGR, LAR, SLA and LAD which led to a greater DB. In terms of the interaction of S * N, snap bean with shadow and N, was more efficient in forming leaf area per g of DW inverted in the

leaf (SLA), the canopy was more efficient to produce DM thus generating higher biomass (DB). The control (without shade and N) showed the lowest indices. This indicates that under conditions of high temperature, the use of shading-mesh with N supply, it is advisable for a greater production of DM of snap bean cv.Strike.

Table 1. Indices analysis of growth of snap bean (*P.vulgaris* l.) cv. Strike in relation to shading and nitrogen.

Shading (S)	Nitrógen	HGR cm d^{-1}	LGR dm^2 d^{-1}	PGR g d^{-1}	NAR g dm^{-2} d^{-1}	LAD dm^2 d	BD g d	SLA cm^2 g^{-1}
S0		0.11b	0.02b	0.02 a	0.53c	24.5b	22.0 b	3.3 c
BS		0.22 a	0.03a	0.01 b	0.95 a	41.4 a	23.2 b	4.8 a
WS		0.20 a	0.03a	0.02 a	0.65b	41.7a	27.0 a	4.0 b
	N+	0.20 a	0.03 a	0.02 a	0.68 b	41.0 a	27.0 a	3.7 b
	n0	0.16 b	0.02 b	0.01 b	0.66 a	30.8 b	21.0 b	4.7 a
Prob F.	N	*	*	*	*	**	**	**
	S	**	*	*	*	**	**	**
	S*N	*	*	NS	**	**	**	**

S0 = without shadow (control); BS = shading with black mesh; WS = shading with white mesh; HGR= plant height growth rate; LGR= leaf area growth rate; PGR = plant growth rate; NAR= net assimilation rate; LAD= duration of leaf area; BD = duration of biomass; SLA= specific leaf area; d=day. *, * indicates F > 0.05 and 0.01, respectively. In columns values with different letter indicates statistical difference (Tukey $\alpha = 0.05$).

CONCLUSIONS

With the shading and nitrogen snap bean cv. Strike presents a higher specific leaf area, greater efficiency of the canopy to produce dry matter, higher growth rates and greater production of biomass.

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IMPACT OF SALINITY AND NITROGEN DEFICIENCY ON CONTAMINATION OF NITRATE NITROGEN IN COMMON BEAN (*PHASEOLUS VULGARIS* L.) ROOTS

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INTRODUCTION

Main goal of plant living strategies is to overcome tough environmental conditions, and to maximize the effectiveness of nutrition uses. One of this type situations is symbiosis of bean plants with bacteria from *Rhizobium* family. It allows both sides to optimalyse their living conditions – bacteria can obtain necessary macro- and microelements from plant, and plants can use nitrogen assimilated by bacteria (Yang et al. 2009). Many authors point bad nitrogen fertilization as cause of: lower quality and quantity of yield, longer vegetation period or problems with overcoming witner. Nitrogen as structure-building nutrient takes a part in synthesizing not only cell organella, but also whole tissues, what makes it one of the most important macroelements (Starck 2008). Another major problem is too high salinity of soil, which usually occurs in early spring – during snowmelts. Too high concentration of sodium chloride in medium causes occurrence of water stress symptoms, leading to degradation of cells and tissues and deepening the toxicity of sodium ions (Kaymakanova et al. 2010). The experiment scope was to determine connections between contamination of nitrate nitrogen in roots of common bean, and nitrogen dose as also presence and doses of sodium chloride (salinity) in medium.

MATERIALS AND METHODS

Seeds of common bean cultivar 'Erla' were germinated in perlite (watered with H_2O_d), arranged in the phytostatic chamber with controlled conditions: light intensity of 300 μmol $m^{-2} \cdot s^{-1}$, temperature (24°C at day, and 18°C at night), photoperiod 14h/10h (day/night) and relative air humidity - 65%. After developing first pair of true leaves plants were moved into hydroponic cultures (using full Hoagland's medium as control, and medium without 50% of nitrogen as experimental variant). NaCl was also added as second experimental factor: control – 0 mM·kg $^{-1}$ (0 g·kg $^{-1}$); first concentration - 30 mM·kg $^{-1}$(1,755 g·kg $^{-1}$) and second concentration 50 mM·kg $^{-1}$ (2,925 g·kg $^{-1}$). After two weeks of adaptation period, analysis of nitrate nitrogen in plants roots were taken. 1 gram samples of fresh weight were homogenised with 10 ml of distilled water. Next the samples were placed in boiling water bath for 10 minutes, and then putten to cool down. Obtained samples were filtered, and 0,1 ml of extract was taken. Extract was carefully mixed with 0,4 ml of salicylic reagent. After 20 minutes 9,5 ml of 2-mol NaOH was added. Absorbance of so made extracts was determined at 420 nm wavelength (using MarcelMini spectrophotometer) (Bielecki et al. 2001). The results were developed in the program "Statistica 10.0". The Duncan's test was used (at significance level $\alpha = 0.05$) to evaluate the differences between control and experiment variants.

RESULTS AND DISCUSSION

Statistically significant differences in contamination of nitrate nitrogen in roots of bean plants grown in different medium were found. The highest values of nitrate nitrogen content were noted in plants grown in control conditions (0,0686 µmol NO_3^-·cm^3), while the lowest (0,0011 µmol NO_3^-·cm^3) - in plants grown in medium deprived of 50% nitrogen from composition and with addition of 50 mM NaCl. Leidi and Rodriguez-Navarro (2000) in their research on nitrogen content in soil and nitrogen content in bean organs have shown similar data. Total nitrogen contet in plant was decreasing with decreasing nitrogen content in substrate. Similar trend was obtained by Bruning and Rozema (2012) in studies on the effect of salinity on nitrogen content in plants – with increasing salinity, nitrogen content in plant significantly decreased.

On the base of obtained results it can be clearly stated that combined stress of salinity and lower nitrogen content in medium has the greatest impact on reduction nitrate nitrogen content in the roots of common bean.

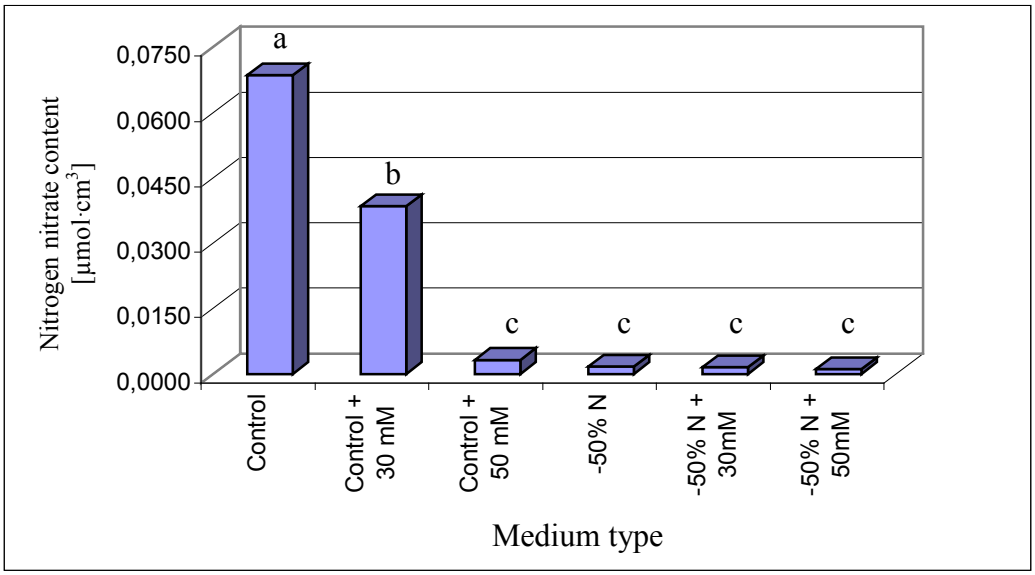

Figure 1. Average content of NO_3^- ions [µmol·cm^3] in bean roots depending on salinity and nitrogen content in medium (letters above charts = homogeneous groups)

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IMPACT OF BIOSTIMULATORS ON WATER RELATIONS IN BEAN PLANTS (*PHASEOLUS VULGARIS* L.) GROWN UNDER SALINITY STRESS

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INTRODUCTION

Despite many efforts to reduce the salinity of the soli in environment, it is still considered to be one of the biggest problems in today's agriculture and horticulture. Another problem is the continuous reduction of available fresh water in the world, therefore research is carried to use mixes of salt water with fresh water to irrigate crops. Unfortunately, adverse effects of NaCl on growth, development and yield of plants have already been proven, and thus attempts to neutralize it are often made - either through addition of other salts, or by the application of various types of preparations.

One of the major damage caused by an excessive amount of sodium ions in the medium is a direct increase of osmotic pressure in the body and thereby plant water management disorder. The aim of the study was to determine the impact of biostimulators on the water balance of bean plants grown under salinity conditions.

MATERIALS AND METHODS

Experience with common bean cv. 'Erla' was conducted in July and August 2012, in the Department of Plant Physiology phytotron chamber, with controlled photoperiod 12h/12h (day/night) and light intensity of about 200 μmol $s^{-1} \cdot m^{-2}$. Initially, the plants were grown in perlite. After the evaluation first pair of true leaves, plants were removed from the perlite, carefully cleaned and transferred to containers with a capacity of 1000 ml filled with the appropriate type of medium. The experiment was conducted in 3 replications, having regard to the full Hoagland medium - as control and with addition of 30 and 50 mM NaCl as the experimental variant. After 10 days of adaptation, plants were sprayed with preparations (Asahi or Pentakeep). Measurements were performed 7 days after treatment.

In order to determine RWC and WSD indicators, selected leaves were weighed on an analytical weight with an accuracy of 0,001 g. After that they were placed for 24 hours in a glass vessel filled with distilled water, next they were removed and dried with tissue paper. Leaves were weighed again (weight of leaves at full saturation). Subsequently the plant material was dried in an oven to constant weight (at 80°C for 24 h), and weighted again. The obtained data were converted by patterns of two indicators (Bandurska 1991, Kopcewicz, Lewak 2002). The results were developed in the program "Statistica 10.0" produced by Statsoft. The Fisher's test was used (at significance level $\alpha = 0.05$) to determine the differences between control and stressed variants.

RESULTS AND DISCUSSION

A significant effect of salinity and preparations used on the indicators of water balance in bean plants was found. The highest relative water content in the leaf (RWC) were observed in plants grown under control conditions and treated with Pentakeep preparation (89.5%). In turn the highest water saturation deficit in the leaf tissue was found in the plants grown in medium with the largest (50 mM) NaCl addition (WSD = 22.5%) - Fig.1.

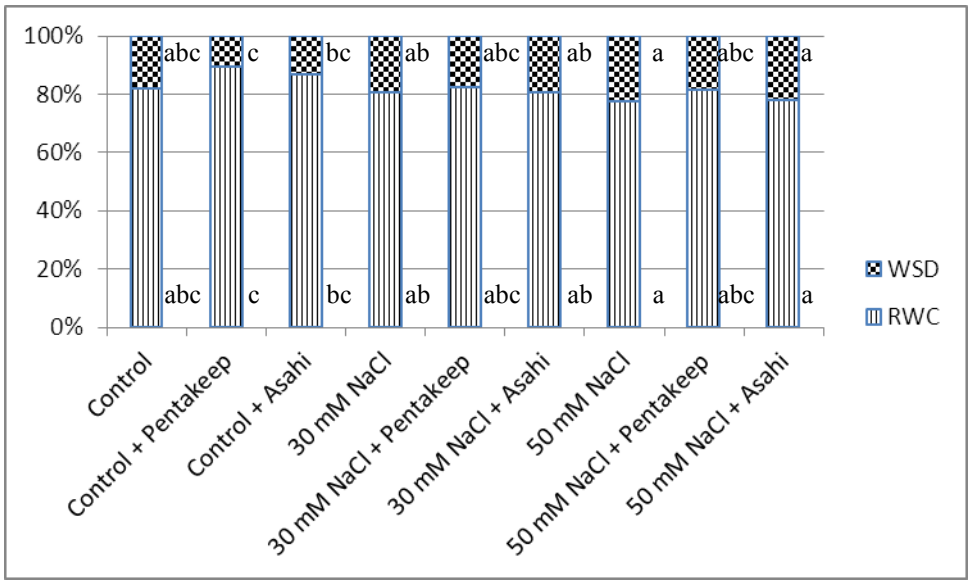

Figure 1. Average values of water balance indicators (RWC, WSD) of common bean depending on salinity and used biostimulators (letters above charts = homogenous groups)

Based on obtained data an increased water content in the leaf tissue of plants treated with Asahi and Pentakeep preparations was found. Preparations from the biostimulators group are based on biologically active compounds (Asahi - nitrophenols, Pentakeep - 5-aminolevulinic acid), which have a significant impact on increasing the biomass of plants (Szot et al. 2009).
The consequence of this situation is that area of plant transpiration and the intensity of this process is increased. However, the maintenance of the relatively high water content in the leaf can be explained by increased water uptake by plants from the soil. This phenomenon has been corroborated by other researchers such as Przybysz et al. (2010), and Wrochna et al. (2008), who obtained similar results.

CONCLUSIONS

1. Pentakeep and Asahi preparations had a positive effect on increasing water content in the leaf tissue, in both control and salinity conditions.
2. Treatment with Pentakeep almost completely abolished the negative effect of small (30 mM) and medium (50 mM) salinity on water balance of bean plants.

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BIOCOMPATIBILITY OF TOMATO (*SOLANUM LYCOPERSICUM* L.) AND COMMON BEAN (*PHASEOLUS VULGARIS* L.) IN AN ORGANIC CULTURE SYSTEM

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INTRODUCTION: The growth and development of organic vegetables depend on soil biological activity which in turn depends on soil properties such as, soil texture and structure, pH, organic matter, nutrient contents, and cultural practices. Particularly, organic farming systems have notable differences in nitrogen (N) sources, cycling and management strategies compared to conventional systems with high inputs of synthetic N fertilizer [1]. In organic farming, nitrogen availability can be limiting especially in the absence of livestock and causes vegetables yield depressions, lower protein and nutrient contents. Indeed, the slow turnover of organic amendments in organic farming may become a limiting factor for optimal crop productivity, particularly in greenhouse production where the nutrient requirements such as nitrogen are higher than those of field crops, mostly due to increased yield which, in the case of tomatoes, can be up to ten times more inside the greenhouse compared to outside field conditions. For these reasons, integrating legumes with symbiotic fixation of atmospheric N2 is essential for balancing nitrogen exports from the system. Intercropping (IC) vegetables and legumes is a practice for eco-functional intensification, which is considered a mean to enhance yields and soil productivity in organic farming [2]. The objective of this study was to determine the effects of intercropping of common bean inoculated with *Rhizobium* strains (CIAT 899, and 4H41) on chemical contents, yield and growth of tomato (*Solanum lycopersicum* L.) vegetable species conducted in biological mode.

MATERIAL AND METHODS: The experiments were conducted at the experimental organic farm of Higher Institute of Agronomy of Chott Mariem between December and April of 2011 and 2012. The soil characteristics before sowing were: pH 8.37, percentage organic matter 1.12, total N 0.13%, available P (ppm) 85 and available K (ppm) 450. The effects of common bean plant inoculation by CIAT 899 and 4H41 strains and the intercropping effects of fruit in tomato were evaluated on 10 plants by determining growth and yield compound. The micro-Kjeldahl procedure was applied for determination of N after wet digestion of dried and ground subsamples in a H2SO4-Se-salisilic acid mixture. All data were subjected to a one-way analysis of variance (ANOVA) and separated by Duncan's multiple range tests using SYSTAT 8.0.1.

RESULTS AND DISCUSSION: The effects of bacterial application on mineral (N) contents of tomato fruit and common bean seed were significant at $p<0.05$ (Tables 1). All bacterial applications particularly affected on increasing in N contents of the tomato and common bean plant. The highest N contents were obtained from CIAT 899 applications in the both species.

The higher mineral contents in the bacteria treated plant may have resulted from the producing plant hormones [3] ability of these bacteria, as reported that many kinds of bacteria had given same results on different plant species in previous studies [4]. These authors stated that increasing mineral contents in plants results in greater uptake of nutrient elements from soil. We suggested that increasing N contents in tomato plant intercropped with common bean, particularly in the CIAT 899 bacteria-treated common bean plants, which may be explained by higher concentration of N2 stimulated by bacterial application and resulted from the producing plant hormone. Growth promoting effects of bacterial application on common bean growth and yield parameters were significant, but its effect on some parameters such as shoot fresh weight, root fresh weight, and number of pods per plant was not significant in both of the *Rhizobium* strains application (Table 2). Effects of intercropping of common bean and bacterial application on fruit weight per plant, plant length, total soluble sugars and acidity of tomato fruit were significant (Table 3). The highest average fruit weight per plant (696,57g) and plant length (154,57cm) were obtained from CIAT 899 applications as compared to that of the other applications. This is the first report on effects of intercropping of common bean inoculated with Rhizobium strains on growth and yield of tomato conducted in biological mode. However, similar reports were obtained in different plant species. Researchers stated that mixed cropping of wheat and chickpea can stimulate growth and increase yield [5]. The reason of growth promoting effect of intercropping and the use of bacterial strains on plant growth is that they affect on fixation capacity of (N) and are one of the most plausible mechanisms of action affecting plant growth in organic farming. Thus, our finding is in good agreement to previous studies mentioned above.

Table 1. Effect of intercropping culture on N (%) content (CB, common bean; CBS1, CB inoculated with CIAT 899; CBS2, CB inoculated with 4H41; TV1, tomato variety number 1; TV1CB, TV1 intercropped with CB; TV1CBS1, TV1 intercropped with CBS1; TV1CBS2, TV1 intercropped with CBS2; TV2, tomato variety number 2; TV2CB, TV2 intercropped with CB; TV2CBS1, TV2 intercropped with CBS1; TV2CBS2, TV2 intercropped with CBS2; TV3, tomato variety number 3; TV3CB, TV3 intercropped with CB; TV3CBS1, TV3 intercropped with CBS1; TV3CBS2, TV3 intercropped with CBS2)

	CB	CB S1	CB S2	TV 1	TV1C B	TV1C BS1	TV1C BS2	TV 2	TV2C B	TV2C BS1	TV2C BS2	TV 3	TV3C B	TV3C BS1	TV3C BS2
N (%)	5,7a	7,7b	5,9a	8,3c	9,7d	11,3e	9,2f	9,2f	10,5g	11h	8,8i	6,3j	5,7a	5,6a	6k

Table 2. Effects of inoculation on different plant parameter of common bean

	CB	CBS1	CBS2
Plant height (cm)	36,4 ± 2,07 a	72,2 ± 7,19 b	51,4 ± 3,14 c
Shoot fresh weight (g)	18,60 ± 4,5 a	75,40 ± 8,96 b	57,80± 7,31 b
Root fresh weight (g)	5,50 ± 1,41 a	7,30 ± 2,01 b	7,20 ± 1,52 b
Shoot dry weight (g)	5,42 ± 1,21 a	14,11± 2,35 b	10,45 ± 1,51 c
Root dry weight (g)	3,4± 0,39 a	1,66± 0,35 b	1,88± 0,43 b
Root/Shoot ratio	0,63± 0,1 a	0,11± 0,019 b	0,178± 0,056 b
Number of nodule/plant	0,2± 0,44 a	76± 7,035 b	35,8± 5,8 c
Number of pods/plant	9,2± 2,86 a	15,6± 2,88 b	14,8± 3,03 b
Number of seeds/pod	2,32± 0,17 a	3,08± 0,16 b	3,56± 0,08 c
100-seeds weight (g)	83,5± 1,11 a	77,5± 1,18 b	75,10± 0,89 c

Table 3. Effects of mixed cropping on different plant parameter of tomato

	Plant height (cm)	Yield/plant (g)	Acidity (g/100 ml)	Total soluble sugars
TV1	129,71± 8,96 a	521,29± 59,07 a	0,63± 0,016 a	4,89± 0,089 a
TV1CB	123,85± 6,54 a	503± 35,51 a	0,6± 0,013 a	4,07± 0,075 b
TV1CBS1	154,57± 6,26 b	696,57± 53,53 b	0,59± 0,025 a	4,97± 0,125 a
TV1CBS2	136,28± 8,93 a	555,43± 53,36 a	0,55± 0,009 b	4,37± 0,138 c
TV2	116,28± 7,13 c	476,71± 46,16 a	0,48± 0,028 c	4,91± 0,063 a
TV2CB	123,42± 7,20 ac	479,71± 22,24 a	0,61± 0,019 a	4,89± 0,146 a
TV2CBS1	131± 5,25 a	599± 44,83 ac	0,5± 0,015 cd	5,81± 0,106 d
TV2CBS2	122,71± 6,07 ac	569± 38,55 a	0,66± 0,017 e	6,31± 0,254 e
TV3	121± 3,16 ac	409,86± 42,76 d	0,45± 0,036 c	4,94± 0,078 a
TV3CB	128,28± 6,04 ac	549,57± 42,91 a	0,43± 0,016 cf	4,54± 0,222 c
TV3CBS1	125,42± 8,4 ac	524,71± 45,48 a	0,39± 0,039 g	4,46± 0,151 c
TV3CBS2	115,71± 8,03 dc	515,29± 55,93 a	0,36± 0,024 g	5,31± 0,106 f

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POSSIBILITIES FOR CONTROL OF BEAN WEEVIL (*ACANTHOSCELIDES OBTECTUS* SAY) IN COMMON BEAN (*PHASEOLUS VULGARIS* L.) IN OPEN FIELD CONDITIONS

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INTRODUCTION: Bean crops are attacked by numerous pests in all growing stages but the devastations caused by bean weevil (*Acanthoscelides obtectus* Say) are of economical importance. The control of this pest is difficult and it is required a complex method that should include: (i) crop protection potential (Staneva 1993) and (ii) use of pest tolerant cultivars as cost-efficient and environmental-friendly assay (Tomlekova 2012). It should start on the bean fields as it is directed against the beetle adults and it is performed for a very short period before harvesting (Schmale *et al* 2002). Generally in Bulgaria this period includes the second half of June and August during the pods ripening (Staneva 1993, Tsvetkov 2000). Some organophosphorus and pyrethroids insecticides could be applied in field conditions for control of this pest (Staneva 1995, Săpunaru *et al* 2006, Sofkova & Yankova 2011). New products such as neonicotinoids are used recently. Their innovative mechanism of action combines contact and systematic effect. It was established that product Mospilan 20 SP 0,02% (a.i. acetamyprid) possesses high toxicity towards bean weevil in laboratory conditions and good effectiveness under field conditions (Yankova, 2006). The objective of the study was to establish the effectiveness of the plant protection products against bean weevil in three snap bean varieties possessing different pest tolerance, applied individually and in combination under field conditions.

MATERIALS AND METHODS: Plots were set in the experimental field at Maritsa VCRI during 2011-2012 cropping seasons in randomized block design in three replications with three snap bean genotypes (*Phaseolus vulgaris* L.) and natural infestation ground of *Acanthoscelides obtectus* Say. Varieties Tangra and Pagane - previously developed in the Institute, possessing different level of pest tolerance (Poryazov *et al* 2008) and var. Starozagorski cher was used as susceptible control. Test insecticides: neonicotinoids - Mospilan 20 SP 0.02% (a.i. acetamyprid) and Actara 25 WG 0.03% (a.i. thiamethoxam) and pyrethroid – Decis 2.5 EC 0.04% (a. i. deltamethrin). Treatment was performed in the beginning of pods ripening at density of 2 adults caught for 100 strokes of the entomology bag with control untreated plants. The following indexes were calculated: % infested seeds, index of infestation (%) (by Mc Kinney) and effectiveness (%) (by Abbot). Three treatments have been carried out with the products – individually and by rotation with an interval of seven days. Duncan's multiple range tests (1955) was used to compare different genotypes.

RESULTS AND DISCUSSION: Tangra and Pagane genotypes performed significantly low infestation from bean weevil compared to Starozagorski cher according to the results obtained from the control treatment (Table 1). That statement confirmed previous studies of Sofkova and Yankova, (2011). The best results for the three genotypes were obtained when rotation of the insecticides treatment was used. In this treatment a maximal value (100 %) of the effectiveness was achieved in Tangra during the two experimental years, followed by Pagane (Fig. 1).

Mospilan 20 SP 0,02% and Actara 25 WG 0,03% demonstrate very good biological activity towards the bean weevil in field conditions that is higher than recorded in Decis 2,5 EC 0,04%. Thus, the performed high effectiveness of neonicotinoides along with pyrethroides treatment combined with suitable pest tolerant bean varieties can significantly decrease the pest risk of attack under field conditions.

Table 1. Bean weevil infestation in *P. vulgaris* L. varieties treated with plant protection products during 2011-2012

Treatment	Variety	Damaged seeds, %	Index of infestation, %
Control (untreated)	Starozagorski cher	3,10 a	1,83 a
	Tangra	1,19 b	0,44 b
	Pagane	1,94 ab	0,82 ab
Mospilan 20 SP 0,02% (threefold treatment)	Starozagorski cher	0,51 a	0,15 a
	Tangra	0,10 b	0,02 b
	Pagane	0,18 b	0,05 b
Decis 2,5 EC 0,04% (threefold treatment)	Starozagorski cher	0,78 a	0,27 a
	Tangra	0,20 b	0,05 b
	Pagane	0,32 b	0,11 b
Actara 25 WG 0,03% (threefold treatment)	Starozagorski cher	0,22 a	0,06 a
	Tangra	0,10 a	0,03 a
	Pagane	0,09 a	0,03 a
Rotation treatment: Mospilan 20 SP 0,02% Decis 2,5 EC 0,04% Actara 25 WG 0,03%	Starozagorski cher	0,21 a	0,05 a
	Tangra	0,00 b	0,00 b
	Pagane	0,08 ab	0,02 ab

a, b.....degree of significance at P≤0,05 by Duncan's Multiple Range Test

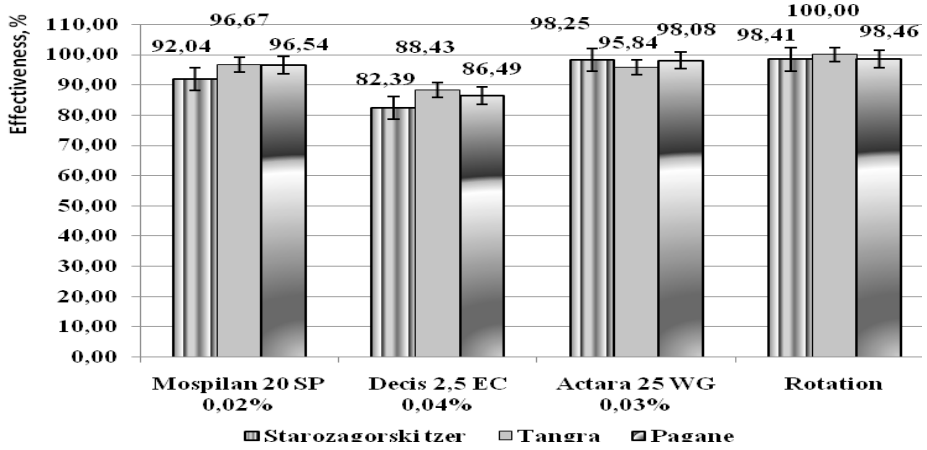

Fig. 1. Effectiveness of plant protection products against *Acanthoscelides obtectus* Say in the field

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EFFECT OF INJURY AND FECAL DROPPINGS ON THE FEEDING BEHAVIOR OF *CEROTOMA ARCUATA* (OLIVIER, 1791) (COLEOPTERA: CHRYSOMELIDAE) ON COMMON BEAN

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is the most widely cultivated species among others of the genus, contributing about 95% of the world production (YOKOYAMA et al 1996).

Cerotoma arcuata (Oliver, 1791) adults are brown beetles, with dark spots on the dorsum, measuring 5-6 mm length. The adults feed on the plant leaves, reducing the photosynthetic capacity, being able to transmit phytopathogenic virus (QUINTELA, 2002). This work aimed to evaluate the feeding behavior of *C. arcuata* on common bean leaves, which suffered injuries through holes and impregnation with fecal droppings.

MATERIAL AND METHODS

The assay was carried out at Faculdade de Ciências Agrárias e Veterinárias – UNESP, Jaboticabal SP, Brazil, Departamento de Fitossanidade, Laboratório de Resistência de Plantas a Insetos, under controlled conditions of temperature (25 ± 1 ^{o}C), relative humidity (60 ± 10%) and photoperiod (12 hours).

A randomized block design was used with five replications, using the common bean cultivar BRS-Supremo. The treatments adopted were: Leaves with one hole; Leaves with three holes; Leaves with one hole and fecal droppings; Leaves with three holes and fecal droppings; Leaves without holes and with fecal droppings; Control without holes and fecal droppings.

The holes were performed manually, using a metal puncher of 0.3 cm². The leaves were impregnated with 0.03 g of *C. arcuata* fecal droppings, per plant, being moistened with 0.03 ml of water, for better setting on the plant. The plants were placed into glass cages, equidistantly from center, subsequently releasing two adults per treatment, into the cage center. The attractiveness was evaluated at 1, 3, 5, 10, 15, 30 minutes and 1, 2, 6, 12, 24, 36 and 48 hours after the insects release. Later the adults were removed, measuring the leaf intake through the device LI-COR®, model 3100, discounting the values of the holes made.

The data obtained through the tests were submitted to the analysis of variance (ANOVA) by F test, and their averages compared by Tukey test, at 5% probability. For statistical analysis, data were transformed to $(x + 0.5)^{1/2}$.

RESULTS AND DISCUSSION

Regarding the attractiveness and leaf intake (cm²) on the leaves with holes and impregnated with fecal droppings, in free-choice test, significant differences were not verified at any of the times assessed (Table 1) and leaf intake (Figure 1). In conclusion, the results showed that holes and fecal droppings on the leaves of BRS-Supremo did not influence in the attractiveness and feeding of *C. arcuata*.

Table 1. Attractiveness on common bean leaves with or without the presence of holes and fecal droppings of *Cerotoma arcuata*. Jaboticabal, SP, 2011.

TREATMENTS	Minutes					
	1	3	5	10	15	30
Control	0.0 a	0.0 a	0.2 a	0.2 a	0.8 a	0.8 a
One hole without fecal droppings	0.0 a	0.0 a	0.2 a	0.2 a	0.4 a	0.2 a
Three holes without fecal droppings	0.2 a	0.0 a	0.4 a	0.4 a	0.2 a	0.4 a
Without holes with fecal droppings	0.0 a	0.0 a	0.2 a	0.4 a	0.2 a	0.4 a
One hole with fecal droppings	0.0 a	0.0 a	0.2 a	0.2 a	0.0 a	0.2 a
Three holes with fecal droppings	0.0 a	0.2 a	0.6 a	0.4 a	0.8 a	0.6 a
F (Treatment)	1.00 ^{ns}	1.00 ^{ns}	0.63 ^{ns}	0.23 ^{ns}	1.43 ^{ns}	0.33 ^{ns}
C. V. (%)	13.05	13.05	28.37	30.61	32.74	39.48
TREATMENTS	Hours					
	1	2	6	12	24	36
Control	0.8 a	0.8 a	1.0 a	1.4 a	1.4 a	0.8 a
One hole without fecal droppings	0.4 a	0.0 a	0.4 a	0.6 a	0.8 a	1.2 a
Three holes without fecal droppings	0.6 a	0.6 a	1.2 a	1.4 a	1.6 a	1.6 a
Without holes with fecal droppings	0.4 a	0.6 a	0.8 a	1.0 a	1.0 a	0.8 a
One hole with fecal droppings	0.2 a	0.2 a	0.4 a	0.4 a	0.6 a	0.8 a
Three holes with fecal droppings	1.0 a	0.6 a	0.6 a	1.2 a	1.2 a	1.6 a
F (Treatment)	0.72 ^{ns}	0.65 ^{ns}	0.50 ^{ns}	0.71 ^{ns}	0.52 ^{ns}	0.38 ^{ns}
C. V. (%)	35.58	40.28	41.51	40.61	37.60	40.43

[1] Means followed by the same letter in column did not differ significantly by Tukey's test at 5% probability.
For analysis data were transformed to $(x + 0,5)^{1/2}$. ns = not significant

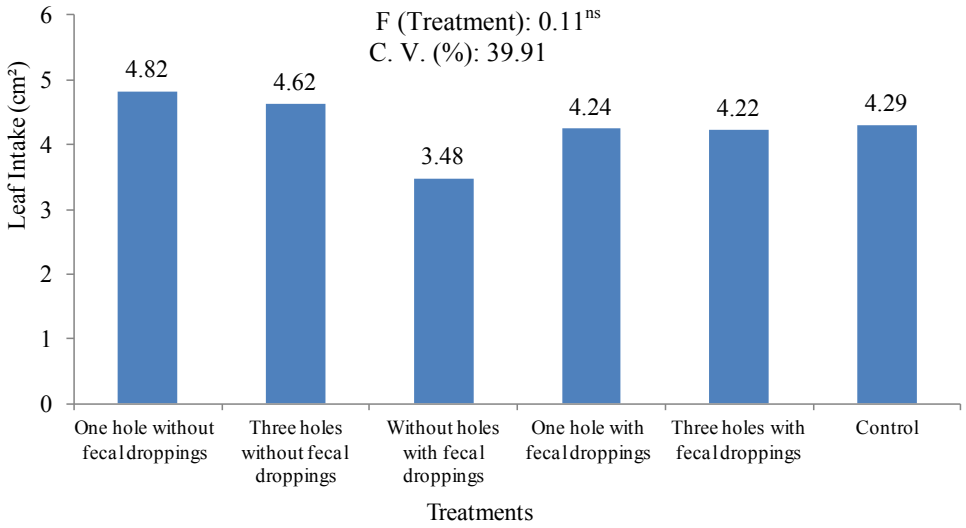

Figura 1. Leaf Intake on common bean leaves (cm^2) by *Cerotoma arcuata*, with and without the presence of holes and fecal droppings. Jaboticabal, SP, Brazil, 2011.

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EFFECT OF INJURY AND FECAL DROPPINGS ON THE FEEDING BEHAVIOR OF *DIABROTICA SPECIOSA* (GERMAR, 1824) (COLEOPTERA: CHRYSOMELIDAE), ON COMMON BEAN

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INTRODUCTION

Brazil is the largest consumer and producer of bean (*Phaseolus vulgaris* L.*)*, with average production in the crop of 2011/12 of 2918.4 thousand tons (CONAB, 2013), however, its productivity is low. Amid the responsible factors by the frequent losses are the pests, which depending on the cultivar and sowing epoch, may occasion total loss in the yield (ABREU, 2005).

The defoliator pests may cause significant losses in the photosynthetic area. In this group, the species *Diabrotica speciosa* (Germar, 1824) stands out, which is widespread in the whole national territory, being considered the main or secondary pest in several crops (LORENZATO, 1984) among them, the common bean. In laboratory trial, Hohmann and Carvalho (1989) estimated that *D. speciosa* may consume 10.32 cm² during its cycle. The occurrence of two adults per plant, in the first week after emergence, may cause losses above 50% in the production and even the plants death (MAGALHÃES e CARVALHO, 1988). Thus, this work aimed to assess the effects in the attractiveness and leaf intake of *D. speciosa* on injured and/or impregnated plants with fecal droppings.

MATERIAL AND METHODS

The experiment was conducted at Laboratório de Resistência de Plantas a Insetos, Departamento de Fitossanidade of UNESP/FCAV. The assays were carried out under controlled conditions, at temperature of 25 ± 2 ^{o}C, relative humidity of 70 ±10% and photoperiod of 12 hours. A randomized blocks design was used, with six treatments constituted by common bean plants of BRS-Supremo cultivar, with one hole, three holes, one hole and fecal droppings, three holes and fecal droppings, only fecal droppings, and the control without holes and fecal droppings, with five replications.

The holes were performed with a puncher of 0.83 cm² when the plants showed the cotyledonary leaves. For impregnation on the leaves 0.025 g of *D. speciosa* fecal droppings were used per treatment, being moistened with 0.03 ml of water, for better setting on the leaves. These were placed into glass cages equidistantly from center, where two *D. speciosa* adults were released per treatment, totaling 12 adults per cage. The attractiveness was evaluated at 1, 3, 5, 10, 15, 30 minutes and 1, 2, 6, 12, 24, 36, 48, 60 and 72 hours after the insects release. When 75% of leaf intake (L.I) was observed, at least in one replication, the adults were removed and the leaves detached, for subsequent measurement of leaf intake, through the device LI-COR®, model 3100.

The data obtained were transformed to $(x + 0.5)^{1/2}$ and submitted to the analysis of variance by F test, and their averages compared by Tukey test, at 5% probability.

RESULTS AND DISCUSSION

Significant difference was observed in the attractiveness at 60 and 72 hours, where the treatment with one hole without fecal droppings attracted more insects than the treatments without holes with fecal droppings, and with one hole and fecal droppings, at 72 hours. In the leaf intake (L.I), significant difference was not verified (Table 1). In conclusion, the injuries caused by the holes and the fecal droppings did not influence in the attractiveness and leaf intake of *D. speciosa.*

Table 1. Attractiveness and leaf intake of *Diabrotica speciosa* on common bean leaflets with or without the presence of holes and fecal droppings. Jaboticabal, SP, 2011.

TREATMENTS	Minutes[1, 2]							
	1	3	5	10	15	30	60	120'
Control	0.0 a	0.4 a	1.0 a	1.4 a	1.8 a	1.8 a	2.0 a	1.8 a
One hole without fecal droppings	0.0 a	0.2 a	0.4 a	0.6 a	0.8 a	0.6 a	1.2 a	1.2 a
Three holes without fecal droppings	0.0 a	0.0 a	0.0 a	0.4 a	0.2 a	0.6 a	1.0 a	1.4 a
Without holes with fecal droppings	0.0 a	0.4 a	0.4 a	0.8 a	1.2 a	0.6 a	1.0 a	1.6 a
One hole with fecal droppings	0.0 a	0.0 a	0.0 a	0.0 a	0.4 a	0.6 a	0.4 a	1.2 a
Three holes with fecal droppings	0.0 a	0.0 a	0.2 a	0.4 a	0.8 a	1.0 a	1.2 a	1.0 a
F (Treatment)	-	0.89^{ns}	1.36^{ns}	0.76^{ns}	0.90^{ns}	0.42^{ns}	0.42^{ns}	0.12^{ns}
C. V. (%)	-	17.17	22.26	31.62	31.61	33.97	36.84	38.91

TREATMENTS	Hours[1, 2]						Mean[1, 2]	L.I[1, 2]
	6	12	24	48	60	72		
Control	2.4 a	1.8 a	1.8 a	2.6 a	0.4 ab	1.4 ab	21.2 a	18.8 a
One hole without fecal droppings	1.6 a	1.2 a	1.2 a	1.0 a	1.8 b	3.0 b	15.6 a	18.1 a
Three holes without fecal droppings	1.6 a	1.4 a	1.4 a	1.2 a	0.4 ab	1.4 ab	12.0 a	8.9 a
Without holes with fecal droppings	1.6 a	1.6 a	1.2 a	1.6 a	0.8 ab	0.4 a	14.4 a	11.7 a
One hole with fecal droppings	1.0 a	1.2 a	1.2 a	1.4 a	0.0 a	0.6 a	8.8 a	13.1 a
Three holes with fecal droppings	1.2 a	1.0 a	0.8 a	0.4 a	0.4 ab	1.4 ab	10.6 a	4.4 a
F (Treatment)	0.25	0.13^{ns}	0.29^{ns}	1.93^{ns}	3.00*	3.12*	0.56^{ns}	1.21^{ns}
C. V. (%)	39.50	36.42	25.60	26.06	22.68	25.11	46.90	47.94

[1] Means followed by the same letter in column did not differ significantly by Tukey's test at 5% probability.
[2] For analysis data were transformed to $(x + 0.5)^{1/2}$. ^{ns} not significant; * significant at 5%.

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EFFECT OF INJURY TIME ON ATTRACTIVENESS AND CONSUMPTION OF *SPODOPTERA FRUGIPERDA* (J. E. SMITH) ON BEAN PLANTS

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INTRODUCTION

Bean plants undergo several problems throughout the cultivation period of the crop, and amongst them, defoliating pests such as *Spodoptera frugiperda* (J. E. Smith) stands out (CRUZ, 2009). This work aimed to evaluate the feeding behavior of *S. frugiperda* larvae on bean leaflets submitted to injuries in different hours.

MATERIALS AND METHODS

The assay was performed at Faculdade de Ciências Agrárias e Veterinárias – UNESP, Departamento de Fitossanidade, Laboratório de Resistência de Plantas a Inseto, Jaboticabal, SP, Brazil, under controlled conditions of temperature (25 ± 1 ^{o}C), relative humidity (60 ± 10%) and photophase (12 hours). Randomized blocks design and completely randomized blocks design were used respectively for free-choice and non-choice tests, with six treatments and six replications.

Bean cultivars IAC Harmonia (resistant) and IAPAR 81 (susceptible) (SOUZA et al., 2012) were used, and the following treatments were adopted: (1) leaflets submitted to larvae injury 48 hours before the tests beginning; (2) 24 hours before; (3) 12 hours before; (4) 6 hours before; (5) 3 hours before; and (6) control (leaflets without injury). For injury obtainment, four seven days-old larvae were confined during three hours on bean leaflets, into a cage constituted by a 1 cm height circular plastic tube, covered by an anti-aphid screen on one end and with a paper rectangle on the other.

In free-choice test, circular glass arenas were used (23.0 cm diameter), lined with filter paper softly moistened with distilled water, where the leaflets were placed equidistantly from the center, and one larva per treatment was released. For non-choice test, the same methodology was used, however, the leaflets were individualized into Petri dishes (9.0 cm diameter). Attractiveness assessments were done at 1, 3, 5, 10, 15, 30 minutes and 1, 2, 6, 12, 24, 36 and 48 hours after larvae release. Leaf consumption was measured through a leaf area measurer device, model LI-COR 3100A®, before the beginning and after the closure of the experiment, thus obtaining the leaf area consumed (cm^2) by the insects. Data obtained from the assays were submitted to the analysis of variance (ANOVA) by F test, with means compared by Tukey's test, at 5% probability.

RESULTS AND DISCUSSION

Regarding the attractiveness in free-choice and non-choice tests in the cultivar IAC Harmonia, significant difference was observed only in the period of 60 to 2580 minutes for free-choice test, where the injury time of 6 hours provided the highest number of larvae attracted. For this same test yet, plants submitted to injury 6 hours before the assay beginning were the most consumed by third-instar *S. frugiperda* larvae, and possibly this is the period plant express its higher susceptibility (susceptible plant) or higher resistance induction (resistant plant).

For the cultivar IAPAR 81, set as the susceptible pattern, significant difference was found in the period of 1 to 30 minutes after larvae release in non-choice test, where the injury time of 12 hours and control (leaves without injury) were significantly less attractive to *S. frugiperda* larvae than plants' leaves submitted to injury in a period of 3 hours before test beginning.

Table 1. Number of *Spodoptera frugiperda* larvae attracted and leaf consumption (cm²) on bean leaflets in free-choice and non-choice tests. Jaboticabal, SP, Brazil, 2013.

IAC Harmonia						
Injury (I)	Free-choice			Non-choice		
	1' to 30'	60' to 2580'	Consumption	1' to 30'	60' to 1440'	Consumption
48 h	0.83	0.67 ab	1.99 a	0.83	0.67	0.33
24 h	0.67	0.33 a	0.85 a	0.17	0.50	0.50
12 h	0.72	0.60 ab	0.98 a	0.50	0.67	1.00
6 h	0.83	1.14 b	7.26 b	0.50	0.50	1.00
3 h	0.72	0.48 a	0.61 a	0.67	0.83	0.50
Control	0.05	0.50 a	2.87 a	0.33	0.67	1.67
F (I)	0.69^{ns}	4.05**	9.60**	1.36^{ns}	0.36^{ns}	1.67^{ns}
C.V. (%)	38.58	14.60	32.54	26.50	25.49	35.66
IAPAR 81						
Injury (I)	Free-choice			Non-choice		
	1' to 30'	60' to 2580'	Consumption	1' to 30'	60' to 1440'	Consumption
48 h	0.56	0.81	3.18	0.33 ab	0.50	2.00
24 h	0.83	0.88	4.37	0.83 ab	1.00	3.83
12 h	0.58	0.60	2.37	0.17 a	0.67	1.00
6 h	1.27	0.71	4.69	0.50 ab	0.67	1.50
3 h	0.19	0.78	4.36	1.00 b	0.50	3.83
Control	0.80	0.69	5.10	0.17 a	0.67	3.33
F (I)	1.43^{ns}	0.43^{ns}	1.02^{ns}	4.13**	0.86^{ns}	0.96^{ns}
C.V. (%)	34.07	14.92	30.62	22.60	23.76	51.03

Means followed by the same letter in column did not differ significantly by F test, at 5% probability. For statistical analysis, data were transformed in $(x + 0.5)^{1/2}$. ^{ns} = not significant, * = significant at 5%, ** = significant at 1%.

We concluded leaves from the resistant cultivar submitted to injury 6 hours before free-choice test beginning influenced larvae attractiveness and feeding, indicating it may be the period spent by plant to produce compounds to induce resistance to *S. frugiperda*.

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EFFECT OF INJURIES ON BEAN LEAVES ON ATTRACTIVENESS AND CONSUMPTION OF *SPODOPTERA FRUGIPERDA* (J. E. SMITH) (LEPIDOPTERA: NOCTUIDAE)

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INTRODUCTION

Pest insects can significantly decrease bean yield, however, there are various management tactics that may be used to control them, and host plant resistance stands out as one of these methods, once some plants may hold structural, physical or chemical features that express some resistance degree against the insects attack (GALLO et al., 2002).

Moreover, there are plants with susceptibility traits, which after being attacked by insects they can alter their defense phenotype by producing chemical compounds or changing some structures that confer them some kind of protection or resistance (AGRAWAL, 1999). Considering these information, the aim of this work was to assess the effect of different injury percentage on attractiveness and foliar consumption of *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) larvae on bean cultivars with resistance and susceptibility features.

MATERIALS AND METHODS

The experiment was carried out at FCAV/UNESP, Departamento de Fitossanidade at Laboratório de Resistência de Plantas a Insetos, under temperature of 25 ± 2 ^{o}C, relative humidity of 70 ± 10% and photophase of 12 hours. Randomized blocks design and completely randomized blocks design were used for free-choice and non-choice tests, respectively, with four treatments consisted of different injury types on leaves, corresponding to 75%, 50%, 25% and control (without injury) on two common bean cultivars, IAC Harmonia (resistant) and IAPAR 81 (susceptible) (SOUZA et al., 2012), with six replications for each test.

Injuries were occasioned by third-instar larvae of *S. frugiperda*, confined into 1.0 cm height circular plastic cages, covered with anti-aphid screen clipped on the leaflets and a paper rectangle placed on the other side of the leaflet. Cages were removed 24 hours before the assays, and the injured leaflets remained on plants until the tests beginning, where they were detached and then their leaf area was measured through the device LI-COR®, model 3100A. Next, they were placed into Petri dishes (9.0 cm diameter) in non-choice test, where one larva was release per plate. Free-choice test was conducted into circular glass arenas (23.0 cm diameter) lined with filter paper softly moistened where the leaflets were placed equidistantly from the center, where four larvae were released per arena.

Attractiveness assessments were done at 1, 3, 5, 10, 15 and 30 minutes and 1, 2, 6, 12, 24, 36, 48 and 75 hours after larvae release. Tests were finished when one of the replications exhibited 95% of its leaf area consumed. Data obtained from the assays were transformed in $(x + 0.5)^{1/2}$ and subjected to the analysis of variance by F test, with means compared to Tukey's test at 5% probability.

RESULTS AND DISCUSSION

In non-choice test, there was significant difference only for the mean of evaluation attractiveness between 1 to 12 hours for the cultivar IAC Harmonia, where the treatments with higher injury percentages were the least attractive to larvae, however, there was no significant difference in leaf area consumed (Table 1).

Significant differences were not found for attractiveness in any cultivars in free-choice test (Table 1), however, IAPAR 81 was the least consumed in treatments with 25 and 75% injuries, differencing significantly from control (without injury), with the highest leaf area consumed. These results evidence plants of the susceptible cultivar IAPAR 81 may be released some allelochemical due to larvae attack, forcing them consume lower leaf area, despite the treatment 50% did not differ from the other treatments.

Table 1. Mean number of *Spodoptera frugiperda* larvae attracted and leaf area consumed (cm^2) on leaflets with different injury percentages in free-choice and non-choice tests. Jaboticabal, SP, Brazil, 2013.

Treatments	IAC-Harmonia				IAPAR 81			
	Non-choice				Non-choice			
	[1]Attractiveness			[1]Leaf area consumed	[1]Attractiveness			[1]Leaf area consumed
	Minutes	Hours			Minutes	Hours		
	1 - 30	1 - 12	24 - 48		1 - 30	1 - 12	24 - 42	
75%	0.47 a	0.33 a	0.28 a	1.85 a	0.11 a	0.08 a	0.61 a	5.01 a
50%	0.42 a	0.29 a	0.39 a	3.75 a	0.28 a	0.50 a	0.66 a	5.10 a
25%	0.56 a	0.71 b	0.39 a	2.22 a	0.19 a	0.29 a	0.78 a	7.89 a
Control	0.39 a	0.42 ab	0.28 a	2.14 a	0.17 a	0.17 a	0.78 a	5.60 a
F(Treatments)	0.27^{ns}	5.58**	0.68^{ns}	2.19^{ns}	0.26^{ns}	1.52^{ns}	0.55^{ns}	1.32^{ns}
CV (%)	17.92	10.17	12.10	22.20	22.65	23.66	11.90	21.70
Treatments	Free-choice				Free-choice			
	[1]Attractiveness			[1]Leaf area consumed	[1]Attractiveness			[1]Leaf area consumed
	Minutes	Hours			Minutes	Hours		
	1 - 30	1 - 12	24 - 72		1 - 30	1 - 12	24 - 48	
75%	0.53 a	0.50 a	0.25 a	5.05 a	0.50 a	0.71 a	0.33 a	2.03 a
50%	0.75 a	0.88 a	0.08 a	6.93 a	0.39 a	0.83 a	0.44 a	3.64 ab
25%	0.50 a	0.63 a	0.46 a	3.98 a	0.39 a	0.25 a	0.44 a	0.79 a
Control	0.25 a	0.75 a	0.42 a	6.93 a	0.33 a	0.75 a	0.44 a	6.59 b
F(Treatments)	0.65^{ns}	0.33^{ns}	1.57^{ns}	1.35^{ns}	0.08^{ns}	1.41^{ns}	0.21^{ns}	6.81**
CV (%)	28.80	30.65	20.27	27.40	25.90	25.43	20.18	32.39

[1]Means followed by the same letter in column did not differ significantly by F test at 5% probability. For analysis, data were transformed in $(x + 0.5)^{1/2}$. ^{ns} not significant; **significant at 1%.

We concluded different injury percentages on bean leaves affect negatively the consumption of *S. frugiperda* larvae on the susceptible cultivar IAPAR 81.

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INFESTATION OF *BEMISIA TABACI* (GENN.) BIOTYPE B ON BEAN CULTIVARS IN "WATER SEASON"

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INTRODUCTION

The whitefly *Bemisia tabaci* (Genn., 1889) biotype B (Hemiptera: Aleyrodidae) is considered the major pest of common bean *Phaseolus vulgaris* L. The insect causes direct damages due to its feeding on phloem, weakening plant by sucking its nutrients, in addition to inject it toxins, occasioning physiological problems in bean plants, and indirect damages, which occur because of the sugary excretion of honeydew, inducing the development of saprophytic fungi, generally from *Capnodium* genus, on leaves, flowers and fruits, prevent gas changes and photosynthesis, and hence reducing yield. However, the most serious damage cause by the whitefly on bean plants is the virus transmission, such as the bean golden mosaic virus (BGMV), which can cause economical losses ranging from 30 to 100% (SALGUERO, 1993).

In this scenario, host plant resistance can be used as one more control tactic within the integrated pest management concepts, aiming to attenuate damages caused by *B. tabaci* biotype B, as it supports the reduction of the pest population to certain levels below economical injury, does not cause environmental imbalance, has cumulative and persistent effects, does not overtax production costs and does not demand specific knowledge by the farmer (LARA, 1991). Thus, this study aimed to evaluate the natural infestation of *B. tabaci* biotype B on nine bean cultivars in "water season" in Jaboticabal, SP, Brazil.

MATERIALS AND METHODS

The study was conducted in an experimental area from Departamento de Fitossanidade of Faculdade de Ciências Agrárias e Veterinárias – FCAV/UNESP, located in Jaboticabal, SP, Brazil, where six samplings were done in "water season". Nine bean cultivars were used: IPR Eldorado, IPR Siriri, BRS Cometa, BRS Requinte, Guará, IAPAR 81, IAC Alvorada, IAC Centauro and Pérola.

The evaluation of *B. tabaci* biotype B eggs and nymphs were performed in laboratory, through a stereoscope. Samplings of adults were done on 10 plants per plot, evaluating 10 leaflets from the upper part of the plants through visual inspection, using the turned leaf technique, consisted by holding leaf by the petiole and turning it carefully in order to not let the insects fly away. Data collected from the mean number of eggs, nymphs and adults of *B. tabaci* biotype B were transformed in $(x + 0.5)^{1/2}$, submitted to the analysis of variance by F test and means were compared by Tukey's test, at 5% probability.

RESULTS AND DISCUSSION

There was significant difference for the infestation of *B. tabaci* biotype B eggs. The cultivars IAPAR 81 and Guará exhibited the least number of eggs (3.10 and 4.00, respectively), differing

from the cultivar IPR Eldorado, which was the most preferred for oviposition, with the mean of 9.02 eggs on 10 leaflets (Table 1). The other cultivars behaved intermediate. Studies of JESUS et al. (2010a), when evaluating the infestation of the whitefly in "water season", also in Jaboticabal, SP, Brazil, obtained as the main results that the cultivars Pérola and IAC Alvorada were the least oviposited by the insect.

For nymphs' infestation, the cultivar IAC Centauro stood out as the least preferred, and IPR Siriri, BRS Cometa, Guará, IPR Eldorado and Pérola showed the highest infestation of *B. tabaci* biotype B nymphs. For whitefly adults' infestation, significant differences were not observed in the experimental field (Table 1), with an amplitude from 0.46 (cultivar IAC Centauro) to 1.00 (cultivar Guará) adults. Different results were found by JESUS et al. (2010b), when evaluating the behavior of *P. vulgaris* cultivars against the whitefly's attack in Catalão, GO, Brazil, where significant differences were not verified among the 14 cultivars tested for the infestation of eggs and nymphs, also in "water season". These contradictory results may be attributed to the different environmental conditions related to sowing location.

We highlight as the main conclusions the cultivars IAPAR 81 and Guará were the least preferred for *B. tabaci* biotype B oviposition; the cultivar IAC Centauro exhibited the lower mean of nymphs; and there was no preference by the adults among the cultivars.

Table 1. Number of eggs, nymphs and adults of *Bemisia tabaci* biotype B on 10 leaflets obtained from plants of nine bean cultivars, in six samplings in "water season". Jaboticabal, SP, Brazil, 2011.

Cultivar (C)	Eggs	Nymphs	Adults
1 – IAC Centauro	5.08 ab	1.33 b	0.46
2 – IAC Alvorada	5.29 ab	3.23 ab	0.58
3 – BRS Requinte	5.77 ab	4.08 ab	0.81
4 – BRS Cometa	5.29 ab	8.81 a	0.56
5 – IPR Siriri	5.02 ab	9.48 a	0.75
6 – IPR Eldorado	9.02 a	5.81 a	0.58
7 – IAPAR 81	3.10 b	4.37 ab	0.58
8 – Guará	4.00 b	7.71 a	1.00
9 – Pérola	5.06 ab	5.77 a	0.73
F (C)	1.87*	3.62**	1.50^{ns}
C.V. (%)	24.87	32.43	21.35

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INFESTATION OF *BEMISIA TABACI* BIOTYPE B IN THREE SOWING SEASONS IN JABOTICABAL, SP, BRAZIL

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INTRODUCTION

The whitefly *Bemisia tabaci* (Genn., 1889) biotype B (Hemiptera: Aleyrodidae) is one of the major pests of common bean. Amongst the main control methods adopted to its management, plants with resistant traits can constitute an important tool against the pest, with the advantages to reduce pest population below economic injury levels, not overtax production costs, in addition to be compatible with other pest management tactics (BOIÇA JÚNIOR et al., 2012).

Sowing season on crop setup comprises a factor of great importance which may influence resistance expression, affecting plant physiology, population density of the pest insect, among others (LARA, 1991). Thus, we aimed with this study to evaluate the infestation of the whitefly *B. tabaci* biotype B in three sowing seasons, under the conditions of Jaboticabal, SP, Brazil.

MATERIALS AND METHODS

The assay was conducted from August 2010 to June 2011 at an experimental field (760 m^2) from Departamento de Fitossanidade of FCAV/UNESP, Jaboticabal, SP, Brazil. Population surveys of *B. tabaci* biotype B were done weekly, from 25 to 60 days after emergence (DAE) of plants, totaling six evaluations per sowing season (winter, water and dry), in 76 points at random in the area, where in each of them, 10 leaflets were sampled on bean plants, cultivar Carioca. For adults assessment, turned leaf technique was used, and for eggs and nymphs, leaflets were collected at random and taken to laboratory, where their number were counted through a stereoscope. During the experiment conduction no insecticide application was sprayed on the area. In order to compare the infestation of eggs, nymphs and adults of *B. tabaci* biotype B among the three sowing seasons, the analysis of variance (ANOVA) was performed and Tukey's test ($P = 0.05$) was applied for means comparison.

RESULTS AND DISCUSSION

The infestation of the whitefly's eggs in water season showed the highest value (101.7 eggs), differing significantly from the winter season (49.8 eggs), whereas the dry season stood out as the sowing season with the least mean (1.6 eggs) for this parameter (Table 1).

For nymphs infestation, the behavior was similar to eggs infestation, being the water season the most infested (99.0 nymphs), differing significantly from the winter season (6.8 nymphs), and from the dry season (0.5 nymphs) (Table 1).

Regarding the population survey of adults, the winter season (18.0 adults) was the most infested, differing from the water season (12.9 adults) and dry season (1.3 adults), the latter with again with the lowest infestation (Table 1).

Contradictory results to the present study were found by JESUS et al. (2010), evaluating the infestation of *B. tabaci* biotype B on 19 common bean genotypes, also in the conditions of Jaboticabal, SP, Brazil. The authors observed dry season was the most suitable for eggs

infestation, followed by winter and dry seasons, respectively. For nymphs, the same authors did not find significant differences among the sowing seasons.

In the present study comparing to JESUS et al. (2010), low infestations of the whitefly were observed in the experimental field, which associated with the different climatic conditions may explain the different results obtained.

From the results obtained in the present study, we emphasize the importance to evaluate resistance of cultivars taking into account the sowing season, where the whitefly infestation and the cultivars behavior varied among the different seasons, despite bean plants has grown in the same experimental field.

Table 1. Infestation (± SE) of the whitefly *Bemisia tabaci* biotype B in three sowing seasons. Jaboticabal, SP, Brazil, 2010/2011.

Sowing season	*Bemisia tabaci* biotype B		
	Eggs	Nymphs	Adults
Winter	49.8 ± 5.37 b	6.8 ± 1.01 b	18.0 ± 1.12 a
Water	101.7 ± 20.60 a	99.0 ± 10.80 a	12.9 ± 3.23 b
Dry	1.6 ± 0.51 c	0.5 ± 0.24 c	1.3 ± 0.20 c
F	8.30*	75.70**	7.60*
C.V.%	27.59	29.56	31.13

Means followed by different letters in column differed significantly by F test ($P = 0.05$). For statistical analysis, data were transformed in $(x + 0.5)^{1/2}$. SE = standard error of means. * = significant at 5%. ** = significant at 1%.

Overall, water season exhibited the highest infestation of eggs and nymphs, and winter season had the highest infestation of *B. tabaci* biotype B adults. For the present study, we observed low infestation of the whitefly for dry season.

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APHIS CRACCIVORA KOCH 1854 (HEMIPTERA: APHIDIDAE) PREFERENCE FOR CULTIVARS AND BEAN PLANT PARTS, IN DIFFERENT EPOCHS, IN THE WATER SEASON

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INTRODUCTION

Aphis craccivora Koch 1854 (Hemiptera: Aphididae) is a pest broadly distributed in different continents (Capinera, 2001). This species was described in approximately, 50 cultivated species pertained to 19 families (Blackman & Eastop, 2007), being associated, mainly, to species from Fabaceae family, such as common beans, *Phaseolus vulgaris* L. In the tropics, these aphids reproduce without perform the copulation and the colonies are entirely constituted by females (Schreiner, 2000). The host plant resistance is an alternative control method of these insects, which offers a series of advantages, including the perfect integration with other integrated pest management programs (Lara, 1991).

This study aimed to assess the aphids preference for different cultivars and bean plant parts, in different epochs, in the water season.

MATERIAL AND METHODS

The experiment was conducted from January 13, 2011 to February 22, 2011, in an experimental area pertained to Departamento de Fitossanidade of Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista (UNESP), Jaboticabal-SP, in the soil type known by dark red latosol (Embrapa, 1999).

Each parcel was constituted by four lines of five meters in length, with 0.50 m spacing between lines, keeping 12 plants per linear meter. In the sowing application of fertilizer 430 $kg.ha^{-1}$ of the formula 04-14-08 was used, and the cultural treatments such as manual weeding, irrigation, etc. were performed when necessary.

The treatments were disposed in a randomized block design, which correspond to 19 cultivars described in Table 1, using four replications. The evaluations were performed in different epochs, from 25 to 60 DAE (days after emergence), on the superior and inferior plant parts, assessing the number of insects on ten leaflets, per plot.

The data obtained were transformed to $(x + 1)^{1/2}$ and submitted to the analysis of variance by F test, and their averages compared by Tukey test, at 5% probability.

RESULTS AND DISCUSSION

Significant differences were observed to epoch (E) (F = 37.90) and plant part (P) (F = 20.81) (Table 1). Regarding the epoch, the highest aphids number was verified at 25 DAE (days after emergence), with mean number of 2.32 individuals, while from 32 DAE a decrease in the insects number was observed. As regards the plant part, the highest insects number was found on the leaves from inferior part, with mean value of 0.91, against 0.37 registered insects on the leaves from superior part. In conclusion, the pest showed, in the water season, population peak at 25 DAE, preferring to feed on the leaves from plants inferior part, regardless of genotype.

Table 1: Number of *Aphis craccivora* nymphs on different cultivars and bean plant parts, in different epochs, in the water season. Jaboticabal, SP, 2011.

Cultivars (C)	Number of aphid nymphs
IAC Harmonia	0.27 a
BRS Cometa	0.54 a
IAC Diplomata	0.79 a
IPR Eldorado	0.38 a
IAC Tybatã	0.29 a
Pérola	0.69 a
Guará	0.79 a
IAPAR 81	0.25 a
BRS Supremo	0.65 a
IPR 139	0.25 a
BRS Requinte	2.15 a
IAC Alvorada	0.31 a
BRS Pontal	0.50 a
IAC Galante	0.85 a
IAC Centauro	0.67 a
IAC Carioca Eté	0.40 a
IAC Una	0.35 a
IPR Siriri	0.77 a
IAC Formoso	0.52 a
Epoch (E)	
25 DAE	2.32 a
32 DAE	0.54 b
39 DAE	0.26 b
46 DAE	0.09 b
53 DAE	0.09 b
60 DAE	0.56 b
Plant part (P)	
Superior	0.37 b
Inferior	0.91 a
C.V.(%)	35.11

[1] Means followed by the same letter in column did not differ significantly by Tukey's test at 5% probability. For analysis data were transformed to $(x+1)^{1/2}$.

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INFESTATION OF *APHIS CRACCIVORA* KOCH ON PARTS OF BEAN CULTIVARS PLANTS' CANOPY IN DIFFERENT EPOCHS IN DRY SEASON, IN JABOTICABAL, SP, BRAZIL

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INTRODUCTION

The black aphid *Aphis craccivora* Koch (Hemiptera: Aphididae) is a cosmopolite pest which feeds on several plant species, especially on Fabaceae, and it is considered the major sucking insect pest of alfalfa and bean crops (RAKHSHANI et al., 2005). Adults and nymphs of the aphid also cause directly and indirectly economical losses to cowpea plants (OBOPILE, 2006). Plant-insect interaction studies are of great importance for the use of resistant plants, however, little information is known about the black aphid effect on common bean cultivars. Thus, this study aimed to evaluate the infestation of *A. craccivora* on parts of plants' canopy of bean cultivars in different sampling epochs in dry season, in the conditions of Jaboticabal, SP, Brazil.

MATERIALS AND METHODS

The experiment was carried out at an experimental field of Departamento de Fitossanidade da Faculdade de Ciências Agrárias e Veterinárias/UNESP, Jaboticabal, SP, Brazil, from 23/05/2011 to 28/06/2011. Each plot consisted of four rows with 5 m length spaced in 0.5 m, where 12 plants were maintained after thinning. For sowing fertilization, 430 kg ha^{-1} of NPK 04-14-08 was used, and cultural practices of manual weeding and irrigation were done when necessary. No applications of insecticide were sprayed in the experimental field.

Treatments were set in split plots randomized blocks design, corresponding to 19 cultivars, six sampling epochs and two parts from plants' canopy (Table 1), with four replications. Assessments were done weekly, from 25 to 60 days after emergence (DAE), by collecting 20 leaflets per plot, which were packed into paper bags and taken to the laboratory, where the number of the aphids' nymphs was quantified through a stereoscope. The obtained data were transformed in $(x + 1)^{1/2}$ for normalization and submitted to the analysis of variance (ANOVA) by F test, and means were compared to Tukey's test, at 5% probability.

RESULTS AND DISCUSSION

According to the obtained data presented in Table 1, significant differences were found on the infestation of *A. craccivora* nymphs for cultivars ($F = 3.14^{**}$), época ($F = 41.10^{**}$) and parts of the plant ($F = 9.10^{**}$). The most infested cultivar was IAC Centauro (5.21 nymphs), followed by IPR Eldorado, which exhibited intermediate infestation (3.00 nymphs) and the other cultivars had the lowest numbers of aphids. For the sampling epochs, the population peek of *A. craccivora* occurred at 39 DAE (4.41 nymphs). In regarding to the part of plant, higher number of insects was found on leaflets from the lower part of plants' canopy, with mean of 2.17 nymphs, against 1.77 aphids reported on leaves from the upper part.

Table 1. Number of *Aphis craccivora* nymphs on parts of plants' canopy of bean cultivars in different sampling epochs in dry season. Jaboticabal, SP, Brazil, 2011.

Cultivars (C)	Number of aphids
IAC Harmonia	2.42 b
BRS Cometa	1.58 b
IAC Diplomata	2.10 b
IPR Eldorado	3.00 ab
IAC Tybatã	2.13 b
Pérola	1.46 b
Guará	1.21 b
IAPAR 81	1.98 b
BRS Supremo	2.19 b
IPR 139	1.77 b
BRS Requinte	1.90 b
IAC Alvorada	1.58 b
BRS Pontal	1.35 b
IAC Galante	1.46 b
IAC Centauro	5.21 a
IAC Carioca Eté	1.27 b
IAC Una	1.48 b
IPR Siriri	1.23 b
IAC Formoso	2.19 b
Epoch (E)	
25 DAE	1.14 cd
32 DAE	2.51 b
39 DAE	4.41 a
46 DAE	2.16 bc
53 DAE	0.73 d
60 DAE	0.89 d
Part of canopy (P)	
Upper	1.77 b
Lower	2.17 a
C.V.(%)	41.06

Means followed by the same letter in column did not differ significantly by F test at 5% probability. For analysis, data were transformed in $(x+1)^{1/2}$.

We concluded *A. craccivora* nymphs showed the highest infestation on the cultivar IAC Centauro, with its population peek at 39 DAE, preferring to feed on leaves from the lower part of bean plants' canopy in dry season, in Jaboticabal, SP, Brazil.

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EFFECTS OF CULTIVARS AND BEAN PLANT PARTS, IN DISTINCT EPOCHS, IN THE INFESTATION OF *APHIS CRACCIVORA* KOCH 1854 (HEMIPTERA: APHIDIDAE), IN THE WINTER CROP

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INTRODUCTION

Aphis craccivora Koch is a cosmopolite pest which feeds on several host plants, especially from Fabaceae family (Chen et al., 1999). It is also considered the main sucking pest of economically important cultures, as alfalfa and bean (Rakhshani et al., 2005). On cowpea crop, adults and nymphs of this aphid cause significant economic damages, both direct as indirectly (Obopile, 2006). These insects attack the crops in short time after the plants emergence, and disperse quickly (Schreiner, 2000).

This work aimed to evaluate the effects of different cultivars and bean plant parts, in different epochs, in the pest infestation.

MATERIAL AND METHODS

The experiment was conducted from August 03, 2010 to November 09, 2010, in an experimental area pertained to Departamento de Fitossanidade of Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista (UNESP), Jaboticabal-SP, in the soil type known by dark red latosol (Embrapa, 1999).

Each parcel was constituted by four lines of five meters in length, with 0.50 m spacing between lines, keeping 12 plants per linear meter. In the sowing application of fertilizer 430 $kg.ha^{-1}$ of the formula 04-14-08 was used, and the cultural treatments such as manual weeding, irrigation, etc. were performed when necessary.

The treatments were disposed in a randomized block design, which correspond to 19 cultivars described in Table 1, using four replications. The evaluations were performed in different epochs, from 25 to 60 DAE (days after emergence), on the superior and inferior plant parts, assessing the number of insects on ten leaflets, per plot.

The data obtained were transformed to $(x + 1)^{1/2}$ and submitted to the analysis of variance by F test, and their averages compared by Tukey test, at 5% probability.

RESULTS AND DISCUSSION

According to the data shown in Table 1, significant differences were verified only to the epoch (E) ($F = 4.29$). The aphids population peak occurred at 46 (DAE), with average of 1.22 insects, indicating the insect preference for this period, considering further that the epochs which preceded (53 DAE) and anteceded (39 DAE) the population peak, presented, numerically, the second and third higher insects values, with 1.01 and 0.97 individuals, respectively. Regarding the cultivars and plant parts, the infestation was similar in all treatments.

Table 1: Number of *Aphis craccivora* nymphs on different cultivars and bean plant parts, in different epochs, in the winter crop. Jaboticabal, SP, 2011.

Cultivars (C)	Number of aphid nymphs
BRS Cometa	0.90 a
IAC Diplomata	0.95 a
IPR Eldorado	0.63 a
IAC Tybatã	0.92 a
Pérola	1.02 a
Guará	0.75 a
IAPAR 81	1.08 a
BRS Supremo	0.50 a
IPR 139	0.60 a
BRS Requinte	0.83 a
IAC Alvorada	0.92 a
BRS Pontal	0.92 a
IAC Galante	1.13 a
IAC Centauro	1.13 a
IAC Carioca Eté	0.83 a
IAC Una	1.00 a
IPR Siriri	1.32 a
IAC Formoso	0.67 a
Epoch (E)	
25 DAE	0.56 c
32 DAE	0.62 bc
39 DAE	0.97 abc
46 DAE	1.22 a
53 DAE	1.01 ab
60 DAE	0.96 abc
Plant part (P)	
Superior	0.89 a
Inferior	0.89 a
C.V.(%)	32.13

[1] Means followed by the same letter in column did not differ significantly by Tukey's test at 5% probability. For analysis data were transformed to $(x+1)^{1/2}$.

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INFLUENCE OF ABIOTIC FACTORS ON *APHIS CRACCIVORA* KOCH INFESTATION ON BEAN CROP IN THREE SOWING SEASONS, IN JABOTICABAL, SP, BRAZIL

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INTRODUCTION

The black aphid *Aphis craccivora* Koch (Hemiptera: Aphididae) stands out as one of the pests that infest bean crops, limiting its yield directly by sap sucking on branches, sprouts and leaves and indirectly through virus transmission (BLACKMAN & EASTOP, 2007). Studies aiming to elucidate the role of environmental resistance exerted by abiotic factors, such as temperature, relative humidity and rainfall, plays on insect fluctuations are of great interest, as they may prevent the populations to reach the numerical expression that its biotic potential allow (CARVALHO, 1996).

Thus, this work aimed to evaluate the influence of the abiotic factors temperature, relative humidity and rainfall on *A. cracciora* infestation on bean plants during three sowing seasons, in the conditions of Jaboticabal, SP, Brazil.

MATERIALS AND METHODS

The assay was carried out in an experimental field (760 m^2) of Departamento de Fitossanidade of Faculdade de Ciências Agrárias e Veterinárias – FCAV/UNESP in Jaboticabal, SP, Brazil from August 2010 to June 2011, using 19 common bean, *Phaseolus vulgaris* L., cultivars. No application insecticide was sprayed on the field during the experiment conduction.

Population assessments of *A. craccivora* was done weekly, from 25 to 60 days after emergence (DAE) in three sowing seasons (winter, water and dry) in 76 points of the experimental field, where in each of them, 20 leaflets were collected at random, packed into paper bags, taken to the laboratory, and through a stereoscope, the number of the aphids' nymphs was counted.

Average monthly climatic data of temperature (ºC), relative humidity (RU) and rainfall (mm) in the samplings dates were obtained from the weather station of FCAV/UNESP, located near the experimental field, and simple linear correlation (Pearson) was used to analyze the infestation of *A. craccivora* nymphs with these abiotic factors.

RESULTS AND DISCUSSION

From the results obtained through the simple linear correlation, there was a significant negative correlation ($y = 1.3599x + 22.936$, $r = -0.4950^{*}$) between the temperature and the number of *A. craccivora* nymphs throughout the three bean sowing season in Jaboticabal, SP, Brazil (Figure 1A). There was a trend to increase the number of the black aphid with the reduction of the temperature, so that the lowest mean number of insects (0.09 nymphs) was observed when the temperature reached 25.8 ^{o}C and the highest number (4.41 nymphs) with 18.6 ^{o}C (Figure 1A).

Regarding the relative humidity ($y = 0.2249x + 60.237$, $r = 0.0138^{ns}$) and rainfall ($y = -3.646x + 19.588$, $r = -0.1310^{ns}$), there was no significant correlation between the number of *A. craccivora* nymphs and these abiotic factors, and the population fluctuation of the black aphid

throughout the year in the area may be attributed to other factors, such as nutritional quality, allelochemical or morphological differences of the cultivars used, or due to ecological interactions as parasitism, predation and competition with other organisms.

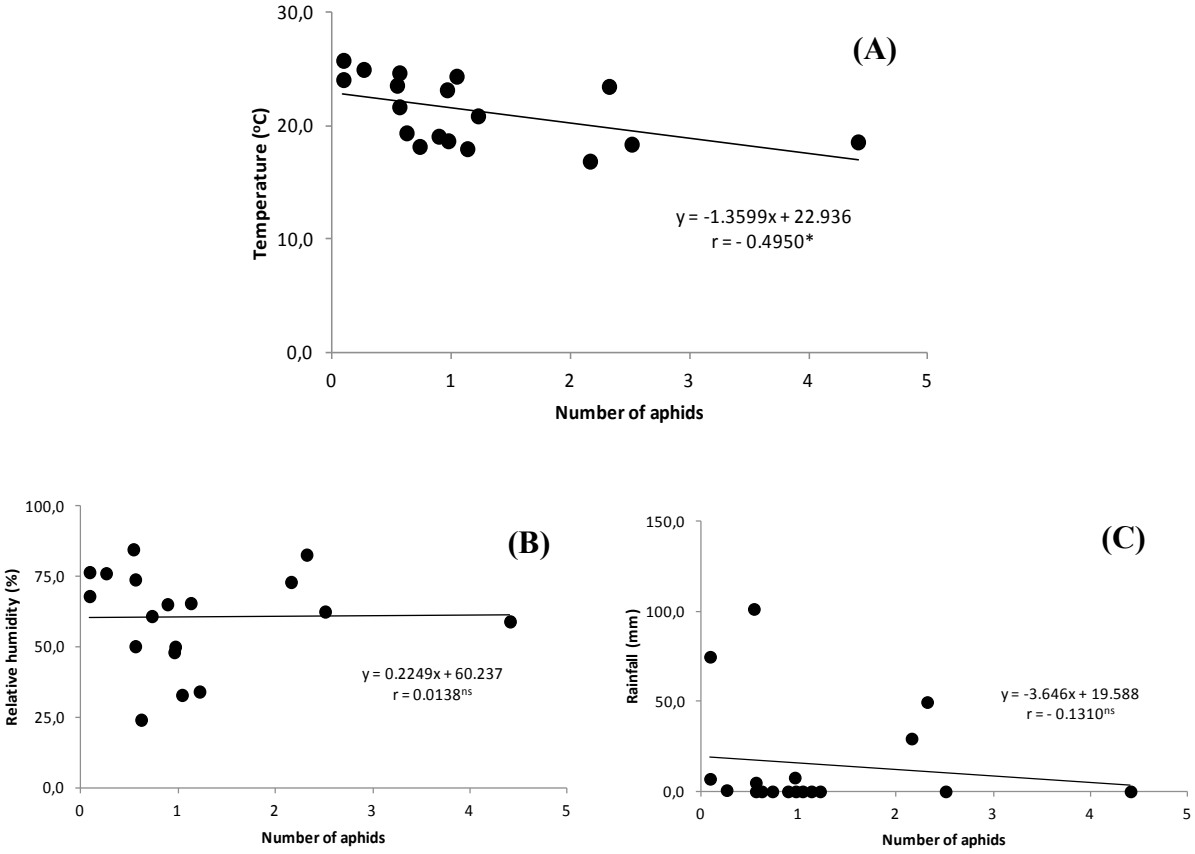

Figure 1. Simple linear correlation between the average number of *Aphis craccivora* nymphs and temperature (A), relative humidity (B) and rainfall (C) during three bean crop sowing seasons (winter, water and dry) in Jaboticabal, SP, Brazil, 2010/2011.

We can conclude temperature influences *A. craccivora* infestation on bean plants, whereas the abiotic factors relative humidity and rainfall seem not to affect the black aphid fluctuation in the conditions of Jaboticabal, SP, Brazil, and the differences of the populational density may be attributed to other factors.

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EFFECT OF AGES OF BEAN PLANTS ON FEEDING PREFERENCE OF *DIABROTICA SPECIOSA* (GERMAR) ADULTS

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INTRODUCTION

Among the factors that may affect resistance expression, the utilization of plants with different ages in resistance assays may exert fundamental influence on the result of cultivars screening (SMITH, 2005). Thus, the aim of this work was to evaluate the effect of different ages of bean cultivars plants on feeding preference of *Diabrotica speciosa* (Germar) adults.

MATERIALS AND METHODS

The experiment was carried out at Laboratório de Resistência de Plantas a Insetos of Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, SP, Brazil, under controlled environmental conditions. Adults of *D. speciosa* used in the assays were from the laboratory maintenance rearing, which were fed on bean plants, cultivar Pérola.

Leaves from the resistant and susceptible bean cultivars to lepidopterous larvae, IAC Harmonia and IAPAR 81, respectively (SOUZA et al., 2012), from the upper part of plants with 15 and 35 days after emergence (DAE) were collected and then leaf discs (2.5 cm diameter) were prepared to be utilized in feeding preference assays. For free-choice test, Petri dishes (14.0 cm diameter) lined with filter paper softly moistened with distilled water were used, within of which the leaf discs from plants with different ages of both cultivars were distributed equidistantly from each other, whereas the non-choice test was constituted of Petri dishes (9.0 cm diameter) containing one leaf disc (treatment) per plate.

Next, one *D. speciosa* adult per treatment was released into the plates in both tests, and the leaf beetles attractiveness was evaluated at 10, 15 and 30 minutes and 1, 2, 6, 12 and 20 hours after their release, in addition to the leaf area consumed (L.A.C.) at the end of the assays, through an electronic leaf area measurer device, model LI-COR 3100A®. The experiments consisted of four treatments in a 2 x 2 factorial scheme (two cultivars x two ages of plants) in randomized blocks design and completely randomized blocks design for free-choice and non-choice tests, respectively, with 10 replications both. Data obtained from insects attractiveness and leaf area consumed were transformed in $(x + 0.5)^{1/2}$ and subjected to the analysis of variance (ANOVA) by F test, with means compared to Tukey's test ($P = 0.05$).

RESULTS AND DISCUSSION

In free-choice test (Table 1), we verified no significant differences in the number of insects attracted between the cultivars in any age of plants as well as in the leaf area consumed. However, at 6 hours after the beginning of the experiment, the cultivar IAPAR 81 with 15 DAE was more attractive to the leaf beetles; both cultivars with 15 DAE were more attractive at 12 hours; and IAC Harmonia, also in this age, exhibited higher number of insects attracted in relation to 35 DAE. Moreover, both cultivars were significantly more consumed when leaf discs from younger plants were used (Table 1).

There were no significant differences in the attractiveness of the leaf beetles between the cultivars in any time assessed or leaf consumption in non-choice test (Table 2). Nevertheless, plants of the cultivar IAC Harmonia with 15 DAE was significantly more attractive to *D. speciosa* at 20 hours after the insects' release (Table 2).

Table 1. Number of *Diabrotica speciosa* adults attracted in time periods and leaf area consumed (L.A.C.) of leaf discs from bean cultivars plants with different ages, in free-choice test.

Cultivars (C)	10'	15'	30'	1h	2h	6h	12h	20h	L.A.C. (cm^2)
Plants with 15 DAE (A)									
IAC Harmonia	0.40aA	0.60aA	0.70aA	0.60aA	0.30aA	0.50aA	0.80aB	0.80aB	2.31aB
IAPAR 81	0.30aA	0.40aA	0.50aA	0.60aA	0.50aA	0.50aB	0.90aB	0.40aA	1.66aB
Plants with 35 DAE (A)									
IAC Harmonia	0.50aA	0.50aA	0.60aA	0.60aA	0.30aA	0.10aA	0.10aA	0.00aA	0.49aA
IAPAR 81	0.10aA	0.30aA	0.20aA	0.30aA	0.20aA	0.00aA	0.00aA	0.20aA	0.65aA
F (C)	2.87^{ns}	0.49^{ns}	1.14^{ns}	0.89^{ns}	0.01^{ns}	0.19^{ns}	0.04^{ns}	0.11^{ns}	0.50^{ns}
F (A)	0.12^{ns}	0.14^{ns}	0.90^{ns}	0.42^{ns}	0.43^{ns}	8.35**	12.35**	5.35*	25.07**
F (C x A)	1.03^{ns}	0.11^{ns}	0.23^{ns}	0.42^{ns}	0.43^{ns}	0.05^{ns}	0.10^{ns}	1.98^{ns}	1.44^{ns}
C.V.(%)	27.58	37.15	36.39	35.29	34.26	29.11	35.67	35.06	26.12

Means followed by lower case letter between cultivars within the same age of plants and by upper case letter between different age of plants within the same cultivar did not differ significantly by F test ($P = 0.05$).

Table 2. Number of *Diabrotica speciosa* adults attracted in time periods and leaf area consumed (L.A.C.) of leaf discs from bean cultivars plants with different ages, in non-choice test.

Cultivars (C)	10'	15'	30'	1h	2h	6h	12h	20h	L.A.C. (cm^2)
Plants with 15 DAE (A)									
IAC Harmonia	0.30aA	0.40aA	0.40aA	0.70aA	0.40aA	0.40aA	0.70aA	0.70aB	1.80aA
IAPAR 81	0.10aA	0.30aA	0.30aA	0.40aA	0.60aA	0.40aA	0.50aA	0.40aA	1.14aA
Plants with 35 DAE (A)									
IAC Harmonia	0.30aA	0.30aA	0.40aA	0.20aA	0.30aA	0.40aA	0.40aA	0.20aA	0.95aA
IAPAR 81	0.10aA	0.10aA	0.20aA	0.40aA	0.30aA	0.40aA	0.50aA	0.10aA	0.92aA
F (C)	2.40^{ns}	1.08^{ns}	0.95^{ns}	0.11^{ns}	0.40^{ns}	0.00^{ns}	0.10^{ns}	2.06^{ns}	1.16^{ns}
F (A)	0.00^{ns}	1.08^{ns}	0.11^{ns}	2.65^{ns}	1.60^{ns}	0.00^{ns}	0.85^{ns}	8.23**	2.89^{ns}
F (C x A)	0.00^{ns}	0.12^{ns}	0.11^{ns}	2.65^{ns}	0.40^{ns}	0.00^{ns}	0.85^{ns}	0.51^{ns}	0.76^{ns}
C.V.(%)	26.07	27.81	28.73	27.13	28.31	29.24	27.17	25.70	25.72

Means followed by lower case letter between cultivars within the same age of plants and by upper case letter between different age of plants within the same cultivar did not differ significantly by F test ($P = 0.05$).

Overall, *D. speciosa* adults preferred to feed on bean plants with 15 DAE in free-choice test, and the cultivar IAC Harmonia does not hold resistance to the leaf beetle in any ages of plants used in the assays.

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ACTION OF AZADIRACHTIN ON REPELLENCY AND CONSUMPTION OF *DIABROTICA SPECIOSA* (GERMAR) ADULTS ON *PHASEOLUS VULGARIS* L.

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INTRODUCTION

In Brazil, *Phaesolus* genus has wide climatic adaptation, allowing it to be grown throughout the year (WANDER, 2007). However, this feature enables the increase the number of insects, and *Diabrotica speciosa* (Germar), commonly known as bean leaf beetle, represents an important defoliating pest.

The efficiency of natural products to control the insect has been observed by Migliorini (2010), and according to Schmutterer (1990), azadirachtin shows great insectistatic activity. Thus, the aim of this work was to evaluate the action of different concentrations of neem oil, commercial product with 1.2% azadirachtin, on repellency and consumption of *D. speciosa*.

MATERIALS AND METHODS

The effect of azadirachtin on repellency and deterrence of *D. speciosa* adults was evaluated under controlled environmental conditions at Laboratório de Resistência de Plantas a Insetos of Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, SP, Brazil. To perform the experiment, adults of *D. speciosa* were collected in peanut crop, cultivar IAC Runner 886, and remained without feeding for 24 hours before the beginning of free-choice and non-choice tests.

Four doses of neem oil Azamax® (azadirachtin 1.2 EC) were assayed: 75, 150, 225 and 300 mL ha^{-1}, and a treatment used as control, with the application of distilled water. Leaf discs were used, which were extracted through a metallic puncher (2.5 cm diameter), from trefoils of 30 days-old bean plants, cultivar Pérola. These discs were immersed into emulsions during one minute, and next exposed to environmental conditions for 30 minutes to be dried, protected from the light in order to avoid the product degradation. After drying, for the free-choice test, leaf discs were distributed equidistantly into Pedri dishes (14.0 cm diameter), whereas, in the non-choice test, the discs were individualized into Petri dishes (9.0 cm diameter) lined with filter paper softly moistened with distilled water.

Adults of *D. speciosa* were released in the center of the plates, in the proportion of one insect per treatment, totaling five insects per plate in free-choice test and one insect per plate in non-choice test. At 5, 10, 15 and 30 minutes and 1, 2, 6, 12, 24 and 30 hours after the insects release, *D. speciosa* attractiveness was assessed in relation to the treatments, and at the end of the tests, the leaf area consumed (L.A.C.) was quantified through an electronic leaf area measurer device, model LI-COR 3100A®.

The experiments consisted of five treatments set in randomized blocks design and completely randomized blocks design for free-choice and non-choice tests, respectively, with 10 replications each. Data obtained from insects attractiveness and leaf area consumed were transformed in $(x + 0.5)^{1/2}$ for normalization and submitted to the analysis of variance (ANOVA) by F test, and means were compared to Tukey's test ($P = 0.05$).

RESULTS AND DISCUSSION

Regarding the insects attractiveness to bean leaf discs treated with different concentrations of neem oil in free-choice (Table 1) and non-choice (Table 2) tests, significant differences were observed. At 10, 15 and 30 minutes and 1, 2 and 6 hours after the insects' release, higher number of *D. speciosa* adults was found on control treatment, and this preference may indicate azadirachtin repellency effect to the insect. However, this characteristic did not remain over the entire test period, possibly due to the need of *D. speciosa* adults to feed. Significant differences were not noted in leaf area consumed among the treatments.

Table 1. Number of *Diabrotica speciosa* adults attracted in different times and leaf area consumed (L.A.C.) of *Phaseolus vulgaris* leaf discs treated with doses of neem oil, in free-choice test. Jaboticabal, SP, Brazil, 2013.

Treatments	5'	10'	15'	30'	1h	2h	6h	12h	24h	30h	L.A.C. (cm^2)
Control	0.30	0.70a	0.80a	0.80a	1.00a	1.60a	1.10a	0.10	0.00	0.10	1.29
75 mL ha^{-1}	0.20	0.00b	0.00b	0.10b	0.30b	0.40b	0.10b	0.20	0.30	0.20	1.19
150 mL ha^{-1}	0.10	0.10b	0.00b	0.00b	0.10b	0.10b	0.10b	0.00	0.40	0.20	0.97
225 mL ha^{-1}	0.10	0.20b	0.10b	0.10b	0.00b	0.00b	0.00b	0.00	0.20	0.10	0.77
300 mL ha^{-1}	0.00	0.00b	0.00b	0.00b	0.00b	0.00b	0.00b	0.30	0.20	0.10	1.40
F (Treatments)	0.74^{ns}	6.99**	9.26**	5.24**	11.48**	14.59**	7.71**	0.96^{ns}	0.76^{ns}	0.21^{ns}	0.74^{ns}
C.V.(%)	25.29	21.33	21.80	26.59	22.52	27.03	28.42	25.18	31.12	24.94	27.57

Table 2. Number of *Diabrotica speciosa* adults attracted in different times and leaf area consumed (L.A.C.) of *Phaseolus vulgaris* leaf discs treated with doses of neem oil, in non-choice test. Jaboticabal, SP, Brazil, 2013.

Treatments	5'	10'	15'	30'	1h	2h	6h	12h	24h	30h	L.A.C. (cm^2)
Control	0.30	0.40a	0.40a	0.50a	0.50a	0.80a	0.70a	0.40	0.60	0.10	1.15
75 mL ha^{-1}	0.10	0.00b	0.00b	0.10ab	0.20b	0.20bc	0.60a	0.60	0.60	0.30	1.43
150 mL ha^{-1}	0.20	0.20ab	0.20ab	0.30ab	0.40ab	0.50ab	0.60a	0.30	0.60	0.20	1.26
225 mL ha^{-1}	0.00	0.00b	0.00b	0.00b	0.00b	0.00c	0.20b	0.30	0.60	0.20	1.05
300 mL ha^{-1}	0.00	0.00b	0.00b	0.00b	0.10b	0.10bc	0.20b	0.40	0.20	0.40	0.76
F (Treatments)	1.66^{ns}	3.60*	3.60*	3.85**	2.62*	7.30**	2.58*	0.59^{ns}	1.29^{ns}	0.68^{ns}	1.99^{ns}
C.V.(%)	21.52	20.06	20.06	22.61	25.25	22.71	25.94	28.50	26.45	27.22	17.38

We concluded azadirachtin in the tested doses causes repellency to *D. speciosa* adults, however, this effect does not remain over time, not affecting leaf consumption.

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INFLUENCE OF PARTS OF BEAN PLANTS ON FEEDING PREFERENCE OF *DIABROTICA SPECIOSA* (GERMAR) ADULTS

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INTRODUCTION

The effects inherent to the plant which may influence the evaluation results of host plant resistance in laboratory, greenhouse or field, must be previously determined before any precipitate conclusions regarding its resistance or susceptibility to a specific arthropod pest (SMITH, 2005). Thus, this work aimed to assess the effect of different parts of plants on the feeding preference of *Diabrotica speciosa* (Germar) (Coleoptera: Chrysomelidae) adults on bean cultivars.

MATERIALS AND METHODS

The experiment was carried out at Laboratório de Resistência de Plantas a Insetos of Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, SP, Brazil, under controlled environmental conditions. Adults of *D. speciosa* used in the assays were from the laboratory maintenance rearing, which were fed on bean plants, cultivar Pérola.

Leaves from the resistant and susceptible bean cultivars to lepidopterous larvae, IAC Harmonia and IAPAR 81, respectively (SOUZA et al., 2012), were detached from the upper and lower parts of plants at 30 days after emergence, and then leaf discs (2.5 cm diameter) were prepared to be utilized in feeding preference assays. For free-choice test, Petri dishes (14.0 cm diameter) lined with filter paper softly moistened with distilled water were used, within of which the leaf discs from the upper and lower parts of both plants cultivars were distributed equidistantly from each other, whereas the non-choice test was constituted of Petri dishes (9.0 cm diameter) containing one leaf disc (treatment) per plate.

Next, one *D. speciosa* adult per treatment was released into the plates in both tests, and the leaf beetles attractiveness was evaluated at 10, 15 and 30 minutes and 1, 2, 6, 12 and 24 hours after their release, in addition to the leaf area consumed (L.A.C.) at the end of the assays, through an electronic leaf area measurer device, model LI-COR 3100A®. The experiments consisted of four treatments in a 2 x 2 factorial scheme (two cultivars x two parts of plants) in randomized blocks design and completely randomized blocks design for free-choice and non-choice tests, respectively, with 10 replications both. Data obtained from insects attractiveness and leaf area consumed were transformed in $(x + 0.5)^{1/2}$ and subjected to the analysis of variance (ANOVA) by F test, with means compared to Tukey's test ($P = 0.05$).

RESULTS AND DISCUSSION

Basing on data obtained from free-choice feeding preference test (Table 1), we observed no significant differences in the number of *D. speciosa* adults attracted between the cultivars IAC Harmonia and IAPAR 81 using leaf discs from the upper or lower parts of bean plants throughout the evaluation time of the experiment. Leaf area consumed in this assay did not differ significantly between the cultivars for any part of the plant's canopy either, despite the cultivar IAC Harmonia has shown a numerically lower value for this parameter. Likewise, in non-choice

test (Table 2), significant differences were not found for either beetles attractiveness or consumption between the leaf discs from the different parts of bean plants cultivars.

Table 1. Number of *Diabrotica speciosa* adults attracted in different times and leaf area consumed (L.A.C.) of cultivars leaf discs from the upper and lower parts of plants, in free-choice test. Jaboticabal, SP, Brazil.

Cultivars (C)	10min	15min	30min	1h	2h	6h	12h	24h	L.A.C. (cm^2)
Upper part (P)									
IAC Harmonia	0.10aA	0.20aA	0.30aA	0.20aA	0.40aA	0.30aA	0.10aA	0.20aA	1.09aA
IAPAR 81	0.20aA	0.30aA	0.40aA	0.40aA	0.50aA	0.20aA	0.10aA	0.10aA	1.88aA
Lower part (P)									
IAC Harmonia	0.20aA	0.30aA	0.50aA	0.20aA	0.30aA	0.00aA	0.30aA	0.20aA	1.19aA
IAPAR 81	0.30aA	0.50aA	0.30aA	0.20aA	0.10aA	0.10aA	0.00aA	0.10aA	1.45aA
F (C)	0.63^{ns}	0.97^{ns}	0.05^{ns}	0.32^{ns}	0.01^{ns}	0.00^{ns}	2.19^{ns}	0.22^{ns}	2.74^{ns}
F (P)	0.63^{ns}	0.97^{ns}	0.05^{ns}	0.32^{ns}	1.91^{ns}	3.09^{ns}	0.24^{ns}	0.00^{ns}	0.30^{ns}
F (C x P)	0.00^{ns}	0.11^{ns}	0.68^{ns}	0.32^{ns}	0.69^{ns}	0.77^{ns}	2.19^{ns}	0.00^{ns}	0.69^{ns}
C.V.(%)	25.48	28.51	29.76	29.51	34.13	23.75	21.50	30.70	25.01

Means followed by lower case letter between cultivars within the same age of plants and by upper case letter between different age of plants within the same cultivar did not differ significantly by F test ($P = 0.05$).

Table 2. Number of *Diabrotica speciosa* adults attracted in different times and leaf area consumed (L.A.C.) of cultivars leaf discs from the upper and lower parts of plants, in non-choice test. Jaboticabal, SP, Brazil.

Cultivars (C)	10min	15min	30min	1h	2h	6h	12h	24h	L.A.C. (cm^2)
Upper part (P)									
IAC Harmonia	0.50aA	0.40aA	0.30aA	0.60aA	0.60aA	0.30aA	0.00aA	0.20aA	1.41aA
IAPAR 81	0.20aA	0.40aA	0.30aA	0.40aA	0.60aA	0.30aA	0.10aA	0.00aA	1.68aA
Lower part (P)									
IAC Harmonia	0.40aA	0.40aA	0.50aA	0.50aA	0.60aA	0.10aA	0.00aA	0.00aA	1.30aA
IAPAR 81	0.20aA	0.20aA	0.30aA	0.30aA	0.20aA	0.20aA	0.10aA	0.30aA	1.44aA
F (C)	2.78^{ns}	0.41^{ns}	0.41^{ns}	1.53^{ns}	1.64^{ns}	0.13^{ns}	2.00^{ns}	0.24^{ns}	0.68^{ns}
F (P)	0.11^{ns}	0.41^{ns}	0.41^{ns}	0.38^{ns}	1.64^{ns}	1.21^{ns}	0.00^{ns}	0.24^{ns}	0.49^{ns}
F (C x P)	0.11^{ns}	0.41^{ns}	0.41^{ns}	0.00^{ns}	1.64^{ns}	0.13^{ns}	0.00^{ns}	2.08^{ns}	0.06^{ns}
C.V.(%)	28.05	28.81	28.81	28.14	26.50	27.11	15.79	21.50	17.61

Means followed by lower case letter between cultivars within the same age of plants and by upper case letter between different age of plants within the same cultivar did not differ significantly by F test ($P = 0.05$).

We concluded *D. speciosa* adults do not exhibit specific feeding preference for the upper or lower part of bean plants, and the resistant cultivar to lepidopterous larvae must not be set as the resistance pattern in non-preference for feeding tests to this leaf beetle.

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INFLUENCE OF BEAN PLANTS AGE ON RESISTANCE EXPRESSION TO *SPODOPTERA FRUGIPERDA* (J. E. SMITH)

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INTRODUCTION

The influence of plants age on arthropod feeding preference has been approached in several studies. Overall, nitrogen content trend to be reduced in leaves of older plants (MATTSON JR., 1980), whereas allelochemical production may increase or decrease (PANDA & KHUSH, 1995) depending on the host plant, previous feeding by other insects, pathogens infection, etc. This work aimed to evaluate the influence of different ages of bean plants on non-preference for feeding-type resistance expression to *Spodoptera frugiperda* (J. E. Smith) larvae.

MATERIALS AND METHODS

The experiment was carried out at Laboratório de Resistência de Plantas a Insetos of Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, SP, Brazil, under controlled environment conditions. Larvae of *S. frugiperda* used in the assays were from the stock rearing maintained in laboratory, fed on artificial medium, according to the methodology of GREENE et al. (1976).

Bean plants of the resistant and susceptible cultivars IAC Harmonia and IAPAR 81, respectively (SOUZA et al., 2012), were grown in 5 L volume pots with soil, sand and manure, in the proportion of 2:1:1 and placed into a greenhouse. When they were 20 or 40 days after emergence (DAE), leaf discs (2.5 cm diameter) were prepared from the upper part of the cultivars plants which were used in the free-choice and non-choice non-preference for feeding tests. The former assay was constituted by Petri dishes (14.0 cm diameter) lined with filter paper softly moistened with distilled water within which the leaf discs from the plants of both cultivar in different ages were distributed equidistantly from each other, whereas the latter test was carried out into Petri dishes (9,0 cm diameter) containing one leaf disc (treatment) per plate.

Next, one eight years-old *S. frugiperda* larva per treatment was released in the center of the plates, totaling four larvae per plate in free-choice test and one larva per plate in non-choice test. Larvae attractiveness was assessed at 5, 10, 15 and 30 minutes and 1, 2, 6, 12 and 24 hours after their release, and the leaf area consumed (L.A.C.) was quantified at the end of the assays through an electronic leaf area measurer device, model LI-COR 3100A®. The experiments consisted of four treatments in a 2 x 2 factorial scheme (two cultivars x two ages of plants), in randomized blocks design and completely randomized blocks design, respectively, for free-choice and non-choice tests, with 10 replications for both. Data obtained from attractiveness and leaf area consumed were transformed in $(x + 0.5)^{1/2}$ and subjected to the analysis of variance (ANOVA) by F test, and means were compared to Tukey's test ($P = 0.05$).

RESULTS AND DISCUSSION

In free-choice test, we observed significant differences in the attractiveness of *S. frugiperda* larvae between the bean cultivars with 40 DAE after 1 hour of their release ($F = 5.75$*), at 6 hours for plants with 20 DAE ($F = 9.58$**) and at 2 hours for both ages ($F = 15.29$**), so that, in these evaluation times, the cultivar IAC Harmonia was the least attractive. The resistant cultivar

was also significantly less attractive to the insects at 10 $F = 7.02$*) and 15 minutes ($F = 9.99$**) and 1 hour ($F = 6.04$*) after the beginning of the assay for plants with 20 DAE, and at 30 minutes ($F = 36.75$**) and 2 hours (27.66**) for plants of both ages.

Regarding the leaf area consumed in free-choice test (Figure 1A), the resistant cultivar IAC Harmonia was less preferred than the susceptible IAPAR 81 using plants with 20 and 40 DAE, and significant differences were not found in the consumption for any cultivar in different ages (Figure 1A). For non-choice test (Figure 1B), leaf consumption differed significantly only between the cultivars plants with 20 DAE. None of the cultivars differed significantly in regard to the leaf area consumed between the different ages of the plants (Figure 1B).

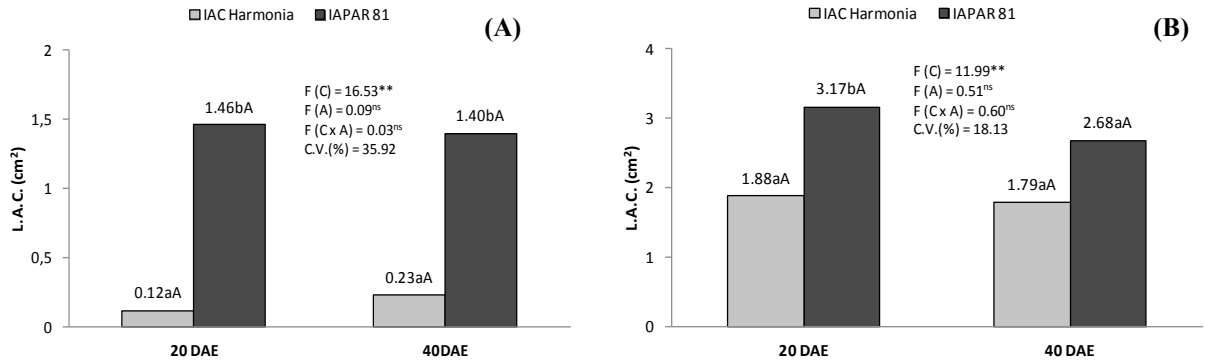

Figure 1. Leaf area consumed (L.A.C.) of bean plants cultivars with different ages in free-choice (A) and non-choice (B) tests. Means followed by the same lower case letter between cultivars of the same part of plants and upper case letter for the same cultivar between different parts of plants did not differ significantly by Tukey's test ($P = 0.05$).

We concluded the age of bean plants influenced resistance expression in the cultivar IAC Harmonia in non-choice test, and the utilization of plants with 20 DAE provided better differentiation of the resistance degrees between the resistant and susceptible cultivars in non-preference for feeding of *S. frugiperda* larvae.

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INFLUENCE OF PARTS OF BEAN PLANTS ON RESISTANCE EXPRESSION TO *SPODOPTERA FRUGIPERDA* (J. E. SMITH)

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INTRODUCTION

Since it is a genetic trait, resistance in plants express under determined conditions, and, amongst the factors inherent to plants, its different parts may be more or less preferred for feeding by arthropod pests (SMITH, 2005). Thus, this study aimed to assay the influence of different parts of bean plants on non-preference for feeding-type resistance to larvae of the fall armyworm *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae).

MATERIALS AND METHODS

The experiment was carried out at Laboratório de Resistência de Plantas a Insetos of Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, SP, Brazil, under controlled environment conditions. Larvae of *S. frugiperda* used in the assays were from the stock rearing maintained in laboratory, fed on artificial medium, according to the methodology of GREENE et al. (1976).

Bean plants of the resistant and susceptible cultivars IAC Harmonia and IAPAR 81, respectively (SOUZA et al., 2012), were grown in 5 L volume pots with soil, sand and manure, in the proportion of 2:1:1 and placed into a greenhouse. When they were 30 days after emergence, leaflets from the upper and lower parts were detached from the cultivars plants and then leaf discs (2.5 cm diameter) were prepared, which were used in the free-choice and non-choice non-preference for feeding tests. The former assay was constituted by Petri dishes (14.0 cm diameter) lined with filter paper softly moistened with distilled water within which the leaf discs from the different parts of both cultivars were distributed equidistantly from each other, whereas the latter test was carried out into Petri dishes (9,0 cm diameter) containing one leaf disc (treatment) per plate.

Next, one eight years-old *S. frugiperda* larva per treatment was released in the center of the plates, totaling four larvae per plate in free-choice test and one larva per plate in non-choice test. Larvae attractiveness was assessed at 5, 10, 15 and 30 minutes and 1, 2, 6, 12 and 24 hours after their release, and the leaf area consumed (L.A.C.) was quantified at the end of the assays through an electronic leaf area measurer device, model LI-COR 3100A®. The experiments consisted of four treatments in a 2 x 2 factorial scheme (two cultivars x two parts of plants), in randomized blocks design and completely randomized blocks design, respectively, for free-choice and non-choice tests, with 10 replications for both. Data obtained from attractiveness and leaf area consumed were transformed in $(x + 0.5)^{1/2}$ and subjected to the analysis of variance (ANOVA) by F test, and means were compared to Tukey's test ($P = 0.05$).

RESULTS AND DISCUSSION

From the results obtained in free-choice test, significant differences were found in the attractiveness of *S. frugiperda* larvae after 6 ($F = 10.45$**) and 12 hours ($F = 18.65$**) of their release, so that, in the first test, lower number of insects was observed on the resistant cultivar IAC Harmonia only for the lower part of plants, while in the second, this cultivar was less

attractive than the susceptible cultivar IAPAR 81 for both parts of plants. In non-choice test, there were significant differences for the number of larvae attracted between the bean cultivars at 10 minutes (F = 6.39*) and 2 (F = 6.04*) and 24 horas (F = 5.73*) after the beginning of the experiment. At 10 minutes and 24 hours, the cultivar IAC Harmonia was less attractive only for the lower part of plants, and at 2 hours, only for the upper part. Moreover, in the times of 1 (F = 10.27**) and 2 hours (F = 6.04*), there were significant differences for the parts of plants. After 1 hours of larvae release, the cultivar IAPAR 81 exhibited number of larvae significantly higher when the leaf discs were from the upper part of the plants, and at 2 hours, IAC Harmonia was less attractive for this part of the plant's canopy.

With respect to the leaf area consumed, for free-choice (F = 33.17**) and non-choice (F = 20.90**) tests (Figures 1A and 1B), significant differences were found between the resistant and susceptible cultivars for both upper and lower parts of plants, although differences for the parts of plant's canopy within the same cultivar did not occur.

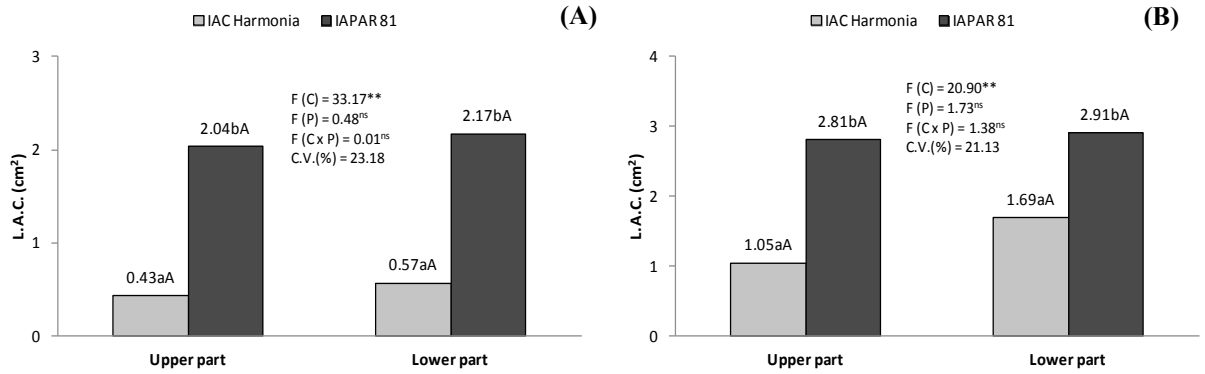

Figure 1. Leaf area consumed (L.A.C.) of discs from the upper and lower parts of bean plants cultivars in free-choice (A) and non-choice (B) tests. Means followed by the same lower case letter between cultivars of the same part of plants and upper case letter for the same cultivar between different parts of plants did not differ significantly by Tukey's test (P = 0.05).

We conclude the upper and lower parts of bean plants do not influence non-preference for feeding on the resistant cultivar IAC Harmonia to *S. frugiperda*, and any of the parts from plant's canopy can be used in the assays to evaluate this resistance category to the fall armyworm.

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ACTION OF AZADIRACHIN ON REPELLENCY AND CONSUMPTION OF *SPODOPTERA FRUGIPERDA* (J. E. SMITH) LARVAE ON *PHASEOLUS VULGARIS* L.

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INTRODUCTION

Azadirachtin is as bioactive compound extracted from neem plant *Azadirachta indica* A. Juss (Meliaceae), which has insecticidal action on first-instar larvae of *Spodoptera frugiperda* (J. E. Smith, 1797) (RIBEIRO et al., 2012), and according to Schumetterer (1990), this substance also exert antifeeding effect. Thus, this study aimed to evaluate the antifeeding effect of different concentrations of neem oil, commercial product with 1.2% azadirachtin, on *S. frugiperda* larvae.

MATERIALS AND METHODS

Azadirachtin antifeeding effect on *S. frugiperda* larvae was assayed under controlled environmental conditions at Laboratório de Resistência de Plantas a Insetos of Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, SP, Brazil. To perform the experiment, *S. frugiperda* larvae were obtained from the maintenance rearing in laboratory, fed on artificial medium according to the methodology of GREENE et al. (1976).

Four doses of neem oil Azamax® (azadirachtin 1.2 EC) were assayed: 75, 150, 225 and 300 mL ha^{-1}, and a treatment used as control, with the application of distilled water. Leaf discs were used, which were extracted through a metallic puncher (2.5 cm diameter), from trefoils of 30 days-old bean plants, cultivar Pérola. These discs were immersed into emulsions during one minute, and next exposed to environmental conditions for 30 minutes to be dried, protected from the light in order to avoid the product degradation. After drying, for the free-choice test, leaf discs were distributed equidistantly into Pedri dishes (14.0 cm diameter), whereas, in the non-choice test, the discs were individualized into Petri dishes (9.0 cm diameter) lined with filter paper softly moistened with distilled water.

Larvae of *S. frugiperda* (eight days-old) were released in the center of the plates, in the proportion of one insect per treatment, totaling five larvae per plate in free-choice test and one larva per plate in non-choice test. At 5, 10, 15 and 30 minutes and 1, 2, 6, 12 and 24 hours after the insects release, *S. frugiperda* larvae attractiveness was assessed in relation to the treatments, and at the end of the tests, the leaf area consumed (L.A.C.) was quantified through an electronic leaf area measurer device, model LI-COR 3100A®.

The experiments consisted of five treatments set in randomized blocks design and completely randomized blocks design for free-choice and non-choice tests, respectively, with 10 replications each. Data obtained from insects attractiveness and leaf area consumed were transformed in $(x + 0.5)^{1/2}$ for normalization and submitted to the analysis of variance (ANOVA) by F test, and means were compared to Tukey's test ($P = 0.05$).

RESULTS AND DISCUSSION

From data obtained in free-choice test, significant differences were observed in attractiveness of *S. frugiperda* larvae. In the first evaluation, the insect's preference for the treatment without azadirachtin was already observed, and this preference remained in most time periods assessed

(Table 1). In non-choice test, significant difference among treatments only occurred at 15 minutes (Table 2). With respect to leaf area consumed, for free-choice (Table 1) and non-choice (Table 2) tests, significant differences were not found among bean leaf discs treated with the different doses of azadirachtin.

Table 1. Number of *Spodoptera frugiperda* larvae attracted in different times and leaf area consumed (L.A.C.) of *Phaseolus vulgaris* leaf discs immersed into doses of neem oil, in free-choice test. Jaboticabal, SP, Brazil, 2013.

Treatments	5'	10'	15'	30'	1h	2h	6h	12h	24h	L.A.C. (cm^2)
Control	0.80a	0.60a	0.50	0.80a	0.60	1.00a	0.90a	0.80a	0.40	1.64
75 mL ha^{-1}	0.10b	0.75ab	0.40	0.40ab	0.30	0.40ab	0.20ab	0.20ab	1.30	0.89
150 mL ha^{-1}	0.30ab	0.10ab	0.20	0.10b	0.20	0.10b	0.30ab	0.10b	0.80	1.03
225 mL ha^{-1}	0.00b	0.00b	0.00	0.00b	0.00	0.00b	0.00b	0.20ab	0.50	1.04
300 mL ha^{-1}	0.00b	0.20ab	0.20	0.00b	0.00	0.00b	0.20ab	0.00b	0.40	0.77
F (Treatments)	4.58**	2.53*	1.13^{ns}	4.87**	1.52^{ns}	4.72**	3.55*	4.45**	1.97^{ns}	1.36^{ns}
C.V.(%)	27.65	27.00	31.54	28.16	33.69	32.58	30.40	28.25	34.69	25.42

Table 2. Number of *Spodoptera frugiperda* larvae attracted in different times and leaf area consumed (L.A.C.) of *Phaseolus vulgaris* leaf discs immersed into doses of neem oil, in non-choice test. Jaboticabal, SP, Brazil, 2013.

Treatments	5'	10'	15'	30'	1h	2h	6h	12h	24h	L.A.C. (cm^2)
Control	0.30	0.40	0.50a	0.40	0.40	0.40	0.60	0.50	0.60	2.08
75 mL ha^{-1}	0.10	0.20	0.20ab	0.30	0.30	0.30	0.20	0.30	0.30	1.51
150 mL ha^{-1}	0.00	0.00	0.10ab	0.00	0.10	0.10	0.20	0.10	0.30	1.46
225 mL ha^{-1}	0.10	0.20	0.10ab	0.10	0.10	0.20	0.50	0.30	0.40	2.18
300 mL ha^{-1}	0.00	0.00	0.00b	0.10	0.20	0.10	0.60	0.40	0.70	2.42
F (Treatments)	1.73^{ns}	2.25^{ns}	2.82*	1.93^{ns}	0.97^{ns}	0.97^{ns}	1.80^{ns}	0.99^{ns}	1.34^{ns}	0.66^{ns}
C.V.(%)	20.08	23.12	23.42	24.20	26.42	26.42	27.05	27.96	27.20	34.46

Thus, we concluded azadirachtin, in the assessed doses, does not cause antifeeding effect on *S. frugiperda* larvae.

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CONCENTRATION AND ACCUMULATION OF PHOSPHORUS IN *PHASEOLUS VULGARIS* SUBJECTED TO SOIL PHOSPHATE LEVELS AND FOLIAR-APPLIED PHOSPHORUS FORMS

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INTRODUCTION

Aim of this study was to investigate action produced by soil phosphate levels and foliar-applied phosphorus forms on concentration and accumulation of P in shoot and root in common bean (*Phaseolus vulgaris* cv. Radiante) plants.

MATERIALS AND METHODS

Study was implemented in Departamento de Ciência do Solo of the Universidade Federal de Lavras, Brazil. Plants remained in glasshouse environment under natural conditions day/night. Substrate used was composed by Oxisol placed in plastic pots with capacity of 6 L (Table 1). For plant material was used common bean (*Phaseolus vulgaris* cv. Radiante) plants.

Table 1. Chemical, physical and mineralogical compositions of Oxisol.

Chemical [(1)]															
pH	P	K	Zn	Cu	Mn	Fe	EP	Ca	Mg	Al	H+Al	T	m	V	MPAC
	mg dm^{-3} of soil						mg L^{-1}	cmol$_c$ dm^{-3} of soil					%		mg kg^{-1}
5.4	0.9	22	0.5	0.7	0.4	27.4	20.5	0.1	0.1	0.1	1.7	2	28	13.3	396

Physical [(2)]			
Sand	Silt	Clay	OM
%			
60	17	23	0.8

Mineralogical [(3)]										
SiO_2	Al_2O_3	Fe_2O_3	TiO_2	P_2O_5	Fe_d	Fe_o	Ct	Gb	Ki	Kr
g kg^{-1} of clay										
95.1	97.4	36.2	6.2	0.0	10.8	0.1	752.0	63.0	0.98	0.71

[(1)] pH in water (1:2.5), P and K by Mehlich I extraction, Mg and Al extractable by 1 M KCl solution; P in the equilibrium solution (EP); T = Cation exchange capacity at pH 7.0; m = Aluminum saturation index; V = Base saturation index and MPAC = maximum P adsorption capacity.
[(2)] The soil granulometry was determined by the pipette method.
[(3)] Ct is kaolinite and Gb is gibbsite; Ki = SiO_2 / Al_2O_3 and Kr = SiO_2 / (Al_2O_3 + Fe_2O_3).

Experiment was organized in factorial scheme completely randomized using 2 soil phosphate levels (Pi-starved and Pi-sufficient), combined with 3 nutrient sources applied via foliar (KH_2PO_3, KH_2PO_4, and KCl used as control), and 2 foliar application numbers (single and two applications). This study had 3 replicates, and each experimental unit consisted of one pot containing two plants, and all variables measured were expressed as mean of two plants.

Shoot and root dry mass were ground and analyzed for total P content colorimetrically (Murphy and Riley, 1962) after nitric-perchloric digestion of the plant material (Johnson and Ulrich, 1959). Data from shoot and root dry wt and total P concentration were used to calculate the P accumulation. Results were submitted to variance analysis and applied to Tukey test at 5% level, as well as the standard errors were calculated in all evaluated points.

RESULTS AND DISCUSSION

Common bean plants grown under limiting phosphate availability (Pi-starved) showed decreased concentrations and accumulations of P in shoot and root (Figure 1).

Foliar-applied potassium phosphite did not affect P nutrition of phosphate-sufficient plants, but increased concentration of P in shoot of phosphate-starved plants. However, accumulation of P was not significant varied among foliar application treatments ($p > 0.05$), showing that this increased concentration of P was not due directly to the P from the foliar-applied phosphite, but likely to "concentration effect", which is confirmed by the lower shoot biomass yield (data not shown). When biomass yield decreases, the "concentration effect" for some nutrients may occur, which is the elevation of their concentration in the tissues without having there being an alteration in the quantity of nutrient taken up. In addition, foliar-applied potassium phosphate did not significantly affect ($p > 0.05$) concentrations and accumulations of P in common bean, when compared with the control. This shows that either one or two foliar applications of phosphate were not sufficient to affect the plant P status.

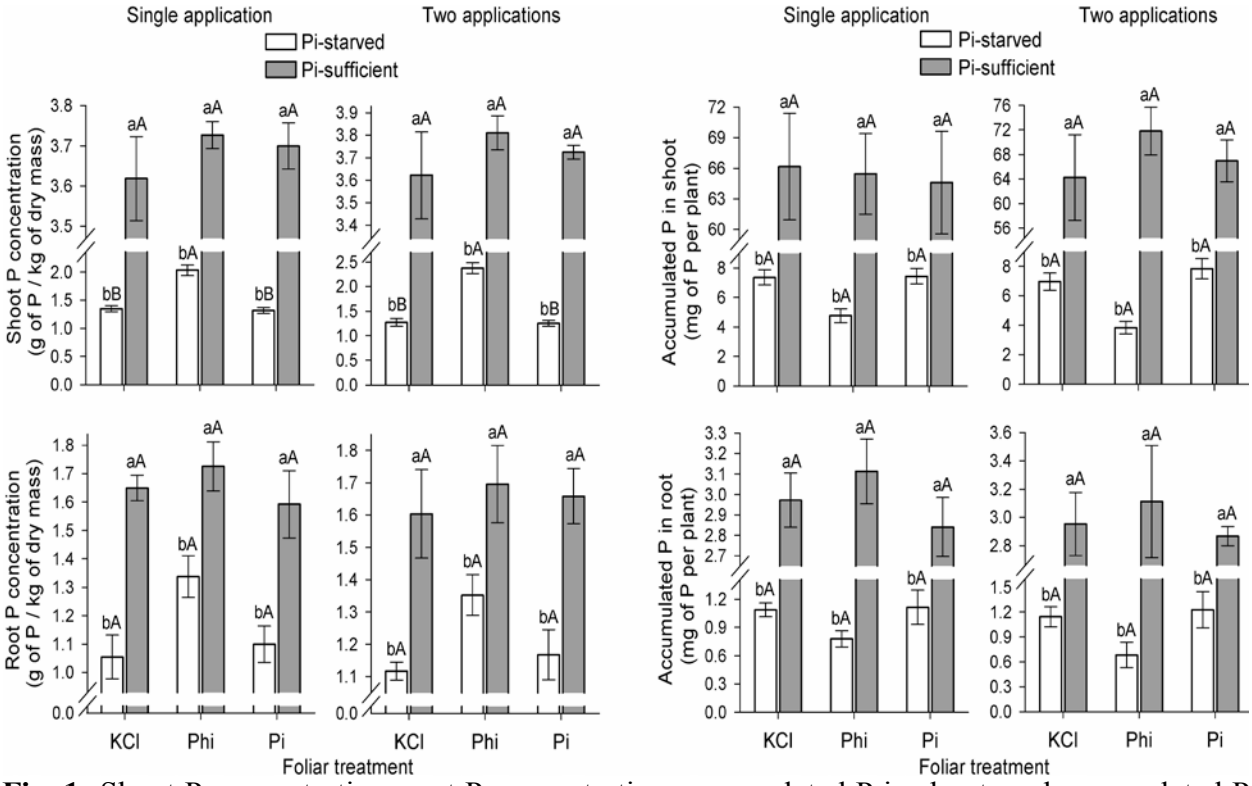

Fig. 1: Shoot P concentration, root P concentration, accumulated P in shoot, and accumulated P in root in common bean grown in Oxisol under 2 soil phosphate levels (Pi-starved and Pi-sufficient), 3 nutrient sources supplied via foliar application (KH_2PO_3, KH_2PO_4, and KCl), and 2 foliar application numbers (single and two applications). Averages followed by the same lowercase letter within soil phosphate levels and uppercase letter among foliar application for each soil phosphate level, do not differ among themselves by the Tukey test at 5% of probability. The bars represent the mean standard error.

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TISSUE P CONCENTRATION AND TOTAL P ACCUMULATION IN COMMON BEAN PLANTS GROWN IN NUTRITION SOLUTION UNDER PHOSPHATE AND PHOSPHITE TREATMENTS

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INTRODUCTION

Phosphate (Pi) and phosphite (Phi) are the two main P forms used in agriculture, in which there are several P forms present in the environment. Phosphate is the main phosphorus (P) source used for plant nutrition, while Phi have been used as fungicide. Aim of this study was to investigate action of Phi anion on tissue P concentration and total P accumulation in common bean (*Phaseolus vulgaris* L.) plants grown in nutrient solution under limiting and non-limiting Pi conditions.

MATERIALS AND METHODS

Study was implemented in Departamento de Ciência do Solo of the Universidade Federal de Lavras, Brazil. Plants remained in glasshouse environment under natural conditions day/night. For plant material was used common bean (*Phaseolus vulgaris* L.) crop. Plants were grown in plastic pots containing 3 L of modified Hoagland's solution with the phosphite (Phi) and phosphate (Pi) treatments. Phosphite used in the experiment was obtained by the reaction of phosphorous acid with potassium hydroxide, resulting in potassium Phi.

Experiment was organized in a completely randomised experimental design with 3 replicates, being 7 Phi levels (0, 16, 32, 64, 128, 256 and 512 μM) and 2 Pi levels (80 and 800 μM, these levels considered Pi-starved plants and Pi-sufficient plants, respectively) in nutrient solution. Each experimental unit consisted of one common bean plant per pot.

Plants were harvested at full flowering stage, the stage in which common bean exhibits high metabolic activity. Shoot and root dry mass were ground and analyzed for tissue P concentration colorimetrically (Murphy and Riley, 1962) after nitric-perchloric digestion of the plant material. Results from shoot and root dry weight and tissue P concentration were used to calculate the total P accumulation. Data were submitted to variance analysis ($p \leq 0.05$) using the SAS software (SAS Institute, 1996). Standard errors were calculated for all means.

RESULTS AND DISCUSSION

Tissue P concentration and total P accumulation in shoot and root of Pi-sufficient plants were not significantly ($p > 0.05$) affected by Phi treatments applied in nutrient solution (Fig. 1 A, B, C and D). Nevertheless, in Pi-starved plants, shoot and root exhibited a progressive increase in tissue P concentration from 32 and 128 μM Phi, respectively. At the highest Phi level (512 μM Phi) there was a substantial increase, corresponding to 7.2-fold in shoot and 11.7-fold in root in tissue P concentration of these Pi-starved plants (Fig. 1 A and C), compared with the control. Total P accumulation in shoot and root (Fig. 1 B and D) of Pi-starved plants were also increased from 128 and 256 μM Phi, respectively; but the differences were of smaller magnitude than those found for the tissue P concentrations. At the highest Phi level, the values of total P accumulation

in shoot and root of these plants were 4.5- and 8.7-fold higher, respectively, compared with the control.

At the control treatments (without application of Phi in nutrient solution), Pi-sufficient plants exhibited much higher tissue P concentration and total P accumulation than Pi-starved plants. However, in the treatment with 512 µM Phi, the tissue P concentration in shoot was higher for Pi-starved plants, and tissue P concentration in root did not differ between Pi-sufficient and Pi starved plants. These effects were mainly due to large increase of tissue P concentration in Pi-starved plants.

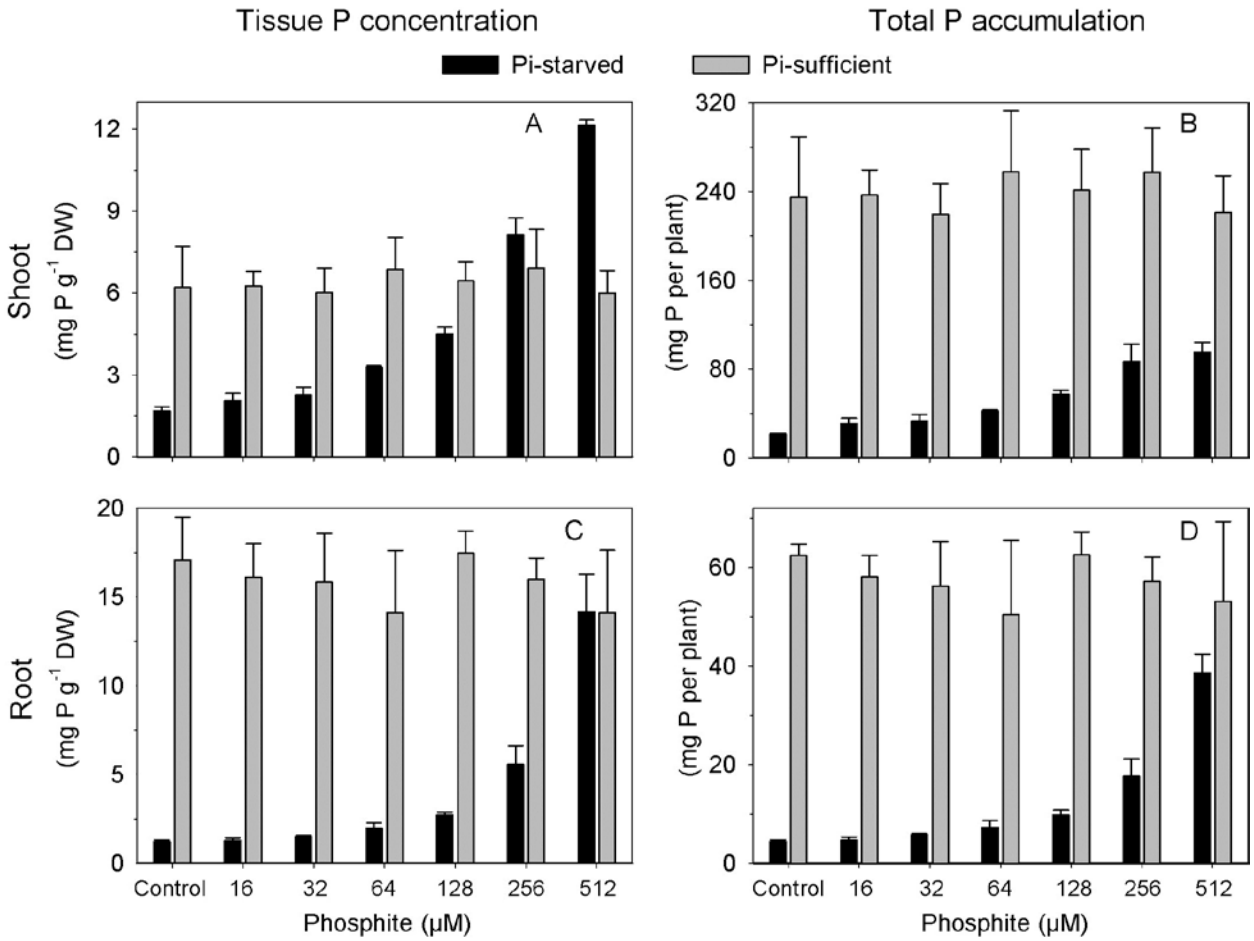

Fig. 1: Shoot P concentration (A), shoot P accumulation (B), root P concentration (C), and root P accumulation (D) at flowering stage of *Phaseolus vulgaris* plants grown in nutrient solution under 2 phosphate levels (Pi-starved and Pi-sufficient plants) and 6 phosphite (Phi) levels + control (without Phi supply). Values represent the mean value of 3 replicates ± SD (Standard deviation).

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SHOOT AND ROOT PRODUCTION IN COMMON BEAN GROWN IN NUTRITION SOLUTION UNDER DIFFERENT PHOSPHATE AND PHOSPHITE LEVELS

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INTRODUCTION

Aim of this study was to investigate action of Phi anion used as P source on shoot and root dry weight and shoot to root ratio in common bean (*Phaseolus vulgaris* L.) plants grown in nutrient solution under different Pi and Phi levels.

MATERIALS AND METHODS

Study was implemented in Departamento de Ciência do Solo of the Universidade Federal de Lavras, Brazil. Plants remained in glasshouse environment under natural conditions day/night. For plant material was used common bean (*Phaseolus vulgaris* L.) crop. Plants were grown in plastic pots containing 3 L of modified Hoagland's solution.

Experiment was organized in a completely randomised experimental design with 3 replicates, being 7 Phi levels (0, 16, 32, 64, 128, 256 and 512 μM) and 2 Pi levels (80 and 800 μM, these levels considered Pi-starved plants and Pi-sufficient plants, respectively) in nutrient solution. Common bean plants were evaluated at 2 different growth stages: flowering and mature grain stages. For plants harvested at the mature grain stage, two more treatments (additional treatments) were added: –P = no P supply in nutrient solution; and +Phi = all the P (800 μM) from nutrient solution was supplied only as Phi. Each experimental unit consisted of one common bean plant per pot. Phosphite used in the experiment was obtained by the reaction of phosphorous acid with potassium hydroxide, resulting in potassium Phi. Shoot and root dry weight and shoot to root ratio of these plants were measured. Data were submitted to variance analysis ($p \leq 0.05$). Standard errors were calculated for all means.

RESULTS AND DISCUSSION

Shoot and root weights of Pi-sufficient Phaseolus vulgaris plants were not affected by Phi levels in nutrient solution (Fig. 1 A, B, C, and D). However, high Phi levels (256 and 512 μM Phi) decreased significantly shoot and root dry weight in Pi-starved plants.

Pi-sufficient common bean plants exhibited much higher shoot dry weight than Pi-starved common bean plants, and there was no significant variation of shoot dry weight between the two growth stages (Fig. 1 A and B). Root dry weight was increased in Pi-sufficient common bean plants at mature grain stage (Fig. 1 D), but interestingly root dry weight did not vary between Pi-starved and Pi-sufficient plants at flowering stage, with the exception of plants grown under 512 μM Phi.

In additional treatments, which were applied only in plants evaluated at mature grain stage, no P supply and P supply using only Phi (800 μM Phi) decreased the shoot and root dry weight by around 93 and 81% (Fig. 1 B and D), respectively, compared with plants grown under 800 μM Pi (Pi-sufficient plants).

Root to shoot ratios (Fig. 1 E and F) of common bean plants were not affected significantly by Phi supply in nutrient solution. Root to shoot ratio was 3-fold higher in Pi-starved plants than Pi-sufficient plants at flowering stage, but this ratio did not differ significantly when the plants were evaluated at mature grain stage. Interesting, Pi-starved plants and Pi-sufficient plants exhibited higher and lower values of root to shoot ratio, respectively, at flowering and mature grain stages. No P supply and P supply only as Phi in nutrient solution (additional treatments) increased root to shoot ratio by around 3.3- and 2.2-fold respectively, compared with Pi-sufficient plants grown under control treatment (Fig. 1 F).

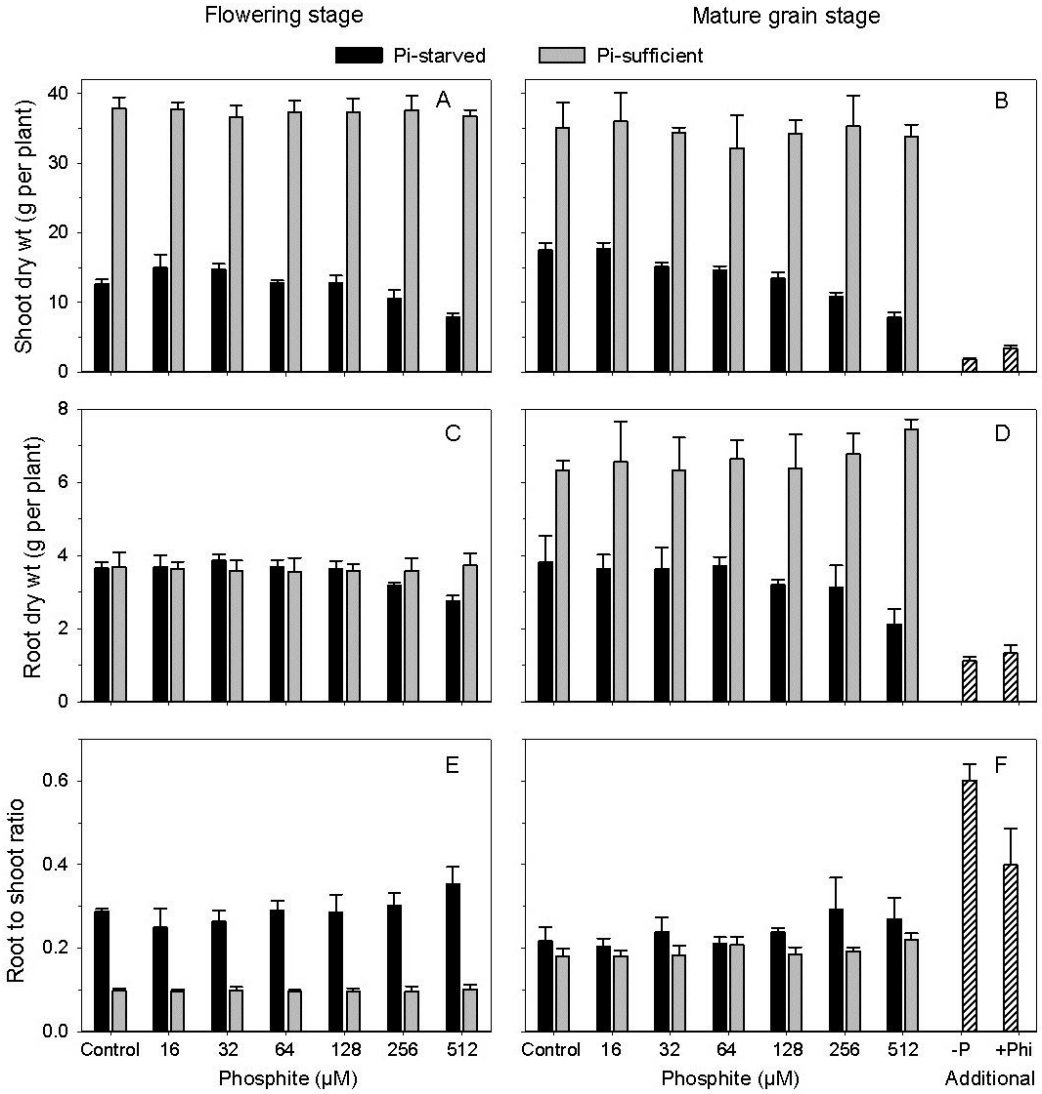

Fig. 1: Shoot dry weight (A and B), root dry weight (C and D), and root to shoot ratio (E and F) at 2 different growth stages (flowering and mature grain stages) of *Phaseolus vulgaris* plants grown in nutrient solution under 2 phosphate levels (Pi-starved and Pi-sufficient plants) and 6 phosphite (Phi) levels + control (without Phi supply). For plants harvested at mature grain stage, additional treatments are: –P = no P supply in nutrient solution; and +Phi = all the P (800 µM) from nutrient solution was supplied only as Phi. Values represent the mean value of 3 replicates ± SD (Standard deviation).

NUTRIENTS IN SHOOT OF COMMON BEAN UNDER DIFFERENT PHOSPHORUS FERTILIZATIONS

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INTRODUCTION

Objective of this study was to measure concentrations of N, K, Ca, Mg, S and micronutrients in common bean (*Phaseolus vulgaris* cv. Radiante) shoot under different phosphorus fertilization.

MATERIALS AND METHODS

Study was implemented in Departamento de Ciência do Solo of the Universidade Federal de Lavras, Brazil. Plants remained in glasshouse environment under natural conditions day/night. Substrate used was composed by Oxisol placed in plastic pots with capacity of 6 L. For plant material was used common bean (*Phaseolus vulgaris* cv. Radiante) plants.

Experiment was organized in factorial scheme completely randomized using 2 soil phosphate levels (Pi-starved and Pi-sufficient), combined with 3 nutrient sources supplied via foliar application (KH_2PO_3, KH_2PO_4, and KCl used as control), and 2 foliar application numbers (single and two applications). This study had 3 replicates, and each experimental unit consisted of one pot containing two plants, and all variables measured were expressed as mean of two plants.

Concentrations of N, K, Ca, Mg, S and micronutrients in common bean shoot were determined after nitric-perchloric digestion (Johnson and Ulrich, 1959) as follows: S by turbidimetry; K by flame photometry; Ca, Mg, Cu, Mn, Fe, and Zn by flame atomic absorption spectroscopy. Total N was determined using the Kjeldahl method after sulphuric digestion, and B by colorimetry using the Azomethine-H method after dry digestion, with ash content obtained in muffle furnace by 1 h at 550 ºC. Results were submitted to variance analysis and applied to Tukey test at 5% level, as well as the standard errors were calculated in all evaluated points.

RESULTS AND DISCUSSION

Concentrations of N, K, Ca, Mg, S and micronutrients in plant shoot tissues were not significant affected ($p > 0.05$) by foliar application numbers (Table 2). Apart from the K, common bean grown under limiting phosphate availability exhibited higher concentrations of nutrients in shoot, regardless of the foliar-applied treatments.

Foliar application of potassium phosphite increased concentrations of N, K, Mg, B, Cu, Mn and Fe in shoot tissues of phosphate-starved plants. However, this increased concentration of nutrients coincides with the decreased shoot dry mass production (data not shown), which may suggest that it is involved with the "concentration effect". When biomass production decreases, the "concentration effect" for some nutrients may occur, which is the elevation of their concentration in the tissues without having there being an alteration in the quantity of nutrient

taken up (Marschner, 1995). Indeed, the applied treatments did not increase accumulation of nutrients in shoot (data not shown), supporting the suggestion above.

Table 2. Concentrations of nutrients in common bean shoot grown in Oxisol under 2 phosphate levels (Pi-starved and Pi-sufficient), 3 nutrient sources supplied via foliar application (KH_2PO_3, KH_2PO_4, and KCl), and 2 foliar application numbers (single and two applications).

Application numbers	Soil P status	Foliar treatments	Macronutrients					Micronutrients				
			N	K	Ca	Mg	S	B	Zn	Cu	Mn	Fe
			g kg^{-1}					mg kg^{-1}				
A single foliar application timing	Pi-starved	KCl	47 bA	19 bA	13 aA	6 bA	2 aA	32 abA	57 aA	6 bA	61 bA	347 bA
		Phi	57 aA	22 aA	15 aA	9 aA	2 aA	37 aA	61 aA	8 aA	77 aA	511 aA
		Pi	46 bA	18 bA	14 aA	6 bA	2 aA	30 bA	58 aA	5 bA	58 bA	381 bA
	Pi-sufficient	KCl	33 aB	17 aA	9 aB	4 aB	1 aB	17 aB	30 aB	4 aB	39 aB	131 aB
		Phi	33 aB	17 aB	9 aB	4 aB	1 aB	17 aB	29 aB	3 aB	42 aB	183 aB
		Pi	31 aB	18 aA	8 aB	3 aB	1 aB	16 aB	35 aB	4 aB	37 aB	167 aB
Two foliar application timings	Pi-starved	KCl	46 bA	20 bA	14 aA	6 bA	2 aA	31 abA	52 aA	5 bA	55 bA	334 bA
		Phi	55 aA	23 aA	16 aA	8 aA	2 aA	36 aA	53 aA	7 aA	73 aA	559 aA
		Pi	45 bA	19 bA	13 aA	6 bA	2 aA	29 bA	51 aA	5 bA	62 abA	310 bA
	Pi-sufficient	KCl	29 aB	18 aA	9 aB	3 aB	1 aB	17 aB	32 aB	4 aB	40 aB	186 aB
		Phi	34 aB	19 aB	8 aB	4 aB	1 aB	16 aB	34 aB	3 aB	38 aB	176 aB
		Pi	32 aB	19 aA	7 aB	4 aB	1 aB	16 aB	36 aB	3 aB	41 aB	148 aB
Source of variation												
Foliar application numbers (A)			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Soil P status (B)			***	***	***	***	***	***	***	***	***	***
Foliar treatments (C)			***	*	ns	***	ns	**	ns	***	*	***
A × B			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
A × C			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
B × C			*	*	ns	**	ns	*	ns	***	*	***
A × B × C			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

In each number of foliar application (a single application timing and two application timings), lower case compare the foliar application products (KCl, Phi e Pi) for each soil phosphate level (Pi-starved and Pi-sufficient), and upper case compare the soil phosphate levels for each foliar application product. Means followed by same letter are not different by Tukey's test ($p \leq 0.05$). *, **, ***, and ns corresponding to $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, and non-significant, respectively, by F test.

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EFFECT OF PHOSPHITE ON GRAIN YIELD OF COMMON BEAN PLANTS GROWN IN NUTRITION SOLUTION UNDER PHOSPHATE STARVATION

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INTRODUCTION

Phosphate (Pi) anion is the major form of phosphorus (P) utilised by plants for their adequate growth and development, while phosphite (Phi) anion is effective in controlling some important plant diseases, such as *Phytophthora* sp. Nonetheless, recently Phi-based products have also been marketed in the world as fertilizers for foliar spray, fertigation and direct soil application.

Aim of this study was to investigate action of Phi anion used as P source on grain yield in common bean (*Phaseolus vulgaris* L.) plants grown in nutrient solution under Pi starvation.

MATERIALS AND METHODS

Study was implemented in Departamento de Ciência do Solo of the Universidade Federal de Lavras, Brazil. Plants remained in glasshouse environment under natural conditions day/night. For plant material was used common bean (*Phaseolus vulgaris* L.) crop. Plants were grown in plastic pots containing 3 L of modified Hoagland's solution with the phosphite (Phi) and phosphate (Pi) treatments. Phosphite used in the experiment was obtained by the reaction of phosphorous acid with potassium hydroxide, resulting in potassium Phi.

Experiment was organized in a completely randomised experimental design with 3 replicates, being 7 Phi levels (0, 16, 32, 64, 128, 256 and 512 μM) and 2 Pi levels (80 and 800 μM, these levels considered Pi-starved plants and Pi-sufficient plants, respectively) in nutrient solution. Two more treatments (additional treatments) were added: –P = no P supply in nutrient solution; and +Phi = all the P (800 μM) from nutrient solution was supplied only as Phi. Each experimental unit consisted of one common bean plant per pot.

Plants were harvested at mature grain stage and grain dry weight of these plants was determined. Data were submitted to variance analysis ($p \leq 0.05$) using the SAS software (SAS Institute, 1996). Standard errors were calculated for all means.

RESULTS AND DISCUSSION

Grain dry weight of Pi-sufficient common bean plants did not vary significantly with any of the levels of Phi in nutrient solution (Fig. 1). However Pi-starved common bean plants exhibited decreased grain dry weight when grown under 32 μM Phi, and these plants did not produce grains when grown from 64 μM Phi. Treatment under 16 μM Phi did not influence the grain yield of the Pi-starved plants. Common bean plants grown under both additional treatments (-P and +Phi) also did not produce grains. In general, grain yield in Pi-sufficient plants was 4-fold higher than Pi-starved plants grown under control treatment (zero μM Phi).

Toxicity symptoms of Phi on grain yield of Pi-starved common bean were also supported by the visual appearance of these plants. Development of pods in Pi-sufficient plants was not altered by Phi treatments. On the other hand, Phi-starved common bean exhibited much more pods per plant in treatments with 64, 128, 256 and 512 μM Phi, but these pods were small and

malformed that resulted in no-filled grains (Fig. 2). This harmful effect of Phi on development of pods in Pi-starved plants was increased with increasing the levels of Phi in nutrient solution.

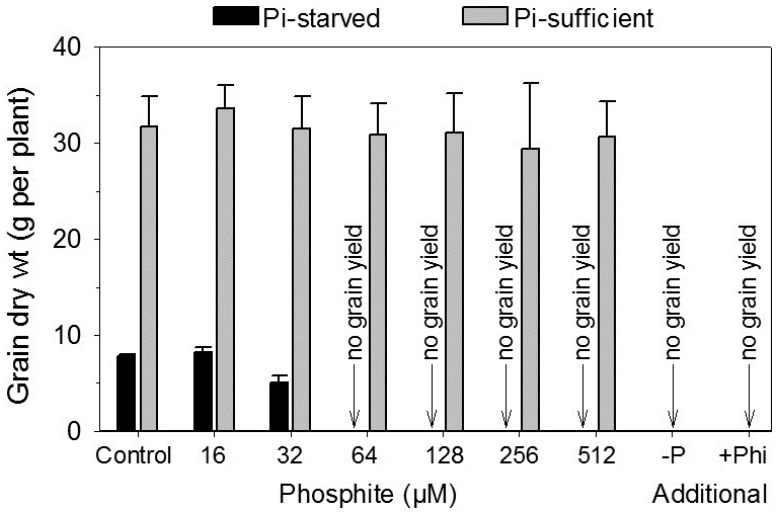

Fig. 1: Grain dry weight at mature grain stage of *Phaseolus vulgaris* plants grown in nutrient solution under 2 phosphate levels (Pi-starved and Pi-sufficient plants) and 6 phosphite (Phi) levels + control (without Phi supply). Additional treatments are: –P = no P supply in nutrient solution; and +Phi = all the P (800 µM) from nutrient solution was supplied only as Phi. Values represent the mean value of 3 replicates ± SD (Standard deviation).

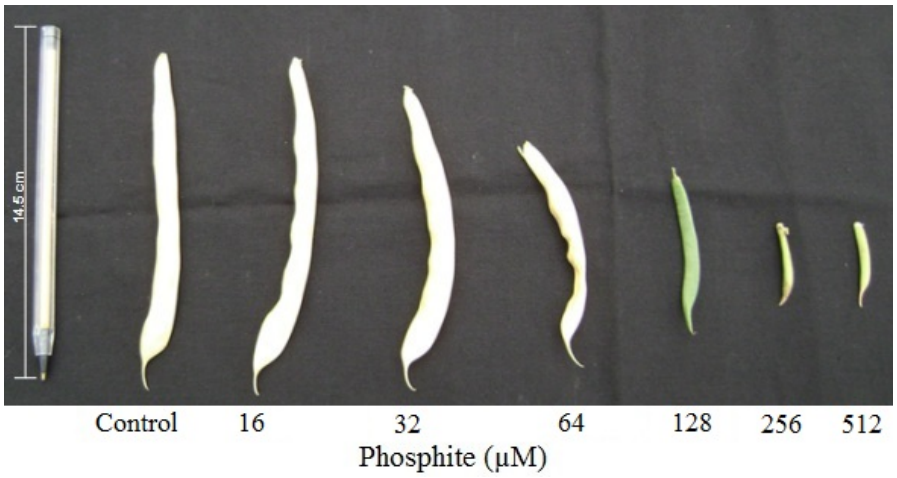

Fig. 2: Toxicity symptoms on grain yield at *Phaseolus vulgaris* plants grown in nutrient solution under low phosphate level (Pi-starved plants), as affected by 6 phosphite (Phi) levels + control (without Phi supply).

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RELATIONSHIPS AND PREDICTIONS AMONG EMERGENCE-TIME, SEED WEIGHT, AND EARLY VEGETATIVE GROWTH

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a good nutritive-value food and one of the most economically important cultivated legumes worldwide. During the last two decades, common bean has become a significant cash crop in North Dakota, which is the US leading producer. Obtaining good yield depends on seed quality and a uniform emergence in a short time is a good indicator of the future plant growth. Early monitoring of vegetative growth can help in identifying stand quality and diseases in order to ensure high seed yields. The relationship of the bean life cycle and seed yield can be used to develop an economic growth rate. Crop models help enhance productivity and agricultural research. Previous dry weight simulations in common bean (Adikua et al. 2001) suggested good predictions under water stress conditions. Knowledge of the phenological stages influencing common bean growth and development is crucial to obtain reliable yield estimates and help optimizing management and production practices. This study aims to establish a) the relationship between seed weight and emergence time, and b) to predict the growing rates and phenologic stages of 164 common bean recombinant inbred lines (RILs) and the parents ('Negro Jamapa' and 'ICA-Calima') at five locations. The results from the field trial at North Dakota are reported.

MATERIALS AND METHODS

In summer 2012, the experiment was conducted at the Prosper Research Site located ~ 30 km NW of Fargo, ND. Rainfall for the period was 157.0 mm and the average temperature was 20.0 ^{o}C during the growing season. Hundred seeds of each RIL were weighted (g) before planting and emergence time were recorded when fifty percent of the plot were germinated. Phenological data were collected twice a week from six flagged plants over 164 plots replicated three times in a resolvable row-column design. Dry weight of the main stem (DWMS), hypocotyl (DWH), and the primary leaves (DWU) were used to predict bean growth at vegetative stages. Growth rates were determined by using the Blackman (1919) equation: $W=W_0\ e^{r(t-t0)}$, were W = biomass (g); W_0 = initial biomass at t_0 (g); r = growth rate (g day^{-1}); t = time (days); and e = natural logarithm (Ln). Data were analyzed (ANOVA) using the PROC MIXED procedure (SAS Institute, 2008). Predictions were evaluated by using the square root mean square error [RMSE (Willmott, 1985)]: $\frac{\quad}{\quad}$ P = predicted and O = observed.

RESULTS AND CONCLUSIONS

Seed size and yield of common bean are negatively associated (White et al., 1992). Bigger seed size cultivars have better quality and germination. Large-seed size cultivars have greater stored food reserves to support early seedling and development. The results obtained showed a

significant relationship between seed weight and germination time. However, only 21% of the weight variable can be explained by the germination time model. Each gram of seed increase delayed the emergence for less than one day. As a result, the parents Calima and Jamapa are significantly different in germination time (15 and 13 days) when weighing 46.5 and 25.5 g for hundred seeds, respectively. The germination can be influenced by seed size, soil-weather conditions, and also the optimum planting depth. Even though the temperature at Prosper was adequate after planting, delay of rainfall caused the soil to dry out quick and develop a crusty surface, making emergence more difficult in this particular trial.

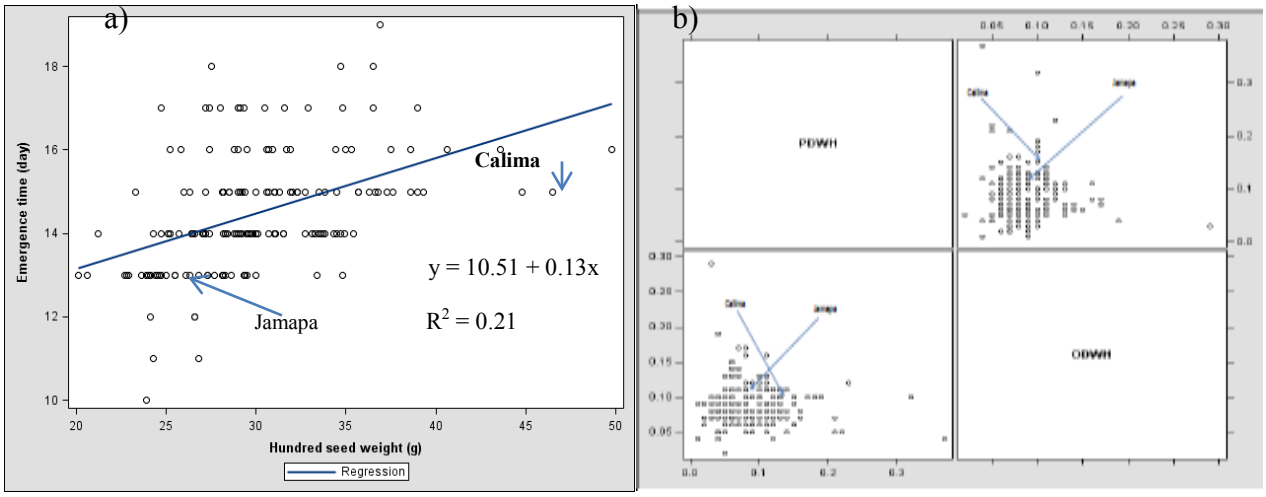

Figure 1.a) Relationship between seed weight and emergence-time; b) Predicted (PDWH) and observed (ODWH) dry weight of hypocotyl.

RMSE values close to 0 indicate good predictions. DWH was predicted better with RMSE (0.06) than DWU (0.11) and DWMS (0.24). Early vigor of the selected traits was predicted more or less accurately. DWH of Jamapa was predicted better than Calima**.** The evaluation indicates good predictions for common bean early vegetative growth. DWH can be used as direct selection criteria for early vigor following by DWU and DWMS. These preliminary results will be useful in the near future and the data set can be adjusted and matched with QTL for developing a gene-based crop model including all locations. Results across other locations (Florida, Puerto Rico and two locations in Colombia) are currently being analyzed and will allow for accurate estimates of genotype by environment interaction.

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DOUBLE ROW ARRANGEMENT ENHANCES PINTO BEAN PRODUCTION FOR UPRIGHT CULTIVARS

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INTRODUCTION

A three-year study was conducted at the Colorado State University (CSU) research farm north of Fort Collins, CO. The study compared the performance of three pinto bean cultivars with varying growth habit grown under furrow irrigation to determine if double row arrangement on seed beds increased seed yield or altered seed size compared to single row arrangement on seed beds. Upright type II cultivars Croissant (CSU release) and Stampede (North Dakota State University release) were compared to the prostrate type III cultivar Montrose (CSU release).

A 4-bed mechanical planter was used to plant 1 or 2 rows per bed with rows planted 15 cm apart on the bed), and beds spaced 75-cm apart at 207,500 seed/hectare in 2010, 2011, and 2012. Three or four replicates were used each year and experimental units consisted of 4-beds, 8 meters in length. Standard grower practices were applied for fertilizer, irrigation, weed, disease and insect management. Data included plant emergence, node height, biomass, yield, and seed size. All data were analyzed statistically with PC SAS combined over years.

RESULTS AND DISCUSSION

Growing conditions were favorable for plant development with trace infections of common bacterial blight [caused by *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Dye, Syn. with *X. campestris*] and insect pests each year. Adequate furrow irrigation water and fertilizer supported optimum plant development and pod set. Significant interactions were noted among factors year, entry, and rows per bed.

Yield varied from 2900 to 3800 kg/hectare and 200-seed weight varied from 62 to 83 grams, depending on entry and row arrangement during 2010 to 2012. Upright growth habit cultivars Croissant and Stampede had 5% higher yield under double row arrangement compared to single row arrangement, while the prostrate cultivar Montrose showed no response to row arrangement (Tables 1 and 2).

Mean seed weight among cultivars did not differ between row arrangements (Table 2). These results suggest that pinto bean growers should be able to increase yield and maintain desirable seed size using double row arrangement over traditional single row arrangement on the planting bed. Growers should carefully choose cultivars with appropriate agronomic and disease resistance characteristics to enhance yield in a traditional production system with an integrated pest management strategy.

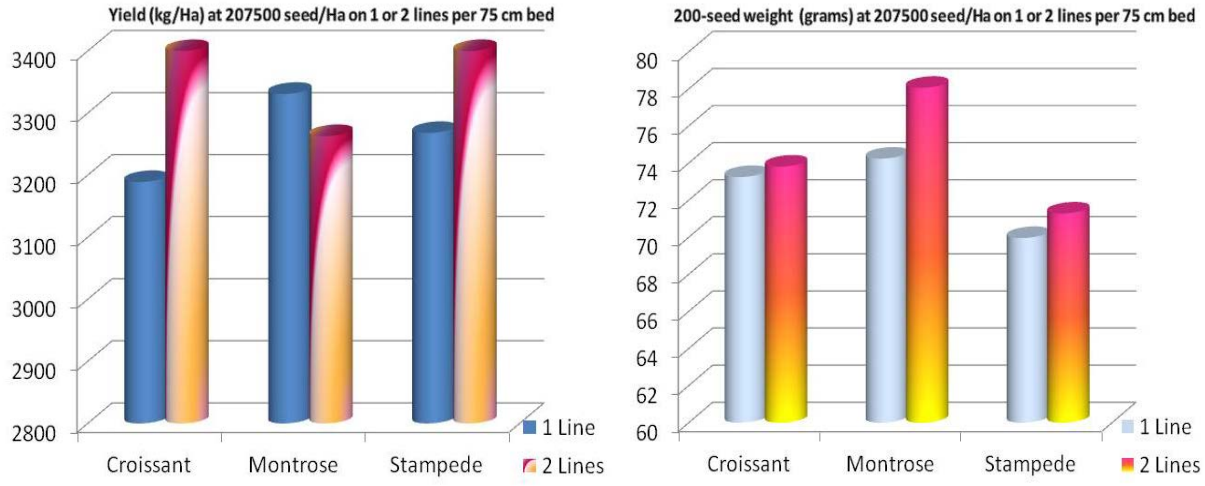
Table 1. Summary of yield for three cultivars evaluated on 75-cm wide beds, grown with 1 or 2 rows per bed during 2010 to 2012 at Fort Collins, Colorado.

		Year			
		2010	2011	2012	Average
Entry	**Rows/bed**	----------Yield (kg/hectare)----------			
Croissant	1	2918	3460	-	3189
	2	3187	3813	3239	3413
Stampede	1	3121	3414	-	3268
	2	3220	3570	3413	3401
Montrose	1	3118	3543	-	3331
	2	2765	3386	3638	3263

Table 2. Summary of seed weight for three cultivars evaluated on 75-cm wide beds, 1 and/or 2 rows per bed during 2010 to 2012 at Fort Collins, Colorado.

		Year			
		2010	2011	2012	Average
Entry	**Rows/bed**	Seed Weight (grams/200 seed)			
Croissant	1	70.29	76.20	-	73.25
	2	68.77	76.00	76.66	73.81
Stampede	1	67.32	72.60	-	69.96
	2	62.38	72.00	79.46	71.28
Montrose	1	71.47	77.00	-	74.24
	2	70.45	80.00	83.73	78.06

Figure 1. Yield response when three pinto cultivars were planted on 1 and 2 rows per bed under furrow irrigation during 2010 to 2012.



ACKNOWLEDGEMENTS: The authors gratefully acknowledge funding for this research provided by Colorado State University, Colorado Department of Agriculture, Colorado Dry Bean Administrative Committee, and edible bean producers and handlers in Colorado

EFFICIENCY OF PHENOTYPIC RECURRENT SELECTION FOR PLANT ARCHITECTURE IN COMMON BEAN

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INTRODUCTION

The Federal University of Lavras (Universidade Federal de Lavras - UFLA) has been conducting a recurrent selection program for the purpose of obtaining upright common bean lines. As of cycle III, recurrent selection came to be phenotypic – mass selection – performed visually by a grading scale. The purpose of this study is to assess the efficiency of recurrent selection using $S_{0:3}$ and $S_{0:4}$ progeny assessment data from cycles CV and CVIII.

MATERIALS AND METHODS

The details of obtaining the population and conducting it up to the third recurrent selection cycle were presented by Cunha et al, 2005. After the recombination of the third cycle, the S_0 (F_2) population was sown and, at the beginning of flowering, the most upright plants, identified visually, were crossed. The F_1 seeds were then multiplied and, in the F_2 generation, the process was repeated. After the fifth cycle (CV) and eighth cycle (CVIII) of recurrent selection, progenies were obtained and assessed up to almost complete homozygosity, being that progenies $S_{0:3}$ and $S_{0:4}$ of CV were evaluated in winter and in the rainy season of 2009, respectively; whereas the progenies $S_{0:3}$ of CVIII were evaluated in the rainy season of 2011, and the progenies $S_{0:4}$ in the dry season of 2012. All experiments were conducted using three replicates, and the plot was formed by two lines of two meters. Using mean data of 47 progenies of $S_{0:3}$ and $S_{0:4}$ from CV and CVIII which were assessed together with two common controls in two locations, genetic progress was estimated. Experimental details of assessment of progenies are shown in Table 1.

Data of seed yield ($kg.ha^{-1}$) and assessment of growth habit were obtained, the latter using a one to nine grading scale in which one indicates prostrate plants and nine upright plants. Since the same controls were used in both cycles, combined analysis of variance was undertaken (PIMENTEL GOMES, 2009). This analysis allowed obtaining mean values corrected according to common controls. Based on the corrected mean values, genetic progress (GP) per cycle was estimated, using the following estimator:

GP =

Table 1: Estimates of genetic progress per cycle, obtained from recurrent selection for upright plant architecture and high seed yield:

	Plant architeture (Scores 1 to 9)	Seed yield ($kg.ha^{-1}$)
Mean value of the progenies CV (MCV)	5.95	1991
Mean value of the progenies CVIII (MCVIII)	6.24	2399
Overall mean value	6.10	2195
Total Progress [	4.86	20.43
Progress per cycle (%) [	1.62	6.81

The population that is being subjected to recurrent selection still has enough genetic variability for the continuity of genetic progress from selection for plant architecture and grain yield, as may be verified by means of the heritability estimates obtained in CVIII (Table 2).

Table 2: Estimates of the h² of joint analysis of CVIII:

Trait	$S_{0:3}$ Progenies		$S_{0:4}$ Progenies	
	h²	IC	h²	IC
Yield	0.74	(0.63 – 0.81)[1]	0.66	(0.44 – 0.78)
Plant Architeture	0.37	(0.12 – 0.54)	0.61	(0.35 – 0.75)

[1]Confidence interval of h^2, using the expression from Knapp et al. (1985) at the level of ($\alpha \leq 0.05$).

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POPULATION DENSITIES FOR ALTERNATIVE CULTIVARS OF BEAN PLANT IN SOUTHERN AND CENTER-WESTERN MINAS GERAIS

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The most consumed bean in Brazil is the carioca. However, there has been interest of the consumer for other sorts of grains, the growing of which will be able to represent new income option to the farmers. For this purpose, it is necessary to adapt the current systems of crop production inclusive the recommendations about population density. So, the aim of this work was studying the performance of the alternative bean plant cultivars in the South and Center-West regions of Minas Gerais by means of better plant populations for its cultivation.

MATERIALS AND METHODS: Two field experiments (rainy season 2006/2007) were conducted in Lavras and Bambuí (respectively, in the South and Center-West of the state of Minas Gerais, Brazil). The statistical design was of randomized blocks with three replications and factorial scheme 2x4x5, involving the two places; cultivars Radiante, Ouro Vermelho, Bolinha and Novo Jalo and five population densities (100, 200, 300, 400 and 500 thousand plants ha^{-1}). Sowing was by hand at the spacing of 0.5 m inter-rows and densities enough to obtain the wished populations. At sowing, 400 kg ha^{-1} of NPK fertilizer 8-28-16 were applied and at top-dressing, 30 kg ha^{-1} of N, source urea according to the Technological Level NT3 (RIBEIRO et al., 1999). At harvest, the final stand (EF) and grain yield (REND) with it primary components: number of pods per plant-VP, number of grains per pod-GV and weight of one hundred grains -P100 were evaluated. The data were submitted to the individual and joint variance analysis for each site, the effects of cultivars being evaluated by means of the means clustering by the Scott-Knott test and the effects of the population densities, when significant by the F test, studied by means of the regression analysis.

RESULTS AND DISCUSSION: There was a significant effect of the factor cultivar (C) on all the characteristics, of population (P) on the VP and P100 and of site (L) on the GV and P100. The triple interaction CxPxL and the double interactions CxP and PxL were not significant in any situation, while the interaction CxL was always significant.

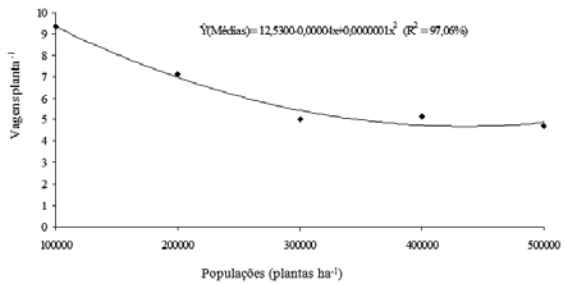

Figura 1. Number of pods per bean plant in relation to the plant populations

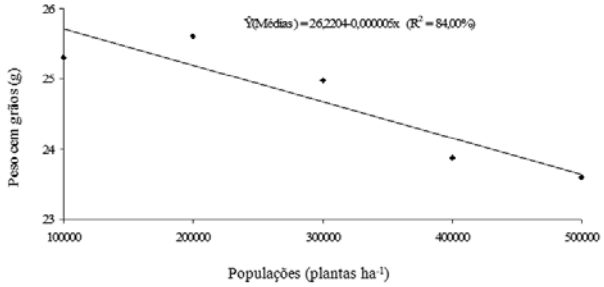

Figura 2. Average weight of one thousand grains of the bean plant in elation to the plant populations.

The cv. Radiante presented the greatest VP in the two sites and in Lavras it did not differ from the cv. Ouro Vermelho. But in Bambuí, this latter produced the lowest VP, which can mean that the most limiting environment furthered the shortest cycle cultivars, which would have defined the number of pods earlier. The VP decreased further with the increase of the population (Figure 1) and that reduction possibly occurred by the inclusion of cultivars with type III growth habit, with poorer adaptation capacity in larger populations (LOLLATO,

1997). As to the GV, there was prominence of the cv. Ouro Vermelho in the two localities, but in Bambuí, it did not differ from the cv. Novo Jalo (Table 1).

Table 1. Average values of final stand (EF), number of pods per plant (VP) and of grains per pod (GV), average weight of one hundred grains (P100) and grain yield (REND) of the bean plant in relation to cultivars.

Treatment	VP	GV	P100	REND
	-------(nº)-------		(g)	(kg ha^{-1})
		Lavras		
Radiante	7.0a	2.6c	29.13b	639b
Novo Jalo	5.0b	3.0b	30.76a	694b
Bolinha	5.0b	3.1b	27.96b	548b
O. Vermelho	7.0a	4.7a	19.92c	914a
		Bambuí		
Radiante	9.0a	2.8b	30.17a	991a
Novo Jalo	6.0b	3.4a	25.23b	804b
Bolinha	7.0b	2.6b	21.42c	591c
O. Vermelho	4.0c	3.7a	12.69d	248d
		Locais		
Lavras	6.0a	3.4a	26.9a	699a
Bambuí	6.0a	3.1b	22.4b	659a
Means	6.2	3.2	24.7	678

Within each factor, means followed by different letters in the columns belonging to distinct groups by the Scott-Knott test.

The cultivars Novo Jalo (Lavras) and Radiante (Bambuí) presented the heaviest grains. In both the localities, the lowest P100 was obtained by the cv. Ouro Vermelho. In general, in both the localities, the P100 lay below those reported for the two cultivars, which allows us to deduce which unfavorable factors acted in this crop, likely shortening the grain-filling period. The cv. Radiante, of shortest cycle and type 1 habit, was the one presented greatest reduction in the grain weight. That variable proved further affected by the plant populations with a decrease in the P100 with increasing plant population (Figure 2).

The REND little ranged in the two places and lay below the expected for the NT3 (RIBEIRO et al., 1999). For concerning trtar-se sowing in the rainy season, the cliamte conditons exercised strong influence, pressing the yield downwards. In Bambuí, the cv. Radiante, of greatest VP, was the highest yielder and in Lavras outyielded the cv. Ouro Vermelho.

Although, there has been reduction of the VP with increasing of the population (Figure 2), that was not enough to reduce gain yield. Those results show that under the conditions of the study, there was no advantage in increasing the population density of the bean plant, mainly because that would represent additional cost of seeds. They pointed out**,** therefore, the use of populations close to the ones recommended, of the order of 240 thousand plants ha^{-1}, since smaller populations could mean insufficient close of the, with serious consequences for the weed management.

CONCLUSIONS: 1) The cultivars Radiante, Novo Jalo, Bolinha and Ouro Vermelho did not present good performance under the limiting conditions of rainy season crop. 2) The increase of the plant population in the interval studied reduces the number of pods per plant and the weight of one hundred grains of the bean plant, but grain yield is not influenced.

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EFFECT OF SOWING SYSTEM ON GRAIN YIELD OF TWO DRY BEAN VARIETIES IN AGUASCALIENTES

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INTRODUCTION: Dry bean producers in Aguascalientes State usually sow the bean crop in 0.76 m wide furrows with a single plant row. However, using this sowing method does not allow taking advantage of the new bean varieties having a compact growth habit, but more efficient in producing grain. On the other hand, compact, reduced growth and low leaf area index genotypes do not to cover the entire area in such wide rows, wasting solar energy and losing water by evaporation of soil water. This situation can be improved by reducing the width between sowing rows and make more efficient use of the land allowing a great canopy cover capturing more solar energy and also covering ground quickly. The objective of the present work was to evaluate different sowing systems on the grain yield of two dry bean varieties of contrasting morphological development.

MATERIAL AND METHODS: Sowing systems evaluated were: a) furrows at 0.76 m in single-row and a plant density of 90 thousand pl ha^{-1}, b) beds of 1.52 m with three rows and a density of 145 thousand pl ha^{-1}, c) beds of 1.52 m with four rows and a density of 188 thousand pl ha^{-1} and d) beds to 1.52 m with six rows with a density of 260 thousand pl ha^{-1}. Sowing systems having more than one row, the separation between plant rows was 0.4, 0.3 and 0.2 m, respectively. The dry bean varieties evaluated were: Pinto Saltillo and Flor de Mayo Eugenia. The experimental unit consisted of 4, 6, 8 and 12 rows of 60 m length for each variety. Grain yield was recorded on plots of 2 m wide and 5 m in length. Data was analyzed based on an experimental split plots design with four replications, where varieties were the main plots and sowing methods the subplots.

RESULTS AND DISCUSION: During growth season 209 mm of rain were recorded where the most of the rainfall occurred before the grain filling. Pinto Saltillo "PS" variety lasted 40 days to flowering and 85 days to physiological maturity, while Flor de Mayo Eugenia "FME" flowering was 45 days after sowing and reached physiological maturity 95 days after sowing. It is important to point out that both materials did not present rust problems or other pathogens. Table 1 shows the performance of the bean genotypes evaluated, finding that FME had greater grain yield as compared to PS and this difference represents an increase of 40% in FME over PS. On the other hand regarding to sowing systems, 1.52 m beds with six-rows showed the highest yield surpassing all other treatments evaluated. This results are in agreement with those reported by Alves et al., (2008) mentioning that greater canopy cover and capturing more solar energy since early growth stages results in an increase in crop performance and this could be achieved by reducing the distance between furrows and increasing plant density. Increasing plant densities from 90 to 260 thousand plants ha^{-1} significantly affect some grain yield components. A reduction in the number of pods per plant and weight of 100 seeds was

observed in the treatments with four and six rows, as compared to the treatments of one and three rows. However, grain yield was not influenced by the plant density.

Table 1. Grain yield, number of pods pl^{-1}, weight of 100 seeds (g) and harvest index of two dry bean varieties under four sowing systems. CEPAB-AGS. 2012.

Treatments	GY (t ha^{-1})	NVP	PCS	IC (%)
Varieties:				
Flor de Mayo Eugenia	1.78 a	10.03	32.84	57.75a*
Pinto Saltillo	1.32b	11.56	30.67	54.38b
Media	1.55	10.80	31.76	56.07
DMS_{05}	0.3024	NS	NS	4.3841
Sowing Methods:				
0.76 Single row	1.12 c	12.63 a	33.45 a	54.25 c
1.52 m bed and triple plant rows	1.36 bc	11.10 ab	32.05 ab	56.00 ab
1.52 m bed and four plant rows	1.69 b	9.58 b	30.72 b	56.00 bc
1.52 m bed and six plant rows	2.02 a	9.88 b	30.81 b	58.00 a
Media	1.55	10.80	31.75	56.06
DMS_{05}	0.3219	1.8748	1.8651	4.3841

GY- grain yield; NVP-number of pods per plant; PCS- weight of 100 seeds and IC- harvest index; *Means followed by the same letter within columns are not statistical different. ($LSD_{0.05}$).

Highest values of harvest index were found in the 1.52 m bed with six rows, which overcome statistically to all other treatments. Flor de Mayo Eugenia showed higher grain yield than Pinto Saltillo, with FME outperforming PS at all sowing systems (table 2). The differences in yield between genotypes were explained based on the weight of seeds and harvest index values. Both dry bean varieties showed a grain yield increase when sowed in 1.52 m beds, showing an increase of 63% and 109% in FME and PS, respectively when sowed in 1.52 m bed with six rows as compared with furrows at 0.76 m and single row.

Table 2. Grain yield of two dry bean varieties cultivated under four sowing systems. Pabellon, Ags. 2012.

Sowing system	Grain yield t ha^{-1}	
	FME	PS
0.76 m single row	1.37	0.86
1.52 m bed and triple plant rows	1.66	1.07
1.52 m bed and four plant rows	1.86	1.53
1.52 m bed and six plant rows	2.23	1.80
Mean	1.78	1.32

FME-Flor de Mayo Eugenia, PS- Pinto Saltillo

CONCLUSION: Both dry bean genotypes produced higher yield when sowed at narrow row spacing and high population density. Thus, this technology could be used by farmers at the region growing dry bean under rainfall conditions.

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EFFECT OF THE ESSENTIAL OIL ON THE GERMINATION OF BEAN SEEDS (*PHASEOLUS VULGARIS* L.)

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INTRODUCTION

Allelopathy is an interference mechanism, in which the are release chemical substances from plant materials, which inhibit or stimulate the associated plant growth. Several classes of allelopathic substances, such as monoterpenes and phenols, are produced naturally by most plant species. These compounds are usually synthesized in the leaves, which fall to the ground during periods of stress [Jefferson, Pennacchio 2003].
In organic agriculture the are used this process. Plant ethereal oils the are one of the organic seed treatment methods in recent years [Kaczmarek 2006; May et al. 1990].
The aim of study was to determine the impact of different essential oils on the germination of bean seeds cultivar: ‘Blauhilde.

MATERIALS AND METHODS

Experience with common bean cv. 'Blauhilde’ was conducted in November and December 2012, in the Department of Horticulture. The experiment was conducted in two series and three replications of 25 seeds for each treatment were placed in petri dishes on moistened cotton wool with distilled water. In the middle of each petri dish were placed paper disk (10 mm diameter) soaked with appropriate dose of pure essential oil. Control object was treated with pure water only. In experiment three different oils (thyme oil, basil oil, mint oil) were used in three different doses (2.5 µl, 5.0 µl, 10.0 µl).
Energy and capacity of germination were recorded of each day for seven days, and also the effect of oils on the rate of germination was investigated. The numbers of normal, abnormal seedlings, dead and healthy ungerminated seeds were counted. Germination energy was a measure of the rapidity of germination expressed as the percentage of seeds germinating [Bewley et al. 2006].

RESULTS AND DISCUSSION

Analyzing, the results it was found that some essential oils have significant effect on seed germination. The highest percentage of germinated seeds found in the variant treated by thyme oil, especially using the dose 5.0 µl (100% seed germinated). Significantly the lowest content of germinated seeds was observed in seeds treated by 10.0 µl of mint oil (67% seed germinated).
The number of germinated seeds in the variant using the dose 5.0 µl of thyme oil was the same as the control, but it was significant difference (33%) in germinated seeds between control object and variant treated by 10.0 µl of mint oil.
Accordingly, germination energy reflects germination rate, uniformity, vigour and viability [Bewley et al. 2006]. Theoretically, no seeds in a population are immune from the effects of aging. Therefore, seeds in conducted experiments with low germination energy might have experienced deterioration process. The essential oil can be toxic to the seeds of plants that may affect the inhibition of germination. This was confirmed by Liu et al. [2006] that camphor tree ethereal oil was significantly toxic to wheat grains at 500 µg/ml. Kaborek et al. [2006] came

to similar conclusions. They proved that ethereal oils of Citrus citratus and C. giganteus were slightly phytotoxic to seeds. The carried out experiment did not bring any convincing proof that the used solutions of ethereal oils were toxic to seeds, which then was expressed by lowering their germination. The best dose of the tested oils was 5,0 µl.

Tab.1. Effect of the essential oil on the germination on the bean seeds (T-thyme oil, B-basil oil, M-mentha oil)

Variant	Observations						
	1th day	2nd day	3th day	4th day	5th day	6th day	7th day
Control	20%	40%	20%	20%	0%	0%	0%
T 2.5 µl	13%	30%	20%	7%	17%	0%	0%
T 5.0 µl	7%	7%	53%	33%	0%	0%	0%
T 10.0 µl	0%	7%	27%	20%	30%	13%	0%
B 2.5 µl	0%	53%	20%	7%	7%	0%	0%
B 5.0 µl	0%	33%	33%	13%	13%	0%	0%
B 10.0 µl	0%	27%	33%	0%	13%	10%	0%
M 2.5 µl	0%	33%	7%	7%	13%	13%	7%
M 5.0 µl	0%	33%	33%	23%	7%	0%	0%
M 10.0 µl	0%	0%	20%	20%	10%	10%	7%

CONCLUSIONS

1. The significant effect on germination seeds had the thyme oil compared to the oils of mint and basil.
2. The best dose of the tested oils was 5.0 µl in each of the variant of ethereal oil.

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MORPHOAGRONOMIC CHARACTERIZATION OF LIMA BEAN POPULATIONS – BOCA DE MOÇA LANDRACES

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INTRODUCTION

Lima bean (*Phaseolus lunatus* L.) is one of four species of the genus *Phaseolus* commercially exploited, with the potential to provide vegetable protein. In Brazil, mainly in the Northeast region, is a income and alternative food source for the population. Boca de moça landrace is among the most widely consumed in the Piauí State, due to color pattern characteristic of their seeds. This study aimed to characterize lima bean populations of the Boca de moça landrace, based on agronomic traits, to verify the genetic diversity and to select individual plants with potential for improvement.

MATERIAL AND METHODS

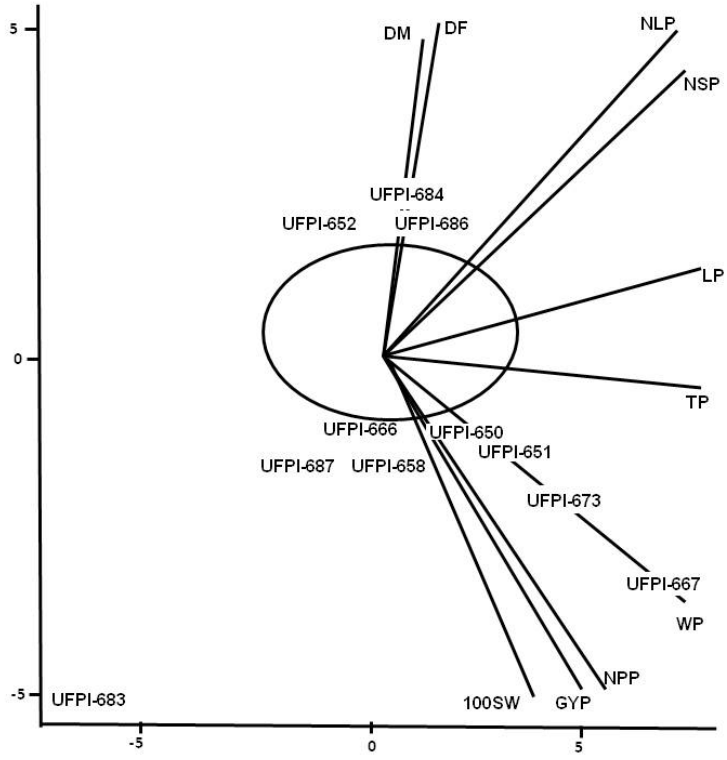
Lima bean subsamples from BAG UFPI were collected in conties of the Piauí State. The experiment was conducted in Teresina,PI county, during the period January-September 2010. Were evaluated the following traits: days to flowering and maturity (DF and DM), number of pods per plant (NPP); length, width and thickness pod (LP, WP and TP respectively); number of locules per pod (NLP); number of seeds per pod (NSP); one hundred seed weight (100 SW), grain yield per plant (GYP). Mean Euclidean distance and component principal analysis were performed by software SAS (SAS INSTITUTE, 1989) and GENES (CRUZ, 2001).

RESULTS AND DISCUSSION

In the principal component analysis (PCA), the first three principal components explained about 74.57% of the variation. The traits that accounted for the highest effect on the first principal component were length (0.43), width (0.38) pod and grain yield per plant (0.37), indicating that represents production components. Thus, genotypes with high values for the principal component 1 present high average for production components. Number of days to flowering and maturity were the descriptors with the highest impact on the second principal component, which represent the culture life cycle. Figure 1 illustrates the first two principal components of diversity for production components and lyfe cycle. UFPI-666, UFPI-650, UFPI-651, UFPI-687, UFPI-658, UFPI-673 and UFPI-667 subsamples were the earliest and presented high values for production components. UFPI-667 and UFPI-682 subsamples were most divergent by mean Euclidean distance. Since the new hybrids to be established must be based on the magnitude of their dissimilarities and potential *per se* of the parents, therefore the subsamples mentioned can be potential combination, because presented high means for desirable traits. Based on the individual plant production, were selected 13 plants earliest and greater production among UFPI-701, UFPI-653, UFPI-673 e UFPI-667 subsamples.

CONCLUSIONS

UFPI-666, UFPI-650, UFPI-651, UFPI-687, UFPI-658, UFPI-673 and UFPI-667 showed life cycle early and higher average for production components. The subsamples UFPI-667 and UFPI-682 showed greater dissimilarity and high complementarities for desirable traits.



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GENETIC DIVERGENCE AMONG SUBSAMPLES OF LIMA BEAN FROM GERMPLASM ACTIVE BANK FROM UFPI

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INTRODUCTION

Phaseolus lunatus L. known as lima bean, is a tropical legume specie known for high genetic diversity and yield potential (MAQUET et al., 1999). The analysis of genetic diversity is needed for better conservation. Estimating genetic diversity, by multivariate analysis, enhances the efficiency of germplasm collection management and genetic improvement. This study was conducted to investigate the genetic diversity in Lima Bean Active Germplasm Bank (BAG) from Federal University of Piauí (UFPI) based on quantitative traits of seeds

MATERIAL AND METHODS

This work was performed in the county of Teresina, Piauí, Brazil, in 2011, using 226 subsamples from Lima Bean BAG from UFPI. Were evaluated seed descriptors: length, width and thickness seed (LP, WP and TP respectively) and one hundred seed weight (100 SW). Genetic divergence among subsamples was estimated by Euclidean distance, establishing the Tocher grouping. All analysis were performed using software GENES (CRUZ, 2001)

RESULTS AND DISCUSSION

Genetic dissimilarity showed lower limit of 0.027 (UFPI-528 and UFPI-549) and upper limit of 3.814 (UFPI-217 and UFPI-688). Subsample UFPI-688 presented highest averages for length and one hundred seed weight, besides high averages for other descriptors evaluated. Twenty one were formed by Tocher method (Table 1). Group I contained 81 subsamples that showed high values for length (over 16.00mm), width (greater than 11.00mm) and thickness (greater than 6.00mm) and one hundred seed weight (greater than 60 g); Subsamples UFPI-503 (Group XV) presented lowest average thickness (6.77mm); UFPI-688 composed Group XVIII. In the Group XIX occurred UFPI-613 subsample, which present high values for width (13.38mm), length (20.24mm) and one hundred seed weight (99.67g). Subsamples more divergent were found groups XVIII (UFPI-688) and XX (UFPI-217). The relative contribution by Singh method (1981) showed that one hundred seed weight contributed with 97.42% of the difference.

CONCLUSIONS

High variability among subsamples in lima bean Active Bank Germplasm from UFPI, especially between UFPPI-217 and UFPI-688 subsamples. One hundred seed weight was the most important trait for genetic divergence.

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Table 1. Grouping by Tocher method of 226 subsamples of lima bean. Teresina, PI, 2011.

Groups	Subsamples
I	UFPI-528 UFPI-549 UFPI-212 UFPI-652 UFPI-664 UFPI-717 UFPI-522 UFPI-657 UFPI-464 UFPI-698 UFPI-693 UFPI-673 UFPI-577 UFPI-653 UFPI-663 UFPI-605 UFPI-670 UFPI-33 UFPI-582 UFPI-281 UFPI-578 UFPI-675 UFPI-694 UFPI-696 UFPI-651 UFPI-268 UFPI-669 UFPI-680 UFPI-689 UFPI-690 UFPI-267 UFPI-655 UFPI-721 UFPI-701 UFPI-679 UFPI-686 UFPI-695 UFPI-718 UFPI-676 UFPI-2 UFPI-720 UFPI-697 UFPI-668 UFPI-617 UFPI-677 UFPI-722 UFPI-187 UFPI-685 UFPI-678 UFPI-519 UFPI-674 UFPI-666 UFPI-491 UFPI-661 UFPI-684 UFPI-662 UFPI-683 UFPI-1 UFPI-598 UFPI-472 UFPI-705 UFPI-470 UFPI-654 UFPI-671 UFPI-504 UFPI-692 UFPI-667 UFPI-691 UFPI-702 UFPI-656 UFPI-160 UFPI-659 UFPI-708 UFPI-703 UFPI-626 UFPI-658 UFPI-277 UFPI-700 UFPI-699 UFPI-650 UFPI-285
I	UFPI-218 UFPI-244 UFPI-238 UFPI-250 UFPI-234 UFPI-290 UFPI-232 UFPI-243 UFPI-224 UFPI-229 UFPI-219 UFPI-585 UFPI-537 UFPI-222 UFPI-647 UFPI-237 UFPI-513 UFPI-252 UFPI-242 UFPI-231 UFPI-221 UFPI-239 UFPI-240 UFPI-256 UFPI-236 UFPI-253 UFPI-728 UFPI-538 UFPI-233 UFPI-616 UFPI-615 UFPI-225 UFPI-247 UFPI-255 UFPI-584 UFPI-627 UFPI-223 UFPI-261 UFPI-468 UFPI-612 UFPI-594 UFPI-257 UFPI-591 UFPI-473 UFPI-588 UFPI-649 UFPI-251 UFPI-264
III	UFPI-26 UFPI-621 UFPI-586 UFPI-466 UFPI-611 UFPI-608 UFPI-712 UFPI-714 UFPI-719 UFPI-523 UFPI-189 UFPI-713 UFPI-518 UFPI-492 UFPI-465 UFPI-121 UFPI-715 UFPI-202 UFPI-141 UFPI-278 UFPI-579
IV	UFPI-471 UFPI-602 UFPI-707 UFPI-467 UFPI-589 UFPI-516 UFPI-681 UFPI-590 UFPI-274 UFPI-500 UFPI-723 UFPI-463 UFPI-709 UFPI-710 UFPI-629 UFPI-711 UFPI-271 UFPI-704 UFPI-507 UFPI-607 UFPI-517 UFPI-682 UFPI-587 UFPI-515
V	UFPI-609 UFPI-619 UFPI-262 UFPI-648 UFPI-614 UFPI-134 UFPI-624 UFPI-599 UFPI-216 UFPI-596 UFPI-625
VI	UFPI-593 UFPI-600 UFPI-623 UFPI-214 UFPI-495 UFPI-280 UFPI-129 UFPI-706 UFPI-540 UFPI-482
VII	UFPI-228 UFPI-628 UFPI-235 UFPI-230 UFPI-220 UFPI-245
VIII	UFPI-604 UFPI-618 UFPI-601
IX	UFPI-597 UFPI-716
X	UFPI-493 UFPI-620 UFPI-595
XI	UFPI-276 UFPI-672 UFPI-508 UFPI-592 UFPI-486 UFPI-622
XII	UFPI-273 UFPI-494
XIII	UFPI-282
XIV	UFPI-687
XV	UFPI-503
XVI	UFPI-665
XVII	UFPI-166
XVIII	UFPI-688
XIX	UFPI-613
XX	UFPI-217
XXI	UFPI-610

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BREEDING OF THE CARIOCA BEANS AFTER TWO RECURRENT SELECTION CYCLES

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INTRODUCTION

The Carioca bean is the most cultivated and consumed in Brazil, reason by which the chief bean plant breeding programs in Brazil have given emphasis to the breeding of this sort of grain. The great difficulty in the autogamous plant breeding is finding two parents which join together all the phenotypes of interest. But, it is not always possible to associate in the wished extent in a single individual, the phenotypical expressions of the traits under selection, aiming to solve the problems at a single time. In this case, the alternative would be to promote the recurrent selection, namely, successive selection cycles and intercrossing of the best individuals or of the best families.

The objective of the work was estimating the genetic gain of yield in two recurrent selection cycles in the carioca bean breeding.

MATERIAL AND METHODS

The experiments were carried out in the Experimental Station of the municipality of Coimbra, belonging to the Plant Science Department of the Federal University of Viçosa (Universidade Federal de Viçosa (UFV)), in the winter and rainy crops of 2011. Both fertilization and cultural practices were conducted according to the one recommended to the bean culture in the region. The genetic gain estimate (PG) was undertaken by comparing simultaneously the forty (40) best families obtained in each cycle (C_0 e C_I). The experiment was conducted in randomized blocks with three replicates and plots of 2 rows of 2 meters. The gain was estimated on the basis of the following estimator:

$$PG(\%) = \left(\frac{\overline{X}_{C_I} - \overline{X}_{C_0}}{\overline{X}_{C_0}} \right) x100$$

In which: $\overline{X}_{C_I}$: mean of 40 families of C_I;

$\overline{X}_{C_0}$: mean of 40 families of C_0.

RESULTS AND DISCUSSION

The summary of the joint variance analyses of the experiment of the winter and rainy crops of 2011, concerning the simultaneous evaluation of the 40 best families of each cycle showed that the family x crop interactions and FC_0 *versus* FC_I x crops was non-significant for yield. The families of the C_I showed behavior superior to those of the C_0 as to grain yield. The behavior of the 10 best families of the C_0 and C_I cycles as to yield is presented in Table 1. It is found that the those families had behavior similar to the controls Pérola, BRSMG Majestoso and BRSMG Madrepérola.

Taking into account the mean of the two crops, the genetic gain for yield was 8.6%, which is equivalent to 293 kg/ha. In the literature genetic gain estimates with recurrent selection in bean for yield ranged from 3.3 to 55% (RAMALHO et al., 2005; MENEZES JÚNIOR et al., 2008; SILVA et al., 2010).

Table 1 – Means of yield (kg/ha) of the 10 best families of C_0 and C_I, evaluated simultaneously in the winter and rainy crops of 2011, Coimbra-MG.

Treatments	Yield
42-C_I	4483 abc
24-C_0	4230 abc
48-C_I	4189 abc
68-C_I	4131 abc
47-C_I	4087 abc
80-C_I	4054 abc
33-C_0	4040 abc
74-C_I	3984 abc
44-C_I	3983 abc
59-C_I	3974 abc
Pérola	3215 a
BRSMG Majestoso	3309 b
BRSMG Madrepérola	3106 c

CONCLUSION

Recurrent selection proved effective in the carioca bean breeding, as a gain of 8.6% for grain yield was observed with two selection cycles.

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YIELD RESPONSE IN BREEDING LINES OF DIFFERENT COMMON BEAN COMMERCIAL CLASSES AT THREE LOCATIONS IN NORTHERN MÉXICO

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INTRODUCTION: Several common bean (*Phaseolus vulgaris*) breeding lines have been selected at INIFAP's breeding program in North-Central México. Seed lines commercial classes include pinto, black (opaque and shiny), flor de mayo (pink), flor de junio (pink yellow-stripped) and azufrado (sulfur). Traditional breeding lines selection has been made using earliness, yield and disease resistance as the main criteria in order to obtain enhanced seed yield. New options include seed size and stability of seed weight across environments. Larger seed size (35-45 g 100^{-1} seeds) is preferred in pinto and azufrado; while small seeds (18-22 g 100^{-1} seeds) are common in opaque black seeded cultivars. Shiny black, flor de mayo and flor de junio commonly shows 25-35 g per 100 seeds weight. Significant reductions for seed weight have been observed among environments for pinto (25 g 100^{-1} seeds) and opaque black (16 g 100^{-1} seeds) cultivars. The objective was to select improved lines adapted in Northern-México using yield, disease resistance and seed size as selection criteria.

MATERIAL AND METHODS: In 2012, a set of 220 (F_6 and F_8) breeding lines was planted at three locations in Northern-México (Durango, F. I. Madero, Dgo. and General Terán, N. L.). Lines were grouped in five commercial classes: pinto (142), black (48), sulfur (14), flor de mayo (10) and flor de Junio (6). Commercial checks were also included such as: Pinto Saltillo, Pinto Bravo, Negro San Luis, Flor de Mayo Media Oreja and Flor de Junio Victoria. In General Terán, N. L., local check known as "Pinto Americano" was also included. Experimental plot consisted in a row of 5 m in length and row spacing varied between 0.81 m in Durango to 0.85 m in N. L. In Durango fertilization dose 25-35-00 (N-P_2O_5-K_2O) was used, while in Nuevo León only foliar fertilizer (Bayfolán ®) was applied twice, at a rate of 800 mL ha^{-1}, to counteract chlorosis observed in most of the lines. Fertilizer plant spraying was performed at 32 (pre-flowering) and 48 (flowering) days after planting (DAP). Data were recorded for the number of days to flowering, disease reaction (0-9 scale), days to maturity, yield and 100 seeds weight (CIAT, 1987). In each location data were analyzed to obtain means by commercial classes and lines, using three subsamples.

RESULTS AND DISCUSSION: Pinto lines showed earliness to flowering and physiological maturity (Table 1). Common bacterial blight was the main disease affecting common bean plantings in Northern-México due to reduced genetic base of germplasm used in breeding programs. Tolerance to *Macrophomina phaseolina* (*Mp*) was observed in

flor de junio and black commercial classes in General Terán, N. L. In contrast most of pinto and azufrado lines showed susceptibility to this disease. Black seeded lines showed higher yield across environments registering average from 858 kg ha^{-1} (Durango) to 1,122 kg ha^{-1} (F. I. Madero, Dgo). Outstanding results were also observed for pinto beans in both locations in the State of Durango (F. I. Madero= 1,107 kg ha^{-1} and Durango= 872 kg ha^{-1}). In General Terán, N. L. high average yield was observed for the flor de junio group (1,025 kg ha^{-1}), showing also tolerance to *Mp*. Selection performed using seed weight as selection criteria favored greater seed weight in pinto lines (31.4 g-39.5 g per 100 seeds weight), therefore considerable reduction of seed weight was observed across environments. This is an undesirable trait observed in rainfed plantings reducing market acceptance in most of commercial classes. Other commercial classes such as black maintained seed weight at both locations in Durango and an increased value were observed under irrigation at General Terán, N. L. (35.4 g 100 seeds^{-1}). In Durango, Dgo. highest yield was obtained by Pinto Bravo (2,083 kg ha^{-1}) and NGB10058 (1,822 kg ha^{-1}). In F. I. Madero, Dgo. outstanding cultivars were Pinto Bravo (2,535 kg ha^{-1}) and Jamapa (2,091 kg ha^{-1}). High yielding lines at General Terán, N. L. were PT12055 (1,352 kg ha^{-1}) and NGB10050 (1,312 kg ha^{-1}). Significant advances have been made in breeding programs for pinto and black commercial classes, obtaining wide adaptability and early to intermediate maturity. Disease resistance (CBB and *Mp*) and seed weight stability need to be improved under rainfed and irrigated conditions.

Table 1. Traits registered in common bean breeding lines planted at three locations in Northern-México. 2012.

Commercial Class (n)	*DF	DPM	CBB	*Mp*	Yield kg ha^{-1}	100 seeds Wt (g)
				Durango, Dgo.		
Azufrado (14)	48	105	6	--	287	29.7
Flor de Junio (6)	48	101	5	--	590	30.7
Flor de Mayo (10)	49	101	6	--	695	27.9
Black (48)	48	104	5	--	858	27.5
Pinto (142)	47	99	6	--	872	33.0
				F. I. Madero, Dgo.		
Azufrado (14)	45	102	6	--	430	28.9
Flor de Junio (6)	42	100	6	--	664	30.1
Flor de Mayo (10)	44	102	5	--	857	29.1
Black (48)	46	99	5	--	1,122	27.4
Pinto (142)	43	97	5	--	1,107	31.4
				General Terán, N. L.		
Azufrado (14)	46	111	5	5	294	31.4
Flor de Junio (6)	44	108	6	2	1,025	37.3
Flor de Mayo (10)	46	111	6	4	677	29.5
Black (48)	42	100	6	3	871	35.4
Pinto (142)	43	102	5	5	790	39.5

*DF= days to flowering, DPM= Days to physiological maturity, CBB= common bacterial blight, *Mp= Macrophomina phaseolina*.

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PATERNITY TEST FOR IAPAR 139 CULTIVAR OF COMMON BEAN

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INTRODUCTION AND METHODOLOGY

Progenies and lines derived from common bean breeding programs are usually evaluated in field trials aiming to select the best one to be delivered as cultivar. Despite being an autogamous species, insects are able to promote crossings among the common bean genotypes in those trials and could result in the selection of recombinant lines, derived from the crosses, becoming new cultivars. However, the male parent can be any progeny or line that is under evaluation. That was the case of the IPR 139 cultivar which has the IPR Juriti cultivar as female parent, but the male parent was still unknown. In the field trials of IPR Juriti, from which the IPR 139 was selected, the cultivar Pérola was also under evaluation. This cultivar became a male parent candidate because its flowers are more expose at the top of the plant canopy, they are larger and more visited by insects, its flowering is more prolonged and also because of seed similarities between IPR 139 and Pérola.

One way to check if Pérola cultivar is the male parent of IPR 139 is by analyzing the DNA of the three genotypes, using preferentially a codominant marker like simple sequence repeat (SSR). So 16 seedling from each of the three cultivars were used in order to test the paternity of IPR 139 cultivar. One young leaf per plant was taken to make a bulk per cultivar, and was grinded for DNA extraction through the CTAB procedure (Doyle and Doyle, 1987). 199 SSR primers (www.css.msu.edu/bic) were utilized, and the products of the PCR reaction were separated in an 8% polyacrylamide gel electrophoresis stained with silver (Creste et al., 2001).

The common bean coat color has maternal inheritance. Thus, when the plant that derived IPR 139 cultivar was discovered it was probably from the F_2 generation, whose seeds have coat color of F_1 generation. Its phenotype was similar to the seeds of the female parent (IPR Juriti) and also to the seeds of the candidate male parent (Pérola). Using the polymorphic SSR loci, the expected probability of finding the genotype of IPR 139 in the F_2 (P=

Considering the original plant that derived IPR 139 cultivar was from the F_2 generation, from a natural crossing, the expected probability of its genotype per locus (P_i) (Table 1), as well as the expected probability for all loci (i=11) is P=3.725 x 10^{-7}. Therefore, the PE=99.99% confirms that Pérola cultivar is the male parent of IPR 139. This line was tested in 13 field trials, and exhibited similar grain yield to IPR Juriti cultivar, although a lighter seed color which is preferred by the consumer. The usefulness of knowing the parents as well as some agronomical and commercial phenotypes is mainly for registering the cultivar.

Table 1. Marker genotypes of parents, F_1, and the expected frequencies in F_2 (P_i) of the genotypes that derived IPR139.

SSR primer	**IPR Juriti**	**Pérola**	**F_1**	**IPR 139 (P_i)**
BMD19	A^1A^1	A^2A^2	A^1A^2	¼ A^1A^1
PVTTC002	B^1B^1	B^2B^2	B^1B^2	¼ B^2B^2
AJ416401	C^2C^2	C^1C^1	C^1C^2	¼ C^1C^1
PVM13A	D^2D^2	D^2D^3	½ D^2D^2, ½ D^2D^3	¼ D^2D^3
PVM17	E^2E^2	E^1E^1	E^1E^2	¼ E^1E^1
BM205	F^2F^2	F^1F^2	½ F^1F^2, ½ F^2F^2	5/8 F^2F^2
PV107	G^2G^2	G^1G^1	G^1G^2	¼ G^1G^1
PV131	H^1H^1	H^1H^2	½ H^1H^1, ½ H^1H^2	1/8 H^2H^2
PV31	I^1I^1	I^1I^2	½ I^1I^1, ½ I^1I^2	¼ I^1I^2
PVESTBR_253	J^1J^2	J^1J^3	¼ J^1J^1, ¼ J^1J^2 ¼ J^1J^3, ¼ J^2J^3	1/8 J^1J^2
PVESTBR_6	K^1K^1	K^1K^2	½ K^1K^1, ½ K^1K^2	5/8 K^1K^1

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RELEASE OF NUÑA BEAN LINES CO49956 AND CO49957 ADAPTED TO TEMPERATE CLIMATES

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The Colorado Agricultural Experiment Station announces the release of CO49956 and CO49957 nuña bean (*Phaseolus vulgaris* L.) breeding lines developed at Colorado State University and tested at three locations in Colorado (Pearson et al., 2012). Nuña beans, also known as 'Pop" beans, are a market class of common bean (*Phaseolus vulgaris* L.) indigenous to the southern region of the Andean mountains. Nuña beans have been grown as an important food crop in high altitudes of Andean South America because they can be cooked by frying in hot oil for two to four minutes, compared to traditional methods of cooking beans by boiling in water for 2 to 4 h. In the U.S., nuña beans have unique potential as a snack food because they are nutritious and have a pleasant "nut-like" taste. Even though nuña bean has potential as a new market class of dry beans in the temperate regions of the USA, germplasm developed in South America are not suitable for production in temperate regions because they are photoperiod sensitive and have a climbing growth habit not suitable for mechanical harvest (Zimmerer, 1992).

To develop nuña beans for production in the temperate zones, we initially made single crosses between nuña bean germplasm and the commercial light red kidney bean cultivar Sacramento to combine popping ability with photoperiod insensitivity, determinate growth habit, and adaptation to temperate environments (Ogg et al., 1998). Most of the $F_{3:4}$ lines derived from the single-crosses Sacramento/PI 293356 and Sacramento/Piemco had low popping frequency (8%); however, two lines had popping frequency greater than 40%. We utilized the two lines from the progeny with high popping frequency as parents to backcross to nuña bean parents PI 316018 and Piemco. All nuña PIs were obtained from the USDA Western Regional Plant Introduction Station, Pullman WA and the parental line we termed "Piemco" was obtained from a package of commercial nuña bean seed purchased in California imported from Peru. The resultant BC_1F_1 plants were increased in the greenhouse and BC_1F_2 progeny were planted in the field at Fruita, CO. Plants in the BC_1F_2 progeny segregated 3:1 for indeterminate:determinate growth habit ($P < 0.05$) as expected because the recessive *fin* allele responsible for determinate growth habit segregated in the populations (Ogg et al., 2008). Ninety BC_1F_2 plants were selected for determinate (Type I) growth habit and photoperiod insensitivity; then planted to progeny rows the following year. Among the 90 $BC_1F_{2:3}$ progeny rows, 34 of the most adapted progeny rows were identified and 10 to 15 plants selected from each row based on early maturity and desirable agronomic traits. Seed from these plants was harvested and pedigree selection was imposed for popping frequency and agronomic traits for two generations at Fruita, CO. Ten $F_{4:5}$ advanced lines were selected and bulked for field testing at three Colorado locations; namely, Fort Collins, Fruita, and Rocky Ford, CO. Mean seed yield among the ten lines varied from 1794 to 1321 kg ha^{-1} (Table 1) and seed size varied from 44 to 34 g 100 $seeds^{-1}$ across environments and years. Two lines, CO49956 and CO49957, had the highest popping frequency (70 and 68%) and are released. Both lines are susceptible to most pathogens that attack dry beans including; *Bean common mosaic virus*, foliar rust [caused by *Uromyces appendiculatus* (Pers.) Unger], and bacterial common blight [caused by *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Vauterin et

al. (Synonym: *X. campestris* pv. *phaseoli* Smith (Dye)]. Additional breeding will be required to reduce or eliminate the susceptibility of these lines to these pathogens and develop agronomical desirable cultivars suitable for the commercial market. Seed is available from Mark Brick, Department of Soil and Crop Sciences, Colorado State University, Fort Collins, CO 80523, 970-491-6551 or Mark.Brick@Colostate.edu.

Table 1. Mean seed yield and popping frequency among ten nuña breeding lines at three locations in Colorado during 2006 and 2007.

Nuña Line	Location				Mean Popping Frequency
	Fort Collins	Fruita	Rocky Ford†	Mean Seed Yield	
	----------Seed yield kg ha^{-1}----------				%
CO49956	1253 b*	1204 ab	1811 ab	1423 B	70 A
CO49957	1495 ab	1030 b	1778 ab	1434 B	68 A
CO49984	1583 ab	1027 b	1728 ab	1446 B	56 B
CO49990	1404 ab	901 b	1816 ab	1374 B	55 B
CO50004	1861 a	1539 a	1982 ab	1794 A	55 B
CO49979	1359 b	1412 ab	1928 ab	1566 AB	49 C
CO49982	1419 ab	1112 b	1550 b	1360 B	49 C
CO49978	1332 b	1396 ab	1912 ab	1547 AB	48 C
CO49961	1358 b	859 b	1745 ab	1321 B	41 D
CO49991	1796 ab	1402 ab	2151 a	1783 A	40 D
Mean	1387	1185	1840	1471	53

*Simple effects least square means separated by different lower-case letters and main effects means separated by different upper-case letters at P≤0.05 according to pdiff command in Proc Mixed analysis in SAS.
†Rocky Ford only includes one year of testing.

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2012 FINANCIAL STATEMENT
BEAN IMPROVEMENT COOPERATIVE

BALANCE AS OF January 1, 2012			**$ 8,817.73**
INCOME			
2012 Dues	$	4,570.00	
Extra Articles for 2012 Report	$	50.00	
2013 Dues	$	90.00	
Back Issues	$	10.00	
Bank Interest	$	182.29	
TOTAL INCOME	$	4,902.29	
EXPENSE			
Labor Charges	$	562.50	
Postage, Copy Charges and Office Supplies	$	1,860.40	
Printing and shipment – Volume 55	$	2,695.06	
Google Checkout and PayPal Fees	$	127.69	
TOTAL EXPENSE	$	5,245.65	
BALANCE AS OF December 31, 2012			**$ 8,474.37**