ANNUAL REPORT OF THE

BEAN IMPROVEMENT COOPERATIVE

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

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BEAN IMPROVEMENT COOPERATIVE

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Coordinating Committee

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SITE REGULARLY UPDATED

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Cover: Row spacing trials of new bean varieties in the Nebraska Panhandle. Photo courtesy of C. Urrea and D. Ostdiek.

THE 52nd 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-fifth Biennial Meeting from October 25 through October 30, 2009 at the Hilton Garden Inn, 2821 East Harmony Road, Fort Collins, Colorado, near the campus of Colorado State University. The local organizers are Howard Schwartz and Mark Brick. In addition, the associated meetings with our colleagues in the North American Pulse Improvement Association (NAPIA), Crop Germplasm Committee, BIC Genetics Committee and the Regional W-1150 Committee are scheduled. 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 website 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** and the **BIC Achievement Award**, and forward your nominations to the Awards Committee Chairperson, Howard Schwartz by May 1, 2009. We will continue to recognize our founding members through the **Frazier-Zaumeyer Distinguished Lectureship**. The Lectureship will be awarded at the meeting in Fort Collins 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 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.

An updated version of the common bean genetic map is posted on the BIC web page. The chromosomes with underlined number designations have been rotated top to bottom from Freyre *et al* (1998) in order for the short arm to be presented on top, based on the new linkage group nomenclature presented by Pedrosa *et al* (2008; BIC 51:106-107).The chromosome nomenclature presented (chromosomes numbers 1-11) corresponds to the B1-B11 designation from the core map. Details are provided in the figure legend.

New guidelines were adopted for the naming of common bean QTL at a recent meeting of BIC Genetics Committee [pg. iii]. BIC members are asked to review the guidelines and follow the same in the naming of future QTL of common bean. This will facilitate the comparison and co-localization of new QTL with previously described QTL and help in identifying regions of genomic interest and functionality associated with traits of economic interest. These guidelines, the 2009 version of the bean core map and an updated bean genes list were recently posted on the BIC website [http://www.css.msu.edu/bic/Genetics.cfm]. Members are asked to review the current gene list before assigning new gene symbols and/or QTL to chromosomes.

In order to reduce mailing costs, the BIC is conducting all 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. The versatility of paying membership dues by credit card through PayPal and Google checkout options has greatly facilitated payment for our international members. We are always open to new ideas and suggestions to make the BIC a more versatile and effective organization and any ideas can be shared with myself or members of the coordinating committee. Looking forward to seeing you all in Fort Collins, Colorado in October…….

Dr. James D. Kelly, BIC President

BIC Committee Membership - 1957 to 2009

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

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

Genetics Committee

2007: Tim Porch (Chair), James Beaver, Matthew Blair, Paul Gepts, Phil McClean, Phil Miklas, Carlos Urrea, Molly Welsh (ex officio).
2008: Tim Porch (Chair), Kirstin Bett, Matthew Blair, Paul Gepts, Phil McClean, Phil Miklas, Carlos Urrea, Molly Welsh (ex officio).

BIC Genetics Committee Minutes

Isabela Substation of the University of Puerto Rico, Feb. 21, 2009

Minutes prepared by Tim Porch, Chair (abstracted- full report available on web)

1. Topic: Genetic map

Tim Porch presented an updated version of the genetic map, where chromosomes have been rotated top to bottom, based on the new linkage group nomenclature presented in a BIC note by Pedrosa et al. (2008). A recommendation was made to add new microsatellite markers and Phil McClean's gene-based markers to the genetic map.

Decisions: The Genetics Committee decided to eliminate the old Roman numeral chromosome numbers at the bottom of the linkage groups. Paul Gepts will check and determine if additional references are needed for the markers and symbols used in the map, which are mentioned in the legend. Additions and corrections to the map can be submitted by March 7th 2009 after which the map will be posted on the BIC website.

2. Topic: QTL nomenclature

Phil Miklas proposed a QTL nomenclature method for common bean publications based on those used in other species including rice, Rosaceae and soybean. The group consulted and decided on a standard nomenclature for future common bean publications.

Decision: *Guidelines for common bean QTL nomenclature:*

1. Capitalize 2-3 letter abbreviation for trait. Capitalized trait name should not be italicized. For example, WM for white mold. A preferred list of abbreviations to use for common traits should be generated, and updated periodically.
2. Each QTL will have a linkage group designation directly after the 2-3 letter abbreviation. For example WM1 indicates a QTL for white mold on linkage group 1.
3. QTL should be listed in chronological order. Thus, new publications on a specific trait will initially need to review and number previous QTL designations in order to arrive at a number for new QTL. For example the first QTL identified on linkage group 1 would be named WM1.1, and the second independent QTL on the linkage group would be named WM1.2, and so forth.
4. The population where the new QTL was identified should be indicated by an abbreviation in caps and non-italicized superscript after the linkage group designation. For example, the first QTL indentified on linkage group 1 for white mold resistance was in the RIL mapping population A55/G122, thus would be designated $WM1.1^{AG}$
5. To distinguish among QTL which co-localize or overlap in the same general region, subsequent population abbreviations would be separated by commas and listed in order of discovery. For example, the overlapping QTL identified first in A55/G122 and subsequently in John/Doe would be designated $WM1.1^{AG,JD}$, and so forth.

Additional provisions:

6. If upon fine mapping in the future two overlapping QTL are proven to be independent, then the subsequent QTL in the example above could be renamed $WM1.1.1^{JD}$ to distinguish it from $WM1.1^{AG}$
7. If two independent QTL (Ex: $WM1.1^{AG,}$ and $WM1.2^{JD}$ in the future are proven to co-localize, then the first QTL identified would retain its original name and the second QTL would be incorporated in the name of the first QTL: $WM1.1^{AG,JD}$. Note if this occurs then original number (2 for this case) representing chronological order is not used again.

3. Topic: Marker development and discovery

Paul Gepts presented a proposal that is being prepared for AFRI funding for the development of a molecular marker toolbox for breeders. This toolbox would integrate the common bean sequence, marker, and linkage map information into an easily accessible web-based interface to assist breeders in marker discovery and development. This resource will use the soybean genome and will:

1. Map targeted *P. vulgaris* sequences to the soybean genome,

2. Use soybean/common bean synteny for the identification of closely linked *P. vulgaris* sequences, and 3. Assist in the development of new markers and the identification of candidate genes.

Paul Gepts has requested input as to the ten most useful target traits and genes to focus on in the proposal. Suggestions were made including regions where QTL for biotic resistance co-localize including regions on chromosomes 2, 4, and 11 as well as the development of additional markers for those SCAR markers that do not function across gene pools. The proposal is integrated with the other two proposals being submitted to USDA involving SNP development and genome sequencing.

4. Topic: Manuscript on blue pattern flower color

A new gene symbol, t^{bp}, for a new allele at the *T* locus conditioning blue patterned flowers in the presence of Prp^{i}*-2* is being proposed in the manuscript "Blue Pattern Flower in Common Bean Expressed by Interaction of Prp^{i}*-2* with a New Gene t^{bp}" by Mark Bassett and Phil Miklas. Jim Kelly suggested that the placement of *Prp-2* on chromosome 7 be reviewed since the bracketed designation [*C prp*] was used while the C locus is located on chromosome 8.

Decision

Through email and the discussion above, the Genetics Committee has accepted the t^{bp} gene symbol and the evidence provided in the manuscript. It was also clarified that the *Prp-2* locus is independent of the [*C prp*] complex locus.

5. Topic: Committee membership

No changes will be made at this time to the committee membership.

6. Topic: Next meeting

The next Genetics Committee meeting will be held during the BIC meeting on October 28th from 11AM-12PM at the Hilton Garden Inn, Fort Collins, Colorado.

Attendance:

	Attendee	University/Agency	Genetics Committee
1.	Paul Gepts	University of California, Davis	Member
2.	Jim Kelly	Michigan State	Member
3.	Phil Miklas	USDA-ARS	Member
4.	Tim Porch	USDA-ARS (Chair)	Member
5.	Carlos Urrea	University of Nebraska	Member
6.	Molly Welsh	USDA-ARS (ex-officio)	Member
7.	Judith Brown	University of Arizona	
8.	Jim Myers	Oregon State University	
9.	Mark Brick	Colorado State University	
10.	Phillip Griffiths	Cornell University	
11.	Shree Singh	University of Idaho	
12.	M.A. Pastor-Corrales	USDA-ARS	
13.	Steve Noffsinger	Seneca Foods Corp.	
14.	Jim Nienhuis	University of Wisconsin	
15.	A.M. Thro	USDA-CSREES	

Recipients of BIC Meritorious Service and 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, Pl. 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**, 1998-2007)
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
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]

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

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 52-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 Coordinating Committee and Awards Committee offers a special award for BIC members who have devoted less time to their "bean careers" than our Meritorious Service Award recipients. 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.

The BIC Coordinating Committee and Awards Committee proudly announce the second **Frazier-Zaumeyer Distinguished Lectureship**. Nomination for this award should be sent to the Awards Committee. These 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-fifth Anniversary of BIC Biennial Meeting in Fort Collins, Colorado from October 25 to October 30, 2009. Please help us select worthy recipients.

BIC AWARD NOMINATION

Return by May 1, 2009 to:

Dr. Howard F. Schwartz
BIC Awards Committee Chairman
Dept. of Bioagricultural Sciences and Pest Management
Colorado State University
Fort Collins, CO 80523-6987, USA
The other Awards Committee members are Dr. George Hosfield and Dr. Shree Singh.

Nominee: Name: _____

Address: _____

_____

_____

_____

Discipline: _____

Nominated for: _____ Meritorious Service Award _____Achievement Award

_____ Frazier-Zaumeyer Distinguished Lectureship

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]

2009 BIC/NAPIA Meetings in Fort Collins, Colorado

The BIC/NAPIA 25th biennial meeting and associated meetings will be held October 25 through October 30, 2009 at the Hilton Garden Inn, 2821 East Harmony Road, Fort Collins, Colorado 80528.Telephone: 970-225-2900 Fax: 970-225-2908 Website: www.fortcollins.stayhgi.com
On-Line Hotel Registration for the BIC and the NPIA can be made at:
http://hiltongardeninn.hilton.com/en/gi/groups/personalized/FNLFCGI-BBN-20091025/index.jhtml
Group Name: Colorado Bean Network – BIC **Group Code: BBN**

Lodging will be available at a reduced rate of $85 at the recently-opened Hilton Garden Inn with numerous amenities and upgrades for your enjoyment and relaxation. It is situated near a wide array of new restaurants and entertainment venues; in addition, the complementary hotel shuttle can transport you to Old Town and other favorite hot spots. Registration information, fees, final meeting agenda and travel arrangements will be made available to members and other interested individuals Spring of 2009.

- **On line registration will be available after May 1, 2009 on BIC web site.**

The Hilton Garden Inn Fort Collins is located at exit 265 (Harmony Road) West off of Colorado Interstate 25 and boasts views of the beautiful Rocky Mountains. It is conveniently located only 1/2 of a mile from the Hewlett Packard Fort Collins office and down the street from Intel. Colorado State University is only 13 miles away and the Anheuser Busch Brewery and Budweiser Event center are only 5 miles away.

Most major airlines provide flights into Denver International Airport (DIA). Car rentals are available, and the SuperShuttle (Shamrock)
http://www.rideshamrock.com/rs_ax_fortcollins_north_smtwtf.php
provides an economical round-trip connection and convenient departure from DIA to Fort Collins and the Hilton Garden Inn.

Tentative meeting plans for the BIC, W1150, PCGC and BIC Genetics Committee:
Oct. 25 - arrival, Registration from 7:00 – 9:00 pm
Oct. 26 - BIC 7 am Registration, Meetings from 8:00 am – 5:00 pm
Oct. 27 - BIC 8:00 am – 5:00 pm followed by evening Awards Banquet
Oct. 28 - BIC 8:00–10:00 am followed by……..
……Phaseolus CGC 10:00–11:00 am; BIC Genetics 11:00 am–noon; W1150 1:30–5:30 pm

If individuals or groups are interested in helping sponsor coffee breaks, printing costs, registration materials, BIC Abstracts and Proceedings, and/or awards for student presentations; please contact the BIC President, **James D. Kelly** (kellyj@msu.edu) or the Local Organizing Committee members: **Howard Schwartz** (howard.schwartz@colostate.edu) and **Mark Brick** (mark.brick@colostate.edu)

NAPIA Arrangements: Please contact Kevin McPhee @ kevin.mcphee@ndsu.edu
Oct. 29 - NAPIA 8 am – 5 pm
Oct. 30 - NAPIA 8 am – 5 pm

First Call for Papers for the BIC

This is the first call to alert authors who desire to present oral or poster papers at the 2009 Biennial Meeting of the BIC and associated meetings. The deadline for receiving abstracts is **Saturday August 15, 2009.** Abstracts may be placed in the poster sessions if the oral sessions have filled up. (Authors will be notified if this placement is necessary). Details about the format of **Abstracts**, **Oral presentations** (1 only per registrant) and **Poster presentations** will only be provided on line by May 1, 2009, as well as information on audiovisual equipment available during the meetings.

IN MEMORY OF CARLOS A. RAVA

Dr. CARLOS AUGUSTIN RAVA, 69, was a researcher from the Embrapa Rice and Beans Center located in Goiás Brazil. He worked on common bean in the area of Phytopathology and Plant breeding with emphasis on disease resistance. Dr. Rava provided significant scientific contribution to disease control, identification of pathogen variability, seed production free of pathogens and the release of more than 37 beans cultivars grown in Brazil. In the last years of his professional career he usually said "I am more a plant breeder than a plant pathologist".

Dr. Rava has born in Montevideo, Uruguay, in 1939, finished his B.S. in Agronomy in 1965 in the University of La República, in the Faculdad de Agronomia, Uruguay. From 1966 to 1970, he did his MS in Agronomy with major in Phytopathology at the University of Agriculture "Luiz de Queiroz", in Piracicaba, São Paulo, Brazil. In 1976, he took the specialist course on common bean disease at International Center for Tropical Agriculture in Cali, Colombia. From 1979 to 1985, he completed his Ph.D. in Agronomy with major Phytopathology at the Federal University of Viçosa, Minas Gerais, Brazil. From 1963 to 1968, he worked as a technical assistant on the University La República, Faculty of Agronomy, Uruguay. From 1968 to 1974, he worked as a technician at the Center of Agrarian Studies Alberto Boerger, in Argentina. From 1974 to 1975, he was an associate professor in the National University from del Comahue in Uruguay. He joined the Embrapa Rice and Beans Center in Goiás Brazil as a researcher in 1975 where he continued until his retirement.

Besides his team work in the bean breeding program at the Embrapa Rice and Beans Center, Dr. Rava was the president of editorial committee of the center during many years. Dr. Rava had many important scientific publications indeed he was editor of 5 books, author of 23 book chapters, 40 scientific papers and 56 papers published as proceedings of scientific meetings. From 1990 to 1991, he was the principal Technical consultant of the project "Producción Artesanal de Semilla Mejorada de Frijol", coordinated by FAO, in Nicaragua. In 1965 and 1991 he received the best publication awards from the journals Beca Artigas, Universidad República Oriental do Uruguay, and Summa Phytopathologica, and Grupo Paulista de Fitopatologia.

Dr. Rava passed away on May 1, 2008. He was a great example of professional dedicated to bean research. He was a generous man with a incredible general knowledge and always willing to help colleagues on any issue from agriculture to questions on how to write more perfect Portuguese. One of life's good lessons always remains. Dr. Rava was a human being that left good examples for all of us and it was a privilege to have a chance to interact with him during his life.

THE USE OF THE SOYBEAN GENOME SEQUENCE FOR MARKER MAPPING AND DEVELOPMENT IN COMMON BEAN

Shelby Repinski and Paul Gepts

Dept. of Plant Sciences / MS1, University of California, Davis, CA

The limiting factor in developing new markers for mapping and tagging in common bean is the dearth of mapped DNA sequences. In the absence of these, researchers have had to convert linked RAPD markers into SCAR markers. Whereas there are some 40 SCAR markers that have been developed in common bean (Miklas 2008: www.css.msu.edu/bic/PDF/SCAR%20Markers%202008.pdf) and have been used in varietal development in common bean, the procedure is cumbersome.

Recent progress in the development of genomic resources in common bean, but also in soybean, provide an alternative, quicker, and potentially more successful approach to marker discovery. In soybean, the new resource is the actual whole-genome sequence (WGS), which is now anchored - since December 2008 - to the 20 chromosomes of soybean (2n = 2x = 40) (www.phytozome.net/soybean). In common bean, there are three new types of sequence resources: a) ~ 90,000 BAC-end sequences from a physical map effort (Schlueter et al. 2008), most of which are unmapped; b) EST sequences arranged in transcript assemblies (TAs: Childs et al. 2007): 21,807 for *P. vulgaris* and 19,746 for *P. coccineus* (http://blast.jcvi.org/ euk-blast/plantta_ blast.cgi); c) sequence-tagged sites (STSs): ~ 300 gm arkers, developed by P . McClean; 79 Bng markers, sequenced by Murray et al. (2002); and 104 cross-legume Leg markers, developed by Hougaard et al. (2008); and ~ 250 SSR markers (Gaitán-Solís et al. 2002; Blair et al. 2003; Mattos Grisi et al. 2007 ; Blair et al. 2008).

One cannot over estimate the importance of the availability of a WGS for a variety of purposes, including gene mapping and tagging. Indeed, such a sequence is actually the ultimate genetic map. Although efforts are under way to obtain such a complete sequence of the bean genome, it is not available yet. An alternative is a WGS of a related species. There are three species with a WGS among legume species. Soybean is the most closely related to common bean as both species belong to the Phaseoleae tribe (Gepts et al. 2005). The last common ancestor between these two species lived some 20 million years ago. *Medicago truncatula* and *Lotus japonicus* also have a WGS but they are more distantly related to common bean as they belong to the Hologalegina clade (last common ancestor with the Phaseoloid clade: ~ 55 million years ago) (Gepts et al. 2005).

There are two caveats to using the soybean WGS as a substitute for a Phaseolus WGS. The first one is that the two species diverged some 20 Mya. Thus, one expects sequence divergence, especially in non-coding regions. This indicates that the soybean sequence *per se* cannot be used directly to design primers for PCR in Phaseolus. Also one may expect changes in gene order and content affecting microsynteny although the full extent of this phenomenon is unknown at this stage. The second drawback is that subsequent to the *Glycine* - *Phaseolus* split (some 15 Mya: Schlueter et al. 2004; Shoemaker et al. 2006), the ancestor of *G. max* underwent a tetraploidization/diploidization cycle. As a consequence, one expects that, for each *Phaseolus* sequence, there will be - on average - two matches in the *G. max* sequence. Nevertheless, one expects that, because of the diploidization of the soybean genome, there may be chromosome rearrangements. Thus, the precise extent of macrosynteny remains to be determined as well.

A preliminary analysis of targeted regions of the bean genome allows us to draw the following conclusions. First, there is extensive micro- and macrosynteny between the *Phaseolus* and soybean genomes; nevertheless, large segments of soybean chromosomes are rearranged compared to the *Phaseolus* genome. Second, *Phaseolus* sequences in most cases have two matches in the soybean genome. However, less-conserved sequences tend to have more matches in the soybean genome. To distinguish between syntenic and non-syntenic locations, determination of synteny of adjacent markers (with more conserved sequences) is recommended. Third, based on the high levels of microsynteny and relative sequence conservation, it is possible to identify - through mapping onto the soybean WGS - closely linked *Phaseolus* sequences. In most cases, these sequences are either BAC-end or EST sequences already mentioned. The latter sequences, which can be visualized in http://www.soybase.org, constitute potential alternative markers after the necessary validation for PCR amplification potential and polymorphism. Fourth, candidate genes from other species such as Arabidopsis can be mapped in *Phaseolus* via the soybean WGS. This mapping provides a preliminary indication of the value of a gene as a candidate gene underlying a specific *Phaseolus* trait.

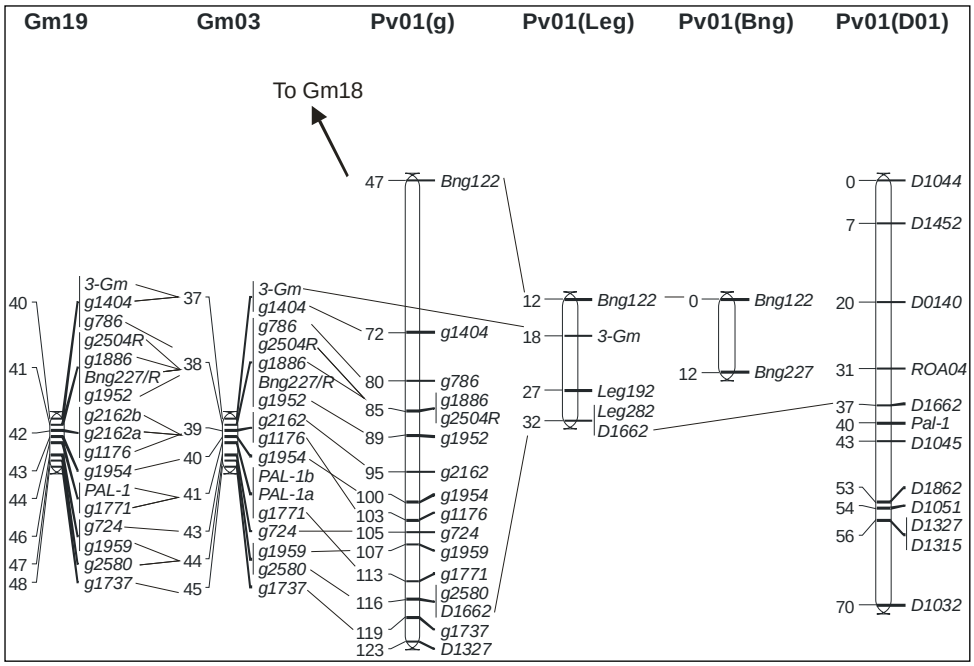

Fig. 1. Synteny between part of linkage group PV01 and corresponding soybean linkage groups. Distances on Pv linkage group are Kosambi cM. Distances on Gm linkage groups are in Mbp.

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IDENTIFICATION OF CANDIDATE GENES ASSOCIATED WITH CBB RESISTANCE IN COMMON BEAN USING CDNA-AFLP

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INTRODUCTION

Common bacterial blight (CBB) of common bean (*Phaseolus vulgaris L.*), incited by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*), is a serious seed-borne disease in both temperate and tropical bean production zones. Yield losses can exceed 40% (Miklas et al., 2006). HR45 is highly resistant to *Xap* infection on leaves and pods in the field and greenhouse. Near immunity response (NIR) to *Xap* was observed on the leaf of HR45 seven days after inoculation. In order to identify resistance genes eliciting NIR against *Xap* in HR45, the gene profiling approach was used to identify the genes that are differentially expressed in the leaves of HR45 at different hours after inoculation.

MATERIALS AND METHODS

The cDNA-amplified fragment length polymorphism (cDNA-AFLP) technology was used in this study for gene profiling. HR45 was inoculated with *Xap* using the multiple needle technique (Park and Dhanvantari, 1987). Leaf tissues were collected from mock-inoculated and inoculated plants at 0, 8, 12, 24 and 120 hours post inoculation (HPI). LI-COR AFLP Expression Analysis Kit (LI-COR Biosciences, Nebraska, Lincoln, USA) was used to perform cDNA-AFLP analysis. The differentially expressed fragments were cloned and sequenced using LI-COR IR2 sequencer (LI-COR Biosciences, Nebraska, Lincoln, USA). Cluster analysis was conducted in the TIGR Microarray Data Analysis System (www.tm4.org).

RESULTS

Thirty four different primer combinations were used in cDNA-AFLP analysis. Two thousands four hundred forty-eight transcript-derived fragments (TDF), ranging from 50 to 1,000 bp were generated. 10.6% of the TDFs had significantly altered expression level at all five time intervals post-inoculation. Majority of the TDFs were up-regulated than down-regulated upon *Xap* infection (Figure 1). Seventy-seven differentially expressed TDFs were cloned and sequenced. They were assembled into 8 contigs and 59 singletons. Thirty-two of them were linked to the TCs (Tentative Consensus sequences) which contain these ESTs in Bean Gene Index (http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/est_report.pl?EST=PvXIS&gudb=p_vulgaris). The 11 clones marked with the asterisk in figure 1 had homology to genes coding for proteins involved in plant defence responses. Whereas, 6 clones marked with the plus sign were *in silico* mapped to the lower region of linkage group 6 making them putative candidate genes for a major QTL associated to CBB resistance (Liu et al, 2008).

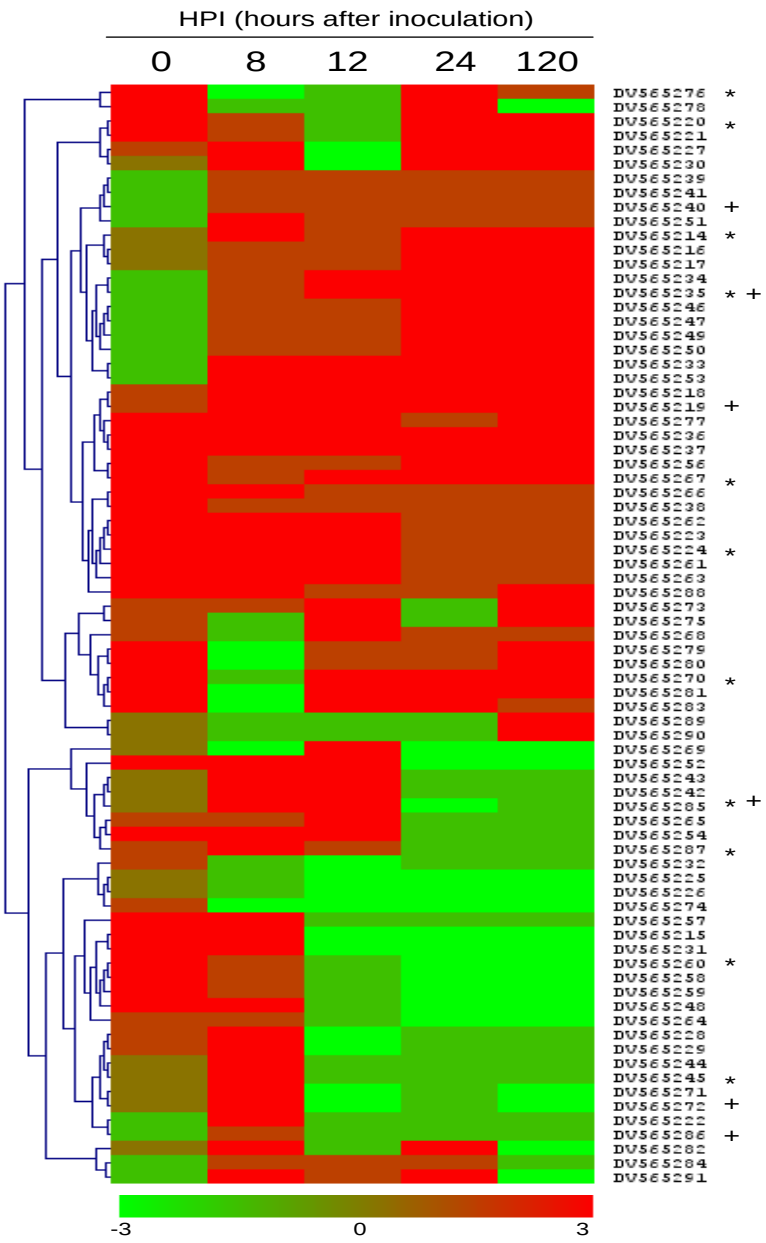

Figure1. Cluster analysis of the time-course expression profiles of 77 transcript-derived fragments. All measurements are relative to the expression level of mock-inoculated leaves at 0 hrs post-inoculation. The color saturation reflects the magnitude of the LOG2 expression ratio for each transcript. Red color marks up-regulated transcript-derived fragments after infection, whereas greens are down-regulated. The color LOG2 scale is provided at the bottom of the figure. * indicates the transcript-derived fragments homologous to proteins involved in plant defence responses. + indicates the transcript-derived fragments *in silico* mapped to the lower region of linkage group 6.

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SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs) IN COMMON BEAN

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INTRODUCTION

Efficient molecular tools for genetic studies in common bean (*Phaseolus vulgaris* L.) are greatly needed. Single nucleotide polymorphisms (SNPs) are highly desirable as molecular markers because they can be used for in-depth genetic analysis. More than 20,000 SNPs have been discovered in soybean (*Glycine max*) via the sequence analysis and comparison of sequence tagged sites (STSs) from a small set of diverse genotypes (Zhu *et al.* 2003, Choi *et al.* 2007). Because of the relatively close relationship of the soybean and common bean genomes (Zhu *et al.* 2005), it has been reported that many of the soybean-derived STS primers are able to amplify single amplicons in common bean. In this work we used available PCR primers designed to amplify soybean shotgun and BAC-end sequences for SNP discovery in common bean. This approach aimed to save time and money spent in the initial steps of primer development. In addition, we have also used some PCR primers designed to *P. vulgaris* genes for detection of SNPs in this species.

MATERIAL AND METHODS

Common bean DNA fragments harboring SNPs were identified in single amplicons via nucleotide sequence analysis of contrasting *P. vulgaris* genotypes of the Andean (Jalo EEP558, G19833, and AND277) and Middle-American (BAT93, DOR364, and Rudá) gene pools. These genotypes are the parents of three common bean RIL mapping populations. The PCR primers were initially used to amplify the DNA of the cultivar Jalo EEP558 at annealing temperatures of 58/48ºC (soybean PCR primers) or 54ºC (common bean PCR primers) followed by DNA sequence analysis of the resulting single amplicons. The two resulting sequence traces derived from opposite ends of each amplicon were analyzed and aligned with the aid of standard DNA analysis software Phred and Phrap. Resulting alignments and trace data were visually inspected in the Consed viewer. When good quality sequence data were obtained, the STS primers were then used to amplify the genomic DNA of the other five genotypes. The resulting PCR products were sequenced and analyzed for SNP discovery with the SNP-PHAGE software (Matukumalli *et al*. 2006).

RESULTS AND DISCUSSION

A total of 1667 primer sets were tested. Out of them, 622 (37.31%) amplified a single PCR product from the common bean genomic DNA. One hundred and ninety-four (11.64%) sets produced high quality sequence data. The total length of aligned sequences was 104,252 bp and the mean length of single amplicons was 537 bp. Five hundred and fourteen SNPs were identified in 129 (7.74%) distinct DNA fragments. The frequency of SNPs was 4.93 SNPs/Kb and the mean nucleotide diversity expressed as Watterson's theta (θ x 1000) was 2.16 (Table 1). The nucleotide diversity in common bean is more than two times higher than that detected in soybean (Choi *et al.* 2007). This work is part of an international effort led by the Soybean Genomics and Improvement Laboratory (USDA-ARS, Beltsville, MD, USA) aimed at detecting SNPs in common bean. The SNPs identified will be useful for genetic mapping, diversity analysis, and synteny studies between legume species.

Currently, SNP discovery approaches using reduced representation libraries and high throughput sequence analysis on the Illumina Genome Analyzer are being undertaken.

Table 1. Detection of SNPs in *Phaseolus vulgaris* DNA fragments generated by common bean and soybean-derived PCR primers. Number of PCR primer sets tested and results of PCR and sequence analysis in six genotypes of *P. vulgaris*.

	Soybean PCR primers		Common bean PCR primers		Total	
	Amount	% of total	Amount	% of total	Amount	% of total
Primer sets tested[a]	1,499		168		1,667	
PCR and agarose gel analysis						
Primer sets producing no product	295	19.68	50	29.76	345	20.70
Primer sets producing multiple bands	691	46.10	9	5.36	700	41.99
Primer sets producing a single band[b]	513	34.22	109	64.88	622	37.31
DNA sequence analysis						
Multiple amplicons	17	1.13	5	2.97	22	1.32
Single amplicon (STS)	128	8.54	66	39.29	194	11.64
Poor or no sequence data	368	24.55	38	22.62	406	24.35
Fragments with at least 1 SNP	81	5.40	48	28.57	129	7.74
Length of aligned sequence						
Total (bp)	66,085		38,167		104,252	
Mean STS length	516		578		537	
SNPs						
Total	277		237		514	
Frequency (SNPs/Kb)	4.19		6.21		4.93	
Nucleotide diversity (θ^c x 1000)	1.84		2.72		2.16	

[a]The primers were initially used to amplify the DNA of the common bean cultivar Jalo EEP558 followed by DNA sequence analysis of the resulting amplicon. When high quality sequence data were obtained, the STS primers were then used to amplify and sequence genomic DNA of the other five genotypes that are parents of three common bean mapping populations: AND277, BAT93, DOR364, G19833, and Rudá. [b]Primer sets that amplified a single band at annealing temperature of 58/48°C (soybean PCR primers) or 54°C (common bean PCR primers). [c]$\theta = K / aL$; where 'K' is the number of SNPs identified in an alignment of 'n' genotypes and 'L' is the total length of aligned sequences in bp; $a = \Sigma\ 1/(i - 1)$, where i = 2-to-6.

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ABORTIVE SEED DEVELOPMENT IN COMMON BEAN (*PHASEOLUS VULGARIS* L.)

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INTRODUCTION

Embryo defective mutants in *Phaseolus vulgaris* obtained by Ethyl methanesulfonate (EMS) induction were studied to determine the structural and molecular basis of seed abortion in this species. In the wild-type, the classical pattern of seed development showed coordinated differentiation of the embryo proper, suspensor, endosperm tissue, and seed coat. On the contrarily, EMS mutant showed disruption in the normal seed development leading to embryo abortion. Most studies are limited to the early stages of embryo development (1). Little is known concerning the maturation process of the embryo or seed development. The present study was therefore undertaken firstly to detail the structural pattern of embryo development and secondly to investigate the structural basis of embryo abortion in common bean.

MATERIAL AND METHODS

The wild-type and mutant EMS (genotype BAT93) seeds were freshly harvested and eventually nicked with a scalpel to facilitate penetration of fixing solutions. Seeds were then embedded in Technovit 8100 resin for two days at 4°C. Sections 3 µm thick were cut on a Zeiss HM 360 microtome fitted with a tungsten-carbide knife. They were stained with an adapted Toluidine blue O and viewed with a Nikon Eclipse E800 fluorescence microscope.

RESULTS AND DISCUSSION

An embryo-defective mutation was identified by screening plant lines treated with EMS (2). EMS mutant embryos development was considerably delayed compared with wild-type embryos. Shortly after fertilization in wild-type, the embryo begins to develop. Approximately 3 days after fertilization (DAF), the embryo reaches the early globular stage of development (Fig 1A). Approximately 8 DAF, the embryo transform into a heart-shaped embryo with the formation of cotyledons (Fig 1C). Cotyledons expand rapidly by vacuolation and gradually extend to produce a torpedo-shaped embryo (Fig 1D). About 12 days after fertilization, the cotyledons continue to expand with a well-defined embryo axis (Fig1E). In EMS mutant (F2 B52 1.1.14), the first stages of seed development are similar to the wild-type genotype. At 3 DAF, embryos are at the early globular stage (Fig 1F). The tissue organization of the EMS mutant embryo appeared to be normal at this early stage of embryogenesis. At 8 DAF EMS mutant embryos did not exhibit the characteristic heart shape and appeared elongated (Fig 1H). Abnormalities begin to appear in the late heart or early torpedo stage. Embryo development appears to be delayed or arrested (Fig 1J). The typical wild-type bent-cotyledon or mature embryo stages were not observed for mature embryos. In EMS mutant, embryo progresses normally until the late globular stage (Fig 1G). Abnormal suspensor development could be observed in some aborted seeds (Fig 1G, H, I, J). In all cases the suspensor appeared to develop but showed dramatic evolution. The early globular stage contains a morphologically normal suspensor and embryo (Fig 1F). Abnormal divisions in the suspensor first appear at the late globular stage (Fig 1G) and are pursued during subsequent growth (Fig 1G, H, I, J). Cell divisions continue in

both the embryo and the suspensor through the torpedo stage, resulting in an elongated embryo and a suspensor often the size of the embryo (Fig 1I). With often the size we demonstrate that in every case, morphological defects in the suspensor precede visible defects in the embryo. This is consistent with that abnormal growth of embryo in mutant EMS is an indirect consequence of a defect in the suspensor. These results suggest that disruption of development in the suspensor can lead to embryo disruption and partial transformation of the embryo.

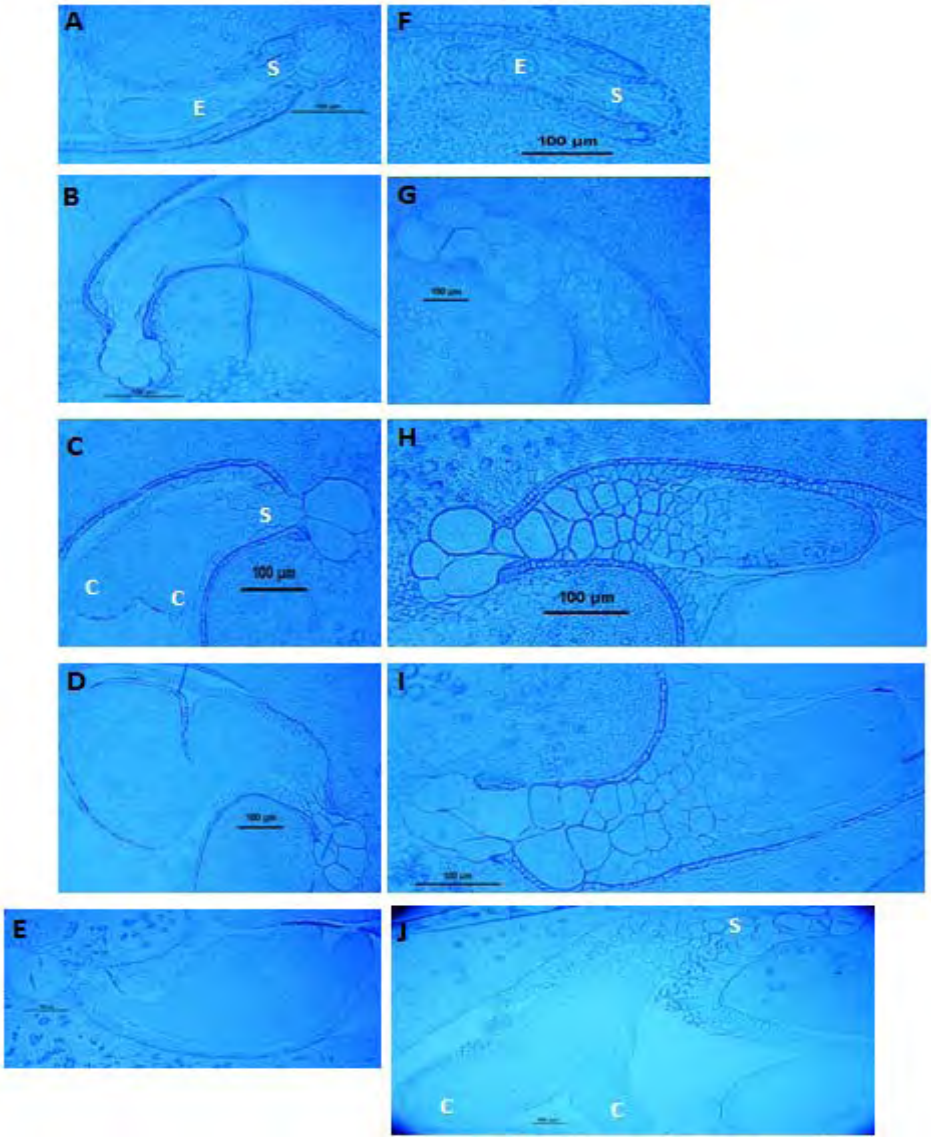

Figure 1. Developmental anatomy of wild-type and EMS mutant embryos. A-F, images of cleared phenotypically wild-type seeds at early globular (A, 3DAF), late globular (B, 7 DAF), heart stage (C, 8 DAF), torpedo (D, 9 DAF), and cotyledonary stage (E, 12 DAF) of embryo development. F-J, images of cleared aborted seed (F, 3 DAF), (G, 7 DAF), (H, 8DAF), (I, 9 DAF), (J, 12 DAF). C, cotyledon; E, embryo; S, suspensor. Scale bars: in A, B, C, D, E ,F ,G, H, I, J, 100 µm.

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RECURRENT SELECTION IN RED BEAN BREEDING

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During the mid 1990s, the Bean Breeding Program of the Universidade Federal de Viçosa (UFV) also included red type bean breeding as part of its research, because of the great importance of this type of bean for Zona da Mata region of Minas Gerais State. This type of grain is well-accepted commercially and is considered the most valuable in this region. However, cultivars available have not met the producers' expectations for being more susceptible to major bean diseases or have not met commercial acceptance. Thus, the development of high yielding lines, resistant to major diseases and displaying well-accepted grains is of fundamental importance. But, to combine into one single line various phenotypes of interest is not an easy task, since it involves a great number of genes. In this case, the main alternative is the use of recurrent selection. This methodology has been applied successfully in bean breeding in the state of Minas Gerais (Ramalho et al., 2005; Silva et al., 2007). Thus, the objective of this work was to evaluate the second cycle of recurrent selection in red bean breeding and to select the best families for recombination.

The experiments were conducted at the Experimental Field of the Department of Plant Sciences - UFV, located in Coimbra-MG, Brazil, at 690 m of altitude (20°45' S and 42°51' W). After the recombination of the best families of the first cycle of recurrent selection, 20 segregating populations were obtained. These populations were advanced in bulk up to the F_5 generation in order to make the red grain pattern uniform, since three lines of the purple type grains (BRS Timbó, VR-2 and VR-3) were introduced into the crossing group. Within each population, 19 plants were selected taking into account the commercial aspect of the grain. These plants constituted the $F_{5:6}$ families, which, together with 20 controls, were evaluated during the 2007 winter season in a simple 20 x 20 lattice. Each plot had one 2 m line. In the next generation ($F_{5:7}$), 2008 dry season, the best families of each population were evaluated together with nine checks in a 13 x13 triple lattice design. Each plot had two 2 m lines. In both trials were evaluated: grain yield, grain aspect and plant architecture (scores from 1 to 5). Rust severity scores were also attributed (1 to 6) during the 2007 winter season as well as angular leaf spot scores (1 to 9) during the 2008 dry season. In the score scale, 1 refers to best grain aspect, best plant architecture and absence of disease. In all the cases, the scores were attributed by more than one appraiser.

There were significant differences ($P<0.01$) among the families for all the characters evaluated. The variability among the families was also confirmed by the estimates of heritability (h^2) that, in all situations, were different from zero, at 95% of probability, with a limit inferior to positive h^2. The family x crop interaction was significant ($P<0.01$), indicating that the families did not present consistent performance in the two seasons for grain yield, plant architecture, and grain aspect. These facts showed the importance of evaluations in different environments.

Table 1 presents the means of 20 families selected for recombination. It is important to emphasize that a family of each population was selected to allow that all genitors are represented in equal proportion. The crossings were performed in a conic scheme so that each family participates in two crossings. Thus, twenty populations will be obtained a gain, constituting the third cycle of evaluation and selection. It is important to emphasize again the occurrence of variability in the population, showing that, after two cycles of recurrent selection, variability is still being observed and that selection progress may be obtained.

Table 1 - Means of grain yield (kg/ha), grain aspect, plant architecture, angular leaf spot and rust severity of the 20 families selected for recombination and acquisition of the third cycle of recurrent selection (C_{II}) compared to the control Ouro Vermelho.

Family	Yield	Grain aspect	Architecture	Angular leaf spot	Rust
284RVCI-2-2	3611	1.8	3.7	5.2	1.5
285RVCI-1-20	3964	1.4	3.8	3.9	3.0
286RVCI-6-44	3754	1.9	3.8	5.8	2.5
287RVCI-5-62	3414	1.7	3.9	4.1	1.2
288RVCI-10-86	4155	1.6	4.2	4.4	2.4
289RVCI-4-99	3717	2.1	4.1	4.0	2.8
290RVCI-12-126	3771	2.3	3.7	5.1	3.0
291RVCI-17-150	4206	1.6	3.6	6.4	2.5
292RVCI-7-159	3438	1.6	4.3	4.5	3.0
293RVCI-17-188	3723	2.0	4.4	4.6	2.6
294RVCI-9-199	3811	2.5	3.0	6.1	2.6
295RVCI-5-214	3968	1.9	3.6	5.2	2.5
296RVCI-16-244	3453	1.7	3.8	3.7	3.0
297RVCI-12-259	4127	2.1	4.3	3.9	2.0
298RVCI-3-269	3701	1.9	3.6	3.6	2.0
299RVCI-11-296	3490	1.7	3.6	5.1	3.4
300RVCI-9-313	3916	1.7	3.8	4.2	2.2
301RVCI-19-342	3285	2.3	3.7	6.8	2.0
302RVCI-12-354	3952	2.7	4.0	5.1	2.5
303RVCI-4-365	3943	1.9	3.5	5.7	1.1
Mean families	3770	1.9	3.8	4.9	2.4
Ouro Vermelho	3427	1.6	3.8	5.3	3.6

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GENERATION ADVANCE IN COMMON BEAN

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INTRODUCTION

Rapid advance of generations toward homozigozity is desirable in breeding programs, especially for traits with low heritability as grain yield. There are evidences in the literature indicating that gains in reduction of life cycle of common bean plants might be accomplished (Costa et al., 1999), what could result in the possibility of advancing a higher number of generations for a given period of time. In order to determine the feasibility of recovering a fertile common bean (*P.vulgaris* L.) plant from seeds starting with 30 days after flowering, the present work was designed.

MATERIALS AND METHODS

The experiment was conducted in 2005 under greenhouse conditions at Lowland Experimental Station, Embrapa Temperate Climate Research Center, Pelotas, Rio Grande do Sul State, Brazil, located at 31° 48' 11"S, 52° 24' 38"W. The land races Guabiju and Chocolate, and the research-derived cultivars TPS Nobre and Carioca were used (Table 1).

Table 1. Seed color, shape and 100-seed weight, plant type and maturity of the cultivars Chocolate, Guabiju, TPS Nobre and Carioca. Temperate Climate Embrapa, Pelotas, RS, 2009.

Cultivar	Seed color	Seed shape[1]	100 seed weight	Plant type[2]	Maturity[3]
Chocolate	Brown	Oblong long	39,4	I	Early
Guabiju	Black	Elliptical	32,9	I	Early
TPS Nobre	Black	Elliptical	21,6	II	Normal
Carioca	Cream brown stripped	Oblong	22,5	III	Normal

[1] According to Antunes et al. (2007)
[2] CIAT, 1987
[3] Early: about 75 days; Normal: about 90 days

Seed harvest time (SHT) was 30, 35, 40, 45 and 50 days after flowering (DAF). SHT 50 DAF constitute the check treatment where pods are already dry. It was performed a split-plot in a RCB design, with cultivar in the main plot and SHT in the sub-plot. The main plot was composed by three 3.0m-rows, 0.5m apart, with 12 plants m^{-1}. At flowering, open flowers were marked to permit collection of pods with equal DAF. Six pods of each treatment were harvested at each DAF and seeds were transplanted to soil. Number of seeds per pod (NSP) was determined and number of seeds that produced plants with new seeds (SSP) were calculated as a function of NSP. Anova and Ancova analyses were performed followed by polynomial regression analysis.

RESULTS AND DISCUSSION

Results have shown that for Chocolate, harvest at 35 DAF is the earlier stage where no loss in SSP was detected (Table 2). For Guabiju, however, the best stage would be 40 DAF. For the cultivars TPS Nobre and Carioca, that displayed a linear evolution for SSP, the best stage would be the one for the best balance between number of days gained in harvest (in comparison to 50 DAF) and the number of plants that produced a new generation(SSP) at that given stage. For TPS Nobre, harvest at 40 DAF, would represent a loss in 39% in SSP with a gain in 10 days in harvest.

Table 2. Variation for number of plants that produced a new generation(SSP) due to cultivar x seed harvest time (SHT) interaction, adjusted for number of seeds per pod (NSP), through polynomial regression analysis and multiple comparison test. Temperate Climate Embrapa, Pelotas, RS, Brazil, 2009.

Cultivar	Model [1]	R^2	Mean [2]	Days after flowering (DAF)				
				30	35	40	45	50
Chocolate	–	–	Transformed	0,81 b	1,21 a	1,37 a	1,48 a	1,42 a
Guabiju	–	–	Transformed	1,16 ab	1,01 b	1,48 a	1,70 a	1,06 b
TPS Nobre	Y = -0,7682 + 0,0532X	0,54	Transformed	0,97	1,00	1,17	1,71	1,95
			Adjusted	0,83	1,09	1,36	1,62	1,89
Carioca	Y = -0,5743 + 0,0493X	0,44	Transformed	0,80	1,30	1,29	1,81	1,78
			Ajusted	0,90	1,15	1,40	1,64	1,89

(1) Chocolate and Guabiju models not shown due to the no significance for orthogonal polynomial components
(2) Transformed means followed by the same letter in the same row, show no difference by Fisher's DMS method (α = 0,05).

This represents a need in seeds of 39% to compensate for the loss in final stand. The implications will reach greenhouse space during winter sowing for a given possible segregating population, an usually important limitation. In summary, the work has shown the existence of variation in cultivar performance for the studied variable -SSP-, that should be considered when generation advance is the goal, mainly for segregating populations where according to the parents used in crossing a greater variation for the best SSP (number of plants that produce a new generation) moment is to be expected.

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INDEX TO SELECT THE BEST SEGREGATING POPULATIONS OF COMMON BEAN

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The breeding programs of common bean in Brazil have aimed to develop highly grain yield varieties with favorable phenotypes for other traits that interest farmers and consumers. By the way, bean breeders have long been interested in modifying plant architecture to obtain plants with erect habit and minimum lodging, as possible while grain yield capacity is maintained or enhanced. In order to selection the best segregation population the breeder must to consider these characteristics simultaneously. Thus this work was carried out to verify if a selection index with equal weight for standardized variables is useful to assessing the genetic values of parents in a diallel crossing.

Partial diallel analysis was used to access the general and specific combining abilities from crosses involving six parents with carioca grain type, commercially acceptable, highly yield but with undesirable architecture (prostate plants) in the group I, with other six parents in the group II that was formed for cultivars with upright plants, but showing some restriction on grain type.

In the diallel matching were obtained 28 $F_{1's}$ of the 36 possible crossing. These 28 combinations, were grew to obtain F_2 and after it, F_3 generations. Trials, with three replications, were conducted in Department of Biology of Universidade Federal de Lavras (UFLA experimental field in order to evaluate these segregating populations, in Lavras-MG, Brazil.

Grain yield (g/plot), plant architecture and lodging (notes ranging 1 to 9 phenotypes for these last characteristics) were obtained and after it, standardized variable Z_{ijk} was obtained by the following estimator:

$$Z_{ijk} = \frac{y_{ijk} - \bar{y}_{.jk}}{s_{jk}} \text{ where,}$$

Z_{ijk} is the standardized value for the character k, in replicate j, for population i; y_{ijk} is the observed value of the character k, in replicate j, for population i; $\bar{y}_{.jk}$ is the overall average of the character k, in replicate j; and, s_{jk} is the phenotypic standard deviation for the character k in replication j.

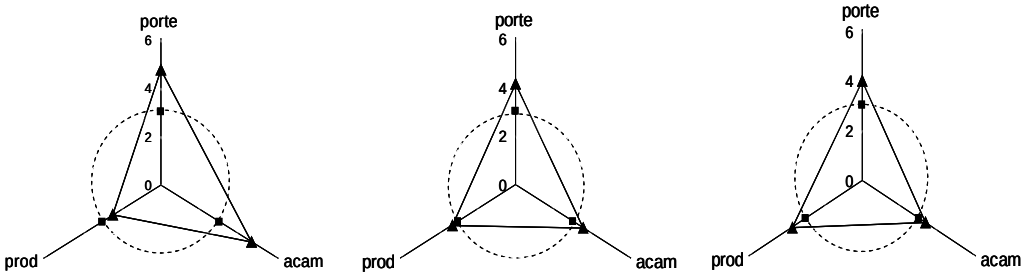
As the value Z_{ijk} assumers positive and negative values was added the value three to obtain only positive estimates, and performed the sum of Z estimated for grain yield, architecture and lodging in order to use it like a index. Diallel analysis were carried out using generations means, according to Griffing's model IV, adapted by Gerald & Miranda Filho (1998) for diallel over lest square method, for index composed by sum of Z.

It was formed that General Combining Ability (GCA) for group I explained 57.5% of total variation among populations. These results allow inferring that the Z index presents predominantly additive genetic control. The higher GCA effect was observed for CVIII8511 (Table 1). Therefore, considering the three characters simultaneously, this cultivar was the best parent overall because its hybrid combinations showed highest performance average. For group II, the highest estimates of GCA were observed for Meia-Noite and RP133 (Table 1)

Table 1 – Average and GCA effects of parents in group I (GCA I) and group II (GCA II) for the standardized variable Z

	RP26	RP133	RP166	Suprema	Valente	Meia-noite	CGCI
CV8511	10.99	9.17	10.93	11.83	9.28	-	1.38
MAII16	8.47	-	6.98	7.54	-	9.01	-1.08
VC3	7.44	8.74	8.63	7.64	8.40	-	-0.64
MAII22	9.55	9.86	7.87	8.46	8.85	10.01	0.13
MAII2	8.68	-	-	10.01	9.48	9.15	0.15
majestoso	8.57	-	8.92	9.46	8.11	-	-0.19
CGCII	0.00	0.18	-0.02	0.02	-0.45	0.57	

Although high average to Z index can be observed for a population it may be deficient in one or more agronomical characters. Therefore, is desirable to associate this estimate with graphic method. In this new situation, each axis of the graphic corresponds to one variable. This procedure was adopted for the three best populations, considering the average of two generations. It is observed in Figure 1, that population CVIII8511 x Supreme showed high estimate of Z due population excellent performance of plant architecture and lodging, however, it was deficient in grain yield. On the other hand, for population 8511CVIII x RP26 and CVIII 8511 x RP166 it was not observed. These latter were much more balanced for these three characteristics in the index.



CVIII8511 x Supremo CVIII 8511 x RP 26 CVIII 8511 x RP 166

Figure 1 - Graphic representation of standardized values for plant architecture (porte), lodging (acam) and yield (prod) by three populations that had high sum of Z.

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COMPARISON OF BREEDING METHODS FOR YIELD SELECTION IN COMMON BEAN SEGREGANT POPULATIONS

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INTRODUCTION

The genetic improvement of plants is among the major contributions of science to the welfare of society. It is estimated that about 50% of the increase in yield of major crops is attributed to the breeding (Raposo et al., 2000). The comparisons among the methods of conducting autogamic plants in segregant populations are very restricted, especially in Brazil. For common beans, the information about it was obtained in other countries (Urrea & Singh, 1994; Ranalli et al., 1996). Thus, it is important to evaluate the relative efficiency of the methods available under the conditions prevailing in the country. The objective of this study was to evaluate and to compare yield in segregating populations of common beans conducted by three breeding methods in order to improve efficiency.

MATERIALS AND METHODS

192 families of common beans with carioca grain type were obtained by hybridization between the lines CNFC 7812 and CNFC 7829. These families were conducted by three breeding methods (SSD, Bulk and Bulk within Family) up to the F_7 generation. For each method, 64 families were evaluated with a common control group of four cultivars. The field trial was conducted at Santo Antônio de Goiás, GO, Brazil. The experimental design used was lattice 14x14, with two replicates and plots were comprised of two rows four meters long spaced apart 0.5 m with 15 seeds per meter. The yield was obtained after harvest and weight of the grains converted to $kg.ha^{-1}$.

RESULTS AND DISCUSSION

The analysis of variance showed that there were significant differences among treatments (Table 1). The bulk and SSD methods showed highly significant differences. There was no significant difference among the controls and controls x methods interaction. The yield had average heritability of 57% that indicates the possibility of success with the selection. The coefficient of variation (22%) indicated a normal experimental precision for beans. It was possible to select twenty best families among the three methods: ten families came from the bulk within the family method, six from the bulk method and four from SSD method and no control was selected. Among the twenty worst families, nine of them originated from the SSD method, six from the bulk method, three from bulk within the family method and two controls. The SSD method was the least efficient method to develop superior families for

yield, since the bulk within families method was the most efficient to develop superior families for this trait. Evaluating families with yield up 2,000 kg.ha^{-1}, it was observed that eleven families came from the bulk within family method, nine from bulk method and eight from SSD method. Considering these results, seems that the bulk within families method is superior when compared to the others, but it is necessary to evaluate these families in a larger number of environments to identify the effect of genotype x environment interaction on the efficiency of breeding methods.

Table 1. Analysis of variance for yield of families evaluated in Santo Antônio de Goiás, GO, Brazil in 2008/2009.

V.S.	D.F.	M.S.
Treatments	**195**	**280403.88****
Bulk within family	63	243582.513*
Bulk	63	320330.9108**
SSD	63	290899.6534**
Controls	3	168391.5676^{ns}
Controls x methods	1	275244.1463^{ns}
Between methods	2	22551.449^{ns}
Error effective	351	131847.8
Efficiency of the lattice (%)		107
CV (%)		22
Mean (kg.ha^{-1})		1647
h^2 (%)		57

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COMPARISON OF METHODS FOR CONDUCTING SEGREGANT POPULATIONS FOR FIBER CONTENT

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INTRODUCTION

Among the autogamous plants, the common beans (*Phaseolus vulgaris* L.) is an outstanding crop in the Brazilian economy especially for its social role (RAMALHO et al., 1993). The nutritional quality of its grains makes it a crop of great importance for the country. The presence of fiber in the food is an important trait due to its direct benefits in human health. Comparisons among the methods of conducting segregant populations of common beans are still scarce and generally their objectives are related to grain yield (RAPOSO, 1999). So, it becomes relevant to quantify fiber content as well as to study the genetic variability of the trait in Brazilian genotypes. (LONDERO, 2005). This research had the following objectives: to quantify the content of crude fiber in populations conducted by three breeding methods, to verify if there is genetic variability among genotypes and to compare the methods utilized.

MATERIALS AND METHODS

192 families of carioca common beans obtained by hybridization between the strains CNFC 7812 and CNFC 7829 were analyzed. These families were conducted by three breeding methods (SSD, Bulk and Bulk within family) up to the F_7 generation. For each method, 64 families were evaluated. The field trial was conducted at the location of Santo Antonio de Goiás, Goiás State, Brazil. The experimental design used was a lattice 14x14, with two replicates and plots were comprised of two rows four meters long spaced apart 0.5 m and seeded with 15 seeds per meter. The harvested grains were utilized for the quantification of crude fiber content by the AOAC (1997) modified method.

RESULTS AND DISCUSSION

The lattice design was of little efficiency (98%), so the experiment was analyzed as a complete randomized block design. A significant difference among families was detected by the F test at the 10% probability level, in the analysis of variance. The coefficient of variation was 10.32%, which indicated a good experimental precision. The heritability estimate was low (16.7%), which lead to the conclusion that the trait is greatly influenced by the environment. There were no differences among family means for each method and so it was possible to compare methods utilizing the mean distribution within each method. It was observed, for the three methods, that genetic variability was present, an indication that it is possible to select superior families (Figure 1). The bulk method was the one that presented the greatest variation in crude fiber content amongst families and that can be observed by the amplitude of the variation of means. It was observed that the number of families obtained with mean crude fiber content greater than 5% (14 families) was similar for the three methods. When the twenty best families were selected across methods, it was realized that five families were obtained through the Bulk method within family, six through the Bulk method and eight through the SSD. The cultivar BRS Cometa was also present within the twenty best populations, showing the

highest value for fiber content (6.02 %). Among the twenty worst families, eight of them originated through the Bulk within family method and six through the other methods. The differences among fiber content and number of superior families among methods were negligible, so it is not possible to identify which method was more efficient for the development of superior families for the fiber content trait. Due to the high environmental influence on this trait, it would be highly advisable to evaluate families over a great range of environments.

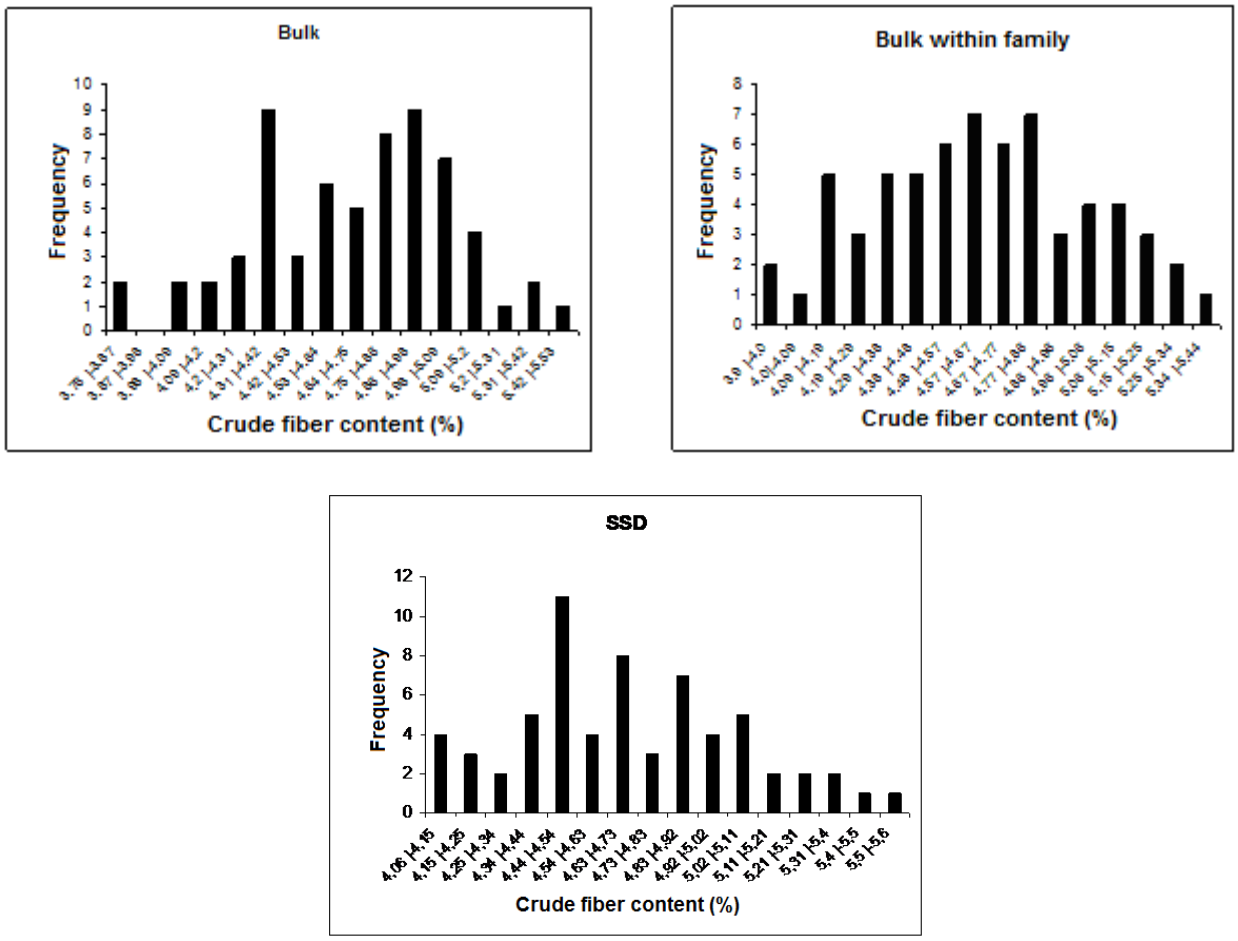

Figure 1. Frequency distribution of crude fiber content obtained in analyses in 2008/2009 by the methods SSD; Bulk and Bulk within family.

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RESPONSE TO GAMETE SELECTION FOR RESISTANCE TO WHITE MOLD IN COMMON BEAN

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INTRODUCTION

White mold [caused by *Sclerotinia sclerotiorum* (Lib.) de Bary] is a serious disease in over 400 species of plants including fruits, vegetables, legumes, and oil-seed crops. In dry and green bean (*Phaseolus vulgaris* L.), white mold is one of the most severe and endemic diseases in North America. Only partial resistance occurs in a few Middle American (e.g., 'ICA Bunsi') and Andean (e.g., A 195, G 122, PC 50, MO 162, Red Kloud, and VA 19) dry bean and green bean (e.g., Cornell 501, NY2060-4). High levels of resistance are found in *P. coccineus* L. and related species some of which has been transferred into common bean. Qualitative and quantitative inheritance of resistance has been reported and >10 QTL with small and large effects distributed across the genome have been identified and mapped in recent years. The availability of only a few dry and green bean and interspecific breeding lines derived from *P. coccineus* with partial resistance, low heritability, unreliable screening methods, architectural avoidance, and large environmental influence have hindered the success in breeding for white mold resistance. Furthermore, breeders often have used only one or two white mold resistant germplasm in crosses and selection methods have been time-consuming or inefficient. The effectiveness of gamete selection (Singh, 1994) for pyramiding and introgressing white mold resistance using multiple-parent populations is not known. The objective of this research was to determine the effectiveness of gamete selection for physiological resistance to white mold in two inter-gene pool double-cross populations.

MATERIALS AND METHODS

Two inter-gene pool double-cross populations, namely Pop I = USPT-WM-1 / CORN 601 // USPT-CBB-1 / 92BG-7, and Pop II = 'Chase' / I9365-25 // ABL 15 / A 195 were developed. For each double-cross, 848 F_1 seeds were produced, using paired plant-to-plant pollinations between male and female single-crosses. F_1 to F_4 of each population were screened in the greenhouse for white mold reaction using the modified cut-stem method (Terán et al., 2006) approximately 30 days after planting. Two mycelial plugs were punched into a 200 ml eppendorf tip and capped over the cut stem. Two separate inoculations on each plant one week apart were made. White mold reaction was scored at 16, 23, and 33 days post inoculation (DPI) using the modified 1 to 9 scale, where, 1-4 = maximum inter-node infection with invasion stopping at the first node, 5-7 = infection past the first inter-node but stopped at the second node, and 8 and 9 = infection past the second node with or without plant death. Eight hundred and forty-eight F_1 plants of each population were screened in the greenhouse. Four hundred and five plants in Pop I and 279 in Pop II, showed intermediate or resistant white mold reaction. Each of those selected plants was harvested separately and their F_1-derived F_2 families ($F_{1:2}$) were evaluated again using six seeds per family. Selection between and within families was practiced and susceptible plants and families were eliminated. Thus, 37 families in Pop I and 16 families in Pop II were selected. All seeds of each selected $F_{1:3}$ family were planted, inoculated, and only resistant plants selected between and within families. Thus, 13 $F_{1:4}$ breeding lines and their four parents from each of Pop I and Pop II were evaluated in a randomized complete

block design with three replicates. Two plants grown in each of three 15 cm diameter pot composed the experimental plot for each replicate. Separate trials were conducted in two different greenhouses in 2008. Data were analyzed with PROC ANOVA procedure in SAS and the mean disease score and Fisher's protected least significant difference (LSD, α=0.05) were calculated.

RESULTS AND DISCUSSION

Despite the low to intermediate levels of resistance to white mold in eight parents, susceptible, intermediate, and resistant plants were detected from F_1 to F_4 in both populations. Thus, in F_1, it was possible to discard 52% of plants in Pop I and 67% in Pop II. Similar segregation patterns also were observed in the subsequent generations, where a marked reduction in the population size was observed. Finally, in F_4, only 1.2% of families from Pop I and 0.9% from Pop II survived the selection process. Thus, gamete selection permitted identification and follow-up of the resistant genotypes and their gametes from their initial occurrence in the F_1 to F_4 (and subsequent generations) in the two inter-gene pool double-cross populations, drastically reducing population size, especially in the F_1 and F_2, even for a quantitatively inherited trait such as white mold resistance although selection method in multiple-parent F_1 was initially proposed for dominant and co-dominant traits (Singh, 1994). The opportunity to progeny-test the selected F_1-derived F_2 families also seemed to have played an important role in eliminating relatively large number of susceptible genotypes between and within families. Had the single plant selection been delayed until the F_2, as in the conventional pedigree method, it would have required screening of 42,400 plants per population with an average fecundity rate of 50 seeds plant $^{-1}$, a monumental and expensive task for most public breeding programs. White mold score for breeding lines in Pop I ranged from 4.1 to 6.2, whereas their parents showed a range from 4.9 to 8.8. In Pop I for the 33 DPI, all breeding lines had significantly lower disease score than susceptible USPT-CBB-1. Four of 13 breeding lines had significantly lower mean disease scores and four were equal to the best resistant parent 92BG-7. Two breeding line with lowest scores of 4.1 and 4.5 had pinto seed color medium seed size and growth habit Type III. White mold scores of breeding lines in Pop II at 33 DPI ranged between 4.5 and 5.7, while scores for parents ranged from 4.6 to 7.5. Ten breeding lines were equal to the best resistant parent A 195. Two breeding lines with score of 4.5 and 4.6 had a pinto seed color despite the fact that no selection for any trait except white mold resistance was practiced from F_1 to F_4.

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SCARLET RUNNER BEAN GERMPLASM ACCESSIONS G 35006 AND G 35172 POSSES RESISTANCE TO MULTIPLE DISEASES OF COMMON BEAN

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Availability of genotypes of cultivated and wild common bean (*Phaseolus vulgaris* L.) and closely related species with resistance to multiple abiotic and biotic stresses would facilitate and expedite genetic improvement of common bean. There are several examples of common bean landraces that possess resistance to multiple stresses. For example, PI 207262 (synonymous with Tlalnepantala 64 and G 1320) from the central highlands of Mexico has moderate to high levels of resistance to angular leaf spot (caused by *Phaeoisariopsis griseola*), anthracnose (caused by *Colletotrichum lindemuthianum*), common bacterial blight (caused by *Xanthomonas campstris* pv. *phaseoli*), powdery mildew (caused by *Erysiphe polygoni*), and rust (caused by *Uromyces appendiculatus*). A breeding line (A 30, a sister line of BAT 93) derived from a double-cross involving PI 207262 contributed to the development and release of popular and widely adapted 'EMGOPA 201-Ouro' (synonymous with A 295) in Brazil in the mid 1980's which had resistance to *Bean common mosaic virus* (BCMV) and all above diseases (Silva et al., 2003). Similarly, Compuesto Negro Chimalteco (synonymous with G 4252), an important source of resistance to rust from the highlands of Guatemala is high yielding and also is resistant to angular leaf spot, anthracnose, root rots, and low soil fertility.

Only partial resistance to *Bean golden mosaic virus* (BGMV), *Bean golden yellow mosaic virus* (BGYMV), and white mold (caused by *Sclerotinia sclerotiorum*) occur in common bean. The BGMV is a severe production problem in common bean in Argentina, Bolivia, and central and southern Brazil, while closely related BGYMV causes severe yield losses in costal Mexico, tropical and subtropical Central America, some Caribbean countries, and southern Florida. Major symptoms of BGMV and BGYMV include plant dwarfing, leaf chlorosis or yellowing, excessive flower and pod abortion, production of deformed and partially filled pods, and severe pod and seed yield reduction. White mold is endemic, widely distributed, and causes severe yield losses in cool and humid regions, especially North America. Some germplasm accessions of the scarlet runner bean (*P. coccineus*), a member of the secondary gene pool of the common bean, are known to possess high levels of resistance to these and other diseases such as angular leaf spot, anthracnose, and Aschochyta blight (caused by *Phoma exigua* var. *diversispora*). The scarlet runner bean germplasm accession G 35006 is resistant to web blight and BGYMV, and G 35172, among others, possess resistance to BGMV and BGYMV.

G 35006 was collected from Guatemala and G 35172 was obtained from Rwanda. Both accessions have a climbing growth habit Type IV, large seeds, and are variable for seed color. G 35172 was first identified to be highly resistant to BGYMV in the field in Guatemala in the 1980's (CIAT, 1986; Singh et al., 2000). The BGYMV resistance of G 35172 was subsequently confirmed in the greenhouse by Dr. F.J. Morales at CIAT (Centro Internacional de Agricultura Tropical), Palmira, Colombia (personal communication, 1987-1988), and the recessive gene *bgm-3* and dominant gene *Bgp-2* imparting resistance to leaf chlorosis and pod deformation, respectively, were transferred in common bean (Osorno et al., 2007). G 35172 was subsequently screened in the greenhouse at University of Idaho, Kimberly and was found to be variable for white mold reaction. That is, some

plants were highly resistant such that after three independent inoculations each using three mycelial plugs, the disease progress stopped at the first node indicating high levels of resistance. On the other hand, some plants exhibited intermediate to susceptible reaction. In the field at Isabela, Puerto Rico, G 35006 was free from angular leaf spot and common bacterial blight. Interspecific backcross inbred lines (IBL) de veloped from G 35006 a nd G 3 5172 w ere s creened f or r eaction t o w hite m old, common ba cterial bl ight, r ust, a nd a nthracnose i n t he g reenhouse. A lso, t he IBL d erived f rom G 35006 w ere s creened f or B GYMV a nd w eb b light r eaction. S ome IBL de rived f rom G 3500 6 exhibited r esistance t o B GYMV, w eb bl ight, anthracnose, w hite m old, a nd r ust. S imilarly, in addition to BGMV and BGYMV resistance, some IBL from G 35172 ha ve exhibited high levels of resistance to anthracnose, rust, and white mold.

Although wide-crosses between cultivated gene pools of and crossing the common bean with wild populations a nd *Phaseolus* species i n t he s econdary a nd t ertiary gene pools ha ve be en r ealized, recovering t he hi ghest l evels of resistance f rom t he di stantly related germplasm ha s s eldom b een achieved. B ecause G 35172 and other accessions of *Phaseolus* species o f t he s econdary gene po ol often are variable for flower color, seed color and size, and reaction to diseases such as white mold, it would be worth screening a relatively large number of plants from each promising scarlet runner bean accession for major traits of interest, and select plants that carry high levels of resistance or trait expression. T hose pl ants s hould t hen be c rossed a nd ba ckcrossed t o a ppropriate c ommon be an genotype. Furthermore, while crossing and backcrossing efforts should be made to produce relatively large number (>75) of F_1 seed of the initial interspecific single-cross. Each plant of the resulting F_1 should be us ed t o de velop a s eparate i nbred b ackcross and/or congruity-backcross (i.e., c rossing alternatively to either parent) family. Because of ten it has be en di fficult to recover hi gh le vels o f resistance to diseases such as BGYMV, white mold, common bacterial blight, and web blight from *P. coccineus* accessions, it ma y be w orth w hile to consider a lternative m ethods t o d evelop interspecific br eeding l ines. O ne a pproach t hat i s of ten us ed i s t o de velop i nterspecific i nbred-backcross and/or congruity-backcross lines without any selection while recurrent- and/or congruity-backcrossing and developing IBL. The second alternative that might merit consideration could be to screen each family for reaction to diseases/traits of interest before backcrossing and to cross only the plants that carry desired resistance. Furthermore, it would be prudent to select and use bulk pollen from a ma ximum num ber of r esistant pl ants w ithin each f amily. This a pproach ma y inc rease th e probability o f de veloping hi gher num ber of IBL w ith hi gh l evels of r esistance. N onetheless, t his approach w ould be c onsiderably m ore l abor a nd r esource i ntensive and may r equire m ore c areful planning. F or e xample, di seases s uch a s BGYMV a nd w eb bl ight c ould not be s creened on the mainland USA. Similarly, white mold and anthracnose could not be screened in Puerto Rico under the field condition. Thus, either a disruptive or bi-directional (tropical versus temperate climate), or alternate s election w ould be r equired t o de velop IBL t hat w ould c arry r esistance t o w eb bl ight, BGMV, BGYMV, white mold, rust, angular leaf spot, and anthracnose.

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IDENTIFICATION OF NEW WILD POPULATIONS OF *PHASEOLUS VULGARIS* IN WESTERN JALISCO, MEXICO, NEAR THE MESOAMERICAN DOMESTICATION CENTER OF COMMON BEAN

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Recent research on microsatellite marker diversity has provided confirmation for two major features of genetic diversity of the Mesoamerican gene pool of common bean (Kwak et al. 2009). First, the Mesoamerican domesticated gene pool resulted from a single domestication and, second, this domestication took place in a small region located in the Lerma-Santiago basin, primarily in the state of Jalisco in west-central Mexico (Fig. 1). Earlier research already had posited a single domestication in the Mesoamerican gene pool based on phaseolin and AFLP data (Gepts et al. 1986; Gepts 1988; Papa and Gepts 2003). Furthermore, phaseolin data also provided evidence for a center of domestication in Jalisco (Gepts 1988).

The identity of the putative region of domestication is of particular interest to understanding the adaptation of Mesoamerican common bean to biotic and abiotic environmental conditions. The domestication area is located between the Transverse Neovolcanic Axis to the south and the southern edges of the Sierra Madre Occidental and Altiplano Mexicano to the north. Altitudes of the different wild populations that are part of the domestication group range from 1400 to 2100 m. The climate of this region is characterized as subtropical (temperature of the coldest month between 5 and 18°C), sub-humid (between 4 and 6 mo of humidity), and semi-warm (average annual temperature between 18 and 22°C) (López Soto et al., 2005). The original vegetation changes gradually from a conifer–oak forest at the western end through a dry deciduous forest in the central part to a drier thorn forest at the eastern end of this area (Rzedowski, 1981).

The authors recently conducted an exploration in the states of Colima and Jalisco to visit wild *P. vulgaris* populations in this area, particularly the westernmost wild population implicated in the Mesoamerican domestication of common bean. A number of findings resulted from this exploration. First, all of the wild bean populations that had been collected in years past in this region are still in existence. This is particularly the case for the westernmost population of the putative domestication area just mentioned. This population, located near the town of Mascota, Jalisco, was collected by D.G. Debouck in 1978. Second, many of the wild bean populations are actually more extensive than what can initially be perceived as shown in Fig. 2. To gauge the actual size of a population, a more extensive local survey needs to be conducted. Third, 13 additional wild bean populations not yet included in germplasm banks (as far as we can tell) were identified (Fig. 1). Thus, wild *P. vulgaris* populations are in a good state of conservation.

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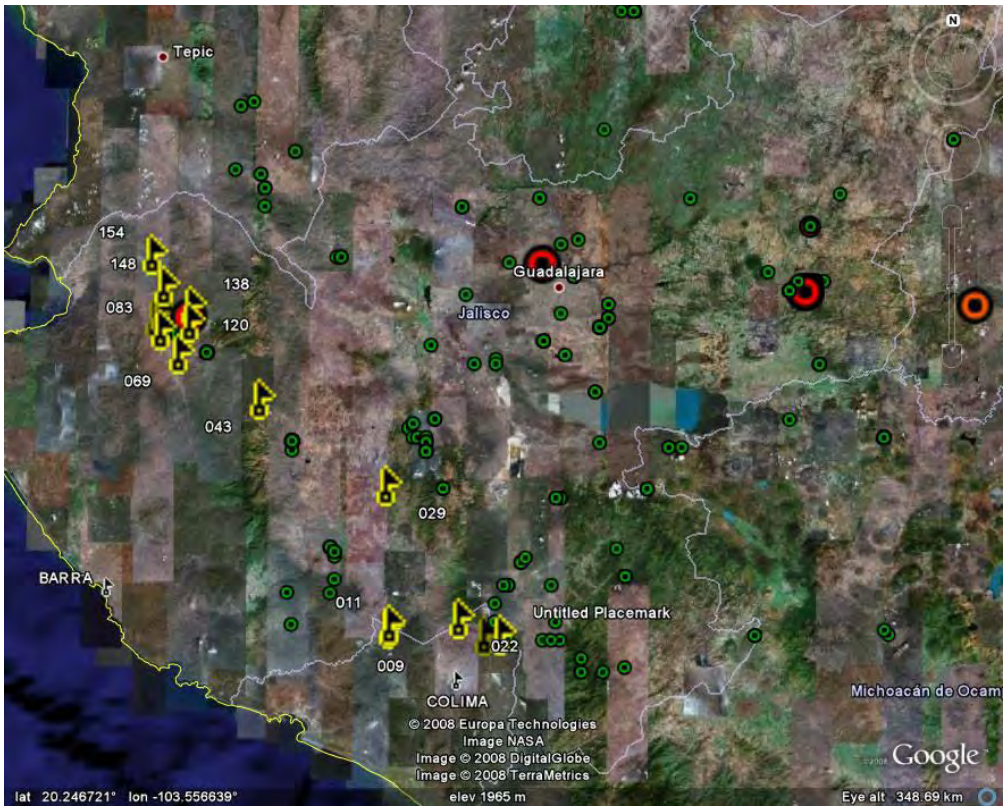

Figure 1. Location of the presumed Mesoamerican domestication area and distribution of newly identified wild *Phaseolus vulgaris* populations in the states of Colima and Jalisco, Mexico. Large (red) dots: presumed wild beans ancestral to the Mesoamerican domesticated gene pool. Smaller (green) dots: other wild bean populations. Flags (yellow): newly identified populations (December 2008).

Figure 2. Distribution of plants (white flags) in a previously undescribed wild bean population encountered in December 08. The horizontal white bar in the left lower corner represents 25 m. The aerial photo shows an extensive distribution of this population. However, the actual boundaries of the population could not be defined because of time limitations.

NUTRITIONAL COMPONENTS VARIATION IN COOKED COMMON BEANS (*PHASEOLUS VULGARIS* L.)

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INTRODUCTION

The common beans is widely consumed by Brazilians, specially the poor people. It is necessarily consumed after cooking in order to develop the aroma and the acceptable consistency demanded by consumers. However, many physical and chemical changes take place in cooked grain. This work aimed to determine the proximal composition of three cultivars of common beans from 'black' and 'carioca' commercial groups (raw and cooked material), the apparent amylose content in raw and cooked grains and in its broth after cooking, in order to observe some variations as a result of cooking process.

MATERIALS AND METHODS

The common beans cultivars from 'black' (BRS-Esplendor) and 'carioca' (BRS-Horizonte, IPR-Juriti) groups were cultivated between May and June/2008 at Capivara farm, Embrapa Rice and Beans, in Santo Antônio de Goiás city, Goiás State, Brazil and were harvested in October/2008.

The harvested grains were milled, sieved (65 *mesh*) and submitted to the following analyses: apparent amylose content estimation (AA) according to JULIANO (1979), proximal composition (moisture, crude protein (microKjeldahl), ash, lipids and dietary soluble and insoluble fiber) based on AOAC INTERNACIONAL (1997), with modifications. The total dietary fiber (TDF) content was obtained from the sum of its soluble (SDF) and insoluble (IDF) fractions values, while the carbohydrates were calculated by the difference between the value 100 and the other components contents (moisture, protein, lipids and ash). The grains were embedded in distilled water for 18 hours at room temperature before cooking in beaker with glass lid on the heating plate at the proportion water:beans (v/v) of 2:1. The cooking time was previously defined by the Mattson cooker (PROCTOR & WATTS, 1987; adapted by Embrapa Rice and Beans). The soluble solids contents were determined in the broth (PLHAK *et al*., 1989), which was separated from the cooked grains and individually dried in the oven (60°C, 42 hours), then, milled and sieved (65 *mesh*), and finally submitted to the AA analysis. For the proximal composition analysis of cooked samples, the dried separated fractions (grains and broth) of each cultivar were joined and homogenized again and considered as a single sample.

RESULTS AND DISCUSSION

The BRS-Esplendor cultivar had the highest cooking time of 27min, when compared to the other ones, BRS-Horizonte (26min) and IPR-Juriti (24min), showing a normal cooking resistance.

The BRS-Esplendor broth was more consistent with soluble solids content of 10,34% while the BRS-Horizonte and IPR-Juriti had 8,94% and 8,77% of soluble solids respectively.

Negative values for AA were found for each broth (BRS-Horizonte: -5,67%; IPR-Juriti: -4,87%; BRS-Esplendor: -3,54%) what means very low contents of amylose, not detected by the limit of the applied method. The cooked grains showed the following values: BRS-Horizonte: 7,84%; IPR-Juriti: 10,47% and BRS-Esplendor: 8,81%. In raw materials the highest amylose content was obtained for IPR-Juriti, 8,47%, followed by BRS-Esplendor, 7,48% and BRS-Horizonte, 6,21%. A similar profile was obtained for cooked samples (broth + cooked grains): IPR-Juriti (10,50%); BRS-Horizonte (7,51%); BRS-Esplendor (9,51%). It could be noticed an increase of AA content after cooking in all cultivars.

According to Table 1, for the three cultivars, it was observed a decrease in carbohydrate, ash and moisture contents after cooking, while the lipids and protein contents increased. This behavior was also mentioned by RAMIREZ-CÁRDENAS (2006). The TDF values were higher in cooked grains of BRS-Esplendor and BRS-Horizonte, while the cooked IPR-Juriti had a decrease resulted from the both fractions (SDF and IDF) contents reduction. The variation observed for BRS-Esplendor is a result of the significant increase in IDF content, while for BRS-Horizonte it was due to SDF more significant increase.

Table 1: Proximal composition of common beans cultivars ($g.100g^{-1}$, dried basis)

Common Bean Cultivar	Carbohydrate	Ash	IDF[1]	SDF[2]	TDF[3]	Lipid	Protein	Moisture
BRS-Esplendor (raw)	65,46	4,21	16,70	6,00	22,70	1,60	22,2	10,74
BRS-Horizonte (raw)	60,89	4,10	19,25	4,45	23,70	1,58	26,9	10,63
IPR-Juriti (raw)	67,94	3,88	19,67	6,29	25,96	1,38	20,5	10,18
BRS-Esplendor(cooked)	61,53	3,00	19,32	5,62	24,94	1,61	24,4	9,46
BRS-Horizonte(cooked)	58,61	3,36	19,49	5,75	25,24	1,93	28,3	7,80
IPR-Juriti (cooked)	64,76	2,50	17,30	5,43	22,73	1,68	21,8	9,26

[1]IDF: Insoluble dietary fiber, [2]SDF: Soluble dietary fiber, [3]TDF: Total dietary fiber.

CONCLUSION

After common beans cooking it could be noticed a variation in nutritional components and apparent amylose contents, although these changes did not take place as a linear tendency. The BRS-Esplendor and BRS-Horizonte grains showed good results for both nutritional and cooking quality aspects.

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MOISTURE AVAILABILITY AND COOKING EFFECTS ON PHENOLICS AND OLIGOSACCHARIDES CONTENT IN BEAN SEED

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In Mexico, different seed classes of beans are produced in different regions and moisture conditions, thus large differences are observed in seed yield and quality, including the seed content of functional phytochemicals. The objective of this work was study the effect of terminal drought and cooking over total phenols and oligosaccharides content in the seed of different bean cultivars. A trial was conducted under full irrigation and terminal water stress by halting irrigation from mid pod filling onwards at two locations, Celaya, Guanajuato in central Mexico (2006) and Los Mochis, Sinaloa in the west coastal lowlands (2006/07). Cultivars included in the test were from different race: Pinto Durango and Pinto Saltillo (Durango), Flor de Mayo Noura, Flor de Mayo Anita, Flor de Junio Marcela and FJB07001 (Jalisco), Azufrado 26 and Azufrado Noroeste (Nueva Granada). After harvest, seed samples were analyzed for condensed tannins, total phenols and oligosaccharides in raw and cook grain.

Under both moisture conditions, average across cultivars the content of total phenols was higher in raw beans from Los Mochis, but the reduction was higher after cooking in comparison with the beans harvested in Celaya. In Celaya the content of total phenols was slightly higher in the seed of PT Zapata, FM Noura, FJ Marcela and FJB 07001 grown under terminal stress. There are reports on the differential effects of the test environment on the seed content of some phytochemicals (Muzquiz *et al*., 1999). In general, cooking affected significantly the content of phenols, causing a reduction higher than 60% on the beans from Los Mochis and of 50% in the beans from Celaya, thus the genotype by environment interaction remains after cooking the beans. Across sites and moisture conditions cultivars in the Azufrado-Yellow class (Nueva Granada), along with the slow darkening bean Pinto Saltillo, show low phenols content.

In regard to oligosaccharides, its content in raw beans was higher in the seed from Los Mochis under irrigation, and the opposite was observed in Celaya, where the content was higher in the beans from the stressed conditions (Table 2). Across sites and moisture conditions, the higher content was shown by cv. Flor de Mayo Anita whereas the lower was in the seed of the Azufrado (yellow) seeded cultivars along with Pinto Saltillo. The relatively high oligosaccharides content in the seed of the Jalisco race cultivars grown under terminal drought at Celaya and after being cook, is remarkably (Table 2), these cultivars were bred at this site. The reduction due to cooking was quite high and differential across cultivars, with Jalisco race cultivars (cream-pink) along with Pinto Zapata displaying higher contents than Azufrado cultivars and Pinto Saltillo. Perhaps that low oligosaccharides content in the seed of yellow cultivars and in Pinto Saltillo has a relationship with its high acceptability by consumers, since these compounds have been related to flatulence. However, we now know that oligosaccharides have a relationship with the control of colon cancer in rats (Rios-Ugalde *et al*., 2007). Reduction of up to 80 % in isoflavones and oligosaccharides content in wild beans after cooking was also reported (Diaz-Batalla *et al*., 2007).

The loss of phytochemicals after cooking may be a concern, but their remnants after cooking have important implications in health. Cultivars of the Flor de Mayo and Flor de Junio seeded types (cream-pinky types, Jalisco race), along with Pinto Zapata displayed higher contents of phenols in

raw and cook grains. The seed of Pinto Zapata has been outstanding on the reduction of colon cancer tumors and blood glucose and cholesterol in experimental male Sprague-Dawley rats; meanwhile Flor de Junio Marcela reduced the survival of rats (Rios-Ugalde *et al*., 2007). The seed of Flor de Junio Marcela used in this experiment showed higher contents of phenols than Pinto Zapata. In spite of numerous assays, up to date we still do not know which are the seed components that confer the beneficial effects on healthy, therefore, we will start working with extracts of the main or suspected health related phytochemicals.

Table 1. Total phenols in the seed of eight bean cultivars grown at two locations under two moisture levels.

	Los Mochis				Celaya			
	Irrigated		Stressed		Irrigated		Stressed	
Cultivar	Raw	Cook	Raw	Cook	Raw	Cook	Raw	Cook
PT Zapata	225±10[1]	96.6±6	231±19	88.7±2.8	199±9.2	86.8±6	191±7	113±3
PT Saltillo	130±13	44.6±3	165±5	57.7±2.5	123±2.9	55.5±2	125±6	74.9±5
FM Noura	267±6	83.7±3	225±15	82.2±2.9	196±8.9	106±4	180±14	100±10
FM Anita	332±21	98.8±2	247±21	101±2.1	238±8.3	98.7±1	343±61	114±3
FJ Marcela	302±1.5	90.5±6	304±17	85.1±3.2	227±17	105±1	214±9	100±5
FJB 07001	254±6.5	92.4±1	254±25	89.2±2.9	201±7.6	102±6	206±6	108±1
Azufrado 26	146±12	60.4±4	147±10	60.9±1.9	115±9.5	72±3	119±1	74.4±2
A. Noroeste	137±3	62±6	145±8	55.8±1.3	89±1.7	62.5±4	111±1	67.3±1
Average	**224.1**	**78.6**	**214.8**	**77.6**	**173.5**	**86.1**	**186.1**	**94**

[1] mg per 100 g

Table 2. Oligosaccharides content in the seed of eight bean cultivars grown at two locations under two moisture levels.

	Los Mochis				Celaya			
	Irrigated		Stressed		Irrigated		Stressed	
Cultivar	Raw	Cook	Raw	Cook	Raw	Cook	Raw	Cook
PT Zapata	296±82[1]	43± 20	300±25	71±6.3	700±28	44±13	865±85	54±26
PT Saltillo	44.2±5	11.9±3	41.7±.3	13±4.7	27.5±8	17±2	102±5.1	8.6 ±4
FM Noura	1458±204	58.1±6	991±105	45±3.5	1133±15	81±19	1406±88	143±39
FM Anita	3357±583	74.8±6	3290±631	23±2.7	1902±221	107±23	1888±100	182±48
FJ Marcela	1541±446	37.1±8	1044±44	31±3.6	1187±51	47±14	1232±155	115±34
FJB 07001	576±66	56.6±1	391± 54	23±7.1	771±69	48.1±9	1048±97	117±33
Azufrado26	5.8±1	5.4±1	21±4	11.3±1	30.9 ±2	7±2.4	18.6±1.9	14.3±1
A.Noroeste	17.4±2	9.9±4	20±2	13.5±3	11.9 ±2	7±2.4	11.4±2.4	18.4±2
Average	**911.9**	**37.1**	**762.3**	**28.8**	**720.4**	**44.9**	**821.4**	**81.6**

[1] mg per 100 g; Key words: phenolics, oligosaccharides, seed types.

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A NEW, VIRULENT, BROAD HOST RANGE BEAN-INFECTING BEGOMOVIRUS FROM PUERTO RICO: RHYNCHOSIA MILD MOSAIC VIRUS

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INTRODUCTION

Members of the genus *Begomovirus* (*Geminiviridae*) are circular, single-stranded (ss) DNA viruses that are naturally infect numerous ruderal, indigenous and introduced dicotyledonous plants in tropical/subtropical habitats. Begomoviral genome arrangement can be bipartite (DNA-A and DNA-B; ~2.6 kb each) or monopartite (~2.8 kb). All New World indigenous begomoviruses have been shown to contain a bipartite genome. Begomoviruses are transmitted by the whitefly vector *Bemisia tabaci* (Genn.) complex, which comprises divergent haplotypes and biotypes that vary in aggressiveness and other behaviors that can influence begomovirus spread (Brown 2007). Some begomoviruses have host-shifted (species-, genus-, family-levels), enabling infection of domesticated plants in which they can cause significant crop loss.

Previously, two begomoviruses were identified in Puerto Rico that infected bean (*Phaseolus vulgaris*), *Bean golden yellow mosaic virus* (BGYMV) (Bird and Sanchez, 1971) and *Macroptilium mosaic Puerto Rico virus* (MaMPRV) (Idris et al., 2003). Also MaMPRV has been identified there in native fabaceous species and in bean. Until recently, BGYMV and its close relatives were prevalent in bean throughout the Caribbean region and in Florida (Blair et al., 1995; Bird Maramorosch, 1978; Faria et al., 1994). However, following the introduction of the Old World B biotype whitefly *Bemisia tabaci* (Genn.) in Puerto Rico during ~1989, BGYMV diminished in prevalence, presumably because this non-native vector does not effectively transmit BGYMV between *P. vulgaris* in which the virus is presumed to have been bottlenecked. Several other begomoviruses infect *P. vulgaris* in the tropical Americas and SW-US, including *Bean golden mosaic virus* (BGMV), *Bean calico mosaic virus* (BCaMV), *Chino del tomato virus* (CdTV), *Cotton leaf crumple virus* (CLCrV), *Melon chlorotic leaf curl virus* (MCLCV), and *Squash leaf curl virus* (SLCV). Also, in Puerto Rico several begomoviruses naturally and/or experimentally infect bean and one more indigenous species, including MaMPRV [*Macroptilium lathyroides*], *Rhynchosia mosaic virus* [*Rhynchosia minima*] and *Jatropha mosaic virus* [*Jatropha gossypifolia*; *P. foetida*].

MATERIALS AND METHODS

R. minima plants exhibiting mild mosaic symptoms reminiscent of begomovirus infection were observed in PR during the summer, 1997. Total nucleic acids were extracted and circular ssDNA was subjected to rolling circle amplification (RCA). RCA products were subjected to restriction endonuclease digestion and *Sac*I was found to linearize both the DNA-A and DNA-B component (~2.6 kb, each). Clone viral inserts (n = 8) subjected to capillary DNA sequencing.

RESULTS AND DISCUSSION

Eight clones bearing a fragment of ~2.6 KB were sequenced using primer walking. Comparative sequence analysis revealed that five and three clones were of the DNA-A and DNA-B components, respectively. The genome organization was similar to that of other bipartite begomoviruses in that

six and two open reading frames (ORF) of characteristic size, position and orientation were identified in the DNA-A and DNA-B components, respectively. The DNA-A (n = 5) and DNA-B components (n=3), respectively, shared 98-99% nucleotide (nt) identity, indicating the presence of a single species. The common region (ca. 200 bases) was 99% identical, indicating that they are cognate components. This newly described begomoviral species is herein referred to as Rhynchosia mild mosaic virus (RhMMV). Phylogenetic analysis of the DNA-A component with well-studied begomoviruses revealed that the closest relative of RhMMV are *Macroptilium mosaic Puerto Rico virus* (MaMPRV) and *Rhynchosia golden mosaic virus* (RhGMV), at 80%. The RhMMV DNA-B component shared 64% and 62% nt identity with RhGMV and *Cabbage leaf curl virus* (CaLCV), respectively.

Biolistic inoculation of *R. minima* and *P. vulgaris* seedlings with the dimeric RhMMV clones indicated the clones were infectious based on symptom development 7-10 days post-inoculation. RhMMV caused severe mottling of 'Red Kidney' bean leaves (Fig. 1), compared to the mild foliar symptoms characteristic of BGYMV. *R. minima* leaves exhibited mild mosaic symptoms reminiscent of those observed in the field, thereby fulfilling Koch's Postulates. This newly described begomovirus has potential to become problematic in cultivated bean in Puerto Rico. In addition, the infectious clones may be useful for germplasm screening, given the extinction of BGYMV where bean varietal screening has been traditionally carried out in Puerto Rico.

Figure 1. Symptoms of RhMMV in 'Red Kidney' bean biolistically inoculated with infectious clones.

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GENETIC VARIABILITY IN POPULATIONS OF *GLOMERELLA CINGULATA* F. SP. *PHASEOLI*

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INTRODUCTION

The causal agent of common bean anthracnose, *Colletotrichum lindemuthianum* (Sacc. & Magn.) Scribner, asexual phase, presents a wide genetic variability that has complicated the development of resistant cultivars (Silva et al., 2007). The mechanisms responsible for this variability are very little understood. Probably, the sexual reproduction is one of those processes that contribute to increase it. In Brazil, the sexual phase *Glomerella cingulata* f. sp. *phaseoli*, has been isolated in laboratory, without the use of inducers, from stems, leaves and pods lesions collected in experimental and yield common bean fields (Camargo Junior et al. 2007). The objective of this study was to make genetic variability available in populations of *G. cingulata* f. sp. *phaseoli*, collected in Brazil, using RAPD analysis.

MATERIALS AND METHODS

Five populations of *G. cingulata* f. sp. *phaseoli* were collected in experimental and yield common bean fields in the states of Minas Gerais (Lavras e Lambari) and Paraná (Guarapuava e Turvo), Brazil, during the year 2006. 40 random ascospores were obtained from each population. A total of 200 isolates were grown in liquid medium M_3 for 5 days in a rotary shaker (110 rpm at 22°C). For RAPD reactions, genomic DNA was performed according to Raeder & Broda (1985). 300 oligonucleotide primers were carried out and only those with highly intensity and reproducible bands were selected for analysis. Amplification products were separated by electrophoresis and visualized under Ultraviolet light. The genetic similarities and clustering analysis were performed using the program GDA (Genetic Data Analysis) for Nei & Li coefficient (1979) and UPGMA, respectively. The diversity inside five populations was measured with Shannon index for each population (Shannon, 1948).

RESULTS AND DISCUSSION

Twenty eight oligonucleotide primers (OP M-1, OP M-2, OP M-3, OP M-4, OP M-5, OP M-6, OP M-7, OP M-8, OP M-9, OP M-10, OP M-11, OP M-12, OP M-13, OP M-14, OP M-15, OP M-16, OP M-17, OP M-18, OP M-19, OP M-20, OP L-1, OP L-2, OP L-3, OP L-4, OP L-5, OP L-6, OP L-7, OP L-8) of Operon kit, among the 300 carried out were employed in the molecular analysis. These primers amplified 128 polymorphic bands. An average of 4.57 bands was generated per primer.

The genetic similarity among the isolates ranged from 0.43 a 1.0. The UPGMA cluster analysis allowed the identification of 73 different groups (Figure 1). Five main groups were found, which showed the five populations. Shannon index's estimates of genetic diversity ranged from 0.0827 (TUR), the least diverse population, to 0.3171 for the most diverse population (VAL). These values found based on populations originated from few plants are compared to studies performed by Silva et al. (2007) with asexual populations originated from a great number of isolates from several locations. Theses results indicate the great potential of the sexual reproduction to generate variability in this pathogen.

Table 1. Genetic variability of isolates of *Glomerella cingulata* f. sp. *phaseoli* for the five populations, Brazil.

Population	Similarity coefficient	Shannon index (*I*)
GUA	0.59-0.97 (0.38)	0.2010
MAJ	0.60-1.00 (0.40)	0.1348
TUR	0.80-1.00 (0.20)	0.0827
VAL	0.77-0.99 (0.22)	0.3171
VCU	0.54-0.97 (0.43)	0.1452

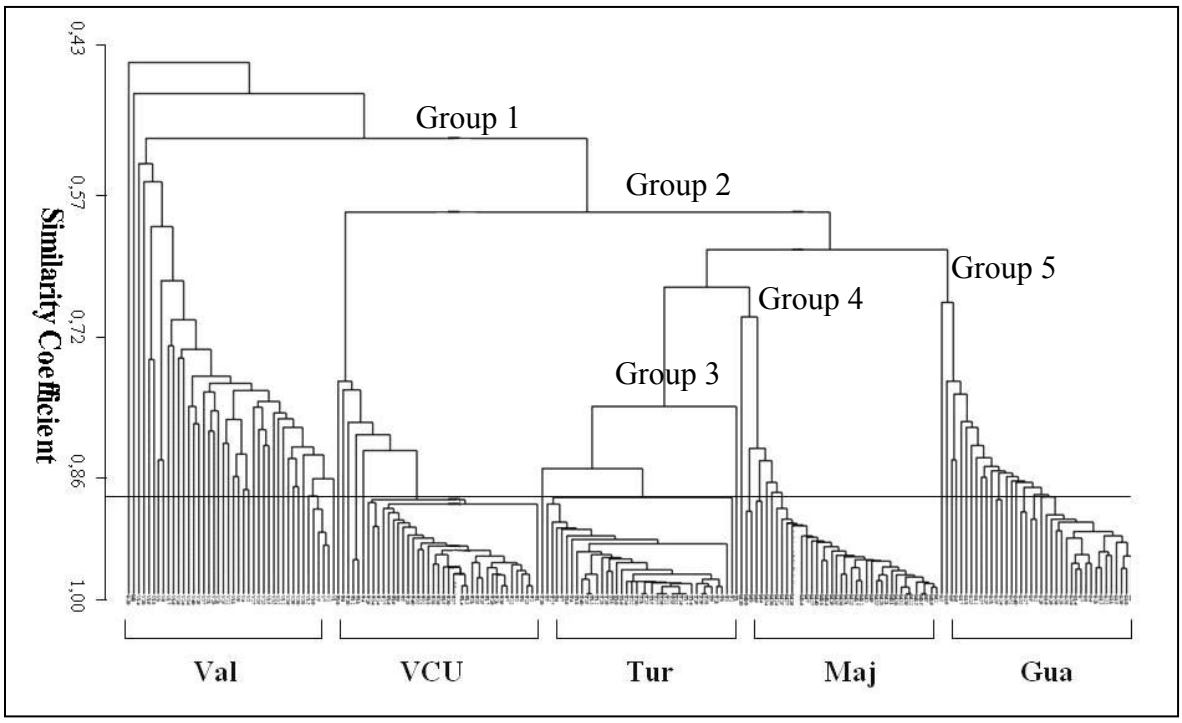

Figure 1. Cluster analysis showing the relationship between all 200 isolates of *G. cingulata* f. sp. *phaseoli*, Brazil.

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RACES OF *COLLETOTRICHUM LINDEMUTHIANUM* IN RHODOPPI MOUNTAINS, BULGARIA AND LANDRACES RESISTANCE

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Common bean (*Phaseolus vulgaris* L.) and runner bean (*P. coccineus* L.) are the most widespread crops in the Rhodoppi Mountains of Bulgaria. The landraces cultivated in this area are large seeded, with white or mottle seeds, climbing habit type and are usually grown in small-scale farms in mixture with maize and potato. The predominantly cold and humid conditions during the period of vegetation, the lack of crop rotation, and usage of seeds of own production favored the occurrence and development of anthracnose. Seven races of the causal agent of the anthracnose, *C. lindemuthianum* have been reported in Bulgaria since 2000 (Kiryakov, 2000; Kiryakov and Genchev, 2004). However, no information has been reported for race diversity of the bean anthracnose pathogen in Rhodoppi Mountains, except the existence of race 6 in the area of Smolyan (Kiryakov and Genchev, 2004). On the other hand, only local landraces are cultivated in this area and there is not information for their resistance to the pathogen races widespread. Study on the anthracnose race diversity will help to evaluate the resistance of the main landraces cultivated in this area with a view of selecting commercial cultivars. Thus, the objectives of this work were to study anthracnose race diversity in Rhodppi Mountains and to evaluate the resistance of landraces to them.

MATERIALS AND METHODS

Thirty-four single-spore isolates were isolated from the pods and seeds with the anthracnose symptoms, which were collected during 2006 from 6 locations in Rhodppi Mountains. The isolates were obtained from primary culture after dilution procedure on PDA and Mathur's medium. Seven-day old seedlings from the 12 standard differential cultivars (Pastor-Corrales, 1991) were inoculated by spraying spore suspension (10^6 spore/ml) from each isolate. The plants were placed in mist chamber for 72h in the greenhouse at 20-22/16-19˚C day/night. The temperature was maintained the same after removing the chamber. Fifteen *P.vulgaris* and eight *P. coccineus* landraces were inoculated by an isolate of races determined in this study following the procedure described above. The landraces were collected from the area of Devin (altitude 710 m), Rakitovo (970 m), Smilyan (680 m), Konstantinovo (970 m), and Gella (1400 m) during 2006-2007. The coding of landraces was formed from the name of the location plus a number. The disease reaction of differential cultivars and landraces was scored 10 days after inoculation by a 9-degree scale (Genchev and Kiryakov, 2005). Score 1 to 3 meant a resistant phenotype in race determination.

RESULTS AND DISCUSSION

Four races were determined on the basis of the virulence of 34 single-spore isolates to the differential lines (Table 1). Race 22 were predominantly isolated (16 isolates) followed by race 6 (9 isolates), race 2 (6 isolates) and race 54 (3 isolates). All of the isolates had a virulent phenotype typical for the Andean-specific isolates of *C. lindemuthianum*. Races 22 and 54 were identified for the first time in Bulgaria, whereas race 6 was isolated previously from samples collected from the area of Smolyan (Rhodoppi Mountains) and Radoil (Rila Mountain) in 2003 (Kiryakov and Genchev, 2004). Race 2 was isolated from the plant samples collected in 2000 from some locations in Pirin Mountain (Kiryakov, 2000).

The results obtained showed a lower diversity of the pathogen despite the higher diversity of the bean genotypes cultivated in each of the studied location. Only two pathotypes were obtained in the area of Devin (races 22 and 54), Grohotno (races 6 and 22) and Sedlarovtzi (races 22 and 6), and one pathotype in Rakitovo, Dospat (race 2) and Smilyan (race 6). Probably, regardless of higher phenotype diversity, the landraces cultivated in the Rhodoppi Mountains had the same or similar resistant spectrum.

Three out of 15 *P.vulgaris* landraces were highly resistant to races 2, 6, 22 and 54 – 'Devin 11', 'Rakitovo 11' and 'Smilyan 27'. These landraces had white seeded coat color, type IVa stem habits and 1000 seed weight in the range of 300-500 g. All of the eight *P. coccineus* landraces, with the exception of two were highly resistant to the investigated races. The resistant landraces had white to motley seeds, climbing habit type (IVa) and 1000 seed weight 1050 to 1600 g. All of the highly resistant landraces from the two *Phaseolus* species were included in the DAI Bean Breeding Program for developing of commercial cultivars appropriate for cropping in the mountain areas in Bulgaria.

Table 1. Races identified on a differential set of 12 bean cultivars, among 34 isolates of *C. lindemuthianum* collected in six locations in Rhodoppi Mountains

Cultivar	Gene Pool	Resistance gene	Binary code	Race 54	Race 22	Race 6	Race 2
Michelite	MA	*Co -11*	1	R*	R	R	R
MDRK**	A	*Co -1*	2	**S**	**S**	**S**	**S**
Perry Marrow	A	*Co -1*^{3}	4	**S**	**S**	**S**	R
Cornell 49-242	MA	*Co -2*	8	R	R	R	R
Widusa	A	*Co -1*^{5}	16	**S**	**S**	R	R
Kaboon	A	*Co -1*^{2}	32	**S**	R	R	R
Mexico 222	MA	*Co -3*	64	R	R	R	R
PI 207 262	MA	*Co -4*^{3}*;Co-9*	128	R	R	R	R
TO	MA	*Co -4*	256	R	R	R	R
TU	MA	*Co -5*	512	R	R	R	R
AB 136	MA	*Co -6;co-8*	1024	R	R	R	R
G 2333	MA	*Co -4*^{2}*;Co-5*$^{2;}$*Co-7*	2048	R	R	R	R
Number of isolates				3	16	9	6

*R-resistant; S-susceptible; **Michigan Dark Red Kidney.

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TOTAL SOLUBLE CARBOHYDRATES AND SUCROSE IN COMMON BEAN CULTIVAR MEXICO 222 INOCULATED WITH *COLLETOTRICHUM LINDEMUTHIANUM* RACE 23

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INTRODUCTION

In plants, the carbon metabolism is composed by several types of sugar, among them sucrose, glucose fructose and starch are normally organic compounds found in higher quantities. These compounds possess the capacity to assume transport, signaling and storage functions; however under abiotic and biotic stress conditions could provide their accumulation in vegetal tissues (Lobato et al., 2008). In common bean crop, the biotic stress due to the incidence of anthracnose, caused by fungus *Colletotrichum lindemuthianum*, is one of the limiting factors of crop yield (Pastor-Corrales et al., 1995; Vieira, 2005). Therefore, the objective of this study was to evaluate the effect of *C. lindemuthianum* race 23 inoculation upon the total soluble carbohydrates and sucrose in foliar tissues of cultivar Mexico 222 seedlings.

MATERIALS AND METHODS

The seeds of common bean Mexico 222 cultivar were sowed in containers and, after 25 days in the greenhouse, the plants were divided in two groups of treatment: inoculated and not inoculated with *C. lindemuthianum* race 23. The completely randomized experimental design with 6 replications was used, being each experimental unit constituted by 1 plant. The plants from inoculation treatments were inoculated with a spore suspension of *C. lindemuthianum* race 23, whereas the non inoculated ones were sprayed only with distilled and sterile water. After the 4th day of inoculation, it was carried out the trifoliate tissue collection of each plant, which later, was submitted to total soluble carbohydrates and sucrose analyses. The total soluble carbohydrates and sucrose contents in 50mg of leaf dry mass of each sample were determined according the methodology proposed by Dubois et al. (1956) and Van Handel (1968).

RESULTS AND DISCUSSION

In Figure 1, A are shown the total soluble carbohydrates contents in *Phaseolus vulgaris* plants Mexico 222 cultivar inoculated and non inoculated with *C. lindemuthianum* race 23. It is observed in inoculated plants that it had occurred an increase of 10.7% in total soluble carbohydrates content (44.4 mg. g DM^{-1}) in relation to plants that were not inoculated (40.1 mg. g DM^{-1}).Increasing of total soluble carbohydrates content in inoculated and non inoculated plants are related to reduction of photosynthesis rate, due to the fact that the resistant gene activates a metabolic route through of oxidant agents accumulation, such as O_2^-, H_2O_2 and OH^-. In relation to sucrose, demonstrated by Figure 1 B, the cv. Mexico 222 plants inoculation with race 23 of *C. lindemuthianum* had not promoted significant alteration of this compound in foliar tissues. The observed values were 7.0 and 7.4 mg. g DM^{-1} in non inoculated and inoculated treatments, respectively. The maintenance of

sucrose contents in foliar tissue of inoculated and non inoculated plants suggests that this sugar does not act in their defense mechanism.

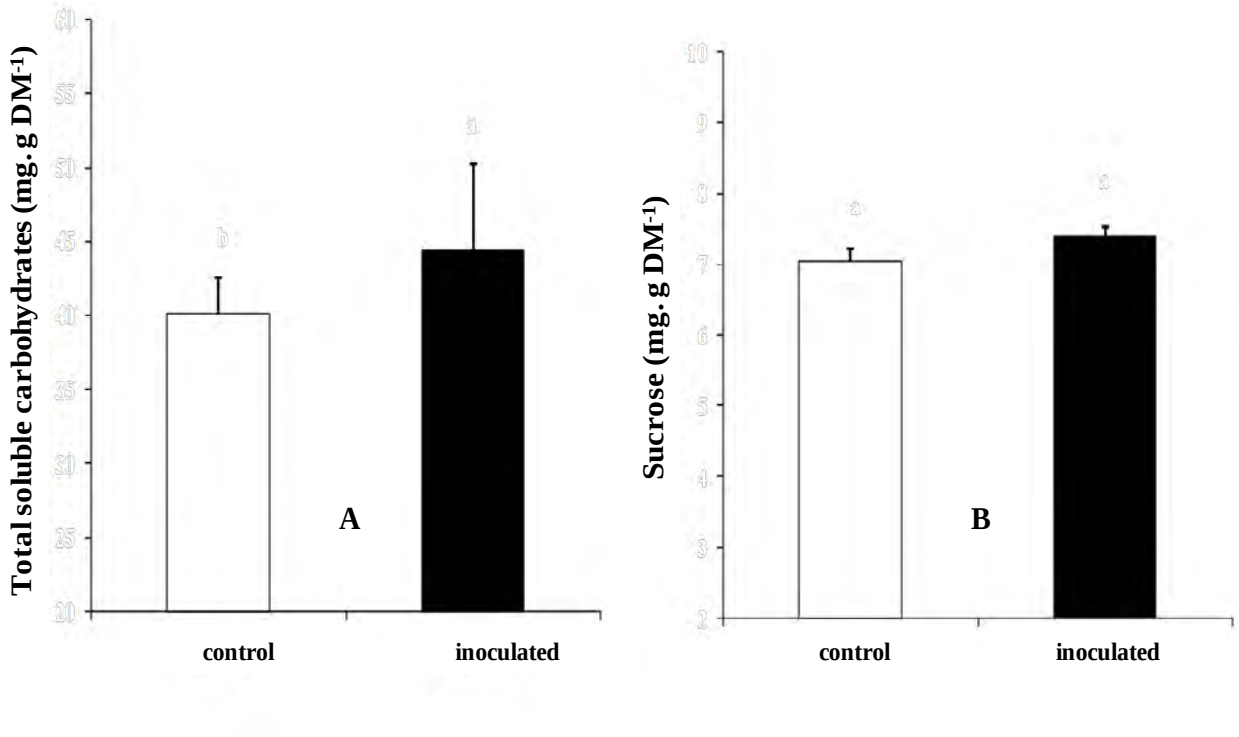

Figure 1 – Total soluble carbohydrates (A) and sucrose (B) contents in *Phaseolus vulgaris* L. plants Mexico 222 cultivar inoculated and non inoculated (control) with *Colletotrichum lindemuthianum* race 23. The bars represent the mean standard errors.

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PHOTOSYNTHETIC PIGMENTS IN COMMON BEAN MEXICO 222 CULTIVAR INOCULATED WITH *COLLETOTRICHUM LINDEMUTHIANUM* RACE 23

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INTRODUCTION

The biotic stresses developed by pathogenic agents are responsible for negative alterations upon vegetal metabolism. The development of these pathogens requires specific environment conditions, such as temperature, humidity, besides the host susceptibility (Kimati et al., 2005). The common bean infection due to *C. lindemuthianum*, causal agent of anthracnose, results in leaves' lesions, which promote the reduction of photosynthetic plant activity, and consequently, decreasing the crop yield (Chaves, 1983). According to Maringoni (2003) there is a strong evidence between absorption and consequently, transference of luminosity energy with grow and adaptation of plants to several environments. This work had the objective to investigate the photosynthetic pigment alterations of *Phaseolus vulgaris* L. plants cv. Mexico 222, after infection with the *C. lindemuthianum* pathogen race 23.

MATERIALS AND METHODS

Seeds from cultivar Mexico 222 of common bean were sowed in containers, and after 25 days in greenhouse, the plants were divided into two groups: inoculated and not inoculated with race 23 of *C. lindemuthianum*. The completely randomized experimental design with 6 replications was used, being each experimental unit constituted by 1 plant. The inoculation treatment had the plants inoculated with a spore suspension of *C. lindemuthianum* race 23, while not inoculated plants were sprayed only with distilled and sterile water. In the 4th day, after inoculation, the trifoliate tissue collection was carried out with each plant for subsequent analysis. The contents of a, b, and total chlorophyll and carotenoids were quantified according to Lichthenthaler (1987). The obtained data were submitted to variance analysis, being the means compared by F test, at 5 % level of probability.

RESULTS AND DISCUSSION

The inoculation of common bean plants, cultivar Mexico 222 with race 23 of C. *lindemuthianum*, had not resulted in significant alterations of chlorophylls a and b in leaves tissues neither in no inoculated plants (control) or inoculated, respectively (Figures 1 A and 1 B). This fact might be plausible due to the resistance of cultivar Mexico 222 to race 23 of *C. lindemuthianum*, since this cultivar has the gene (*Co*-3) that confers resistance to race 23 of the present pathogen (Gonçalves-Vidigal et al., 2008). In relation to carotenoids and total chlorophyll (Figures 1C and 1D), it was observed that the inoculation with C. *lindemuthianum* race 23 provided a significant reduction of these foliar tissue pigments. This fact suggests that carotenoids could be the first foliar pigments to suffer alteration after plant infection with *C. lindemuthianum* pathogens.

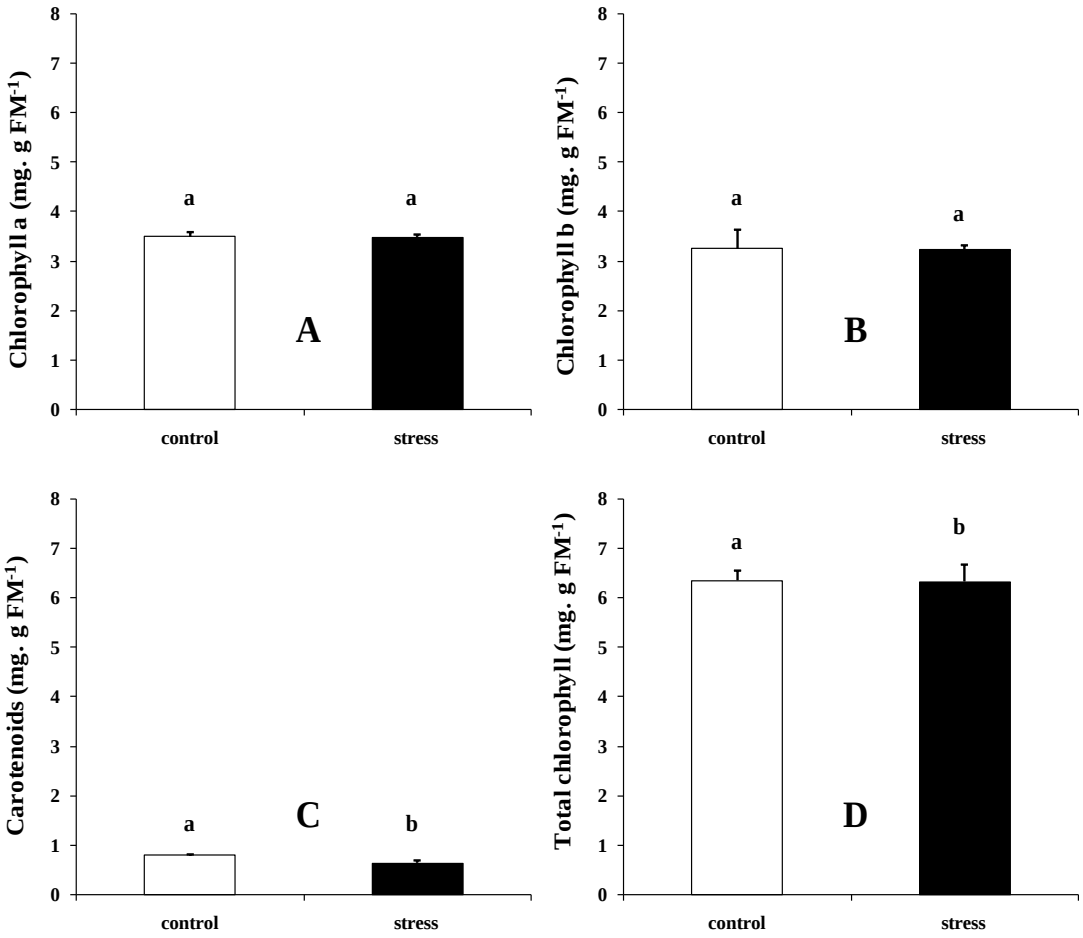

Figure 1 – Contents of chlorophyll a (A), chlorophyll b (B), carotenoids (C) and total chlorophylls (a+b) (D) in *Phaseolus vulgaris* L. plants cv. Mexico 222 inoculated with *C. lindemuthianum* race 23.

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This work was supported by CNPq, Capes, and Fundação Araucária. M.C. Gonçalves-Vidigal and P. S. Vidigal Filho were supported by grants from CNPq. A.K.S. Lobato, C.R. Silva, and L.L. Sousa are recipient of fellowship from Capes.

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RESISTANCE OF DRY BEAN TO SOUTH AFRICAN RACES OF *COLLETOTRICHUM LINDEMUTHIANUM*

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INTRODUCTION

Anthracnose, caused by the fungus *Colletotrichum lindemuthianum*, is an important disease of the common bean in South Africa. Although the disease is well controlled by an efficient disease-free seed scheme, many subsistence and small-scale farmers, as well as some commercial farmers, keep back some of their own crop as seed for the next season. Infected seed gives rise to infected seedlings, and the disease can be particularly aggressive under cool, wet conditions, causing serious yield and quality losses. Popular local cultivars are all susceptible to at least one race, and some are highly susceptible to several races. The aim of the present study was to update the knowledge of local races of the pathogen and to identify suitable sources of resistance as donor parents for a directed resistance breeding programme.

MATERIALS AND METHODS

Diseased material was collected from main production areas in South Africa and 13 single-conidium isolates were made. These were spray-inoculated on the international set of 12 differential bean lines (Michelite, Michigan DRK, Perry Marrow, Cornell 49242, Widusa, Kaboon, Mexico 222, PI 207262, TO, TU and AB 136) with a concentration of 1.2 10^6 conidia ml^{-1}. Disease severity rating was done seven days after inoculation (which included four days incubation in a dew chamber) using the standard 1 to 9 scale (Van Schoonhoven and Pastor-Corrales, 1987). Results were subjected to cluster analysis by UPGMA and principle co-ordinates analysis (PCoA). The races identified were used to screen local cultivars and potential sources of resistance.

RESULTS

Races 3, 65, 80, 81, 83, 81/593 (Koch, 1996); and 3, 6, 81, 323, 390, 593 (Mohammed, 2003) had been previously identified in South Africa. In this study, five races, with the binary codes 7, 81, 83, 89 and 263, were identified. Three of these, namely races 7, 89 and 263, had not been previously collected in South Africa. The differential G 2333 (with anthracnose resistance genes *Co-4*^{2}, *Co-5* and *Co-7*) was resistant to all South African races, as was AB 136 (*Co-6* & *co-8*) and Kaboon (*Co-1*^{2}). Ratings for Kaboon were generally somewhat higher than for AB 136 and G 2333, and races virulent on Kaboon (Leakey & Simbwa-Bunnya, 1972, Uganda, and Alzate-Marin & Sartorato, 2004, Brazil), as well as Kaboon and AB 136 (Mohammed, 2003, Ethiopia) have been reported. The resistance genes in AB 136 and Kaboon, in particular the single gene *Co-1*^{2}, should therefore not be solely relied on but be used in gene pyramiding with additional resistance genes. It is also possible that all South African races have not yet been collected.

For the present study, cluster analysis, supplemented by the PCoA, indicated two groups of races, namely 7 & 263, and 81, 83, & 89. Races 7 & 263 were generally virulent on large seeded material of Andean origin, and races 81, 83 and 89 were generally virulent on small seeded material of Middle-American (MA) origin.

Germplasm accessions also clustered into two main groups, namely those of MA origin or with resistance genes of MA origin, and those of Andean origin. Within these two main groups,

subgroups indicated resistance to zero, one or several races. This information can be used to select genotypes with complementary resistances.

Although, due to moderate reactions, the distinction between races 7 and 263 was not very clear, the South African small seeded cultivar Teebus differentiated these two races clearly. Two isolates were designated as race 83, although ratings for Michigan DRK differed from moderately susceptible to susceptible and for Cornell 49242 and Kaboon from resistant to moderately resistant. These two isolates were clearly differentiated into two different races by the large seeded local cultivars Jenny and RS-4 (Table 1). These results indicate that Teebus, Jenny and RS-4 may contain genes or gene combinations that are not represented in the standard differential set.

CONCLUSIONS

It is clear that, although cultivars of Andean origin are by far the most commonly planted types in South Africa and in Africa generally, races of both Andean and MA origin are prevalent in the region. Although no single gene can be relied upon to give resistance to all local anthracnose races, suitable resistance sources are available. Some germplasm accessions that contain more than one resistance gene have high levels of resistance to all reported SA races, and some local cultivars have complementary resistance, in particular the more resistant accessions from the Andean and MA gene pools (Table 1).

Table 1: Potential sources of resistance to South African races of *C. lindemuthianum*

				Race					
Cultivar	**Seed type**	**Origin**	**Resistance gene(s)**	**7**	**81**	**83a**	**83b**	**89**	**263**
Most local A types	Large	A		S	R	R(S)	S	R	S
Most local MA types	Small	MA		R	S	S	S	S	R
Kaboon	Crème	A	*Co-1*[2]	R	R	R	MR	R	R
PAN 146 (Sani)	Cranberry	A (MA)	Unknown	R	S	S	-	S	R
OPS RS-4	Cranberry	A (MA)	Unknown	S	R	R	S	R	S
RH4-1308C-3-B3	DRK	A(MA)	*Co-4*[2], *Co-1*?, *Co-2*?	R	R	R	-	R	R
Huron	Small White	MA (A)	*Co-2*	R	R	MS	R	MS	R
Ouro Negro	Small Black	MA	*Co-10*	R	S	R	R	MS	R

A: Andean, MA: Middle American, R: resistant, S: susceptible, MR/S: moderately resistant/susceptible

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Financial support by the EISELEN foundation, Ulm, Germany, receipt of the line RH4-1308C-3-B3 from Dr. PN Miklas, USDA, Prosser, and advice on the culture of *C. lindemuthianum* by Dr. S. Koch, ARC-PPRI, Pretoria are acknowledged.

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ANTIFUNGAL PROPERTIES OF MEDICINAL PLANTS AGAINST *COLLETOTRICHUM LINDEMUTHIANUM*

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INTRODUCTION

Anthracnose disease of common bean (*Phaseolus vulgaris* L.), caused by *Colletotrichum lindemunthianum* (Sacc. & Magn.) Scribner, is responsible for extensive yield losses worldwide. Control strategies mainly include, genetic resistance. However, the major limitation for the development of durable resistance in common bean cultivars is the high variability in *C. lindemunthianum* (Silva et al., 2007). Despite that, this disease is currently managed with synthetic fungicides. Nevertheless, there is a growing global concern over the continuous use of synthetic chemicals about food crops because of their potential effects on human health and the environment (Talamini & Stadinik, 2004). In attempt to modify this condition, some alternative methods of control have been adopted. Within this context is the utilization of plants, which are natural sources of antimicrobial substances and whose fungitoxic potential has been referred to in several studies. The goal of this study was to assay medicinal plants from the Alto Rio Grande region, in Minas Gerais State, Brazil, with ability to produce active products in the control of *C. lindemuthianum*.

MATERIALS AND METHODS

Leaves extracts of *Malva silvestris* L., *Ocimum gratissimum* L., *Origamum vulgare* L. and *Tetradenia riparia* (Hochst) NE. Br were prepared as described (Magallanes et al., 2003). In order to assay the extracts fungitoxicity, mycelia growth and percentage of conidia germination were evaluated. Both experiments were performed in a completely randomized statistical design, with four repetitions, in a factorial 4 x 6 design. Four strains of *C. lindemuthianum* were used, two from the 65 race and two from 81 race; four leaves extracts plus two controls, one of Tween 80 at 1% (g/mL) and the other of the fungicide Cercobin 700 WP (IHARABRAS S.A. INDÚSTRIA QUÍMICAS). Briefly, for evaluation of the leaf extracts effect on mycelia growth, 500 µl of plant extracts dissolved in Tween 80 at 1% at the 7.0 g/L concentration were placed on Petri dishes (90 x 15 mm) containing 8 mL culture medium M_3. The plates were inoculated with 9 mm diameter plugs with *C. lindemuthianum* mycelium and incubated at 22°C under 12 h photoperiod for 20 days. Growth inhibition of each fungal strain was measured by colony mycelia diameter after that period (Paulert, 2005). To measure extracts effect on percentage conidia germination, 500 µl plant extracts dissolved in Tween 80 at 1% at the concentration of 6.5 g/L were placed in Petri dishes (60 x 15 mm) with 4.5 mL culture medium agar-water. Afterwards, the plates were inoculated with 200 µl of a 1.2×10^6 spores per milliliter suspension with the four fungal strains and incubated at 22°C under 12h photoperiod for 24 hours. Percentage conidia germination was determined by evaluation of 50 conidia per each plate in light microscope (Pereira, 2006). The statistical significance of differences between mean values was assessed using an ANOVA and Scott Knott range test.

RESULTS AND DISCUSSION

In the ANOVA, for mycelia growth, all sources of variation were significant ($P<0.01$). Although the interaction effect of extracts x strains was significant there was no difference in the classification of extracts for different strains. Then, the means values were analyzed independently of strain. The best extract for inhibition of mycelia growth was *O. vulgare*, which decreased the mycelia growth in 31%

relatively to the control Tween 80 at 1% (Table 1). Castro et al. (2006) reported that *Ricinus communis* L. extracts showed reduction of 36% in the mycelia growth with the same pathogen. However, in this case, the measurements were made daily. In the variance analysis for conidia germination, only the strains x extracts interaction effect was not significant. The best result was observed with the extract of *O. gratissimum*, which showed inhibition of 77% when compared to the control Tween 80 at 1% (Table 1). This number is close to the results reported by Abreu (2005) with aquatic plants extracts, presenting inhibition of 70% of *C. lindemuthianum* conidia germination relatively to the control. The conidia were more sensitive to the plant extracts than the mycelium fungal (Table 1). The causal agent of anthracnose has direct penetration in epidermal cells (Jerba et al, 2005). Then, with the results obtained in the *in vitro* tests, we imply that the best extract in this disease control is *O. gratissimum*. Finally, the results showed that the plant extracts are able to decrease mycelia growth and conidia germination of *C. lindemuthianum*, indicating their potential as an alternative control of that pathogen. However, more studies are necessary, for example, to evaluate the extracts in field conditions.

Table 1. Effect of plant extracts in the mycelia growth (MGR) in centimeters (cm) and percentage of conidia germination (PCG).

Treatment	MGR(cm)	PCG (%)
Cercobin 700	3.2 **A**	3.6 A
Origamum vulgare	5.8 B	25.8 D
Ocimum gratissimum	6.3 C	19.2 B
Tetradenia riparia	6.4 C	21.6 C
Malva silvestris	6.6 D	23.6 C
Tween 80	8.4 E	81.8 E

Means followed by the same letters are not significantly different by the Scott-Knott test at 5% probability.

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RACE 467, A NEW VIRULENT ANTHRACNOSE PATHOTYPE IN CHIHUAHUA, MEXICO

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Co-evolution of anthracnose and the common bean has been observed (Balardin and Kelly, 1998; Sicard *et al*., 1997) as well as the presence of large pathogenic variation. The occurrence of many pathotypes represented by individual isolates in different regions of Mexico and the wide variation in avirulence genes in the populations of this pathogen, suggest that fungal populations are composed of many pathotypes of recent origin and that new pathotypes are being developed (Rodríguez *et al*., 2003). This observation might be related to an increased use of improved cultivars and the movement of seed and grain used as seed across and within regions.

Twenty two isolates of *C. lindemuthianum* collected between 1992 and 1996 in the state of Chihuahua from landraces of the 'ojo de cabra' seed type (Durango race), the predominant type in the 90s, were all classified as race 448 (González *et al*., 1998). Among five isolates collected in 1997 from a single field sown with an American Pinto cv., all were race 448. Among eight isolates obtained from cv. Pinto Villa (Durango race, introduced into the state from 1992 onwards) in 1998, races 448 y 449 were identified, whereas in 1999, also race 467 was identified out of eight isolates from Pinto Villa (Rodríguez-Guerra *et al*., 2003). From a later sampling in 2005, five different races were identified (Table 1) (Mendoza, 2008), taken together these overcome the resistance of the differentials Michelite, Michigan Dark Red Kidney, Widusa, Mexico 222, PI207262, To and AB136. All this new pathogenic variation may have arisen in response to the resistance carried by cv. Pinto Villa against race 448 (Gonzalez *et al*., 2004). Pinto Villa's pedigree shows a Canario line (Andean pool) (Acosta *et al*., 1995) and perhaps carries, in addition to a Mesoamerican resistant gene, an Andean allele (*Co-1*). Therefore, the newly described race 467 overcomes both Mesoamerican and Andean cultivars in the differential set. In Chihuahua, cv. Pinto Villa has already been replaced by Pinto Saltillo, a slow seed darkening cultivar that was hit by anthracnose in 2008 (race has not been defined yet). During 2008 lines from a pinto trial were exposed to pathotypes 467 (from Chihuahua) and 1472 (from Zacatecas) under controlled conditions and standard procedures. This trial included 14 lines plus check cv. Pinto Saltillo and Pinto Durango. A Second trial included 70 lines and bred cultivars Pinto Saltillo, Pinto Villa, Pinto Mestizo and Pinto Durango and was inoculated with pathotype 467. Inoculated plantlets were kept in a moist chamber 72 h and twelve days after inoculation plantlets were scored using a scale from 0 (clean plant) to 4 (dead plant) (Garrido y Romero-Cova, 1988)

In the first trial only few plantlets from lines PTB08004 and PTB08007 (both derived from the single cross (Pinto Durango/MAM 48) resulted resistant to race 467. On the basis of those results, 100 seeds of these lines plus Pinto Durango were sown and plantlets inoculated with race 467 to search for more resistant plants; due to lack of seed MAM 48 was not tested, it is being increased. From this second sowing, a few resistant plantlets were only recovered from PTB08007. Resistant plantlets were transplanted to larger pots to produce seeds. These seeds will be tested again against race 467 and if resistant, they will be further increased to be used in crosses and to develop a new version of the original line. The inoculation with race 1472 in the second trial, showed that out of 74 genotypes, a dozen lines showed heterogeneity in their response. i.e, more that four plants scored 0 out of twelve. Four out of seven lines derived from cross DON38/Azufrado Namiquipa displayed

resistant plants, and five out of eighteen lines derived from P into Bayacora/Pinto Saltillo also produced resistant plants. Those resistant plants were transferred to large pots to increase seed. Since all these lines display grain of high commercial quality, those that resulted heterogeneous, along with parental stocks, will be further tested to recover a resistant version of each. DON38 is a drought resistant pinto line introduced from Malawi throughout the B/C-CRSP, whereas Azufrado Namiquipa was derived from CIAT 326/85, a line introduced from CIAT. One hundred seeds of each parental stock of those lines displaying resistance will be tested along with the other lines. The four pinto cultivars Pinto Saltillo, Pinto Villa, Pinto Mestizo and Pinto Durango, resulted susceptible.

As pointed out by Miklas *et al*. (2006), the development of lines with resistance from both gene pools is a recognized strategy for developing improved, broad-base, long-lasting resistance in common bean. In this particular case, in addition to *Co-4*^{2}, the *Co-1*^{2} allele is also needed. At the moment we do not know what resistant alleles these resistant lines might possess.

Table 1. Anthracnose pathotypes isolated in the main rainfed bean growing area in the state of Chihuahua, Mexico.

	Year				
Pathotype	1992-96	1997	1998	1999	2005
448	X	X	X		X
449			X	X	X
467				X	X
1409					X
1473					X

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IDENTIFICATION OF A MOLECULAR MARKER LINKED TO *CO-11* ANTHRACNOSE RESISTANCE GENE IN COMMON BEAN

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INTRODUCTION

The fungus *Colletrotrichum lindemuthianum*, causal agent of anthracnose, is found in diverse common bean production areas around the world. The using of resistant cultivars is one of most recommended method to control this disease, and it has been demonstrated to be efficient and viable economically (Pastor-Corrales et al., 1995). On the other hand, the identification of resistance genes through molecular markers has shown great importance for breeding programs, once it allows a resistance selection of plants without the presence of the pathogen. The Michelite cultivar, which belongs to the 12 differential group of common bean (*Phaseolus vulgaris* L.), is a significant resistance source to anthracnose (Pastor-Corrales et al., 1995). Previously study had characterized the gene *Co-11* which confers genetic resistance in Michelite (Gonçalves-Vidigal et al., 2007). This gene coffers resistance to the races alpha, beta (130), gamma (102), 0, 2, 4, 6, 36, 38, 64, 128, 13 2, 192, 256, 258, 264, 320, 384, 388, 448, 1088, 13 44, 1472, 1600 and some races named as MA-1 to MA-6, and MA-8 to MA-10 of the Mexican groups (Yerkes Jr. and Ortiz, 1956; Cárdenas et al., 1964; Mahuku and Riascos, 2004). Therefore, in order to assist the selection of resistant genotypes, the present work had as objective to identify molecular markers linked to gene *Co-11*.

MATERIALS AND METHODS

Progeny of F_2 population of Michelite (resistant) with Michigan Dark Red Kidney (MDRK) (susceptible) were harvested to produce $F_{2:3}$ families. The $F_{2:3}$ families of this cross were also used as a mapping population for co-segregation studies with RAPD markers putatively linked to resistance in Michelite. Fifteen plants from each $F_{2:3}$ family were inoculated to confirm the $F_{2:3}$ genotype. Based on the number of susceptible plants observed in individual families, the $F_{2:3}$ families were classified as either homozygous dominant, heterozygous or homozygous recessive. The protocol for inoculation was as follows: 14-day-old bean plants with fully developed first trifoliate leaves were spray-inoculated with a spore suspension (1.2×10^6 spores mL^{-1}) of race 2 of *C. lindemuthianum*. After 7 days of inoculation the seedlings were evaluated for their disease reaction using a scale from 1 to 9 (Pastor-Corrales et al., 1995). Linkage between RAPD marker(s) and the resistance gene in Michelite was tested in 30 $F_{2:3}$ families. Two contrasting bulks were formed using bulked segregant analysis procedure (Michelmore et al., 1991), with DNA from 6 homozygous resistant F_2 individuals, and 6 homozygous susceptible F_2 plants derived from the mapping. The phenotypic segregation was analyzed in the $F_{2:3}$ families derived from the using the Chi-square test. Linkage analyses were performed using the computer software Mapmaker (Lander et al., 1987).

RESULTS AND DISCUSSION

The RAPD marker $OPAZ04_{565}$ (5'-CCAGCCTCAG-3') was found closely linked in repulsion phase to the *Co-11* resistant gene in Michelite (Figure 1). According to Haley et al. (1994), selection based on repulsion–phase markers (linked with the allele conferring susceptibility), yields a greater proportion of homozygous resistant plants, than the selection based on a coupling-phase (linked with

the allele conferring resistance), even at greater recombination frequencies between marker, and resistance loci. The $OPAZ04_{565}$ marker, linked at distance of 1.5 cM, should be useful in marker-assisted selection for the introgression of *Co-11* into susceptible germplasm and could improve the effectiveness of resistance gene pyramiding for anthracnose in bean breeding programs.

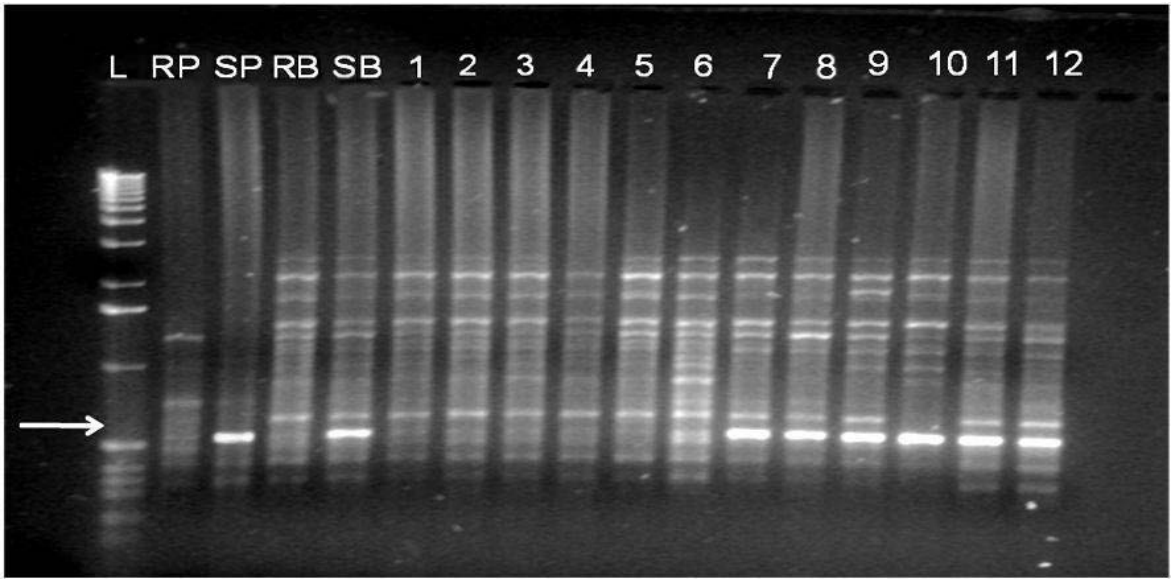

Figure 1 – Electrophoretic analysis of amplification products obtained with $OPAZ04_{565}$ RAPD marker. Lanes are as follows: L, molecular weight marker (100 bp ladder); RP, Michelite (Resistant to race 2); SP, MDRK (Susceptible to race 2); RB, Resistant Bulk; SB, Susceptible Bulk; 1–6, Resistant Plants; 7-12, Susceptible Plants. The arrow indicates a DNA band of 565 bp linked in repulsion phase to the resistance gene in Michelite.

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ALLELIC RELATIONSHIPS OF ANTHRACNOSE RESISTANCE IN MSU 7-1 LINE

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INTRODUCTION

Anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. and Magnus) Briosi and Cavara, is one of the most important fungal disease of common bean (*Phaseolus vulgaris* L.), and has been shown to be particularly harmful in tropical and subtropical regions where temperatures are moderate to cold and the relative humidity is high (Vieira, 2005; Méndez-Vigo et al., 2005).

The control of anthracnose can be achieved through several cultural, chemical, and genetic measures performed in an integrated disease control. The use resistant cultivars to several races of this pathogen is the most economic and effective method, since it reduces production costs and ecological damage. Therefore, to enhance the efficiency of this method, the availability of new anthracnose resistance sources is necessary. At present, 13 anthracnose resistance genes have been identified in common bean and are being used in breeding programs (Kelly and Vallejo, 2004; Gonçalves-Vidigal et al., 2008, 2009).

The search for new resistance sources is constant (Vidigal Filho et al., 2007) due to the wide variability of the pathogen and to the co-evolution of pathogen-host favoring continuous breakdown of resistance in commercial cultivars. MSU 7-1 is a breeding line from Michigan State University, which was derived from the cross of Black Magic x SEL 111 and has the same disease reaction as SEL 111 and SEL 1360 (Vallejo and Kelly, 2009). Thus, the objective of this work was to investigate if the genes in MSU 7-1 are independent of the others previously anthracnose resistance genes described.

MATERIALS AND METHODS

A sample of 10 seeds from a single plant selection of MSU 7-1 was multiplied through four successive generations in order to obtain completely homozygosity. For the allelism tests the MSU 7-1 breeding line was crossed with the cultivars Michigan Dark Red Kidney (MDRK), Widusa, Cornell 49-242, Mexico 222, PI 207262, TU, AB 136, G 2333, Ouro Negro, Michelite, Jalo Vermelho (JV), Jalo Listras Pretas (JLP), and H1 line (*Co-7* from the G 2333). The F_1 and F_2 populations were grown under green-house conditions. The F_2 population from the crosses between MSU 7-1 and the following cultivars: Cornell 49-242, México 222, PI 207262, TU, AB 136 and Ouro Negro were inoculated with race 7. Meanwhile, the F_2 populations of the crosses MSU 7-1 x JV and MSU 7-1 x JLP were inoculated with race 64. The inoculation was made using a spore suspension with adjusted concentration of 1.2×10^6 spores mL^{-1} in distilled and sterilized water. After the inoculation, the plants were kept in a chamber for 96 hours at 20 ± 2ºC temperature and controlled luminosity. Visual assessment for symptoms was carried out 10 days later after inoculation using a scoring scale from 1 to 9. The plants with score of 1 and 3 were considered resistant, meanwhile plants with score of 4 to 9 were considered susceptible.

RESULTS AND DISCUSSION

The F_2 population from the cross MSU 7-1(R) x Mexico 222 (S) was inoculated with race 64 to determine the number of genes segregating for resistance to anthracnose. The progeny segregated in a 3:1 ratio of resistant to susceptible indicating that only one gene is segregating for resistance to race 64 ($p = 0.88$). This fact demonstrated that race 64 of *C. lindemuthianum* overcomes the other resistance gene present in MSU 7-1 (Co-7).

The segregation in the F2 population from the crosses MSU 7-1 x JV and MSU 7-1 x JLP fitted a ratio of 15R: 1S, when inoculated with race 64, indicating the action of two dominant genes (*Co-5* in MSU 7-1, *Co-12* and *Co-13* in JV and JLP, respectively). Meanwhile, the F_2 populations from the crosses between MSU 7-1 and the cultivars Widusa, Cornell 49-242, AB 136, Ouro Negro and Michelite, inoculated with race 7, showed a segregation that fitted a ratio of 63R:1S, demonstrating the presence of two genes in MSU 7-1 and one in each of the other cultivars.

However, segregation was not observed in F_2 population from crosses among MSU 7-1 with Mexico 222, PI 207262, TU, G 2333 cultivars and H1 line. The 104 F_2 individuals derived from the cross MSU 7-1 x TU inoculated with race 7 were all resistant, indicating the presence of allelism. The lack of segregation suggests that the MSU 7-1 (*Co-5* + *Co-7*) and the cultivar TU (*Co-5*) possess alleles at the same locus *Co-5*. Furthermore, there was also lack of segregation among 125 F_2 individuals from the cross MSU 7-1 x Mexico 222 (*Co-3*), inoculated with race 7, indicates that the *Co-7* gene in MSU 7-1 is an allele of the locus *Co-3***.** The results showed in Table 1 indicate that MSU 7-1 has the same allele as Mexico 222, at the *Co-3* and as TU, at the *Co-5* loci. The allelism tests demonstrated that the genes in MSU 7-1 are independent from those previously characterized (*Co-1*[5], *Co-2, Co-6, Co-10, Co-11*, *Co-12*, and *Co-13*). However, the genes providing resistant to race 7 of *C. lindemuthianum* present in MSU 7-1 is allelic to the ones present in the H1 line, PI 207262, Mexico 222, and G 2333 suggesting that those genes are probably located in the same R gene cluster.

Table 1 - Cross, number of plants evaluated and expected phenotypic ratios in F_2 populations for resistance to races 7 and 64 of *Colletotrichum lindemuthianum*

Crosses MSU 7-1 with	Race	Resistance Gene	Observed Ratio		Expected Ratio	χ^2	*P value*
			R[a]	S[b]	R:S		
Widusa	*7*	*Co-1*[5]	140	5	63:1	0.033	0.67
Cornell 49-242	7	*Co-2*	118	2	63:1	0.008	0.93
Mexico 222	7	*Co-3*	125	0	-	-	-
PI 207262	7	*Co-9/Co-3*[3]*, Co-4*[3]	165	0	-	-	-
TU	7	*Co-5*	154	0	-	-	-
AB 136	7	*Co-6*	94	1	63:1	0.161	0.69
G 2333	7	*Co-4*[2]*+Co-5+ Co-7*	156	0	-	-	-
H1	7	*Co-7*	110	0	-	-	-
Ouro Negro	7	*Co-10*	166	2	63:1	0.151	0.70
Michelite	7	*Co-11*	147	6	63:1	0.553	0.20
JV[c]	64	*Co-12*	94	7	15:1	0.079	0.78
JLP[d]	64	*Co-13*	143	9	15:1	0.028	0.87

[a]R = Resistant; [b]S = Susceptible; [c]JV = Jalo Vermelho; [d]JLP = Jalo Listras Pretas.

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PROTEINS AND AMINO ACIDS IN COMMON BEAN CULTIVAR WIDUSA INOCULATED WITH *COLLETOTRICHUM LINDEMUTHIANUM* RACE 23

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INTRODUCTION

Common bean anthracnose caused by the fungus *Colletotrichum lindemuthianum* (Sacc. and Magnus) Briosi and Cavara is the most important disease of common bean in producer regions of the world. Under favorable conditions, both pathogens could cause yield losses ranging from 80 to 100%, and in severe cases it can turn the grain quality inadequate for consumption (Pastor-Corrales et al., 1995).

The nitrogen is an essential element to plant growth and development, which can be absorbed in the forms of nitrate and ammonium, besides the adequate plant nutrition and consequent maintenance of the pathway of nitrogen assimilation and translocation is fundamental to protection and plant defense against pathogen infections (Walters and Bingham, 2007).

The objective of the study was to investigate the responses on total soluble proteins and amino acids in Widusa cultivar (susceptible) of *Phaseolus vulgaris* L. plants inoculated with *Colletotrichum lindemuthianum* race 23.

MATERIALS AND METHODS

The seeds of the Widusa cultivar were placed in containers (length x width x height; 40 x 30 x 10 cm, respectively) that contained the substrate Plantmax®. Twenty seeds were placed in each container and the seedlings were thinned after the 8th day, allowing only 12 seedlings to remain in the container, where the plants remained in the greenhouse.

The plants from inoculation treatments were inoculated with a spore suspension of *C. lindemuthianum* race 23, whereas the non inoculated ones were sprayed only with distilled and sterile water. The control plants were kept in greenhouse conditions for 25 days. All plants remained in mist chamber for three days under the conditions described previously, as well as the leaves were harvested on the 8th day after pathogen inoculation. The completely randomized experimental design with six replications was used, with 2 treatments (control and inoculated), being each experimental unit constituted by one plant.

The amino acids were determined with 50 mg of leaf dry matter powder, which was incubated with 5 mL of sterile distilled water at 100 ºC, as well as the quantification was carried out at 570 nm according to Peoples et al. (1989). The determination of the total soluble proteins was carried out with 100 mg of powder, in which was incubated with 5 mL of extraction buffer (Tris-HCl at 25 mM and pH 7.6), as well as the quantification was carried out at 595 nm in agreement with Bradford (1976). The data were submitted at variance analysis, as well as the standard errors were calculated in all evaluated treatments.

RESULTS AND DISCUSSION

The Figure 1 A showed the amino acids levels, which were 151.8 and 214.9 µmol. g MS^{-1} in control and inoculated plants, respectively. It was observed that the inoculated plants presented significant increase at 41.5%, when compared with control treatment. The accumulation occurred due to mechanism of programmed cellular death, in which it provokes protein breakdowns (Hurst

and Clark, 1993), besides it to reveal that these compounds can be utilized as biochemical indicators due to be very responsible after to plant infection process.

The soluble proteins levels showed not significant changes after inoculation of *C. lindemuthianum* race 23 (Figure 1 B), as well as the control and inoculated plants presented the levels of 6.38 and 6.57 mg. g MS^{-1}, respectively. The maintenance of the total soluble proteins indicates that susceptible plants to anthracnose probably suffer two events simultaneous and opposites, in which the primary response is correlated with the strategy to keep the plant metabolism under normal conditions and it to survive the pathogen invasion increasing the amount of proteins (Wu et al., 2007). However, the secondary response occurs through of the protein breakdown induced by the protease enzymes that promotes the reduction of this nitrogen compound (Lobato et al., 2008).

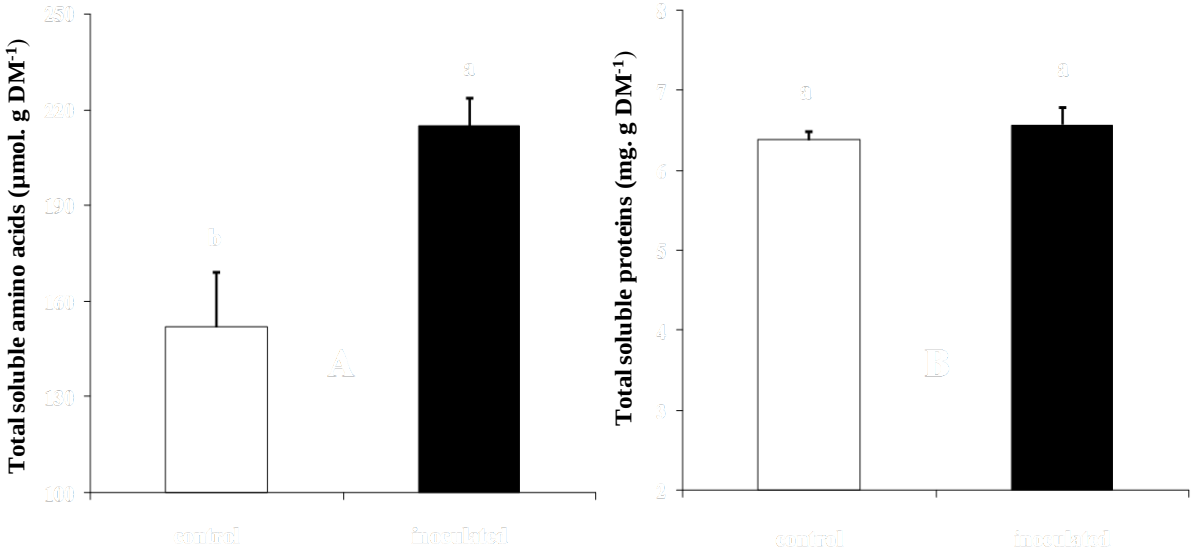

Figure 1 - Total soluble amino acids (A) and total soluble proteins (B) in cultivar Widusa inoculated with *C. lindemuthianum* race 23. Averages followed by the same letter do not differ among themselves by the variance analysis. The bars represent the mean standard error.

ACKNOWLEDGEMENTS

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CHARACTERIZATION OF *COLLETOTRICHUM LINDEMUTHIANUM* ISOLATES FROM MATO GROSSO STATE, BRAZIL

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INTRODUCTION

Anthracnose is one of the most widespread and economically important common bean diseases (Pastor-Corrales, 2005). This disease, caused by the fungus *Colletotrichum lindemuthianum*, is particularly important in sub-tropical and temperate bean production regions (Pastor-Corrales and Tu, 1994). Favorable environmental conditions for bean anthracnose development, such as moderate to cool temperature (between about 15° to 25° C with an optimum of 17°C), high humidity (greater than 90%), gathered to the presence of susceptible bean varieties and the occurrence of early infections, often result in severe anthracnose symptoms, severe reduction in pod and seed quality and yield losses.

Genetic resistance is the most effective, easy to use, and environmentally-friendly common bean anthracnose management strategy (Pastor-Corrales and Tu, 1994; Kelly and Vallejo, 2004). However, the implementation of resistance is challenged by the recurrent appearance of new virulence phenotypes, usually referred as races, of *C. lindemuthianum*. The repeated appearance of new races has resulted in failures of previously anthracnose-resistant commercial varieties (Kelly et al., 1994; Mahuku et al., 2002). Since 1966, several studies have reported the presence of different races of *C. lindemuthianum* in Brazil and approximately 50 races have been characterized (Alzate-Marin and Sartorato, 2004).

The region of Central Brazil have relevant importance in national bean production and the occurrence of anthracnose could represent great a grain yield threat. Therefore, this study was conducted with the objective of characterizing *C. lindemuthianum* isolates from Mato Grosso State by using differential cultivars.

MATERIALS AND METHODS

In 2008 it was observed that several bean commercial cultivars (*Phaseolus vulgaris* L.) were infected by this pathogen in the common bean production field in Primavera do Leste, Mato Grosso state, Brazil. A total of 33 samples of *C. lindemuthianum* were collected on leaves or pods and 10 of them were evaluated.

To distinguish the races derived from different *C. lindemuthianum* isolates, the differential cultivars set was used. This set consisted on 12 cultivars, each with a designated binary number as following: Michelite, 1; Michigan Dark Red Kidney, 2; Perry Marrow, 4; Cornell 49-242, 8; Widusa, 16; Kaboon, 32; Mexico 222, 64; PI 207262, 128; To, 256; Tu, 512; AB 136, 1024; and G 2333, 2048. The sum of the numbers assigned to each infected cultivar of the differential set determined the number or race designation. Cultures from each sample of *C. lindemuthianum* were transferred to petri dishes containing either Mathur's PDA (potato-dextrose agar) or bean pod agar culture medium.

After inoculation, plants were maintained at >95% relative humidity at 21-23°C and 16-h day length (light intensity of 300 micromoles m^{-2} s^{-1} at 1 m height) in a mist chamber for 2 days. After this period, the plants were removed from the mist chamber and transferred to benches in a greenhouse with suitable environment at 22°C with artificial light (12-h day length at 25°C) for seven

days. Anthracnose disease reactions were rated visually using a scale from 1 to 9 (Pastor-Corrales et al., 1995).

RESULTS AND DISCUSSION

The characterization of isolates on the differential cultivars set permitted the identification of two races, both had not been reported previously in Mato Grosso. This is the first occurrence report of races 65 and 81 of *Colletotrichum lindemuthianum* in Mato Grosso. All isolates were compatible to the cultivars Michelite and Mexico 222. Isolates analyzed showed a tendency to infect the Mesoamerican cultivars. Most of the races identified in Paraná and Santa Catarina States have overcome *Co-2*, *Co-3* and *Co-11* genes present in Cornell 49-242, Mexico 222, and Michelite respectively. The races 65 and 81, identified in Mato Grosso, were more frequent and widely distributed in Brazil mainly in Goiás, Santa Catarina, Paraná and Distrito Federal states (Thomazella et al. 2002; Alzate-Marin and Sartorato, 2004; Silva et al., 2007; Gonçalves-Vidigal et al. 2008).

These results are particularly relevant to bean breeding programs that wish to monitor and control the spread of this particular disease through the use of anthracnose resistant cultivars. In this case, to better control the races 65 and 81 of anthracnose the *Co-1*^{2}, *Co-12*, and *Co-13* genes (Andean origin), respectively, present in Kaboon, Jalo Vermelho, Jalo Listras Pretas cultivars, may be used to develop anthracnose resistant bean cultivar by the combination with Mesoamerican origin genes in PI 207262, To, AB 136, and G 2333 cultivars.

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NITROGENOUS COMPOUNDS IN COMMON BEAN MEXICO 222 CULTIVAR INOCULATED WITH *COLLETOTRICHUM LINDEMUTHIANUM* RACE 23

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INTRODUCTION

The nitrogenous compounds are responsible to form essential metabolites, such as amino acids, protein and nucleic acids, considering the nitrate the most important nitrogenous source available for plants (Marschner, 1995). Studies had pointed out the occurrence of nitrogenous content alterations in plants when there is the presence of pathogens (Scarpari et al., 2005). In common bean crop one important biotic factors is anthracnose, caused by fungus *Colletotrichum lindemuthianum*, which can proportionate serious yield and grain quality reduction (Pastor-Corrales et al., 1995). The objective of this study was to evaluate the alterations of amino acids and total soluble proteins in common bean plants Mexico 222, inoculated with *Colletotrichum lindemuthianum* race 23.

MATERIALS AND METHODS

Seeds from common bean cultivar Mexico 222 were sowed in containers, and later 25 days in the greenhouse, the plants were divided into two groups: inoculated and non inoculated with race 23 of *C. lindemuthianum*. The completely randomized experimental design with 6 replications was used, being each experimental unit constituted by 1 plant. The plants from inoculation treatment were inoculated with a spore suspension of *C. lindemuthianum* race 23, whereas non inoculated plants were sprayed with distilled and sterile water. After the 4th day of inoculation, a collection of trifoliate tissue of each plant was carried out for later analysis. The amino acids and total soluble proteins were determined according to Peoples et al. (1989) and Bradford (1976). The obtained data were submitted to variance analysis, being the means compared by F test, with 5% level of probability.

RESULTS AND DISCUSSION

The foliar tissues of inoculated plants (Figure 1 A) demonstrated a total soluble amino acids content equivalent to 186.4 µmol. g MS^{-1}, which means, an increase of 18.3% in relation to non inoculated plants (157.5 µmol. g MS^{-1}). The increasing of total soluble amino acid contents in foliar tissue of Mexico 222 is probably associated to activities of protease enzymes, which carry on effect upon plant proteins in order to provide a synthesis of other amino acids that are used in the activation of resistant gene(s) (Wu et al., 2007). In relation to total soluble proteins content (Figure 1 B), the inoculation with race 23 of *C. lindemuthianum* provided a significant increasing of 38.3% when compared to non inoculated plants. The amplification of total soluble protein content in common bean plants is associated to synthesis of responsible proteins for resistance to pathogen *C. lindemuthianum* race 23, which is induced by resistant gene *Co-3* present in this cultivar (Gonçalves-Vidigal et al., 2008).

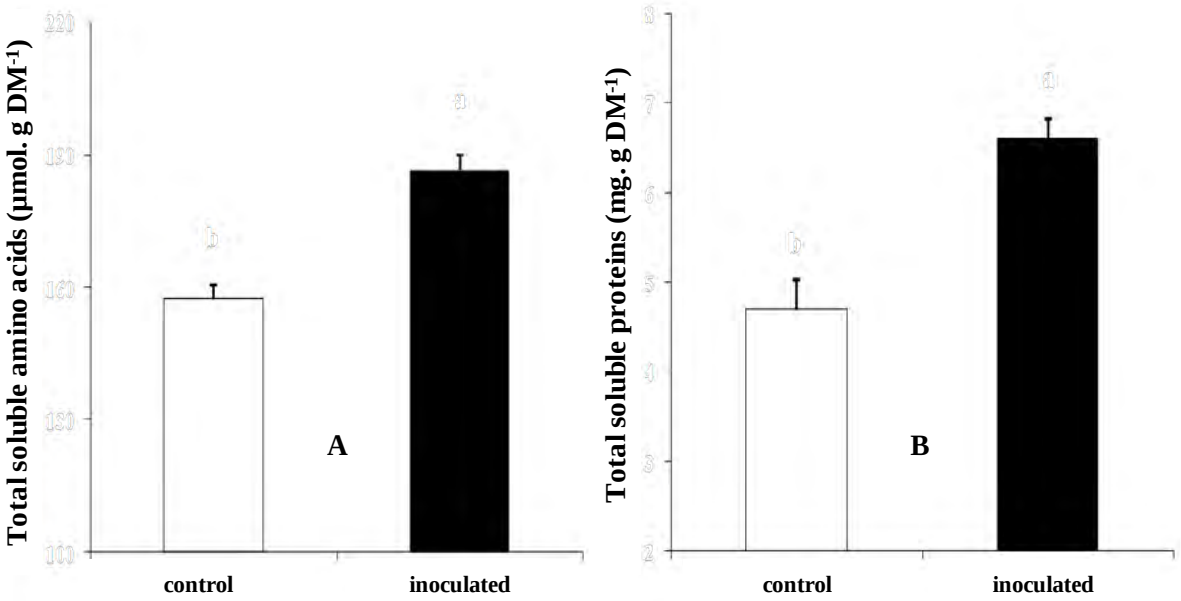

Figure 1 - Total soluble amino acids (A) and total soluble proteins (B) in *P. vulgaris* cv. Mexico 222 inoculated and non inoculated (control) with *C. lindemuthianum* race 23. Averages followed by the same letter do not differ among themselves by the variance analysis. The bars represent the mean standard error.

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CHARACTERIZATION OF *PSEUDOCERCOSPORA GRISEOLA* ISOLATES COLLECTED IN THE STATE OF MINAS GERAIS, BRAZIL

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INTRODUCTION

The common bean is a host to various pathogens. Angular leaf spot (ALS), incited by the fungus *Pseudocercospora griseola* (Sacc.) Crous & U. Braun, is a geographically diverse disease and during recent years has increased in frequency and severity in Latin America (SARTORATO, 2006). Nearly all cultivars planted in Brazil are susceptible to ALS. Therefore, it is important to survey the pathogenic variability in each growing region to aid the selection of effective resistance sources. The objectives of this work were to collect, isolate and characterize the *Ps. griseola* fungus in three of the most important bean growing regions of the state of Minas Gerais, Brazil.

MATERIALS AND METHODS

Bean leaves with ALS symptoms were collected in the following regions of Minas Gerais state: Triângulo Mineiro, Northwest region and Zona da Mata. To obtain monosporic cultures, the methodology described by CORREA-VICTORIA (1987) and PYINDJI (1991) was used. A suspension of conidia and mycelia fragments was prepared for each isolate. The suspension was filtered through gauze, the spore concentration was determined with the aid of a hemocytometer and adjusted to 2.0×10^4 conidia/mL.

To classify the isolates into races 12 plants each of the ALS differential series (PASTOR-CORRALES & JARA, 1995) at the V3 stage were inoculated. The plants were maintained in mist chambers at $24 \pm 2\ ^{o}C$, under a photoperiod of 12 h and relative humidity greater than 95%. After 48 h under these conditions, the plants were transferred to the greenhouse where they remained until symptom evaluation. The severity of the disease was visually verified at 15, 18 and 21 days after inoculation using a nine degree severity scale proposed by PASTOR-CORRALES & JARA (1995). Plants with degrees lower than 3 were considered resistant and those with degrees greater than 4, susceptible.

RESULTS AND DISCUSSION

Bean leaves presenting symptoms of ALS were collected from 33 sites in three growing regions of Minas Gerais state. A total of 78 monosporic isolates of *Ps. griseola* were obtained. So far, 17 isolates have been characterized and classified into 12 distinct races (Table 1), demonstrating the large pathogenic variability of this pathogen. Race 63.63 was the most frequently found, corresponding to four of the 17 isolates characterized. The fact that several isolates were classified as

race 63.63, i. e., they are compatible with all 12 cultivars of the ALS differential series, suggests that this set of cultivars must be reviewed. In the works developed by SARTORATO (2002), SARTORATO (2004) and VITAL (2006), the cultivars Don Timóteo, G11796 and Bolón Bayo showed to be susceptible to approximately 100% of *Ps. griseola* isolates tested. According to these authors these cultivars do not interfere in the discrimination of *Ps. griseola* races, which was also confirmed in this work (Table 1).

Characterization of the other 61 isolates obtained is underway as an effort of the Common Bean Breeding Program of BIOAGRO/UFV to survey the main bean growing regions of the state of Minas Gerais.

Table 1. Characterization of *Ps. griseola* isolates collected in three common bean growing regions of Minas Gerais state, Brazil

Isolate	Virulence phenotype of the Varieties[1]												Race	Collection Region
	A	B	C	D	E	F	G	H	I	J	K	L		
A_1 13	a	b	c	d			g	h	i				15.7	Triângulo Mineiro
A_2 4	a	b	c	d	e	f	g	h	i				63.7	Triângulo Mineiro
B_1 46	a	b	c	d	e	f	g	h	i	j	k	l	63.63	Northwest region
B_3 8	a	b	c	d	e	f	g	h	i	j		l	63.47	Northwest region
B_4 4	a	b	c	d		f	g	h	i			l	47.39	Northwest region
B_4 6	a	b	c	d	e				I				31.4	Northwest region
B_7 50	a	b	c	d	e	f	g	h	i	j	k	l	63.63	Northwest region
C_1 17	a	b					g	h	i		K		3.23	Northwest region
C_1 28	a	b	c	d	e	f		h	i				63.6	Northwest region
C_2 10	a	b	c		e		g	h	i		K		23.23	Northwest region
$CM_1$2	a	b	c	d	e	f	g	h	i	j	k	l	63.63	Zona da Mata
$CM_3$11	a	b	c	d	e	f	g	h	i	j	k		63.31	Zona da Mata
Coimbra 20	a	b	c	d	e	f	g	h	i				63.7	Zona da Mata
Coimbra 21	a	b	c	d	e		g	h	i				31.7	Zona da Mata
SM 32	a	b	c	d	e	f	g	h	i		k		63.23	Zona da Mata
Viçosa 3	a	b	c	d	e	f	g	h	i		k		63.23	Zona da Mata
Viçosa 7	a	b	c	d	e	f	g	h	i	j	k	l	63.63	Zona da Mata

1/ Differential cultivars: A= Don Timóteo, B= G 11796, C= Bolón Bayo, D= Montcalm, E= Amendoim, F= G 5686, G= PAN 72, H= G 2858, I= Flor de Mayo, J= México 54, K= BAT 332 and L= Cornell 49-242. The lower-case letters indicate susceptibility of the variety.

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VARIABILITY AMONG PSEUDOCERCOSPORA GRISEOLA ISOLATES BY RAPD MARKERS

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INTRODUCTION: Angular leaf spot disease of common bean (*Phaseolus vulgaris*), caused by *Pseudocercospora griseola*, is one of the most important of this crop in Brazil. Control strategies include, mainly, the development of resistant cultivars. However, a strategy to control and/or reduce the impact of *P. griseola* requires a previous knowledge of the population structure of this fungus. Understanding the pathogenic variability is a fundamental point in breeding program. Therefore, the purpose of this study was to investigate the genetic diversity and population's genetic structure among *P. griseola* isolates collected in Minas Gerais and Goiás state, Brazil.

MATERIALS AND METHODS: 70 isolates of *P. griseola* obtained from naturally-infected common bean cultivars were used in this study. Isolates were collected in the states of Minas Gerais (Ijací, Lambarí, Lavras and Viçosa) and Goiás (Damolândia), Brazil, as shown in Table 1. *P. griseola* isolates were grown in liquid medium for 15 days (110 rpm at 20°C) and the DNA was extracted according to the methodology developed by Raeder & Broda (1985) with minor modifications. The RAPD reactions were carried out with the *primers* OPAN11, OPAP18, OPAQ01, OPAQ02, OPAQ03, OPAQ04, OPAQ08, OPAS03, OPAS04, OPAS05, OPAS06, OPAS07, OPAS08, OPAS11, OPAS15, OPAS19, OPAT19, OPBB06 e OPBB08 and performed in a final volume of 14 µl containing 4 µl water, 35 ng of genomic DNA, 50 µM of each dNTP and 0.4 µM oligonucleotide *primer*, 50 mM Tris-HCl, pH 8.0, 2.0 mM $MgCl_2$, 20 mM KCl, and 0.6 units Taq DNA polymerase. Amplification was programmed for 1 initial desnaturation cycle (94°C for 2 minutes), followed by 38 cycles of 2 minutes at 94°C, 15 seconds at 37°C and 1 minute at 72°C and a final extension step of 2 minutes. Amplicons were separated by electrophoresis, visualized under UV light and photographed with the Kodak EDA – 290 camera. The genetic similarities and clustering analysis were performed by using the Nei and Li coefficient and UPGMA, respectively. The analysis of molecular variance (AMOVA) was also performed.

RESULTS AND DISCUSSION: The *primers* amplified a total of 76 polymorphic bands, with an average of 4.0 polymorphic bands per *primer*. The genetic similarity among the isolates varied from 0.301 to 0.993 with an average of 0.746. The descriptive analyses revealed a tendency of differentiation of isolates by origin areas. The Shannon diversity index revealed that Viçosa, MG, presented the largest genetic diversity, whereas Ijaci, MG, presented the smallest genetic diversity. The total Nei's genetic diversity was partitioned ($H_T = 0.3535$). The genetic differentiation among the populations was 0.1979 (G_{ST} value). Therefore, 80.21% of the genetic variation observed in this study was due to differentiation within populations. AMOVA demonstrated that 77.51% of the variation was contained within places and 22.49% among places (Table 2). Pairwise comparisons of 76 polymorphic RAPD loci gave disequilibrium values that were all significantly different from zero (Fisher's exact test, $P < 0.05$) for the studied populations, showing that *P. griseola* maintains a genetic structure consistent with asexual reproduction.

Table 1 *Pseudocercospora griseola* isolates (Isol.), counties (C.) and patothypes (Pat.) of Minas Gerais (MG) and Goiás (GO) State, Brazil.

Isol.	C.[1/]	P.	Isol.	C	Pat.	Isol.	C.	Pat.
Ig - 792	DA	63-31	Pg - 16	IJ	63-31	Pg - 52	LM	-
Ig - 799	DA	63-63	Pg - 17	IJ	63-31	Pg - 53	LM	-
Ig - 802	DA	63-63	Pg - 19	LV	63-47	Pg - 54	LM	-
Ig - 806	DA	63-31	Pg - 20	LV	63-63	Pg - 55	LV	-
Ig - 808	DA	63-63	Pg - 21	LV	63-63	Pg - 56	LV	-
Ig - 809	DA	63-31	Pg - 23	LV	63-63	Pg - 57	LV	-
Ig - 822	DA	63-63	Pg - 24	LV	63-63	Pg - 58	LV	-
Ig - 828	DA	63-31	Pg - 25	LV	63-63	Pg - 59	LV	-
Ig - 854	DA	63-31	Pg - 26	LV	63-31	Pg - 60	LV	-
Ig - 860	DA	63-63	Pg - 27	LV	63-31	Pg - 61	LV	
Ig - 865	DA	63-31	Pg - 28	LV	63-63	Pg - 62	LV	-
Ig - 868	DA	63-63	Pg - 31	LV	63-63	Pg - 63	LM	-
Pg - 01	IJ	63-47	Pg - 32	LV	63-31	Pg - 64	LV	-
Pg - 02	IJ	63-15	Pg - 33	LV	63-63	Pg - 65	LV	-
Pg - 03	IJ	63-47	Pg - 34	LV	63-63	Pg - 67	LV	-
Pg - 04	IJ	63-63	Pg - 35	LV	63-63	Pg - 68	LV	-
Pg - 05	IJ	63-31	Pg - 41	LV	63-31	Pg - 69	LV	-
Pg - 06	IJ	63-31	Pg - 45	LV	63-63	Pg - 70	VI	-
Pg - 07	IJ	63-55	Pg - 46	LV	63-63	Pg - 71	VI	-
Pg - 08	IJ	63-15	Pg - 47	LV	63-63	Pg - 72	VI	-
Pg - 09	IJ	63-31	Pg - 48	IJ	63-63	Pg - 73	VI	-
Pg - 10	IJ	63-63	Pg - 50	LM	-	Pg - 74	LM	-
Pg - 12	IJ	63-23	Pg - 51	LM	-	Pg - 75	LM	-
Pg - 15	IJ	63-31						

[1/]County: DA: Damolândia (GO); IJ: Ijací (MG); LM: Lambari (MG); LV: Lavras (MG); VI: Viçosa (MG).

Table 2 Summary of AMOVA for five places of occurrence of *P. griseola*, evaluated by RAPD markers.

S.V.	**DF**	**SS**	**Variance components**	**% Total**	Φ_{ST}	**P**
Among places	4	206.593	3.2463	22.49	0.2249	0.000
Within places	65	727.079	11.1858	77.51		
Total	69	933.672	14.4321	100.00		

CONCLUSIONS: The existence of high variability has been demonstrated by RAPD markers. *P. griseola* maintains a genetic structure consistent with asexual reproduction.

ACKNOWLEDGMENTS: The authors thank the Brazilian National Research Council (CNPq) for a scholarship and for funding the project.

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VARIABILITY AMONG PSEUDOCERCOSPORA GRISEOLA ISOLATES BY ANASTOMOSIS AMONG HYPHAE

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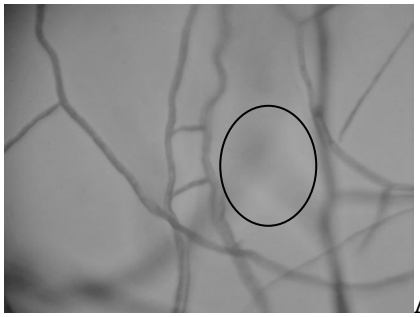
INTRODUCTION: The fungus *Pseudocercospora griseola* is the causal agent of angular leaf spot, a disease of common bean (*Phaseolus vulgaris* L.). Yield losses can be as high as 80%. Breeding for disease resistance is the most effective strategy to control the bean angular leaf spot disease. The successful development of angular leaf spot-resistant cultivars depends on understanding the levels of variability among populations of the pathogen. Traditionally, the high pathogenic and genetic variation has been evaluated by differential cultivars and molecular markers. There is a need to explain the wide pathogenic and genetic variability. The parasexuality has been presented as a mechanism used by assexual fungi and for its ocurrence the formation of anastomosis among hyphae is needed. Anastomosis, i.e. fusion between vicinal hyphae, represents an important communication within a fungal system that leads to the flow of cytoplasmic and genetic material and, consequently, to the increase genetic variability. The anastomosis occurrence among hyphae can be taken into consideration as a trait for population studies. The aim of this work was to identify the variation among isolates of *P. griseola* collected in Minas Gerais state, Brazil, by anastomosis groups.

MATERIALS AND METHODS: A total of 20 isolates of *P. griseola* collected in Minas Gerais State (Ijací, Lambarí and Lavras) and deriving from the culture collection of the Biology Department, Universidade Federal de Lavras (Lavras, MG, Brazil). Each isolate was confronted with itself and all other isolates according to a modified version of the method of Rodríguez-Guerra et al. (2003). Briefly, a sterilized microscope slide was placed on a Petri dish containing water-agar (2%) and covered with a thin layer of M3 medium. Fragments of isolates (5.0 mm) under confrontation were placed on the slide at a distance of 5.0 mm from each other and incubated for 15 days at 22ºC. All confrontations were carried out at least in duplicate. Following incubation, the slide was lifted from the Petri dish and the original fungal fragments were carefully removed in order to leave only the newly formed hyphal masses. Hyphae were stained with a 0.05 % solution of trypan blue-lactophenol, the slide was covered with a cover slip and submitted to examination under the light microscope. Anastomosis was classified as positive (compatible reaction) following observation of fusion of hyphae from the paired isolates.

RESULTS AND DISCUSSION: Anastomosis bridges were observed in H form (Figure 1). Anastomoses were observed for all the isolates (Table 1), however three isolates presented 85.0% of compatibility,. The Pg-48 isolate presented smaller percentile (45.0%) of anastomosis formation with others isolates. The similarity estimates for the anastomosis data varied from 0.15 to 0.85, evidencing high variability with sixteen anastomosis groups being observed. All the groups were formed for a single isolate, except, one group that formed by Pg 01, Pg 02, Pg 07, Pg 08 and Pg 12 isolates. Isolates from different groups can present anastomosis among hyphae. This is the first report of the anastomosis occurrence among hyphae for the *P. griseola* fungus.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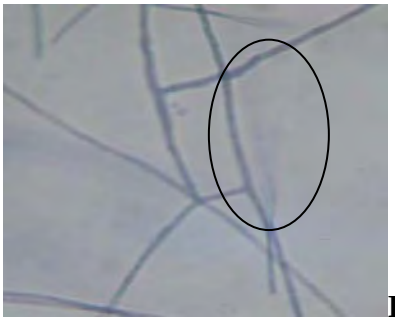
B

FIGURE 1. Anastomosis, in the form of an H-shaped fusion (circled), between hyphae of isolates Pg-01 and Pg-08 (A) and Pg-16 and Pg-52 (B).

TABLE 1. Compatibility reactions (anastomosis among hyphae) among isolates and proportion of compatible reaction (%) for each isolate.

Isolados	Pg 01	Pg 02	Pg 03	Pg 07	Pg 08	Pg 12	Pg 16	Pg 19	Pg 24	Pg 35	Pg 41	Pg 45	Pg 46	Pg 48	Pg 52	Pg 53	Pg 54	Pg 55	Pg 63	Pg 65	%
Pg-01	+	+	–	+	+	+	+	–	+	+	+	+	–	+	–	+	–	+	+	+	75,0
Pg-02		+	–	+	+	+	+	+	+	+	+	+	–	+	–	+	+	+	+	+	85,0
Pg-03			+	+	+	–	–	+	+	+	+	+	+	–	+	+	+	+	+	+	75,0
Pg-07				+	+	+	+	–	+	+	+	+	+	+	–	+	+	+	–	+	85,0
Pg-08					+	+	+	–	+	+	+	+	–	+	+	+	+	–	+	+	85,0
Pg-12						+	+	–	+	–	+	+	+	+	–	+	+	+	+	+	80,0
Pg-16							+	+	–	+	–	–	+	–	+	–	–	+	+	–	60,0
Pg-19								+	+	+	+	+	+	–	–	–	+	+	–	+	60,0
Pg-24									+	–	+	+	+	–	+	–	+	+	+	–	75,0
Pg-35										+	+	–	+	+	+	–	+	+	+	+	80,0
Pg-41											+	–	+	–	+	+	+	+	+	–	80,0
Pg-45												+	+	–	+	–	+	–	+	+	70,0
Pg-46													+	+	+	+	–	+	–	+	75,0
Pg-48														+	–	–	–	+	–	–	45,0
Pg-52															+	+	+	–	+	+	65,0
Pg-53																+	–	–	+	–	55,0
Pg-54																	+	–	+	–	65,0
Pg-55																		+	+	+	75,0
Pg-63																			+	+	80,0
Pg-65																				+	70,0

+ compatible reaction; – incompatible reaction.

CONCLUSIONS: High variability for anastomosis occurrence among hyphae and ausent clustering among isolates for formation of anastomosis among hyphae demonstrating the existence of genetic variability for loci involved with the control of this trait.

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AN EFFICIENT PROTOCOL FOR ISOLATION, SPORULATION AND MAINTENANCE OF *PSEUDOCERCOSPORA GRISEOLA*

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INTRODUCTION – Angular leaf spot (ALS), incited by *Pseudocercospora griseola* (Sacc.) Crous & U. Braun, is present in essentially all common bean growing regions, mainly when the temperature is mild and the relative humidity high. Availability of highly concentrated and homogeneous inoculum is required to validate the monocyclic parameters related to the disease in plants. Deficiencies during the inoculation procedure are among the main causes of the discrepancies among the results of inoculation experiments under controlled conditions (RI BEIRO, 1991). For this reason, the development of a protocol that leads to the production of a large number of mature conidia is needed for obtaining high quality inocula (DALLA PRIA et al., 1997). STENGLEIN et al. (2006) did an extensive review about the dificulties several authors have faced during *Ps. griseola* cultivation *in vitro*. We report here an efficient protocol for cultivation of *Ps. griseola* which will be important for works focusing on ALS genetic control and the physiology of the fungus.

MATERIALS AND METHODS

Isolation – Infected bean leaves should be collected in the field and stored in paper bags at low temperatures (approx. 10°C) for at most 60 days. To obtain monosporic cultures (isolates) it is recommended the following modified procedure based on CORREA-VICTORIA (1987) and PYINDJI (1991): (i) scrape the edge of conidiophores at the abaxial face of contaminated leaves with a needle which has been previously heat sterilized and kept in sterile water; (ii) transfer the conidia attached to the needle to a drop of sterile water on the surface of a 3% agar-agar medium in a petri dish; (iii) spread the drop on the surface of the medium with a Drigalski spatula; (iv) after a 24-hour incubation period at approx. 25°C, the germinated conidia should be visualized with the aid of a stereoscope microscope and transferred individually to test tubes containing 2-3 ml of the following medium prepared with distilled water: 22% commercial tomato sauce, 2% agar-agar, 0.3% calcium carbonate and 10 µg/mL streptomycin. The tubes should be kept for 20-30 days at approx. 25°C.

Sporulation and maintenance – When the colonies reach a satisfactory growth (approx. 1 cm in diameter) after 20-30 days at approx. 25 °C (Figure 1A) the mycelial mass present in tubes should be transferred to petri dishes containing the same type of medium present in the tubes, and kept in a BOD in absence of light at 24ºC for 10-15 days. These petri dishes can now be used for obtaining spores for inoculation or for maintenance of the isolate according to the following steps: (i) a mycelium fragment with approx. 1 cm in diameter should be transferred under aseptic conditions to a test tube containing 2 mL sterile water (Figure 1B); (ii) the mycelium should be macerated with the aid of a 20 cm long sterile wooden stick (Figure 1C); (iii) 0.5 mL of the suspension obtained should be transferred to the center of another petri dish containing fresh medium (Figure 1D); (iv) the plate should be ketp in a BOD in absence of light at 24ºC for 10-15 days. For obtaining spores sterile water should be added to the medium surface and the conidia and mycelium fragments should be scrapped with a sterile spatula (Figure 1E) and filtered through a two layers of gauze (Figure 1F). The spore concentration can now be determined with the aid of a hemocytometer and adjusted with sterile water to the desired concentration.

For maintenance of the isolates steps (i) through (iv) should repeated every three months and the plates kept at 4°C.

RESULTS – In the experiments for obtaining *Ps. griseola* monosporic cultures conducted by the Common Bean Breeding Program of BIOAGRO/UFV mycelia grew in approx. 63% of the tubes used in the isolation procedure. The main factor limiting the isolation success was the presence of contaminating microorganisms. It also seems that the proper positioning of the germinated conidia in the tube during the first steps of isolation is an important factor to consider. The conidia should be in close contact with the medium to allow proper growth. For *Ps. griseola* inoculation procedures, PASTOR-CORRALES & JARA (1995) recommend a final spore concentration of 2.0×10^4 per mL. Using the procedure we described concentrations higher than 1.0×10^5 conidia/mL were obtained. On average, for each plate 100 mL of inoculum containing 2.0×10^4 conidia/mL were obtained, which is enough to inoculate approximately 50 plants at the V3 stage using a de Vilbiss n° 15 atomizer.

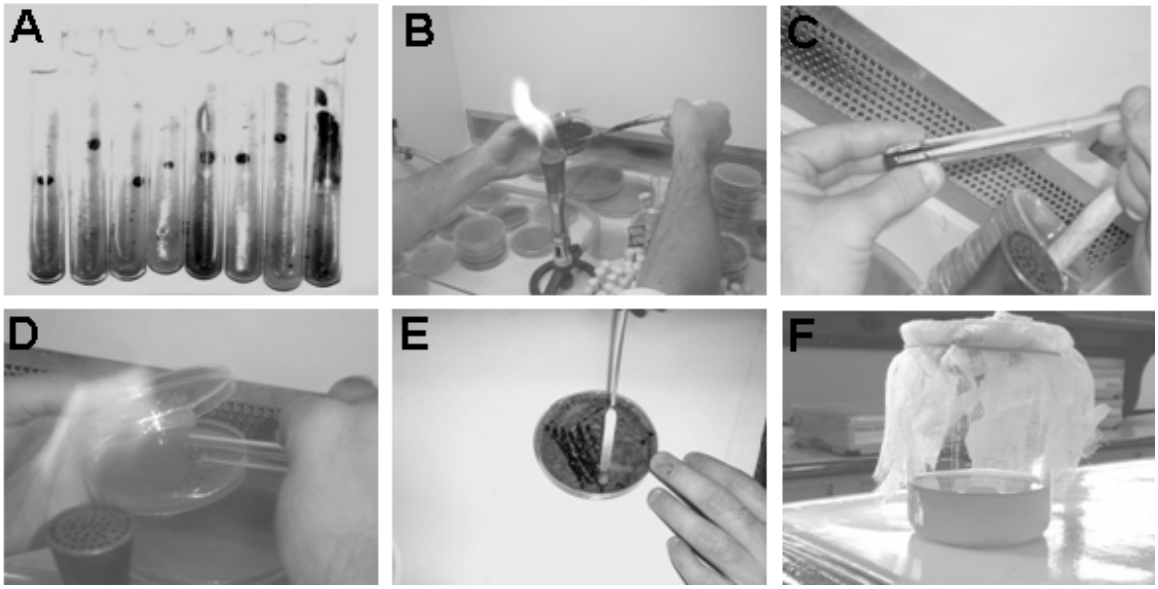

Figure 1. Steps for isolation, sporulation and maintenance of *P. griseola*. (A) Test tubes containing mycelial mass; (B) Fragmentation of mycelium; (C) Maceration of mycelium fragments; (D) Pouring mycelium suspension onto growth medium; (E) Scrapping of medium surface; (F) Filtration of mycelial mass.

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REACTION OF COMMON BEAN CULTIVARS AND ELITE LINES TO ISOLATES OF *PSEUDOCERCOSPORA GRISEOLA*

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INTRODUCTION – Diseases are among the main factors limiting the common bean performance. Angular leaf spot (ALS) incited by *Pseudocercospora griseola* (Sacc.) Crous & U. Braun is one of the most important bean diseases in Brazil. Constant monitoring of prevailing races of the pathogen in different regions, and phenotypic characterization of resistance/susceptibility of the various bean lines and cultivars developed by breeding programs are essential for their success. Therefore, the objective of this work was to determine the reactions of bean cultivars and elite-lines to different isolates of *Ps. griseola*.

MATERIALS AND METHODS – To evaluate the bean genotypes highly virulent and/or highly aggressive isolates maintained by our breeding program were used (Table 1). The severity of the disease was visually verified after 15, 18 and 21 days after inoculation using a nine degree symptom scale proposed by PASTOR-CORRALES & JARA (1995). The group of genotypes evaluated with regard to ALS resistance/susceptibility was composed of important jalo, carioca, black and red bean cultivars and elite-lines developed by the UFV Common Bean Breeding Program (Table 2). Twelve plants from each genotype with a density of three plants per pot were inoculated. Plants with degrees between 1 and 3 were considered resistant and with levels 4 or greater, susceptible.

Table 1. *Ps. griseola* isolates used in this work and maintained at BIOAGRO/UFV

Isolate	Race	Collecting site	Reference
SM 20	63.63	São Miguel do Anta – MG	BALBI (2007)
SM_2-11	63.63	São Miguel do Anta – MG	BALBI (2007)
SM 28	63.63	São Miguel do Anta – MG	BALBI (2007)
B_1-46	63.63	Paracatu – MG	BALBI (2007)
97-2	31.17	Coimbra – MG	NIESTCHE et al. (2000)
158-1	63.23	Goiânia – GO	NIESTCHE et al. (2000)

RESULTS AND DISCUSSION – The results obtained confirm the difficulty of identifying bean genotypes with ample resistance to ALS. Of the 25 genotypes tested, 11 were susceptible to at least five out of the six isolates inoculated. On the other hand, cultivars Majestoso, Diamante Negro and Ouro Negro were resistant to five of the six isolates tested (Table 2). Our results confirm 'Ouro Negro' as highly resistant to ALS (SARTORATO, 2006). The inoculation tests also showed differential reactions of some of the genotypes to distinct isolates, indicating vertical resistance.This work confirms the trend observed by other authors in relation to the small number of bean genotypes with ample resistance to ALS (PAULA-JR & ZAMBOLIM, 1998; NIETSCHE et al., 2001). This observation in addition to the fact that *Ps. griseola* is highly variable indicate that vertical resistance

only is not the best strategy for ALS control. It is therefore suggested that genotypes which present horizontal resistance be used whenever possible as sources of resistance. Another possibility for ALS control is the development and use of multilines (SARTORATO, 2006).

Table 2. Common bean cultivars and elite lines evaluated for their resistance/susceptibility to *Ps. griseola* isolates

Genotypes	Gene pool	Bean type	Elite-Line/ Cultivar	Isolate					
				SM 20	SM_2-11	SM 28	B_1-46	97-2	158-1
BJ-1	A	Jalo	L	7.67*	4.62	3.14	5.00	3.71	1.86
BJ-2	A	Jalo	L	5.50	6.25	2.50	5.60	5.00	2.38
BJ-3	A	Jalo	L	7.17	7.20	1.75	3.40	1.86	2.65
BJ-4	A	Jalo	L	3.60	4.00	2.67	6.75	1.67	5.14
BJ-5	A	Jalo	L	3.60	4.25	2.60	3.50	5.33	2.85
BJ-6	A	Jalo	L	8.60	3.50	4.25	7.00	2.36	2.45
BJ-7	A	Jalo	L	3.60	4.40	1.67	3.83	3.25	4.71
BJ-8	A	Jalo	L	4.50	8.40	2.40	4.43	4.28	2.86
Jalo EEP 558	A	Jalo	C	5.89	7.50	5.50	2.78	2.71	2.13
Jalo MG 65	A	Jalo	C	6.67	7.00	3.50	5.00	5.50	4.75
CAL 143	A	Jalo	C	4.33	3.67	2.33	3.33	1.00	1.43
Rudá	M	Carioca	C	8.85	6.00	5.57	7.86	6.75	5.63
Rudá R	M	Carioca	L	8.67	7.87	7.43	7.17	1.50	1.29
Pérola	M	Carioca	C	2.50	3.60	6.60	5.40	5.00	4.12
Pérola R	M	Carioca	L	5.00	7.67	5.80	5.67	1.75	1.00
Talismã	M	Carioca	C	3.33	3.50	5.25	7.00	2.75	2.25
VC-6	M	Carioca	L	4.33	8.00	7.00	2.25	6.00	3.50
VC-3	M	Carioca	L	4.75	8.75	6.43	8.00	2.14	3.12
Majestoso	M	Carioca	C	1.20	1.20	1.83	1.17	6.30	1.14
Diamante Negro	M	Black	C	2.86	1.14	3.57	1.50	3.00	1.87
Ouro Negro	M	Black	C	3.00	1.85	2.00	2.50	5.43	2.00
Meia Noite	M	Black	C	7.58	7.85	5.87	7.20	6.57	2.86
Valente	M	Black	C	5.50	5.33	7.37	1.50	8.14	3.25
Ouro Vermelho	M	Red	C	8.20	7.25	8.62	4.60	8.88	7.00
Vermelhinho	M	Red	C	8.80	9.00	7.17	8.20	8.38	7.42

A: Andean; M: Mesoamerican; L: Elite-Line from the UFV Common Bean Breeding Program; C: Cultivar; *Average severity based on evaluation of 12 plants of each genotype**.**

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PROGRESS OF HALO AND COMMON BLIGHT IN BEAN GROWN AT TEXCOCO, STATE OF MEXICO

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In the Central Highlands of Mexico diseases are important constraints of bean crop, under rainfed conditions the most frequent are the halo blight (*Pseudomonas syringae* pv. *phaseolicola, Psp*) and the common blight (*Xanthomonas campestris* pv. *phaseoli, Xcp*) (1). The halo blight can induce early defoliation and the death of young plants, *Psp* can affect the yield because of seed abortion. On the other hand *Xcp* can reduce the photosynthetic area due to necrotic symptoms and reduce the yield. Both diseases can induce severe damage to the bean plants and reduce the seed quality, as well they can infect the seeds and initiate a new disease when they are sown (2, 3). The objectives of this research were 1) to study the dynamics of the natural incidence of the diseases bean common and halo blight during the crop cycle at the Valley of Mexico, under rainfed conditions and 2) to identify resistant cultivars to both bacterial diseases.

MATERIALS AND METHODS

Eleven bean cultivars, from different origin and contrasting growth habit, were sown on June 10, 2001 at the Valle de Mexico Experimental Station of INIFAP (19º 20' N, 2240 masl and 640 mm of yearly precipitation) at Texcoco, State of Mexico. The cultivars were sown in two rows of 5 m with six replicates per cultivar in a completely random design. Plants of 1 m were tagged when the initial symptoms of halo and common blight appeared and were evaluated at 67, 85 and 99 days after sowing (das). To record the diseases was used a visual severity scale from 1: healthy plant to 9: highly susceptible (4). Incidence and disease severity were recorded in each date previously mentioned; the incidence rate and area under disease progress curve (AUDPC) were also estimated. The data were processed by variance analysis using the MSTATC program, the Duncan mean separation test, and simple correlation coefficients between evaluated variables were calculated.

RESULTS

All cultivars showed incidence of both diseases, that of halo blight was higher than 50% from the end of flowering onwards, and common blight reached the same level until the beginning of seed filling. Not any cultivars reached up six value in the severity scale for both diseases, for halo blight the higher values were for HHL 9438-56, BAT 477 and Chippata Market in contrast for common blight the higher values were for 97 RS 326, HHL 9438-56, DON 38 and 97 RS 303. On the other hand cultivars with halo blight lower values were Bayo Madero, Negro 8025 and Pinto Villa, as well the symptoms of both diseases were delayed to appear. In the case of common blight cultivars DON 1013, Chippata Market and Pinto Villa had the lower scores, all of them belong to Nueva Granada Race.

There was not relationship between disease susceptibility and cultivars growth habit. Seed yield was negatively affected by the development of both diseases and halo blight had a larger negative impact, with a significant negative correlation between yield and halo blight severity (-0.733), halo blight incidence (-0.836) and area under halo blight progress curve (-0.706). The reduction of seed yield varied from 35 to 68% in the cultivars more affected by halo blight.

The AUDPC described with major precision the effect of the diseases on the bean crop, in comparison with punctual readings of incidence and severity. For the halo blight the best cultivars reached 7 to 19% of the higher AUDPC. Tolerant cultivars against both diseases were Bayo Madero, Negro 8025 and Pinto Villa.

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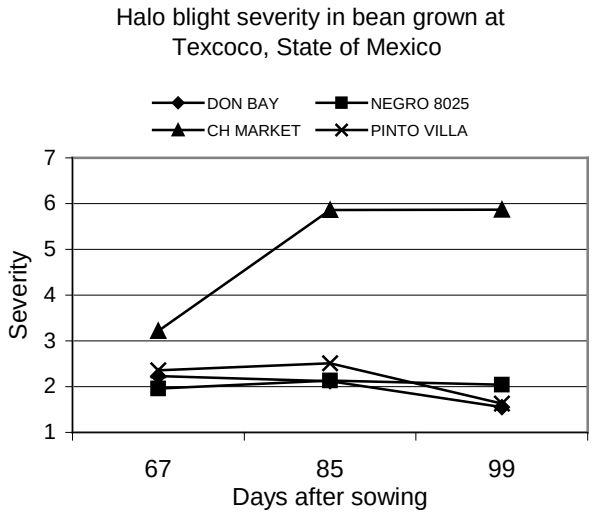

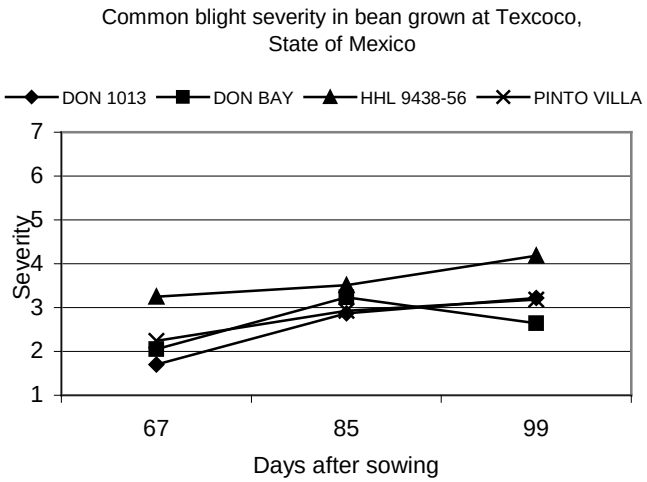

RESISTANCE TO BACTERIAL WILT IN THE PHASEOLUS CORE COLLECTION

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INTRODUCTION

Bacterial Wilt is caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, and has been one of the most problematic bacterial diseases in the USA, particularly throughout the irrigated high plains and Midwest. Bacterial wilt was commonly found in dry bean production in western Nebraska during the 1960's and early 1970's but was largely absent until recently (Harveson et al., 2006). The disease was was widely observed throughout Colorado, Wyoming, and Nebraska from multiple (>200) fields during 2004-2005 (Harveson et al., 2005 and 2006). Affected fields were planted with beans from many different dry bean market classes and seed sources, including yellow, Great Northern, pinto, kidney, black, navy, small red, and Anasazi (Harveson and Schwartz, 2007). CSU researchers recently obtained USDA-ARS funds (58-5348-7-579) via the Phaseolus Crop Germplasm Committee to evaluate accessions from the Phaseolus Core Collections when exposed to representative isolates of bacterial wilt from the region.

MATERIALS AND METHODS

A subset (100 each representing a range of seed colors and origins) of the *Phaseolus vulgaris* Core Collection – Mexican Subgroup accessions and the combined Latin American Subgroup accessions were screened for reaction separately to a yellow and an orange Bacterial Wilt isolate. The core collection subgroups represent the Central/South American *Phaseolus* collection maintained at the Western Regional Plant Introduction Station in Pullman, WA (Miklas et al., 1998).In addition, a subset of currently-grown cultivars (3 to 5 each) from various market classes grown in the United States were evaluated to assess their risk to this pathogen. These included pinto, great northern, small red, light red, yellow, and black market classes.

The accessions and other candidate germplasm were screened using the cotyledonary node inoculation method. Seven to 8 seeds were sown 2.5 cm deep into potting mix in a 15-cm wide plastic pot and thinned to 5 emerged seedlings prior to inoculation. The point of a sterile dissecting needle bearing inoculum was inserted into the stem at the cotyledonary node of 7 to 10 day old seedlings. The inoculated plants and checks (susceptible Myasi and resistant Emerson) were incubated in a greenhouse with daily temperature of 28°C/ 22°C and photperiod 16 hr day/8 hr night and watered as needed. Two pots of 5 plants each served as 1 of 3 replicates for eachisolate of Bacterial Wilt. Ten plants each of the resistant Emerson check and susceptible Myasi check were included with each two month-long series for of one Bacterial Wilt isolate, approximately 100 entries, each with 3 reps, taken from planting to emergence to inoculation to final evaluation 4 weeks later. Approximately 200 entries were evaluated over a 4 to 6 month period per isolate; requiring a total of 9 to12 months to evaluate all entries for reactions to both Bacterial Wilt isolates.

Disease development was evaluated according to a modification of Hseih et al. (2005): 1 = no wilt or discoloration, 2 = wilt or discoloration on one of the primary leaves, 3 = wilt or discoloration on both primary leaves with no symptoms on the 1st trifoliolate leaf, and 4 = wilt or discoloration on the 1st trifoliolate leaf. Data were reported as an average severity for the 10 plants per rep.

RESULTS

The 2 Core Collections from Latin America (100 entries each) have been screened against the Yellow Isolate (B528), Orange Isolate (B557) and Purple Isolate (B597) of Bacterial Wilt in the greenhouse during 2008. Of these 200 accessions, 96, 124 and 104 were rated as resistant (< 2.01) to the yellow, orange and purple isolates, respectively, on the 1 to 4 scale. The highly resistant Emerson check rated 1.00 to 1.25, while the highly susceptible Myasi check rated 3.60 to 4.00. A total of 74 entries from the combined sets of 200 accessions were rated resistant to all 3 isolates of bacterial wilt, and represent a wide range of bean market classes based on seed color and size.

The evaluation of 52 commercial dry bean cultivars has been completed, and 32, 31 and 21 were found to be resistant (< 2.01) to the Yellow, Orange and Purple Isolate, respectively. Only 18 cultivars were resistant to all 3 isolates of Bacterial Wilt, and included: Pinto – Montrose, Othello, Poncho, UI 114; Great Northern – Hungerford, Sawtooth, UI 59, Emerson; Small Red – USRM 20, Merlot; and Pink – Rosalee.

These results will be submitted to GRIN at the conclusion of the Project. It will be interesting to compare stability of resistant cultivars and accessions when exposed to other isolates of Bacterial Wilt by other projects and screening conditions.

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SYMPTOM PATTERN OF COMMON BEAN GENOTYPES INOCULATED WITH DIFFERENT ISOLATES OF *CURTOBACTERIUM FLACCUMFACIENS* PV. *FLACCUMFACIENS*

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INTRODUCTION

Curtobacterium flaccumfaciens pv. *flaccumfaciens* (Cff), the causal agent of the bacterial wilt of common bean (*Phaseolus vulgaris*), is a vascular pathogen of difficult control, first detected in São Paulo State, Brazil in 1995 (Maringoni and Rosa, 1997). Due to the difficulty in controlling this disease, genetic resistance has been the best option for disease management. The aim of this study was to evaluate the disease progress and difference in plant growth of two common bean genotypes considered resistant (Ouro Branco) and susceptible (LMRs 11997) to the bacterial wilt, and the differential interaction between isolates.

MATERIALS AND METHODS

The disease symptoms as wilt (M), flaccidity (F), yellowing (A), leaf burn (BQ) and wizen leaf board (BE) were assessed at 7, 11, 14 e 18 days after inoculation with inoculation of seven Cff isolates in plants at ten days after sowing. The plant height was measured at 14 days after inoculation.

RESULTS AND DISCUSSION

Ouro Branco showed lower intensity of disease and the symptoms of wilt and flaccidity were more frequent. Plants inoculated with the isolate CffCNPAF 03 did not show symptoms of yellowing and wizened boa rd throughout the period of evaluation. It was observed variation in plant height according to the isolates used (Figure 1). UnB 1252, CNPAFCff 01, CNPAFCff 02, CNPAFCff 03 and CNPAFCff 04 isolates were more aggressive, causing further reduction in height, differing from the control (non-inoculated plants) and IAPAR 12771 and IAPAR 14305 isolates were statistically equal to the control. In contrast, the LMRs 11997 showed higher intensity of disease and the plants showed all symptoms evaluated (M, F, A, BE and BQ). The amount of plants that showed yellowing, burning, and wisen leaf board was higher for all isolates (Figure 2) and there was a similar reduction in plant height, where the isolates differed only from control. Therefore, it was possible to observe that the pattern of symptoms and plant growth (height) varied according to the genotype and/or isolate used. The disease scale actually in use for selection of resistant genotypes was adapted from *Fusarium oxysporum* f. sp. *phaseoli*-bean pathosystem by Rava et al. (2003). Therefore, it is necessary to develop a diagrammatic scale for bacterial wilt, which might be used for different genotypes inoculated.

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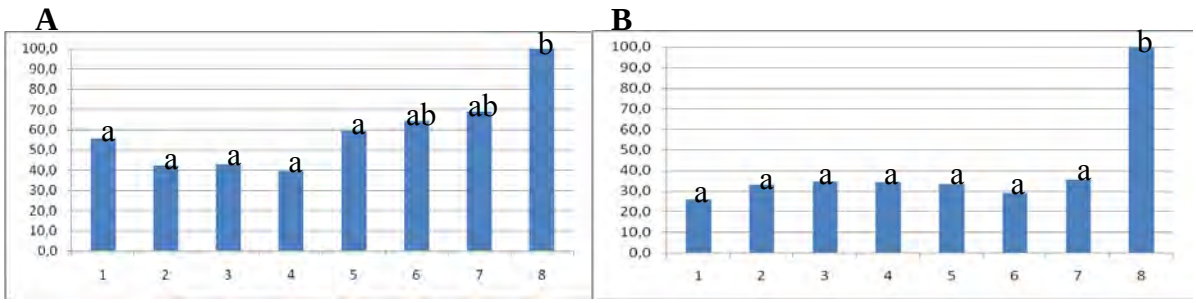

Figure 1. Mean percentage of the plant height of common bean cv. Ouro Branco (A) and line LMRS 11997 (B) inoculated with seven *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* isolates at 14 days after inoculation. Cff Isolates: 1. UnB 1252; 2. CNPAFCff 01; 3. CNPAFCff 02; 4. CNPAFCff 03; 5. CNPAFCff 04; 6. IAPAR 12771; 7. IAPAR 14305; 8. CONTROL.
Columns followed by the same letter are not different by Tukey's test ($p<0.05$).

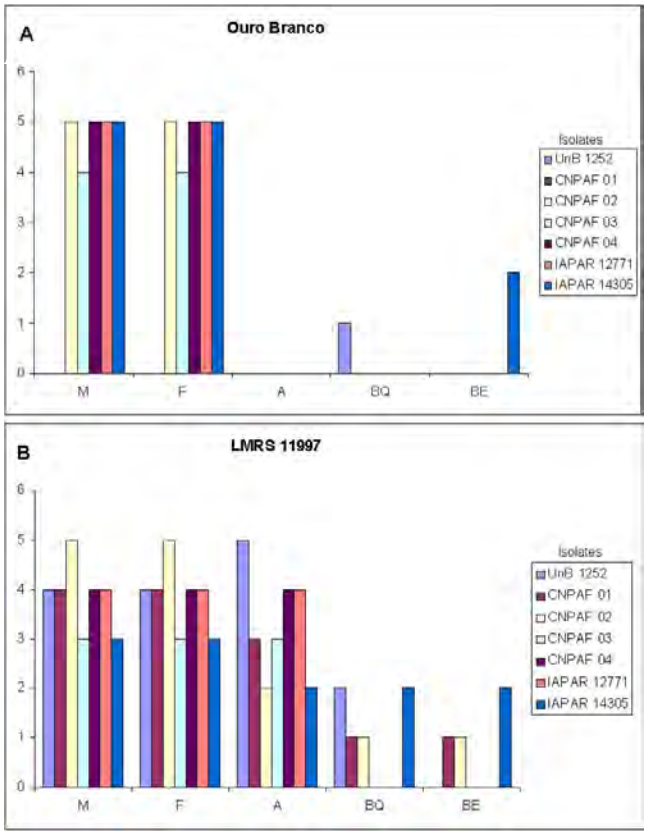

Figure 2. Comparison of symptoms between Ouro Branco - resistant and LMRS 11997 – susceptible (B), at seven days after inoculation with *Curtobacterium flaccumfaciens pv. flaccumfaciens*. The disease symptoms evaluated were wilt (M), flaccidity (F), yellowing (A), leaf burn (BQ) and wizen leaf board (BE).

EVIDENCE FOR A DOMINANT GENE FOR RESISTANCE ON LEAVES OF COMMON BEAN TO THE COMMON BACTERIAL BLIGHT PATHOGEN, *XANTHOMONAS AXONOPODIS* PV. *PHASEOLI*

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The common bean blight pathogen [*Xanthomonas axonopodis* pv. *phaseoli* (Xap)] remains a limiting factor for common (*Phaseolus vulgaris* L.) bean production worldwide and resistance to the pathogen in most commercial bean varieties is inadequate. Variability of the bacterial pathogen has been observed in strains isolated from Puerto Rico and Central America indicating the presence of pathogenic races (Zapata, 1996, Zapata and Beaver, 2005). In previous research, bean lines were identified that had a differential reaction when inoculated with different Xap strains. In an attempt to identify specific genes for resistance to common bacterial blight in common bean, a breeding line that showed a differential reaction on the foliage to Xap strains was crossed with a susceptible parent.

MATERIALS AND METHODS

Two Xap strains (3353 and 1934) that in previous experiments behaved as pathogenic races were selected for the inheritance study. When inoculated with the Xap strains, both PR0313-58 and Rosada Nativa were susceptible to Xap strain 1934. PR0313-58 was resistant and Rosada Nativa was susceptible to strain 3353. A cross between the bean breeding line PR0313-58 x 'Rosada Nativa' was made and advanced a generation to develop a F_2 population. Sixty F_2 plants and 20 plants of each parent were planted in the greenhouse on the University of Puerto Rico, Mayaguez Campus for their response to the selected Xap strains using reactions on two leaflets on the same plant as replications. The method for inoculation has been previously described (Zapata, 2006). Responses were recorded at 14, 21 and 28 days after inoculation and evaluated on a scale from 1 to 10 where 1 = no symptoms, and 10 highly susceptible with systemic infection. For the Chi square analysis two set of data were prepared: Group 1 in which resistant plants were 1-4 and susceptible 5-10 and Group 2 in which 1-3 were resistant and 3.5 to 10 were susceptible. Chi squares tests were used to measure the fit of the results of each group to the expected ratio of 3 Resistant:1 Susceptible plants for a dominant gene in a F_2 population.

RESULTS AND DISCUSSION

The reactions of the F_2 plants using the Xap 3353 strain fit the 3R:1S model for a single dominant gene for resistance to common bacterial blight (Table 1). Both groups of F_2 plants inoculated with Xap 3353 at 14 and 21 days after inoculation had an adequate fit for the 3R:1S model. At 28 days after inoculation, only the F_2 plants in Group 1 fit the 3R:1S model. It should be noted, however, that common bacterial blight readings tend to be less reliable at 28 days after inoculation. When inoculated with the Xap strain 1934, most of the F_2 plants (> 85%) had susceptible reactions at 21 and 28 days after inoculation.

CONCLUSIONS

The resistance derived from the cross PR0313-58 x Rosada Nativa fit the expected ratio of 3 resistant to 1 susceptible plants in the F_2 generation. These results support the hypothesis that resistance to Xap strain 3353 is conferred by a single dominant gene. This suggest the presence of a

corresponding single specific and dominant avirulence gene in Xap 3353 which does not appear to be present in Xap strain 1934. These results represent the first evidence of gene specificity to Xap. We plan to continue to evaluate the reaction to Xap 3353 in the F_3 generation to determine if the patterns of segregation follow the expected distribution for a single dominant resistance gene. If the hypothesis is confirmed we will attempt to identify a molecular marker for the resistant gene. This is the first report of a specific gene for resistance to common bacterial blight in common bean.

Table 1. Leaf reaction of F_2 plants from the cross PR0313-58 / 'Rosada Nativa' to two strains of *Xanthomonas axonopodis pv. phaseoli*.

Group 1

1-4 Resistant (R) Number of plants 3R:1S plants
5-10 Susceptible (S)

Strain	Days after inoculation	Resistant Mean ≤ 4.0	Susceptible Mean > 4.0	Expected resistant	Expected susceptible	X^2	P
3353	14	48	9	42.75	14.25	2.58	0.108
	21	43	14	42.75	14.25	0.0058	0.939
	28	41	15	42.00	14.00	0.0952	0.758
1934	14	36	22				
	21	8	50				
	28	3	55				

Group 2

1-3 Resistant (R) Number of plants 3R:1S plants
3.5-10 Susceptible (S)

Strain	Days after inoculation	Resistant Mean ≤ 3.0	Susceptible Mean > 3.5	Expected resistant	Expected susceptible	X^2	P
3353	14	41	17	43.50	14.50	0.58	0.45
	21	44	13	42.75	14.25	0.15	0.70
	28	31	26	42.75	14.25	12.9*	0.00
1934	14	13	45				
	21	2	56				
	28	1	58				

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VIRULENCE DIVERSITY OF *UROMYCES APPENDICULATUS* IN RHODOPPI MOUNTAINS, BULGARIA

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Bean rust, caused by *Uromyces appendiculatus (*Pers.:Pers.) Unger, is a major disease on common bean (*Phaseolus vulgaris* L.*)* in moderately humid areas of the world, with high virulence diversity in its population. In Bulgaria, this disease has insignificant effect on dry bean production due to its sporadic occurrence in plain areas (Kiryakov, 2004). In the Rhodoppi Mountains, farmers annually grow bean landraces in mixture of *P. vulgaris* and *P. coccineus*, without crop rotation. The environment in this area is favorable for pathogen overwintering, inoculation and dispersal. As a result the disease occurs every year and causes considerable yield losses. Up to now, 7 physiological races have been described in Bulgaria by inoculation of a set of 19 differential bean cultivar (Kiryakov and Genchev, 2003) and 3 pathotypes after inoculation of a new set of 12 differentials - 20-0; 20-2; 20-3 (Kiryakov, 2004). The knowledge about virulence diversity of the rust pathogen in this area will support a breeding program for rust resistant variety.

MATERIALS AND METHODS

During 2006 and 2007 rust-infected leaves including *P. vulgaris* and *P. coccineus* were collected from small farms in four locations in the Rhodoppi Mountains (Table 1). Forty single-uredinium isolates were obtained after tree cycles of inoculation of the susceptible variety Dobrudjanski 7. The virulence phenotype of the isolates was determined by inoculating the new set of 12 differentials cultivars proposed by Steadman *et.al*. (2002). Primary leaves 2/3 expanded (approximately 10 days after sowing) were inoculated with spore suspension (2.0×10^4 uredospores/ml). The spore mass was suspended in Tween 20 (0,1%, v:v) solution for inoculums preparation. The inoculated plants were placed in a mist chamber (20°C, relative humidity >95%) for 18h, and after that they were transferred to a greenhouse (20-25°C) until the symptoms developed. The infection degree was determined approximately 14 days after inoculation when up to 50% of the pustules were sporulating by using a six infection degree scale (Stavely *et.al*., 1983). According to this scale, infection types 1, 2, 3 were considered to be resistant.

RESULTS AND DISCUSSION

Seven physiological races were identified on the basis of the virulence phenotype of 40 single-uredinium isolates on the set of 12 differential cultivars (Table 1). Race 20-3 was the most frequent followed by race 20-0. Fifteen isolates had virulence phenotype typical for race 20-3, and 12 isolates - for race 20-0. Race 20-0 was observed in all investigated locations, and race 20-3 - in tree of them. Third in frequency was race 20-2, which was found in Rakitovo and Smilyan. These races were isolated from both *P. vulgaris* and *P. coccineus* landraces. Least frequent was race 28-1. Two isolates showed virulence which referred them to races 20-1 and 52-3, and tree isolates - to race 20-19. Races 20-1, 28-1, 20-19 were distributed in Devin and race 52-3 in Smilyan. These four races were isolated from *P. vulgaris* landraces.

The virulence diversity of the pathogen was highest in the area of Devin, followed by Smilyan (Table 1). Five races were identified in Devin and 4 races in Smilyan. Races 20-19, 20-1, 28-1 and 52-3 were found only in these locations. Three races were obtained in Rakitovo. The lowest

virulence diversity was observed in Kostandovo. The isolates from this area had a virulence phenotype referring them only to race 20-0.

The results from many investigations confirm the existence of co-evolution between bean and bean rust pathogen. Three groups of isolates have been determined (Sandlin et al., 1999; Araya et al., 2001). All the identified races in our research, except for 20-0, had virulence phenotype typical for the group of Andean-Middle American isolates of the rust pathogen. Race 20-0 overcame only specific resistance genes in the Andean gene pool and had to be referred to the Andean-specific pathotypes of *U. appendiculatus.*

The race specific genes *Ur-4, Ur-13, Ur- 5, Ur-3+, Ur-11* were not overcome by the isolates distributed in the Rhodoppi Mountain. They can be used in the DAI Breeding Program for developing of commercial varieties with pyramidal resistance to the identified *U. appendiculatus* races.

Table 1. Races identified on a differential set of 12 bean cultivars, among 40 isolates of *U. appendiculatus* collected from *P. vulgaris and P. coccineus* landraces grown in four locations in Rhodoppi Mountain.

No of isolates of each races				Race	Gene pool											
					Andean						Middle American					
Devin	Rakitovo	Konstandovo	Smilyan		**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
3	*1*	*2*	*6*	20-0	-*	-	+	-	+	-	-	-	-	-	-	-
6	*3*	-	*6*	20-3	-	-	+	-	+	-	+	+	-	-	-	-
-	*4*	-	*1*	20-2	-	-	+	-	+	-		+	-	-	-	-
3	-	-	-	20-19	-	-	+	-	+	-	+	+	-	-	+	-
1	-	-	-	28-1	-	-	+	+	+	-	+	-	-	-	-	-
2	-	-	-	20-1	-	-	+	-	+	-	+	-	-	-	-	-
-	-	-	*2*	52-3	-	-	+	-	+	+	+	+	-	-	-	-

Andean gene pool: **1)** Early Gallatin (*Ur-4)-1;* **2)** Redland Pioneer (*Ur-13)-2;* **3)** Montcalm (*Ur-?)-4;* **4)** PC 50 (Ur-9;12)-8; **5)** G.G.Wax (*Ur-6*)-16; **6)** PI 260418 (*Ur-?*)-32; **Middle American pool: 1)** G.N.1140 (*Ur-7)-1;***2)** Aurora (*Ur-3) - 2;* **3)** Mexico 309 (*Ur- 5)-4;* **4)** Mexico 235 (Ur-3+)-8; **5)** CNC (*Ur-CNC*)-16; **6)** PI 181996 (*Ur-11*)-32

* - Resistant; + Susceptible

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SOYBEAN RUST RESISTANCE IN THE COMMON BEAN CULTIVAR PI181996

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INTRODUCTION

Soybean rust incited by the highly variable fungus *Phakopsora pachyrhizi* H. Sydow & P. Sydow has been one of the major diseases limiting soybean production in Brazil. The high severity and virulence diversity of the pathogen prevent the effective control of the soybean rust by plant resistance. Currently, all Brazilian soybean commercial cultivars are susceptible to the disease. The host range of *P. pachyrhizi* is broad, including over 90 species, several of them of economical importance. Among these is the common bean (*Phaseolus vulgaris* L.), the food legume most used for direct human consumption in the world. Because soybean rust has been reported in beans gown under field and controlled conditions (Preez *et al*. 2005, Pastor-Corrales *et al*. 2006, Miles *et al*. 2007, Souza *et al.* 2008), many breeders are concerned that this pathogen also becomes a serious problem to the bean crop in endemic areas. Here we report the inheritance of soybean rust resistance in the common bean cultivar PI181996. This cultivar was previously characterized by Souza *et al.* (2008) as an important resistance source to be explored by bean and soybean breeding programs in Brazil.

MATERIALS AND METHODS

Inheritance of soybean rust resistance in PI181996 was studied by crossing this resistance source with the susceptible genotypes US Pinto 111 and Mexico 309. Plants of these cultivars were grown and artificially crossed under greenhouse condition. The F_1 plants were identified using morphological and/or molecular markers and used to obtain F_2 and F_3 populations. Soybean control cultivars CAC-1 and Cristalina, parental lines (PI181996, US Pinto 111, and Mexico 309), F_2 and F_3 plants were inoculated with *P. pachyrhizi* spores maintained by the Common Bean Breeding Program of the BIOAGRO/UFV (Viçosa, MG, Brazil). The primary leaf and the first trifolium of all screened plants were inoculated about 15 days after sowing using a solution containing 3.0×10^5 spores/mL. Soybean rust severity was evaluated at 15, 20, and 25 days after the inoculations using a 1-to-5 scale, according to Souza *et al.* (2008). Plants with degree 1 to 3 (no sporulation or sporulation present but less than 25% of fully sporulating lesions) were considered resistance, whereas those with degrees 4 or 5 (sporulation present and more than 25% of fully sporulating lesions) were considered susceptible. The Chi-squared (χ^2) test was used to define the segregation pattern of soybean rust resistance in the studied populations.

RESULTS AND DISCUSSION

Two hundred and forty-six F_2 plants derived from the cross US Pinto 111 x PI181996 and 46 F_2 plants from the cross Mexico 309 x PI181996 were evaluated. The segregation for soybean rust resistance on both F_2 populations fit a 3 resistant: 1 susceptible ratio (3R_:1rr) with χ^2 values of 0.0487 and 0.0289 and probability (*P*) values of 82.52 and 86.48%, respectively (Table 1). These results support the hypothesis that resistance to *P. pachyrhizi* in the common bean cultivar PI181996 is controlled by a single gene with intra-allelic interaction of complete dominance. In addition, the F_3 resulting populations were also analyzed. A total of 107 (US Pinto 111 x PI181996) and 162 (Mexico 309 x PI181996) F_3 plants were screened. A 5R_:3rr segregation ratio was observed on the F_3 populations with χ^2 values of 0.0006 and 0.5942 and *P* values of 98.01 and 44.08%, respectively (Table 1). The results obtained confirm that soybean rust resistance in PI181996 is monogenic and dominant. Currently, we are working on the identification of molecular markers linked to the resistance gene. We are also conducting additional inheritance studies using *P. pachyrhizi* single-pustule isolates recently obtained by our group.

Table 1. Inheritance of soybean rust (*Phakopsora pachyrhizi*) resistance in the common bean cultivar PI181996.

Population	Cross	No. of plants	Expected ratio[a]	Observed ratio[a]	χ^2	*P*(%)[b]
F_2	US Pinto 111 x	246	3(R):1(S)	183(R):63(S)	0.0487	82.52
F_3	PI181996	107	5(R):3(S)	67(R):40(S)	0.0006	98.01
F_2	Mexico 309 x	46	3(R):1(S)	34(R):12(S)	0.0289	86.48
F_3	PI181996	162	5(R):3(S)	106(R):56(S)	0.5942	44.08

[a]Resistant (R) and susceptible (S) plants.
[b]Percent probability of the Chi-square (χ^2) test.

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MAPPING RUST RESISTANCE GENES ON THE DISTAL PORTION OF BEAN CHROMOSOME 11

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INTRODUCTION

The resistance gene cluster on common bean chromosome 11 contains two of the most important rust resistance (RR) genes, *Ur-3* and *Ur-11*. Other resistance genes in the cluster include the rust resistance gene *Ur-Dorado*-53, the anthracnose resistance gene *Co-2*, and quantitative trait loci (QTL) conditioning resistance to common bacterial blight (CBB) and anthracnose (Freyre et al., 1998; Miklas *et a*l., 2006). Several molecular markers linked to the *Ur-11* and one for *Ur-3* genes have been reported, but none have proved satisfactory. The most useful marker to date has been the SCAR marker $sAE19_{890}$ (de Queiroz *et al.*, 2004), linked in repulsion to *Ur-11* (derived from 'PI 181996'). Liebenberg *et al*. (2008) reported a possible linkage in coupling between this marker and *Ur-3*, in Beltsville lines containing *Ur-3* and *Ur-11*. Awale *et al.* (2008) reported a possible linkage between the SQ4 marker, previously linked to the *Co-2* anthracnose resistance gene, and the *Ur-11* gene. The present study investigates the presence of existing markers linked to genes in this cluster in germplasm carrying *Ur-3*, *Ur-11* and *Ur-(3+11)*, as well as other genes present in this cluster.

MATERIALS AND METHODS

The African rust races RSA-Ua1 and TZ-Ua11 (Liebenberg, 2003) were used to determine rust reactions of important germplasm accessions (Table 1) using previously described methods (Liebenberg & Pretorius, 2004). Genomic DNA was isolated from leaves as according to (Saghai-Maroof *et al.,* 1984). PCR analysis was performed for SCAR markers SK14 (*Ur-3*) (Nemchinova & Stavely, 1999), SQ4 (*Co*-2) (Awale *et al.,* 2008) and SCAreoli (*Co*-2) (Geffroy *et al*., 1998). Results were visualized on 2% agarose gels stained with ethidium bromide.

RESULTS AND DISCUSSION

The SK14 marker (for *Ur-3*) (results not shown) gave erratic results following independent PCR amplifications. Results for the other three markers are shown in Table 1. The marker SQ4 (linked to *Co-2*) was present in all accessions carrying *Ur-(3+11)* and *Ur-11*. It gave a false positive band in Teebus and probably in Teebus-RR 1. The marker SCAreoli (for *Co-2*) was present in accessions carrying *Ur-(3+11)* and some with *Ur-3*. These results are similar to those previously reported for the SCAR marker sAE19, in repulsion with *Ur-11* but in coupling with *Ur-(3+11)* (Liebenberg *et al.,* 2008), also shown in Table 1. It is postulated that the SQ4 marker was introduced into the Bel-lines carrying *Ur-(3+11)* with *Ur-11* from 'PI 181996', and the sAE19 and SCAreoli markers together with the *Ur-3* from 'NEP 2' via 'Kodiak', and that these genes and markers are not widely separated on chromosome 11. The possible association of the *Co-2* markers with the rust genes has been investigated using populations segregating for *Ur-11* (Liebenberg et al., 2009) and will be investigated in populations segregating for *Ur-3*.

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Table 1. Reactions of some important common bean accessions to rust races RSA-Ua1, TZ-Ua11 and molecular markers linked to *Ur-11* and *Co-2* genes

	Resistance gene(s)	SCAR marker			Rust reaction	
		sAE19 (0.89)[a]	**SQ4 (1.44)**	**SCAreoli (1/1.3)**	**Race RSA-Ua1**	**Race TZ-Ua11**
		Ur-11 **In repulsion: *Ur-11*; in coupling: *Ur-3* and *Ur-(3+11)***	***Co-2*** **In coupling: *Co-2*; occurs with *Ur-3* and with *Ur-11***	***Co-2*** **In coupling with *Co-2*, generally occurs with *Ur-3***	**Overcomes *Ur-3* but not *Ur-11*[b]**	**Overcomes *Ur-11* but not *Ur-3*[b]**
Group A: *Ur-3?* + from other sources						
Teebus-RR 1 (bred from Teebus)	*Ur-3? (+)*	0	1 (FP?)	0	R	R
51051	*Ur-3? (+)*	1	0	0	R	VR
Ecuador 299; Mexico 235	*Ur-3? (+)*	0	0	0	MS	VR
Group B: May have *Ur-3* (+)						
Mkuzi (A286); Rudá (A285)	*Ur-3? (+)*	1	0	1	R	VR
Group C: *Ur-3* (+), not Ur-11						
Aurora	*Ur-3*	1	1	1	S	R
Helderberg	*Ur-3* (+)	1	0	1	S	VR
NEP 2 (source of *Ur-3* for Bel-lines)	*Ur-3* (+)	1	0	1	S	VR
Group D: *Ur-3* (from NEP 2) + *Ur-11*						
Bel lines[c] with *Ur-(3+11)*	*Ur-(3+11); Ur-6+*	1	1	1	R	R
Group E: *Ur-11*, not *Ur-3*						
BelDakMi-RR-1 (Bred from PI 151388)	*Ur-6+; Ur-11*	0	1	1	R-MR	S
BelMiDak-RR-9 & -RMR-11 (bred from PI 18199)	*Ur-4; Ur-11*	0	1	0	R-MR	S
Sederberg (bred from BelMiDak-RR-8)	*Ur-11; Ur-13*	0	1	0	R	S
Group F: *Ur-11* (+) [(+) not easily transferable]						
PI 181996	*Ur-11+*	0	1	0	VR	R
PI 151385	*Ur-11+* (?)	0	1	1	ND (similar to PI 181996)	
Group G: Accessions with other genes in the chromosome 11 cluster						
Cornell 49242	*Co-2* , not *Ur-11*	0	1	1	R-MR	R-MR
Huron	*Co-2*, not *Ur-3* or *Ur-11*	0	1	1	S	S
Dorado (DOR 364)	*Ur-Dorado 53*, not *Ur-3* or *Ur-11*	1	0	1	S	S
Group H: Accessions with RR genes not in the chromosome 11 cluster						
Brown Beauty; Kranskop group	Not *Ur-3* or *Ur-11*	0	0	0	S	S
Mexico 309; BelNeb-RR-1	*Ur-5 (+)*	0	0	0	VR	VR
Ouro Negro	*Ur-Ouro Negro* + ?	0	0	0	R	VR
CNC	*Ur-CNC+, not Ur-11*	0	1 (FP?)	0	R	R
Group I: Other accessions with unknown RR genes						
Jalo EEP 558; Bonus	Not *Ur-3* or *Ur -11*	0	0	0	S	S
BAT 93	Not *Ur-3* or *Ur -11*	0	1 (FP?)	0	S	S
Teebus	Not *Ur-3, -11* or *Co-2*	0	1 (FP)	0	S	S

[a] Results from Liebenberg *et al,* (2008).

[b] Some other genes also give resistance to these races.

[c] BelDakMi-RMR-16 & -18; BelMiNeb-RMR-7; sAE19 on 13 Bel-lines.

0 = absence; 1= presence; ND=No data; FP=False positive.
VR=very resistant; R=resistant; MR=moderately resistant; S=susceptible.

A CLOSER LOOK AT THE RESISTANCE GENE CLUSTER ON COMMON BEAN CHROMOSOME 11

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INTRODUCTION

Several resistance genes of international importance are situated within the resistance gene cluster on common bean chromosome 11. These include three genes (*Ur-3, Ur-11* and *Ur-Dorado-53*) conditioning resistance to rust, the anthracnose resistance gene *Co-2*, and quantitative trait loci (QTL) conditioning resistance to common bacterial blight (CBB) and anthracnose (Freyre *et al*., 1998; Miklas *et al*., 2006). Liebenberg *et al*. (2008) reported a possible linkage in coupling between *Ur-3* and the SCAR marker $sAE19_{890}$, (linked in repulsion to *Ur-11* from 'PI 181996') (Johnson et al, 1995; de Queiroz *et al*., 2004) in 13 lines from Beltsville possessing *Ur-3* and *Ur-11;* and Awale *et al.,* (2008) reported a possible linkage between the SQ4 marker for the *Co-2* anthracnose resistance gene and *Ur-11*. Further indications of possible linkages between SCAR markers for *Co-2*, namely SQ4 (Awale *et al.,* 2008) and SCAreoli (Geffroy *et al*., 1998) and *Ur-3* or *Ur-11* were provided by Madubanya *et al*. (2009). The aim of the present study was to investigate the possible association of the *Co-2* markers SQ4 and SCAreoli with *Ur-11*, using populations segregating for *Ur-11*, and so doing, to increase our understanding of the organization of resistance gene(s) in this section of chromosome 11.

MATERIALS AND METHODS

The African rust races RSA-Ua1 and TZ-Ua11 (Liebenberg, 2003) were used to determine rust reactions of three populations ('Jenny'/'PI 181996', 'OPS-RS4'/'PI 181996' and 'Kranskop'/'PI 181996' segregating for *Ur-11* using previously described methods (Liebenberg & Pretorius, 2004). Two additional races (RSA-Ua4 and RSA-Ua10) were inoculated on the 'Kranskop'/'PI 181996' population. Genomic DNA from F_2 plants was isolated from leaves as according to (Saghai-Maroof *et al.,* 1984). PCR analysis was performed for SCAR markers SQ4 (*Co-2;* Awale *et al.,* 2008) and SCAreoli (*Co-2*; Geffroy *et al*., 1998). Results were visualized on 2% agarose gels stained with ethidium bromide. SCAR SQ4, polymorphic in the three populations, was subsequently mapped in these populations using JoinMap 3.0 or MapManager QTX (2002).

RESULTS AND DISCUSSION

Stavely (1990), observed that *Ur-11* is not a simple dominant gene but is made up of a series of tightly linked genes that can segregate as a Mendelian unit. This was confirmed by the present authors. Although the segregation ratio (R:S) for the rust ratings obtained for the Kranskop/PI 181996 population is 3:1 in the F_2, as can be expected with a single dominant gene, portions of the gene are relatively easily lost, so that various nuances of resistance are observed, often characterized by marked, but differing degrees of necrosis with or without sporulating pustules. Not all rust races, however, are able to differentiate these grades of resistance. Certain races have also been observed to differentiate similar nuances of resistance in segregating populations of *Ur-3*, but to a lesser degree. A similar phenomenon has been observed for the anthracnose gene *Co-2*. Using data from

the three segregating populations, SQ4 mapped between 11.9 and 18.4 cM from the *Ur-11* gene. The postulated positions of the markers, the complex nature of the genes concerned, and relative distances observed, have been visualized in Fig. 1, using cM distances determined in this study and by the various SCAR authors. If this representation is correct, the (SCAreoli-*Ur-3*-sAE19) segment was inserted between SQ4 and *Ur-11* by means of a double crossover.

Ur-3 and *Ur-11*, previously linked in repulsion but now available in coupling (Stavely, 1998) are a valuable combination in rust resistance breeding. In order to insert this combination as a unit, two or more markers are necessary to prevent the loss of gene block segments. Existing markers linked to *Co-2* and *Ur-11* appear to be on the *Ur-3* side of *Ur-11* (Fig 1), and their use may prevent loss of *Ur-3*. However, an additional, flanking marker linked to the *Ur-11* gene ('A' in Fig. 1) is necessary to minimize the chances of loss of fragments from *Ur-11*. Future plans also include the validation of all existing markers using a population segregating for *Ur*-3, and the validation of existing markers linked to *Ur-Dorado-53* as well as the CBB and anthracnose QTL. The *Co-2* status of some lines must also be determined. Disadvantages of using this relatively large segment include the possible inclusion of undesirable genes and the probable exclusion of the defeated *Co-2* gene for anthracnose resistance. Other more suitable resistance genes for anthracnose resistance on different linkage groups are, however, available.

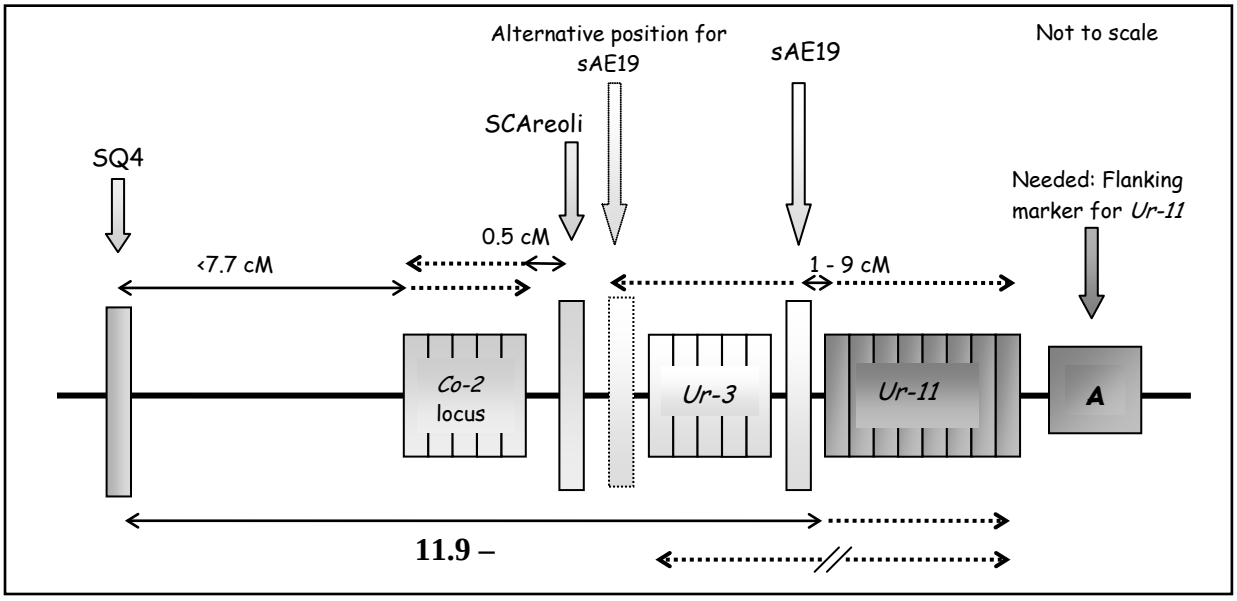

Fig. 1. Postulated positions of some of the resistance genes and existing linked markers for the resistance gene cluster on common bean chromosome 11.

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VIRULENCE OF *UROMYCES APPENDICULATUS* TO THE RESISTANCE GENE *UR-3* IDENTIFIED IN NORTH DAKOTA IN 2008

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INTRODUCTION

Approximately 267,093 hectares of dry edible beans (*Phaseolus vulgaris* L.) are planted in North Dakota (ND) annually (USDA-NASS). Epidemics of common rust (*Uromyces appendiculatus*) were frequent until the 1990's, when the last significant outbreaks occurred in 1994 and 1996 (3). According to loss estimations, statewide yield losses in1994 and 1996 were about 16% and 6%, respectively (3). Since 1996, common rust has been rare in ND, largely due to incorporation of *Ur-3*, an effective resistance gene derived from the cultivar Aurora. Between 1996 and 2000, forty field collections from ND and four from Minnesota were evaluated on 19 differentials (1). Although five races were identified, Aurora was resistant to all isolates (1). Collections after 2000 were rare due to sporadic occurrences of rust, but in 2008, we observed rust late in the season in nearly all dry edible bean fields in a ten to twenty mile wide area centered in Northern Traill County. Rust symptoms were observed in cultivars possessing *Ur-3* previously known to be resistant. The objective of this study was to determine the virulence phenotype of the *U. appendiculatus* isolates found in ND in 2008 and their effect on the current bean varieties grown in this state.

MATERIALS AND METHODS

Rusted leaves were collected from 16 different dry edible bean fields in Traill and Grand Forks Counties in 2008. At least four of those fields were planted with cultivars known to have the resistance gene *Ur-3*. Cultivar names could not be determined for the remaining fields. Urediniospores were harvested, filtered into gelatin capsules, and increased by inoculating field collections on the susceptible pinto cultivar Othello. Urediniospores from seven field collections were re-harvested, suspended in Soltrol 170, quantitated to 500,000 spores/ml, inoculated on the twelve standard differentials (2), and placed into a dew chamber for 24 h (3). The rust reaction was evaluated 14 days post inoculation (2). To assess rust reaction of commercial dry beans cultivars grown in ND, a minimum of four plants of 27 cultivars were inoculated with a combination of the seven field collections and evaluated as described above.

RESULTS AND DISCUSSION

All 2008 field collections were virulent on the Andean differential cultivars Montcalm, and Golden Gate Wax (GGW), and the Middle American cultivars, GN1114 and Aurora (Table 1). The reactions of the differential cultivars to these isolates indicate that they belong to race 20-3. Single pustule isolates are being procured to confirm the race determination. The virulence on Aurora reveals that these rust collections are likely to be virulent on the commonly used dry bean cultivars with resistance gene *Ur-3*. This is supported by the finding that the field collections were virulent on all 26 commercial cultivars tested (Table 2), many of which carry the *Ur-3* gene. If this *U. appendiculatus* population with virulence to *Ur-3* occurs in ND, many dry bean cultivars would be at risk and this will be a significant threat to dry bean production in this state.

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Table 1. Virulence phenotype of seven *U. appendiculatus* field collections isolated from ND in 2008 on the common bean rust standard differentials.

Differentials		Virulence Phenotype[1]						
Gene Pool	Name	1	2	3	4	5	6	7
	Early Gallatin	2	2	2	.	2	1	2
	Redlands pioneer	3	3	3	.	3	3	3
	Montcalm	[2]4,5	4,5	4,5	.	4,5	4,5	5
	PC 50	2	2	3	.	2	2	2
	GGW	4	5	5	.	5	4	6
	PI260418	2	1	3	.	3	3	3
	GN 1140	6	6	6	6	6	6	6
	Aurora	4,5	4,5	4,5	4,5	4,5	4,5	4
Middle	Mexico 309	2	1	1	.	3	2	3
American	Mexico 235	3	3	3	3	3	3	3
	CNC	2	3	3	3	3	3	3
	PI 181996	3	2	2	.	2	1	0
[3]Potential Race Designation		20-3	20-3	20-3	?	20-3	20-3	20-3

[1]Rust reaction was determined according to Steadman *et al.* 2002, when 1 = immune, 2 = hypersensitive flecks, 3 = pustules < 300 µm in diameter, 4 = pustules 300-500 µm in diameter, 5 = pustules 500-800 µm in diameter, and 6 = pustules > 800 µm in diameter. Reactions 1-3 are considered resistant and 4-6 are considered susceptible.
[2]Numbers separated by ","indicate multiple reactions, predominant reaction is presented first.
[3]Race designation determined by adding the binary values assigned to differentials of both gene pool sets, where the six virulent to differentials are valued 1, 2, 4, 8, 16, and 32 in order. Thus, virulence to Montcalm (4), and GGW (16), and GN1140 (1) and Aurora (2) = race designation 20-3.

Table 2. Virulence phenotype of a combination of seven *U. appendiculatus* field collections on 27 bean varieties from multiple market classes.

Pinto		Black		Navy	
Avalanche	5,6	Eclipse	5	Ensign	5
GTS -900	5	Jaguar	5,6	Mayflower	4
La Paz	6	T-39	5	Navigator	4
Lariat	5	Zorro	6	Seahawk	5,6
Maverick	5			Vista	4,3
ND 307	5			Norstar	6
Othello	6	**Kidney**		Aurora	5
Santa Fe	5,4	CELRK	5		
Stampede	4,5	Chinok 2000	4	**Other**	
Winchester	6	Redhawk	4,5	Merlot	6
		Montcalm	4,5	Sedona	6

PERSISTENCE OF A NEW RACE OF THE COMMON BEAN RUST PATHOGEN IN MICHIGAN

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INTRODUCTION

Common bean rust, caused by the hypervariable fungal pathogen *Uromyces appendiculatus* (Pers.:Pers) Unger, severely limits common bean (*Phaseolus vulgaris* L.) production worldwide (Steadman et al., 2002). Many races of this pathogen have been characterized and several resistance genes have also been identified and deployed. Due to co-evolution, both rust resistance genes and pathogen races can be grouped according to their gene pool of origin. Pyramiding multiple resistance genes into a single cultivar should provide the most durable resistance to this pathogen, although few commercially available varieties possess such gene combinations (Stavely, 2000). Previously, the *Ur-3* rust resistance gene has been widely used and effective against all races found in the state of Michigan. However, during 2007 and 2008 rust was observed on the leaves and stems of several previously resistant varieties. The objectives of this study were to further evaluate an isolate of common bean rust collected in Tuscola County, Michigan in 2007 and compare it with an isolate collected in Huron county in 2008 using the rust differentials described by Steadman et al. (2002).

MATERIALS AND METHODS

The 2007 rust isolate was obtained as urediniospores from a previous study described by Wright et al (2008). Samples of infected leaves with sporulating pustules were collected from the varieties 'Merlot', and 'UI-239' by G.V. Varner in Huron county, MI in August 2008. A spore suspension was prepared from these samples and used to inoculate the susceptible variety 'Othello' in the MSU greenhouse. Urediniospores from a single pustule of each isolate were collected and multiplied by additional inoculations of 'Othello'. The differential series proposed by Steadman et al. (2002) was then inoculated using each of these single pustule derived isolates.

RESULTS AND DISCUSSION

Both isolates induced susceptible reactions on the Andean cultivars 'Early Gallatin', 'Redlands Pioneer', 'Montcalm', 'PC-50' and 'Golden Gate Wax' along with the Mesoamerican cultivars 'GN1140', and 'Aurora' (Table 1). Based on the summation of binary values associated with each of these susceptible cultivars in the classification system of Steadman et al. (2002), these reactions identify both isolates as race 31:3. This classification differs from the preliminary identification (22:3) by Wright et al. (2008) that was based on a single inoculation and incorrectly reported the order of the Andean and Mesoamerican differentials (3:22). These data also differ from the data (22:2) obtained when the 2007 isolate was evaluated by M.A. Pastor-Corrales (pers. comm.). These results suggest additional single pustule derived inoculations should be made to determine whether the original field collection could have contained a mixture of several races, leading to different results when only one single pustule derived inoculation was performed. However, these data consistently confirm the virulence of the rust isolates against the widely deployed *Ur-3* gene ('Aurora') that previously conditioned resistance to rust in Michigan. These results also suggest that the isolate collected in 2007 is similar to the 2008 isolate in terms of virulence and that the population of rust possessing virulence to *Ur-3* successfully over wintered and persisted in the environment from 2007 to 2008.

Although observed in MI from one year to the next, this race appeared to exhibit a low fitness in Michigan fields compared to some previously described races. Various races have induced widespread rust epidemics in the past that quickly spread and severely defoliated plants resulting in reduced bean

productivity. In contrast, the isolates from 2007 and 2008 have only been detected in a few localized areas and appear less aggressive in terms of distribution and disease severity. Although a race virulent to *Ur-3* was detected in multiple years, infection was endemic to isolated areas of a field in August when the bean crop was already starting to senesce. In order to significantly impact the crop, infection would need to occur much earlier and in a more widespread manner.

This information should serve as a reminder that varieties which rely on single gene resistance are at risk as pathogen populations shift and new races are introduced by wind, evolve, or become more prevalent in the field. The recent appearance of a similar rust race with virulence to *Ur-3* has also been described by Markell et al., (2009) in North Dakota, suggesting this resistance has lost its effectiveness in these important dry producing states in the U.S. The implications of these evolving pathogen populations remain unclear at this time, but this information serves as a reminder that breeders must continuously seek to broaden the genetic base of disease resistance. Rust resistance genes *Ur-5* and *Ur-11* appear to be the most effective resistance sources to the rust isolates evaluated in this study, and should be considered for pyramiding with *Ur-3* in future cultivars.

CONCLUSIONS

Rust was detected in Michigan in 2007 and 2008 and induced a susceptible reaction on previously resistant cultivars possessing the *Ur-3* resistance gene. Observations to date suggest this rust causes infection later in the growing season, and therefore has a limited effect on the crop. Although similar isolates were obtained from two different years in two different counties, it has only been observed in these isolated locations, suggesting this race may exhibit decreased fitness compared with other indigenous rust races. Regardless of fitness, this race does not appear to spread aggressively even within the major bean producing region of Michigan. However, this knowledge should serve as a reminder that pathogen populations are continually evolving, and maintaining effective genetic resistance to hypervariable pathogens requires persistent effort from plant breeders.

Table 1. Reaction of 12 common bean rust differentials inoculated with rust collected from Tuscola and Huron counties, MI. Reactions scored from 1 to 6 according to Steadman et al. (2002).

Andean	**R-Gene**	**Reaction**		**Mesoamerican**	**R-Gene**	**Reaction**	
		2007	2008			2007	2008
Early Gallatin	*Ur-4*	4,5	4	GN1140	*Ur-7*	6	6
Redlands Pioneer	*Ur-13*	4,5	5	Aurora	*Ur-3*	6	6
Montcalm	*Unknown*	4,5	4	Mexico 235	*Ur-3+*	3	3
PC 50	*Ur-9, Ur-12*	4	3,4	Mexico 309	*Ur-5*	2	3
Golden Gate Wax	*Ur-6*	6	6	CNC	*Ur-CNC*	3/2†	3/2†
PI260418	*Unknown*	3	3	PI181996	*Ur-11*	2	2

† CNC was rated abaxial 3, adaxial 2

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USE OF MULTI SITE SCREENING TO IDENTIFY PARTIAL RESISTANCE TO WHITE MOLD IN COMMON BEAN IN 2008

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The development of bean cultivars with partial resistance and/or avoidance to white mold (*Sclerotinia sclerotiorum*) would reduce disease losses at no cost to producers. The objective of the study is to identify bean germplasm with broad partial resistance to white mold. Bean lines with putative sources of resistance are being developed by bean breeders and were evaluated by greenhouse and field screening methods at seven locations in the United States in 2008.

The field tests consisted of two rows of each entry and a common 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 13 screening tests at nine locations, six field tests and seven greenhouse tests (straw test). Wisconsin field sites were not included due to severe flooding in 2008. The greenhouse screen tested 24 bean lines this year and the field screen tested 12 bean lines. Every location used the same protocol for rating the greenhouse screening method - a modified Petzoldt and Dickson scale for the straw test (Teran et al, 2006) (Table 1). The field nurseries were all evaluated using a 1 to 9 scale (1 = no visible symptoms to 9 = death) which was developed at CIAT (Van Schoonhoven et al., 1987) (Table 2). Spearman and Pearson correlations were used to compare entry WM ratings in the greenhouse and field tests.

The field test results showed a strong correlation between Washington and Michigan (r=0.899 and p=<0.0001); Washington and Oregon (r=0.802 and p=0.0017); Michigan and Oregon (r=0.780 and p=0.0027); and Idaho and Oregon (r=0.754 and p= .0046). The greenhouse tests demonstrated a strong correlation between Washington and Wisconsin (r=0.738 and p=<0.0001); Idaho and Colorado (r=0.675 and p=0.003); and Oregon and Nebraska (r=0.666 and p=0.004).

Bean lines Cornell 605 and A195 were identified as the most resistant using the straw test. Bean lines 11A-39, 37-2, Cornell 603, G122 and 29C-6 demonstrated partial resistance in the greenhouse tests. In the field tests, Cornell 605, 38-4, G122, and 37-2 were identified as the most resistant in the field. Testing bean lines in multiple locations and using field and greenhouse tests have identified Cornell 605 as the most resistant bean line in 2008.

The 2008 screening season continued to use the CIAT rating scale. This change was done to provide a more consistent rating system for all participants of the multi-location screening and provide bean breeders and pathologists with more comparable results.

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Table 1. Mean straw test rating***** with t Grouping****** used to measure white mold resistance in bean lines at seven greenhouse screening locations.

Entry	NE	CO	WA	ID	OR	NY	WI	Mean	t Grouping
Cornell 605 (RK)	4	5	5.9	4	4.4	2.3	7.3	**4.7**	I
A195 (BAYO)	4.3	4.5	5.6	5	5.7	2.7	6.5	**4.9**	I
11A-39 (PTO)	4.1	5.2	5.7	5.9	4.8	3.7	6.2	**5.1**	I
37-2 (PTO)	4.9	4.1	5.8	4.2	5	6.4	5.8	**5.2**	H I
Cornell 603 (RK)	4.3	4.8	6.7	5.6	5.1	2.1	8.2	**5.3**	G H I
G122 (CRAN)	4.1	4.9	7.1	4.8	4.8	3.9	8.1	**5.4**	F G H I
29C-6 (GN)	4.8	4.5	5.3	6.7	4.5	6.9	7.2	**5.7**	E F G H I
Tapia (Romano)	4.7	6.3	5.3	5.8	5.1	8.2	8.1	**6.2**	D E G F H
Stampede (PTO)	4.8	6	6	4.3	6.1	8.5	8.5	**6.3**	C D E F G
NE1-07-12 (GN)	4.9	5.5	7	4	5.9	8.4	9	**6.4**	B C D E F
NE2-06-8 (PTO)	4.6	4.8	7	7.1	5.2	8.5	7.8	**6.4**	B C D E F
Roma II (Romano)	4.2	6.6	5.4	7.4	5.2	9	7.4	**6.5**	B C D E F
38-4 (GN)	4.8	5.9	7.1	4.9	6.6	8.8	9	**6.7**	B C D E
NE2-07-10 (PTO)	4.2	5.7	7.4	9	4.8	7	9	**6.7**	B C D E
B07104 (BLK)	4.9	5.4	6.7	6.8	6.5	8	8.9	**6.7**	B C D E
WM31-2008 (PTO)	5	6	8	5.1	5.5	8.9	9	**6.8**	B C D
P07863 (PTO)	5	6.2	6.1	6.9	5.9	8.6	9	**6.8**	B C D
Ex Rico (Bunsi) (Navy)	4.8	6.7	7.2	6.7	5.1	8.9	9	**6.9**	A B C D
Avalanche (Navy)	4.9	6.8	7.1	7.6	5.1	8.9	9	**7.1**	A B C D
Lariat (PTO)	5.2	6.7	7.5	7.9	5.7	8.6	9	**7.2**	A B C D
NE1-06-12 (GN)	5.8	7.1	7.3	8.2	6.5	7.7	9	**7.4**	A B C
Beryl (GN)	5.5	8.5	7.2	7.8	6.3	7.7	8.8	**7.4**	A B
NE1-07-2 (GN)	5.8	8.6	7.6	9	6.6	8.7	9	**7.9**	A
Eclipse (BLK)	5	8.5	7.8	9	7.4	9	9	**8.0**	A

*****ST rating (1-3 = resistant, 4-6 = intermediate, 7-9 = susceptible); **Alpha = 0.05, LSD = 1.08
BLK = Black, CRAN = Cranberry, GN = Great Northern, PTO = Pinto, and RK = Red Kidney

Table 2. The mean infection rate using the CIAT scale and t Grouping***** in field screening plots from six white mold resistance screening locations.

Entry	NE	WA	ND	MI	OR	ID	Mean	t Grouping
Cornell 605 (RK)	2.0	3.1	5.0	1.0	2.4	4.7	**3.0**	C
38-4 (GN)	2.4	3.0	3.7	3.0	2.6	4.7	**3.2**	B C
G122 (CRAN)	2.0	2.3	6.0	1.5	2.3	5.6	**3.3**	B C
37-2 (PTO)	2.1	2.9	4.7	2.0	3.5	4.7	**3.3**	B C
WM31 (PTO)	3.8	3.3	4.3	2.0	1.6	6.3	**3.6**	B C
Cornell 603 (RK)	2.0	1.8	8.0	1.0	3.2	6.0	**3.7**	B C
B0-7104 (BLK)	4.5	4.0	7.7	2.0	2.3	3.0	**3.9**	B C
P0-7863 (PTO)	2.1	4.1	7.0	3.0	3.2	4.3	**4.0**	B C
11A-39 (PTO)	3.6	2.2	7.0	1.0	4.1	6.3	**4.0**	B C
Ex Rico (Bunsi) (Navy)	3.8	3.6	6.0	2.5	3.4	6.0	**4.2**	B
Orion (GN)	5.0	6.2	8.0	5.0	7.9	8.0	**6.7**	A
Beryl (GN)	4.6	7.2	8.3	4.5	7.7	8.0	**6.7**	A

*****Alpha = 0.05, LSD = 1.17
BLK = Black, CRAN = Cranberry, GN = Great Northern, PTO = Pinto, and RK = Red Kidney

RESPONSE OF TWELVE BREEDING LINES CLASSIFIED IN THE MARKET CLASS FABADA TO WHITE MOLD

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INTRODUCTION

White mold, caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, is a serious disease in common bean causing significant average yield losses. In northern Spain, this disease is endemic and one of the most devastating diseases in the market class fabada. Different plant breeding programs were carried out in the last years in order to protect the cultivar Andecha (market class fabada) against local races of anthracnose (genes *Co-2* and *Co-3/Co-9*), against bean common mosaic virus (BCMV; gene *I*) and bean common mosaic necrosis virus (BCMNV; genes *I+bc-3*), and to modify its growth habit (gene *fin*). A total of twelve near isogenic lines containing different gene combinations and classified in the market class fabada were obtained (Ferreira et al., 2007). Our objective was to investigate the reaction of these twelve lines against one local isolate of white mold. This information can be of most interest in future plant breeding programs focused to increase the resistance degree in the market class fabada.

MATERIALS AND METHODS

A total of 19 accessions maintained in the SERIDA collection were evaluated; (i) the genotypes used as resistance sources: A 252, A 493, BRB130, and Sanilac; (ii) the donor of the determinate growth habit: local accession V203; (iii) the recurrent parent Andecha; (iv) twelve lines derived from cultivar Andecha: A 1220**,** A1258, A 1183, A 1878, A 2806, A 2418, A 2438, X 1319, X1358, X1612, X1633 and Xana;(v) cultivar Cornell 49242, used as susceptible reference.

Resistance tests were developed using the straw method (Petzoldt and Dickson, 1996). The evaluations were carried out using a local isolate obtained from naturally infected bean plants of cultivar Andecha. Plants were evaluated 8-10 days after inoculation, considering the invasion in the main stem. The disease reactions were assessed using a 1 - 9 severity scale, where 1= no symptoms and 9 = total plant collapse (Miklas, 2007). The accessions were classified in three main types according to the average in the disease reaction obtained from three independent evaluations: resistant accessions (disease reaction < 4), susceptible accessions (disease reaction > 6), and accessions showing an intermediate reaction (disease reaction between 4 and 6).

RESULTS AND DISCUSSION

The reaction of the 19 materials against a local isolate of white mold is shown in Table 1. Two types of reactions were identified in the evaluated materials: intermediate and susceptible. Significant differences for the reaction against the isolate were found among cultivar Andecha and the twelve lines derived from this cultivar. The twelve lines showed a higher resistance degree than cultivar Andecha, suggesting that the regions modified as a result of breeding could be implicated in the genetic control of the reaction to white mold. This possibility agrees with the location of quantitative trait loci involved in the resistance to white mold, such as the QTL mapped on linkage group B1 near the *fin* locus (Miklas et al., 2001). The response of the determinate lines Xana; X1612, X1633, and X1358 could be due, in part, to this QTL.

Considering the reaction of the two parents involved in the development of each line, two types of response were found in the twelve lines: (i) lines with an intermediate resistance to that shown by both parents. This is the case of line A 2418 (average reaction against isolate= 6.27), derived from a backcross program where Andecha (7.26) was the recurrent parent and line BRB130

(4.83) the donor parent; (ii) lines with a transgressive response as lines A1258 (4.94) and A1220 (5.21), obtained from a backcross program in which Andecha (7.26) was the recurrent parent and lines A252 (6.30) and A493 (6.95) the donor parents, respectively.

The degree of resistance found in the evaluated lines did not offer a good protection against this pathogen. A new plant breeding program will be necessary in order to increase the resistance degree in the market class fabada.

Table 1. Disease reactions of 19 common bean materials against one local isolate of white mold, using the straw test. Significant differences among materials were revealed by means of a single-factor analysis of variance (ANOVA). Materials with the same letter, the differences are not significant ($p > 0.05$). I = Intermediate reaction, S = susceptible reaction.

				Reaction			
Material	Origin	Introgressed loci	No.	Mean	SE	ANOVA[a]	Type
V203			20	4,75	± 0,27	a	I
BRB130			18	4,83	± 0,33	a	I
A1258	Andecha / A252	*Co-2*	31	4,94	± 0,23	ab	I
A1220	Andecha /A493	*Co-3/Co-9*	29	5,21	± 0,30	abc	I
Xana	Andecha / V203	*fin*	28	5,61	± 0,58	abcd	I
A1183	Andecha / Sanilac	*Co-2*	28	5,79	± 0,23	bcde	I
A2806	A1878/A2418	*Co-2 +I+bc-3*	22	5,82	± 0,23	bcde	I
X1319	Xana/ A1220	*fin+ Co-3/Co-9*	24	5,83	± 0,19	bcde	I
A1878	Andecha / Sanilac	*Co-2 +I*	21	5,90	± 0,21	cde	I
A2438	A1220/A1183	*Co-2+ Co-3/Co-9*	21	5,90	± 0,28	cde	I
X1612	Xana/ A1878	*fin+I+Co-2*	30	5,97	± 0,25	cde	I
X1633	Xana/ BRB130	*fin*	20	6,05	± 0,27	cde	S
A2418	Andecha /BRB130	*I+bc-3*	30	6,27	± 0,21	def	S
A252			20	6,30	± 0,19	def	S
X1358	Xana/ A1183	*fin+Co-2*	31	6,32	± 0,19	def	S
Sanilac			24	6,63	± 0,23	efg	S
A493			20	6,95	± 0,49	fg	S
Andecha			35	7,26	± 0,32	g	S
Cornell49242			33	8,52	± 0,18	h	S

a) Differences are not significant ($p > 0.05$) between materials having the same letter. I = Intermediate reaction, S = susceptible reaction.

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CALCIUM CHLORIDE AND CALCIUM SILICATE DECREASE WHITE MOLD INTENSITY ON COMMON BEANS

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INTRODUCTION

Practices used to manage white mold (*Sclerotinia sclerotiorum*) on common beans include fungicide application, non-infested seeds, low plant population, upright cultivars, crop rotation, biological control, and reduction of irrigation frequency. In Brazil some farmers use calcium fertilizers with alleged good results for white mold control. Commonly, they apply these fertilizers through center pivot. However, there are only preliminary reports about the benefits of calcium on white mold control (Venette, 1998). It is hypothesized that since calcium plays a role on bean plant defense against white mold, its application would reduce the disease intensity. In this work the efficacy of calcium chloride ($CaCl_2$) and calcium silicate ($CaSiO_3$) was evaluated for white mold control on common beans.

MATERIALS AND METHODS

A study was carried out at an experimental area naturally infested with sclerotia. The cultivar Talismã (type III, carioca class) was sown in rows spaced 0.5 m apart. Plots had seven 3 m-long rows. Both $CaCl_2$ and $CaSiO_3$ were applied at 45 days after emergence (DAE) (early bloom) over the plants with a hand sprayer (800 L ha^{-1}) at 100, 200, 300 and 400 mg L^{-1} or at 45 and 55 DAE at 300 mg L^{-1}. These treatments were compared with water (untreated control) and fluazinam applications (0.5 L ha^{-1}) at 45 and 55 DAE. Treatments were replicated four times in a randomized complete block design. An area of 1.2 m^2 in the plots was harvested separately at 100 DAE for disease evaluation. Plants were rated for disease severity index (DSI) by means of a "quarter scale" (Hall & Phillips, 1996), where 0 = no disease present, 1 = 1 % to 25 % of the plant with symptoms, 2 = 26 % to 50 % of the plant with symptoms, 3 = 51 % to 75 % of the plant with symptoms, and 4 = 76 % to 100 % of the plant with symptoms. DSI was calculated on a percentage basis:

$$DSI(\%) = \frac{\sum(scores\ of\ all\ plants)}{4\ x\ (total\ number\ of\ plants)} \times 100$$

Yield data were estimate based on mass of seeds with 12 % moisture (w/w). Data were subjected to variance analysis. Regression analyses were done to test the effect of rates of $CaCl_2$ and $CaSiO_3$ on white mold intensity and yield. Effect of two applications of fungicide and $CaCl_2$ and $CaSiO_3$ were compared to the untreated control by Dunnett's test.

RESULTS AND DISCUSSION

Both incidence and severity of white mold were significantly reduced by one application of $CaCl_2$ and $CaSiO_3$ at early bloom (Fig. 1), but the level of control was not sufficient to increase yield. Two applications of fluazinam decreased white mold incidence and severity ($P < 0.01$) and increased yield ($P < 0.05$) (Table 1). Reduction of disease with two applications of $CaCl_2$ and $CaSiO_3$ was only significant for DSI ($P < 0.05$). Compared to untreated control, fluazinam reduced disease incidence by 52 %, severity by 73 %, and increased yield by 45 % (Table 1). Venette (1998) found that foliar-applied calcium enhanced both disease control and yield. He suggested that calcium may be a nutritional supplement that increases plant resistance to white mold. Nutritional effect is

particularly noticeable in the case of calcium compounds with high water solubility, like $CaCl_2$. As $CaSiO_3$ has very low water solubility, possible effects of its foliar application may also be explained by the establishment of a physical barrier on the host tissue. Moreover, many modifications may occur in the plant surface after calcium application, including increase of pH and changes in the populations of microorganisms.

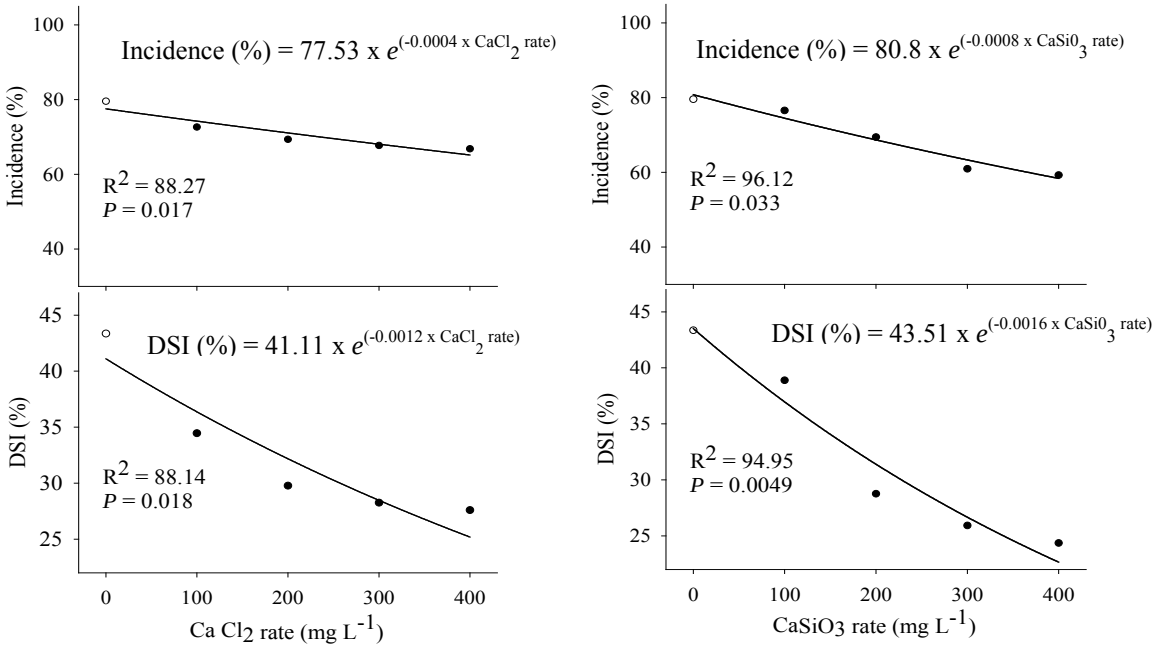

Fig. 1 - White mold incidence and DSI in response to four rates of $CaCl_2$ and $CaSiO_3$ applied at early bloom (45 DAE).

Table 1 – Comparison of untreated control with two applications of fluazinam, $CaCl_2$, and $CaSiO_3$ on white mold intensity and yield.

Treatment[1]	Incidence (%)	DSI (%)	Yield (kg ha^{-1})
Fluazinam	38.4**	11.9**	2253 *
$CaCl_2$ (300 mg L^{-1})	67.9 ns	27.8 *	1505 ns
$CaSiO_3$ (300 mg L^{-1})	72.2 ns	30.4 *	1793 ns
Untreated control	79.5	43.3	1553

Means in the column followed by * and ** are different from unt reated control at 5% and 1%, respectively, by Dunnett's test; ns – non-significant. [1]Applied at both early bloom (45 DAE) and ten days later.

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INFLUENCE OF WATER STRESS AND *MACROPHOMINA PHASEOLINA* IN GROWTH AND GRAIN YIELD OF COMMON BEANS UNDER CONTROLLED AND FIELD CONDITIONS

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Drought stress reduces common bean (*Phaseolus vulgaris* L.) grown and grain yield as well as increases susceptibility to root rot pathogens such as *Macrophomina phaseolina* (Tassi) Goid (Mp) (2, 3). Under controlled conditions water stress rather than Mp affects growth, water relations and grain yield of common beans (2) and resistant cultivars show xeromorphic traits compared with susceptible germplasm (3). Some efforts have been conducted to identify RAPD (6) and QTLs (5) associated to Mp resistance but not to combined resistances. The aim of this work was to compare the response to combined Mp and water stress under both controlled and field conditions of two contrasting common bean cultivars.

Two experiments were conducted during 2008, one under controlled (Reynosa, México; 26° 05' N, 98° 18' W, 38 masl) and the other in field conditions (Rio Bravo, México, 25° 59' N, 98° 06' W, 39 masl). In both them, two bean cultivars (BAT 477, resistant to Mp and water stress and Pinto UI-114, susceptible) (4); two humidity (irrigation through biological cycle and terminal water-stress where irrigation was stopped when flowering started until five days under PWP in greenhouse and until harvest in field experiment) and two inoculation levels (inoculated with rice seeds colonized by a highly virulent isolate of Mp at 5% w/w ratio for greenhouse test and 5 g m^{-1} row in field). Experimental unit was one 20 L pot with three plants (greenhouse) and 2 rows 5 m-length where 100 seeds were sown (field). Treatments (cultivars x humidity levels x inoculation levels) were randomized in RC and RCB designs under controlled (four replications by treatment) and field (three replications) conditions, respectively. In both experiments RWC, charcoal rot severity, dry biomass and grain yield were measured. Data were subjected to ANOVA and means were compared using Tukey test (P=0.05).

Under both controlled and field conditions, water stress and *M. phaseolina* reduced RWC at first and fifth day in PWP (Fig. 1) as well as dry biomass accumulation in vegetative organs and grain yield at harvest, although greatest negative effects was caused by water stress particularly in Pinto UI-114 (2, 3). On the other hand, *M. phaseolina* inoculation favored infection and development of charcoal rot disease in Pinto UI-114 and this effect was aggravated by drought stress (Table 1). Our results indicated that BAT 477 is resistant to both water stress and charcoal rot disease based on water relations as RWC, dry biomass accumulation in the different plant organs and grain yield. In addition, water stress aggravates negative effects by charcoal rot in common bean susceptible germplasm (2, 3). Our current efforts are focused to mapping QTLs associated to both charcoal rot and water stress resistances in BAT 477 and then try to develop molecular markers for MAS (1).

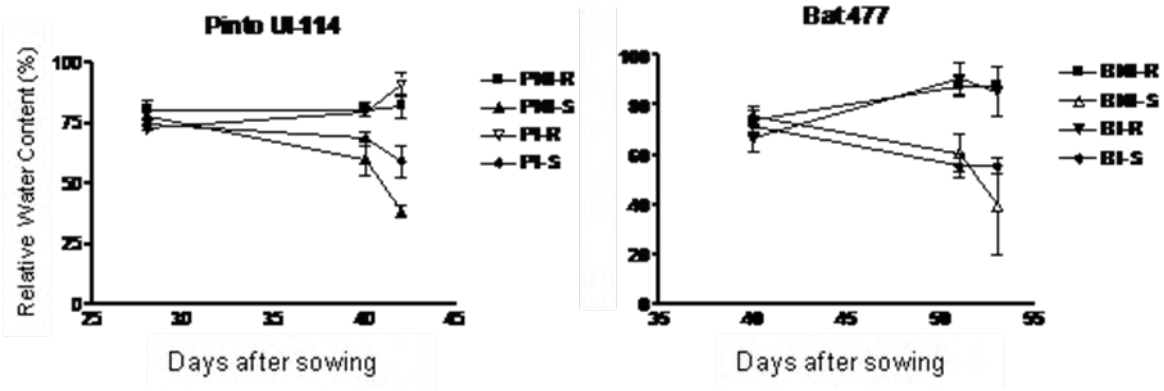

Fig. 1. Relative water content in two bean cultivars growing under two humidity and two *M. phaseolina* inoculation levels under controlled conditions (NI=no inoculated, I=inoculated; R=Irrigated, S=Water-stressed).

Table 1. Charcoal rot severity and dry biomass in two bean cultivars growing under two humidity and two *M. phaseolina* inoculation levels under controlled conditions under controlled and field conditions.

Treatment	Greenhouse				Field			
	SEV[1]	PDB (g)	GY (g)	HI (%)	SEV	PDB (g)	GY (g)	HI (%)
Pinto UI-114								
Irrigated-Inoculated	8.2	6.6	2.7	41	4.3	0.9	0.2	22
Irrigated-No inoculated	1.0	7.2	5.1	71	5.7	1.1	0.3	27
Drought-Inoculated	6.8	1.0	0.5	50	3.0	0.4	0.1	25
Drought-No inoculated	1.0	2.2	0.5	23	3.3	0.4	0.2	50
BAT 477								
Irrigated-Inoculated	5.3	11.0	1.7	15	2.7	3.1	0.6	19
Irrigated-No inoculated	1.0	11.0	1.8	16	3.3	1.4	0.5	36
Drought-Inoculated	6.0	5.2	0.4	9	5.7	1.7	0.2	12
Drought-No inoculated	1.0	3.6	0.6	17	6.3	1.5	0.2	13
HLSD (P=0.05)	1.2	3.5	0.8	25	1.6	0.4	0.2	21

[1]SEV=charcoal rot severity at fifth PWP day; PDB=plant dry biomass; GY=grain yield; HI=harvest index.

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METHODS FOR *MACROPHOMINA PHASEOLINA* INOCULATION IN COMMON BEANS

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The fungus *Macrophomina phaseolina* (Mp) causes charcoal rot in common beans and other crops through Mexico under water stressed and high temperature conditions. Methods for assess pathogenicity of Mp have been developed for specific host species and/or research objectives. Inoculation methods of Mp can be divided into two groups: destructive (p. e. use of colonized seeds and inoculation of seeds and/or seedlings under field or controlled conditions) and non-destructive (p. e. detached-leaf method) (1, 2, 3, 7). Our research group is interested to reproduce Mp symptoms in common beans under specific test conditions in order to develop methods of genomic analysis of Mp-bean interactions and therefore detect those differentially expressed genes during the pathogenesis. The aim of this work was to compare the efficiency of several methods of inoculation of bean seedlings under controlled conditions and to identify an appropriate method for genomic studies in the pathosystem Mp-common beans.

Two experiments were conducted under controlled in Reynosa, México (26° 05' N, 98° 18' W, 38 m asl). Two bean cultivars (BAT 477, resistant to Mp and water stress and Pinto UI-114, susceptible) (5) were included. At first experiment (E-I), four Mp inoculation treatments were included: control (no inoculated), 15 mL of PDB of Mp grown per seven days applied to the pot (≈10^5 cfu); colonized PDA medium from one Petri dish per pot (≈10^4 cfu) (2); and infested wood toothpicks (4) with Mp (one per plant) while at second experiment (E-II) four inoculation treatments (two controls no inoculated with PDA medium or wood toothpicks; colonized PDA medium (≈$2.5X10^3$ and $4.0X10^3$ cfu per pot respectively); and infested toothpicks with Mp (one per plant) were evaluated under tow humidity levels: irrigated plants and water-stressed plants (watering was stopped at 18 das and plants were subjected to PWP for three days). Experimental unit was one 5 L pot with four plants for both experiments. Treatments (bean cultivars, inoculation methods in E-I; cultivars, inoculation methods and humidity levels in E-II) showed factorial arrangements 2x4 (E-I) and 2x5x2 (E-II) and were randomized in RCD designs with four (E-I) and five (E-II) replications. During the development of each experiment charcoal rot severity (SEV) was recorded and (30 das) shoot (SDB), root (RDB) and total (TDB) dry biomass measured at harvest. Data subjected to ANOVA and means compared using Tukey values (P=0.05).

ANOVA detected significant differences ($P \leq 0.05$) among cultivars and inoculation methods for all variables; but for the interaction cultivars x inoculation methods we found differences only for RDB in E-I. For E-II, ANOVA detected significant differences for all sources of variation and measured variables with the exception of cultivars x humidity levels (SEV, SDB) and cultivars x humidity x inoculation (RDB, TDB) (data not shown). In both experiments, BAT 477 showed the highest dry biomass accumulation and the lowest charcoal rot severity compared with Pinto UI-114. In E-II, water stress increased charcoal rot severity and reduced dry biomass production. All inoculation methods increased significantly charcoal rot severity but only infested toothpicks reduced shoot and total dry biomass compared with control in E-I. For E-II the highest *M. phaseolina* damage

was caused by PDA + Mp (≈10^4 cfu) followed by PDA + Mp (≈$2.5X10^3$ cfu) and infested toothpicks. The highest dry biomass accumulation was found in controls (Table 1). Destructive (plants infested by mycelium, microsclerotia, rice or sorghum seeds colonized by the fungus, seeds cultured on PDA medium) and no-destructive (detached-leaf) methods to induce charcoal rot in bean plants have particular advantages depending on specific research aims (p.e. preserve useful bean segregating plants for mapping of resistance genes) (1). Infested toothpicks were ratified as a reliable methodology to induce *M. phaseolina* pathogenesis in bean plants due previous findings was obtained in other crops such as sorghum, strawberry, alfalfa, and white clover (4, 6, 7) and therefore can be used when the researcher needs to start the infective process to specific dates or plant phenological stages. Thus, infested toothpicks are a reliable, fast and easy procedure to assess charcoal rot development in bean plants under variable humidity conditions.

Table 1. Mean comparisons among bean cultivars, inoculation methods of *M. phaseolina* and humidity levels in two experiments established under controlled conditions.

	Dry biomass (g)			
Treatments	SEV	Roots	Shoot	Total
	---------- E-I ----------			
BAT 477	2.8	1.1	1.7	2.8
Pinto UI-114	4.2	0.9	1.5	2.4
DMSH (P=0.05)	0.8	0.1	0.1	0.2
Control	2.6	1.0	1.6	2.6
15 mL PDB + Mp (≈10^5 cfu)	3.2	1.1	1.8	2.9
PDA + Mp (≈10^4 cfu)	3.5	0.9	1.6	2.5
Infested toothpicks	4.7	0.9	1.3	2.2
DMSH (P=0.05)	1.6	0.2	0.2	0.5
	---------- E-II ----------			
BAT 477	3.4	0.7	0.6	1.3
Pinto UI-114	5.7	0.4	0.5	0.9
DMSH (P=0.05)	0.6	0.1	0.1	0.1
Irrigated	4.3	0.7	0.7	1.4
Water-stressed	4.9	0.4	0.4	0.8
DMSH (P=0.05)	0.5	0.1	0.1	0.1
Control (un-inoculated)	1.0	0.8	1.1	1.9
Control (toothpicks)	1.0	0.7	0.7	1.4
PDA + Mp (≈$2.5X10^3$ cfu)	6.7	0.4	0.4	0.8
PDA + Mp (≈10^4 cfu)	8.2	0.2	0.3	0.5
Infested toothpicks	6.1	0.5	0.6	1.1
DMSH (P=0.05)	1.0	0.2	0.1	0.3

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HERITABILITY OF RESISTANCE TO *FUSARIUM OXYSPORUM* F. SP. *PHASEOLI* IN COMMON BEAN

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Root rot disease of common bean has been associated with several soil-borne pathogens, including *Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burkholder) W.C. Snyder & H.N. Hans, *Rhizoctonia solani* Kühn, *Pythium* spp. and *Aphanomyces euteiches* f. sp. *phaseoli*. In Wisconsin, the two most severe root rot pathogens are *P. ultimum* and *A. euteiches* f. sp. *phaseoli*. Fusarium wilt of beans is caused by *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *phaseoli* J.B. Kendrick & W. C. Snyder.(FOP) and is a major limiting pathogen of snap and dry beans, especially in hot dry western environments.

Using a Mexican landrace, Puebla 152 as a donor parent, our laboratory has successfully introgressed resistance to Aphanomyces root rot, based on field screening in naturally infested research plots in the central sands region of Wisconsin and validated in the greenhouse.
The objective of this research was to determine if genotypes previously identified as resistant to Aphanomyces root rot would also express resistance to Fusarium wilt based on greenhouse screening with pure cultures of FOP.

MATERIALS AND METHODS:

Plant Material: A RIL population derived from a cross between Eagle and Puebla 152 was used to conduct a greenhouse screening in 2008. The Eagle × Puebla 152 RIL population consists of 78 F_8 lines developed by single-seed descent, but because of low seed supply only 73 F_{11} lines were used during this experiment.

Screening Trial: Screenings were conducted in a greenhouse with supplemental lighting and temperature control. Plants were inoculated using a root-dip technique described by Pastor-Corrales and Abawi (1990). Inoculum was prepared by scrapping conidia from cultures of FOP into distilled water and quantification of conidia was conducted using a hemocytometer. Ten-day-old bean seedlings were removed from the potting medium, roots were gently washed with tap water, and the distal 1/3 of root system was clipped with a scissors. Seedlings were placed immediately into the inoculum consisting of 1×10^6 conidia/mL of FOP for 5 min. After inoculation, plants were transplanted to a pot with a sterile soil mix. The ATCC90245 (Fop8) isolate of FOP collected from Sedgwick, CO (1988) by Schwartz was used for all inoculations. Disease severity (DS) was rated 3 weeks after inoculation, using the CIAT disease severity index. Visual evaluations of foliar symptoms and vascular tissue lesions were rated from 1 to 9 depending on severity, where 1= no disease symptoms and completely healthy, 3 =10%, 5= 25%, 7= 50% and 9= 75% of leaves wilted, chlorotic or plant death. Plants were classified as resistant, intermediate, and susceptible if rated 1-3, 4-6, and 7-9 respectively.

RESULTS:

Eagle, Puebla 152 and 55% of the Eagle × Puebla 152 RIL were identified as FOP susceptible (DS ≤ 3), 41% as intermediate (DS >3 ≤7), and 4% as resistant (≥7) (Fig.1). A correlation of 0.79 was found between root and foliar data that may be use as FOP resistance predictor. The estimated FOP resistance heritabilities based on foliar and root data are 0.26 ± 0.27 and 0.30 ± 0.27, respectively; these results are in agreement with a previous study by Cross et al.(2000) that estimated FOP resistance heritability between 0.25±0.19 to 0.85±0.34 in five bean populations derived from parents

of r ace M esoamerica i ncluding s usceptible l ines a nd cultivars i ncluding BAT 477, HF465 a nd Crestwood, and resistant cultivars UI1911, Jamapa and Rio Tibagi.

Table 1. Variance components from the analysis of variance for the Eagle x Puebla152 RIL population.

Variance components	Foliar	Root	Signif. Prob
Genotype	13.29	16.91	<.0001
Rep	49.21	239.05	<.0001
Plant(Rep)	18.13	24.22	<.0001
Genotype*Rep	6.82	7.30	<.0001
Experimental Error	2.11	2.48	<.0001
h^2 =	0.26±0.27	0.30±0.27	

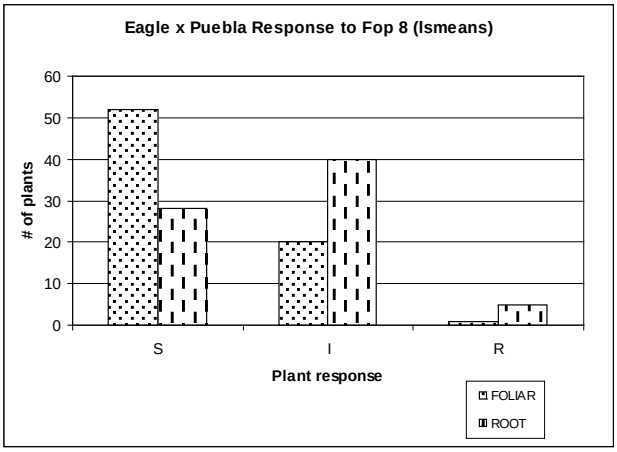

Fig.1 Eagle × Puebla 152 RIL population response to Fop8, where S=Susceptible, I= Intermediate and R=Resistant

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ASSESSMENT OF FOLIAR AND ROOT PATHOGENS OF DRY BEANS CURRENTLY PREVALENT IN NORTH DAKOTA

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INTRODUCTION

North Dakota accounts for nearly half of the dry bean production in the US (USDA-NASS), and diseases are one of the main limiting factors in the state. Economically important foliar and stem diseases reported in the upper Great Plains include; white mold, rust, anthracnose and common bacterial blight (Harikrishnan et al., 2006). Although root rots are economically important, and common to the region, no surveys have been conducted to determine the prevalence of root diseases in North Dakota. A recent survey of dry beans in Minnesota found a very high incidence of root rots (Percich and Sheets, 2006).However, Minnesota production consists largely of kidney beans and under irrigation, while production in North Dakota is largely pintos under dry-land conditions. The objective of this study was to assess the prevalence of foliar and root pathogens on dry beans in the 2007 and 2008 growing seasons.

MATERIALS AND METHODS

Surveys were conducted in the four major dry bean growing counties in North Dakota, namely; Pembina, Walsh, Grand Forks and Steele. A minimum of 30 fields, located at least 3 miles apart, were surveyed in both years. GPS coordinates for each field was recorded. Visual assessment of disease incidence of 20 plants was conducted at 5 locations along a W-transect within each field. The incidence of white mold (*Sclerotinia sclerotiorum* de Bary), rust (*Uromyces appendiculatus* Pers.), anthracnose (*Colletotrichum lindemuthianum* Sacc. & Magnus) and bacterial blights including common blight, halo blight and brown spot caused by *Xanthomonas axonopodis pv. phaseoli* Smith, *Pseudomonas syringae pv. phaseolicola* Burkholder and *Pseudomonas syringae pv. syringae* van Hall respectively, were recorded.. Samples representative of the prevalent diseases in each field were collected and brought back to the laboratory for confirmation. Ten plant samples were collected from every field for root rot assays. Pathogens were isolated into pure culture and identified morphologically. Further confirmation of root pathogens was done by sequencing the TEF 1α or ITS regions. Samples submitted by other university sources were also assayed. Representative isolates of the most prevalent pathogens obtained from these surveys were used for pathogenicity tests to confirm their ability to cause disease on dry beans. Virulence phenotyping of rust field collections was done in the green-house using the standard set of differentials (Steadman et al., 2002).

RESULTS AND DISCUSSION

Foliar diseases: Bacterial blight, white mold and rust were reported in 2007 and 2008 (Fig. 1) Anthracnose was not seen in either of these two years. In 2007, the survey was conducted late in the season and diseases present were at advanced stages. This limited the ability to distinguish between the bacterial blight pathogens. However, relatively severe bacterial blight was seen in all fields sampled. In 2008, sampling was conducted earlier in the season and the bacterial blight pathogens could be distinguished. Common blight was seen in all fields in 2008, and halo blight and brown spot were observed in 55% and 45% of the fields surveyed, respectively. One sample in 2008 tested

positive for bacterial wilt caused by the pathogen *Curtobacterium flaccumfaciens* subsp. *flaccumfaciens*. This disease has not been reported in this region for several years and is being evaluated further.

Allow incidence of rust was recorded in five fields in 2007 in Grand Forks County. However, a significant outbreak of rust occurred in a relatively small geographic area centered in Northern Trail Co. in 2008. Following this finding, rust samples were collected from 17 fields in Trail County and one field in adjacent Grand Forks County. Field collections of rust were virulent on the differential Aurora, which has the commonly used source of the rust resistance gene *Ur-3*. A more detailed report of rust virulence is reported in this issue (Markell et al.).

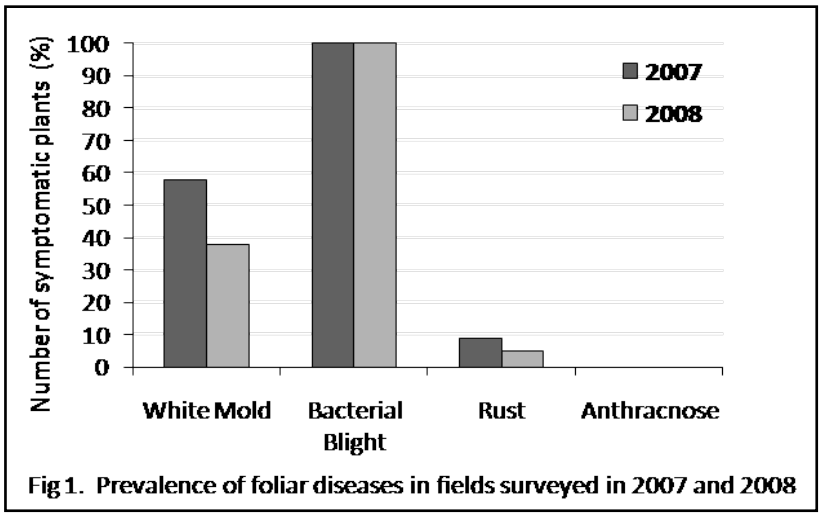

Fig 1. Prevalence of foliar diseases in fields surveyed in 2007 and 2008

Root rots: Plants showing symptoms of root rots were assayed from 17 fields included in the disease survey in 2007 and 39 fields in 2008. Samples from two fields in MN showing high root rot incidence were also included in 2007 pathogen assessment. In both years, *Fusarium* and *Rhizoctonia* were found to be the most prevalent species (Fig 2). In 2007, nearly 50% of the samples plated were from fields planted to kidney beans whereas in 2008 majority were from pinto beans. The most important conclusion from this work is that *Fusarium* species appear to be the major cause of root rots in North Dakota, and *Rhizoctonia spp.*, when present, are often associated with *Fusarium* species. Additionally, numerous *Fusairum spp.* commonly associated with cereals were commonly found and virulent on beans.

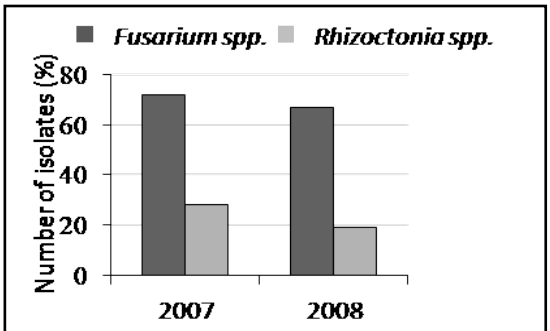

Fig 2. Frequency of *Fusarium* and *Rhizoctonia* species among isolates obtained from plants showing root rot symptoms in 2007 and 2008.

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OSMOTIC ADJUSTMENT AND SEED YIELD OF DRY BEAN UNDER DROUGHT

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INTRODUCTION - Osmotic adjustment has been reported for many crops, among others chickpea (Moinuddin & Khanna-Chopra, 2004), wheat (Silva-Robledo *et al*., 2007) and bean (Villarreal & Larqué-Saavedra, 1983), but its relationship with seed yield under drought has not been reported in dry bean. The objective of the present work was to determine if there is causal relationship between osmotic adjustment and seed yield in several bean cultivars from Central Mexico.

MATERIALS AND METHODS - Seven bred bean cultivars and one landrace (control) of the type III growth habit were tested. Three field experiments were established during the Spring-Summer crop cycle in 2007: one rainfed crop at Celaya, Guanajuato, where it was necessary to apply supplementary irrigation at the flowering and at pod filling stages to avoid the loss of the trial, due to a severe drought. The other two: one watered the other rainfed, both located at Montecillo, State of Mexico. Plants at the flowering stage between 50 to 60 days after sowing (DAS) were sampled to determine water potential (Ψw) and osmotic potential (Ψs) at 12:00 to 13:30 h. These parameters were measured in eight fully expanded leaves of each plot in all four replications. The Ψw was recorded in the field with a Scholander pressure chamber. For Ψs, leaves were sampled in the field, frozen in liquid nitrogen, taken to the laboratory and defrosted at room temperature, and their cell sap obtained by mechanical pressure. Ten µL of this sap was used to determine the Ψs (mmol kg^{-1}) with a pressure osmometer (Wescor 5100 C). Pressure potential (Ψp) was calculated with the following formula: Ψp= Ψw - Ψs. With the Ψs the osmotic adjustment was calculated as proposed by Babu *et al*. (1999) as the difference between Ψs at maximum turgor (values obtained after watering the trial) from the watered plants and plants under water stress. Collected data were analyzed by using the statistical analysis system (SAS, 2006). LSD of Tukey ($p \leq 0.05$) was used for comparison of means.

RESULTS AND DISCUSSION = Water relations. The Ψw, Ψs and Ψp showed differences ($p \leq 0.01$) among cultivars in the average of the three trials and within trials (Montecillo watered and rainfed, and Celaya rainfed) in all samplings. The **Ψw** at the irrigated trial in Montecillo exhibited the highest value ($p \leq 0.05$). In contrast in the rainfed trial at Montecillo the Ψw was the lowest in all the samplings and decreased as the soil water content decreased at the 0-20 and 20-40 cm depths since the difference between the first sampling at 50 DAS and the last one at 68 DAS was - 0.67 MPa. The lowest value (-1.55 MPa) corresponded to 68 DAS and the plants were wilted. In Celaya the lowest Ψw value (-1.02 MPa) occurred at 53 DAS (Fig 1a), a value that did not decrease since at 55 and 65 DAS supplementary irrigation was applied. The **Ψs** between Montillo irrigated and Celaya rainfed were different ($p \leq 0.01$) only at 50 DAS. The lowest value (-1.9 MPa) at Montecillo rainfed corresponded to 62 and 65 DAS (Fig 1b), with a difference of - 0.43 MPa between the first and the last sampling. On the other hand, the highest value (1.1 MPa) for **Ψp** was shown by the sampling taken at Montecillo irrgated. In contrast the lowest values were registered in the rainfed condition at the same site (0.15 and 0.17 MPa) at 53 and 68 DAS (Fig 1c).

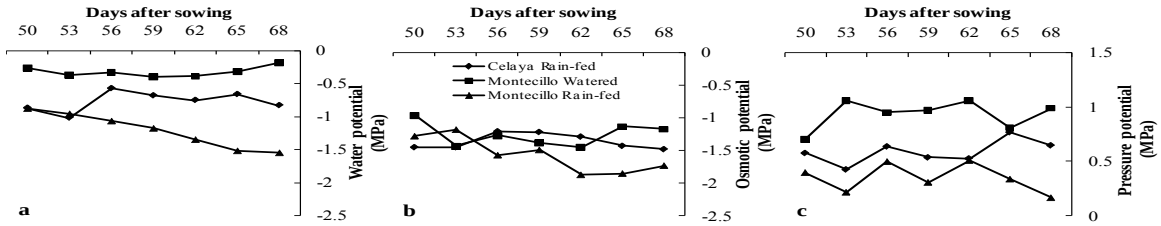

Fig 1. Water potential (a), osmotic potential (b), and pressure potential (c) in fully expanded leaves of bean cultivars. Average of experiments for Montecillo watered and rainfed, and Celaya rainfed. 2007. The vertical lines indicate the least significant difference of Tukey ($p \leq 0.05$) for each sampling DAS.

Table 1. Osmotic adjustment for eight bean cultivars grown in Central Mexico. 2007.

Cultivars	Osmotic potential (MPa)[¶]			SY	Osmotic adjustment (MPa)[†]	
	Montecillo Watered	Celaya Rainfed	Montecillo Rainfed	(g m^{-2})	Celaya Rainfed	Montecillo Rainfed
FM Noura	-1.09	-1.27	-1.84	317.4	0.18	0.75
FM Anita	-1.06	-1.36	-1.94	301.0	0.30	0.88
FM M38	-1.04	-1.35	-1.92	291.8	0.31	0.88
FM Sol	-1.09	-1.48	-1.80	237.5	0.39	0.71
FM Bajío	-1.13	-1.32	-1.72	217.6	0.19	0.59
FM Corregidora	-0.98	-1.51	-1.83	209.9	0.53	0.85
FM 2000	-1.27	-1.34	-1.86	209.5	0.07	0.59
FM RMC	-1.28	-1.38	-1.89	179.1	0.10	0.61
Michoacán 128	-1.23	-1.25	-2.01	142.6	0.02	0.78
General average	-1.13	-1.36	-1.87	234.0	0.23	0.74

[¶]= Maximum turgor for three experiments; [†]= Babu *et al.* (1999); SY= Average seed yield for rainfed Montecillo and Celaya experiments.

Cultivars FM Corregidora and FM M38 showed the highest Ψw and Ψp in Montecillo under irrigation and in Celaya under rainfed plus supplemental irrigation, in most samplings. In contrast the lowest Ψs values were showed by different cultivars across trials.

Osmotic adjustment (OA). Values recorded for OA showed high variability among cultivars. The values in Celaya ranged from 0.02 to 0.54 MPa and in Montecillo under rainfed conditions from 0.59 to 0.88 MPa. In Celaya highest values corresponded to cultivars FM Corregidora, FM Sol, FM M38, and FM Anita. In Montecillo under rainfed conditions highest values were shown by FM Anita, FM M38, and FM Corregidora. The AO values at Celaya were lower than those for Montecillo under rainfed conditions, probably due to more severe water stress Montecillo, accompanied by high values of OA. Correlations between OA and seed yield both for Celaya (R^2= 0.2) and Montecillo under rainfed conditions (R^2=0.41) were not significant.

CONCLUSIONS - Although several high seed yielding cultivars exhibited high values for osmotic adjustment, the correlations did not support a causal relationship between osmotic adjustment and seed yield under drought, i.e. the cases of FM Noura, FM Corregidora and Michoacán 128.

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EVALUATION OF COMMON BEAN GENOTYPES UNDER WATER STRESS

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INTRODUCTION

Common beans in Brazil are grown in the Cerrado region where Latosols are dominant. Annual rainfall in this region is about 1200 to 1500 mm, distributed from October to April. However water deficiency is common from January to April (Steinmetz et al., 1988), which is a major factor responsible for low crop yield. Hence it is recommended that drought tolerant cultivars should be developed. The objective of the work was to study the adaptation of common bean genotypes to water stress, as support for breeding programs with the objective to develop cultivars for areas subject to water deficit.

MATERIALS AND METHODS

The experiment were conducted at the SEAGRO Experimental Station, Porangatu-GO, latitude 13º 18' 31", longitude 49º 06' 47", altitude 391 m, climate Aw, tropical of savanna, megatermic, as Köppen classification. The rainy season occurs from October to April and dry season occurs from May to September. During the experiment conduction did not rain and the relative humidity and the maximum and minimum temperature are presented in the Figure 1.

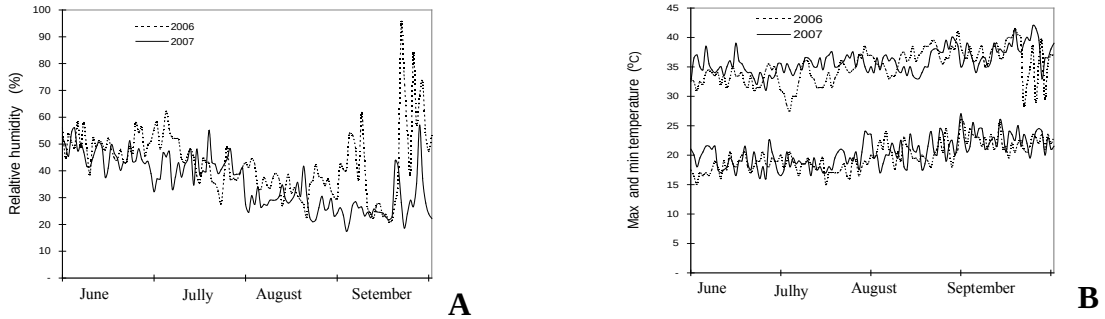

Figure 1. Relative humidity (A) and maximum and minimum temperature (B) during the development of the plants, in 2006 and 2007, at the SEAGRO Exp. Station-Porangatu-GO.

The planting was done on 11th June in 2006 and on 14th June in 2007. Genotypes were planted in four rows, each having four meter length with spacing of 40 cm. It was used the recommended agronomic practices. Forty-nine genotypes were evaluated in the randomized block design, with three replications, under drought and well irrigated conditions. The experiment was well irrigated, with the soil water potential higher than - 0.035 MPa at 15 cm depth (Silveira & Stone, 1994) from planting date to 20 days after emergence, when the water treatments were applied: 1) drought and 2) without drought stress. The irrigation on the second treatment was controlled with tensiometers. The first treatment received about ½ of the water applied at the second treatment. It was evaluated the yield and its components. It was applied the multivariable analysis using de method of Ward (1963) and the genotypes were classified based on the average grain yield of the two experimental years for each treatment.

RESULTS AND DISCUSSION

The genotypes were classified in four groups using the Ward's method based on the grain yield averages of the two experimental years for each water treatment. It was observed that average yield of the groups significant differed in both treatments (Table 1). The average yields of the two experimental years were 863 kg ha^{-1} and 2085 kg ha^{-1}, under drought and without drought stress, respectively. The drought stress decreased 58.6% of the yield.

Table 1. Summary of the multivariable analysis, considering the average yield observed under drought and without drought stress.

F.V	D.F.	Yield (kg ha^{-1})	
		With drought stress	Without drought stress
Group	3	258 207.75**	875 554.98**
Error	45	1 288.65	5031.61
CV (%)		4.16	3.40

** Significant difference at 5% probability, by F test

It was observed that under drought the lower yielding group was formed by the genotypes G 3566, BRA 283983 CIAT G 6492, BAT 1203, BRASIL 0001, RAB 94 VERMELHO 2157, G 983, and BRA 129721 CIAT G 6896, that yielded 657 kg ha^{-1}. Under the same conditions the highest yielding genotypes were BAMBUÍ, FT 84 – 292, G 4280, BAT 304, and BRA 130583 CIAT G 6490, that yielded 1080 kg ha^{-1}, 64.4% higher than the first group. Under well irrigated conditions the lower yielding genotypes were BAT 1203, EMP 86, PIRATÃ 1, G 2475, BRASIL 0001, FE 732007 - XAMEGO, G 4489, FFT 85 - 75 - PORTO REAL, and G 2359, that yielded 1754 kg ha^{-1}. Under the same conditions the highest yielding genotypes were BRA 130583 CIAT G 6490, FT 84 - 292, G 4825, G 20716, IPA 7, G 2358, G 13571, BRA 283983 CIAT G 6492, FT 85 - 79, G 1356, and IAC UNA, that yielded 2429 kg ha^{-1}, 38.5% higher than the first group. The genotypes BRA 130583 CIAT G 6490 and FT 84 - 292 were classified as the most productive when well irrigated or when water stressed, while the genotypes BRA 283983 CIAT G 6492, BRA 129721 CIAT G 6896, and G 983 were classified as productive when well irrigated, but were more susceptible to drought. It was also verified that the number of pods per plant was the most sensitive yield component to water deficit in common bean and significantly affected the yield under drought ($r=-0.413$, $p \leq 0.003$). It was responsible under drought for 22.7% of the yield variability and each one unit of this variable increased 46 kg ha^{-1} of the bean yield.

CONCLUSIONS

The genotypes BRA 130583 CIAT G 6490 and FT 84 - 292 were classified as the most productive when well irrigated. The BRA 283983 CIAT G 6492, BRA 129721 CIAT G 6896, and G 983 were classified as productive when well irrigated, but were more susceptible to drought. It was also verified that the number of pods per plant was the most sensitive yield component to drought in common bean.

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PHENOTYPIC EVALUATION OF A SUBSET OF THE *PHASEOLUS VULGARIS* CORE COLLECTIONS AND THE *P. ACUTIFOLIUS* GERMPLASM COLLECTION, AND ADVANCED COMMON BEAN LINES FOR DROUGHT TOLERANCE IN NEBRASKA

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INTRODUCTION

Drought stress is an important constraint on common bean production worldwide and an increasing constraint on US production (Singh, 2007; Muñoz-Perea et al., 2007). To address this issue, we evaluated exotic common bean and tepary bean germplasm from the NPGS and CIAT collections and from US and international breeding programs for their response to drought stress.

MATERIALS AND METHODS

A total of 277 entries, 128 cultivars and elite lines and 149 accessions of *Phaseolus vulgaris* and *P. acutifolius* from the NPGS and CIAT core collections were screened under terminal drought stress conditions at Mitchell, NE (41°56.6′ N, 103°41.9′ W, 1240 m elevation) during 2008. The entries from core collections were previously selected for insensitivity to photoperiod in 2006 and 2007 in Puerto Rico. The effect of drought using adjacent non-stressed (NS) and drought stressed (DS) blocks, with two replications in each environment, were evaluated as described by Terán and Singh (2002). Within each block, the selected lines were assigned to experimental units using an augmented block design. Beryl-R, Marquis, Orion, Poncho, and SEN 21 were used as reference checks. Each plot consisted of two 7.6-m rows spaced 0.6 m apart. Targeted plant density was 200,000 plants ha^{-1}. Both NS and DS blocks were irrigated until flowering to ensure good plant establishment and normal vegetative growth. Thereafter, the stressed block was not irrigated.

To evaluate plant response to water stress, yield (kg ha^{-1}), 100-seed weight (g), and the number of days to flowering and maturity were determined. To quantify drought severity, the drought intensity index (DII) was calculated. Geometric mean (GM) (Schneider et al., 1977) and the drought susceptibility index (DSI) (Fischer and Maurer, 1978) were determined to predict the performance of a line under DS and NS conditions.

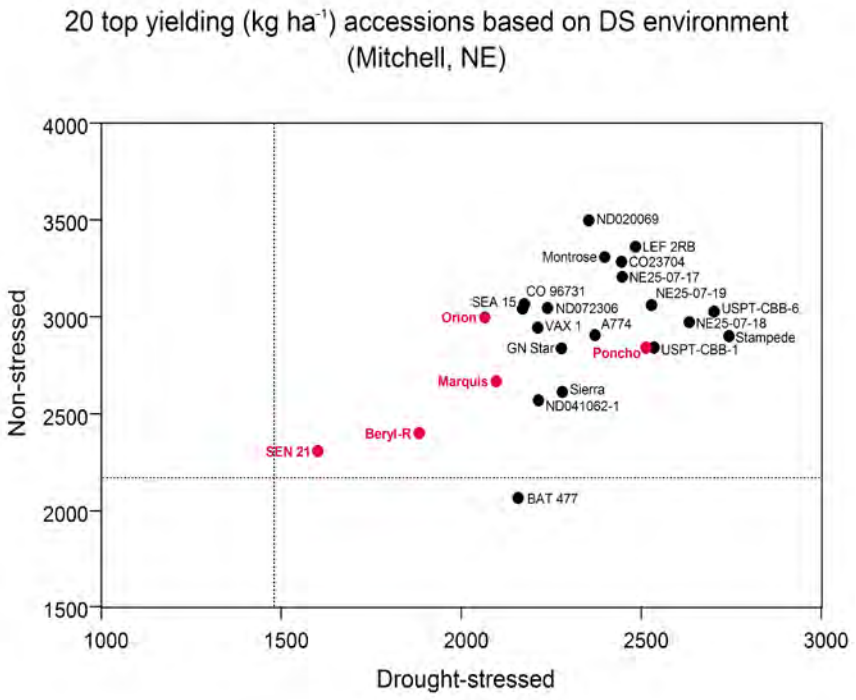
RESULTS AND DISCUSSION

Drought stress was moderate during this study (DII = 0.32) with significant precipitation of 77.8 mm occurring at 53 d after planting. Yield under NS and DS ranged from 735 to 3544 kg ha^{-1}, and from 364 to 2743 kg ha^{-1}, respectively. Under DS conditions yield and 100-seed weight were reduced an average of 32 and 13%, respectively, relative to NS conditions.

Stampede was well adapted to both NS and DS environments and had the lowest PR (5%), smallest DSI (0.2) and the largest GM (2519). USPT-CBB-6 and NE25-07-18 had the second and third highest yield under DS (2703 and 2633 kg ha^{-1}, respectively). Their PR was 11 and 12% respectively, with low DSI (0.3 and 0.4, respectively) and high GM (2554 and 2500, respectively).

Among the reference checks, Poncho had lower yield reduction (12%), lower DSI (0.4) and higher GM (2387) compared to Orion (31%, 1.0, and 2222, respectively) and SEN 21 (31%, 1.0, and 1717, respectively). Yield of the PI accessions (*P. vulgaris* and *acutifolius*) was lower than the elite

lines under both DS and NS environments. This illustrates that progress is being made in breeding for improved adaptation and drought tolerance.



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POTASSIUM, SODIUM, AND CHLORIDE NUTRITION IN SALINISED PHASEOLUS TISSUES AS A BASIS OF SALT TOLERANCE

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Salt tolerance is not exclusively correlated with adaptations to Na^+ and Cl^- toxicity *per se* but also reflects adaptations to secondary effects of salinity such as water deficit and impaired nutrient acquisition (Maathius and Amtmann, 1999). The capacity of plants to counteract salinity stress will strongly depend on the status of the K^+ nutrition. In particular, the crucial role of K^+ homeostasis in salt tolerance mechanisms of salinized plants have been placed centre stage (Kamel and El-Tayeb, 2004). Imposition of salt stress results in a massive efflux of K^+ from cells (Chen et al. 2005) and significantly reduces the intracellular pools of K^+ (Cuin et al. 2003). Mitigation of this loss strongly correlates with the level of salt tolerance. The study reported here represents a contribution to the K^+, Na^+, and Cl^- relations in salinised *Phaseolus* spp.

MATERIALS AND METHODS

Two wild and two cultivated species of *Phaseolus* differing in salt tolerance were used in this study: *P. vulgaris* PI325687, a wild salt-tolerant type (WT); *P. acutifolius* G40169, a wild salt-sensitive type (WS); *P. vulgaris* G04017, a cultivated salt-sensitive type (CS); and *P. acutifolius* G40142, a cultivated salt-tolerant type (CT). Plants were grown in nutrient solution under greenhouse conditions at Universidad Michoacana, Mexico between April and July 2007. Seedlings were allowed to grow with no salinity stress until the emergence of the first trifoliate leaf, when several NaCl treatments were added to the solutions (0, 60 and 90 mM). A randomized complete block design with a split-plot arrangement of salt treatments and six replications was used. Tissue was ashed at 500 ^{0}C for 8 h, followed by dissolution in 1 m M HCl (Basta and Tabatai 1985). Sodium and potassium concentrations were determined by flame emission using an Atomic Absorption Spectrometer (Varian SpectrAA-220FS; Mulgrave, Australia). Chloride concentration was determined colorimetrically using an UV/BIS Spectrometer (Lamda 40 PE; Uberlingen, Germany). Data were analyzed using GLM procedure (SAS, 2002).

RESULTS AND DISCUSSION

Tissue concentration of Cl^- and Na^+ ions increased significantly in response to salt treatments (Table 1). However the magnitude of the Cl^- increments, were always higher than those on Na^+ at all NaCl levels. Saline-induce changes in mineral concentration varied with plant organ and ion. In all species, Na^+ concentration increased almost equally in stems and roots, whereas the concentration of Cl^- increased more in stem and leaves than in roots. Species differed in leaf Na^+ accumulation. *P. acutifolius* CT was able to exclude Na^+ from leaves at 60 mM NaCl. In contrast, all other *Phaseolus* species accumulated Na^+ in their leaves as salt levels increased. Salinity reduced K^+ concentration in the root, stems and leaves of all species. However, decrease in K^+ concentration on stems of *P. vulgaris* species was greater than leaves and roots (Table 1). *P. acutifolius* species had higher K^+ on leaves than *P. vulgaris* species at 60 and 90 mM NaCl. At moderate and high salinity levels, leaf K^+ concentration on *P. acutifolius* species were about 28 to 35% higher at day 20 than those observed on *P. vulgaris* species. Potassium plays a predominantly osmotic role in plants (Maathius and Amtmann

1999), and has high mobility throughout the entire plant through selective K^+ transport mechanisms that can operate at high rates. Moreover, lower concentrations of this nutrient in leaves may reduce their capacity for osmotic adjustment and turgor maintenance, and reduce activation of crucial enzymatic reactions, protein synthesis and homeostasis (Cuin et al. 2003). The maintenance of higher leaf K^+ concentrations in salt-tolerant *Phaseolus* species could be, by far, one of the most important mechanisms underlying superior salt tolerance as reported in *P. filiformis* (Bayuelo et al. 2003).

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Table 1. Effects of external NaCl content on mineral composition of leaves, stem and roots of *Phaseolus* species.

Species/Genotype	Leaves			Stem			Root		
NaCl (mM)	mmol kg^{-1} dry weight								
	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-
P. vulgaris PI325687									
0	66.7c	1381.7a	9.2c	106.7c	1307.5a	15.8c	110.0b	1302.5a	29.2c
60	130.0b	1020.8b	1077.5b	221.7b	1076.7b	658.3b	422.5a	838.3b	662.5b
90	159.2a	746.7c	2152.5a	285.0a	830.8c	1341.7a	460.0a	778.3b	999.2a
P. vulgaris G04017									
0	60.0b	1289.2a	1.7c	71.7b	2267.5a	1.7c	30.8c	1403.3a	25.0c
60	143.3a	849.2b	766.7b	275.8a	1526.7b	671.7b	320.8b	805.8b	480.8b
90	154.2a	726.7b	1895.0a	267.5a	1271.7c	1261.7a	480.0a	702.5b	879.2a
P. acutifolius G40169									
0	49.2c	1430.0a	21.7a	34.2c	1428.3a	31.7c	58.3b	1772.5a	45.0c
60	165.8b	1213.3b	933.3b	257.5b	876.7b	442.5b	376.7a	900.8b	451.7b
90	222.5a	1032.5c	1889.2c	309.2a	704.2c	880.0a	446.7a	495.0c	1040.0a
P. acutifolius G40142									
0	74.2b	1221.7a	21.7c	61.7b	1187.5a	25.8c	85.0b	2157.5a	34.2c
60	90.0b	951.7b	1109.2b	289.2a	1072.5a	417.5b	349.2a	905.0b	630.0b
90	267.5a	926.7b	2200.8a	255.8a	611.7b	842.5a	370.8a	788.3b	965.0a

Values are means of six replicates after 20 days salt exposure. Differences among treatments are given according to Duncan Multiple Range Test. *, **, *** Significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$.

RESPONSES OF *PHASEOLUS* SPECIES TO SALINITY: NUTRIENT ABSORPTION AND UTILIZATION

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In saline habitat, plant growth is likely to be affected by the interactions of salt ions and many mineral nutrients, causing imbalances in the nutrient availability, uptake or distributions within the plant, and also increasing the plant requeriments for essential elements (Grattan & Grieve 1992). In the present study we apply the functional growth analysis approach (Hunt 1990) to highlight the kinetics of the absorption and utilization of mineral elements, how they are affected by salinity, and their relationships to growth rates during the vegetative stage of *Phaseolus* spp.

MATERIALS AND METHODS

Two wild and two cultivated species of *Phaseolus* differing in salt tolerance were used in this study: *P. vulgaris* PI325687, a wild salt-tolerant type (WT); *P. acutifolius* G40169, a wild salt-sensitive type (WS); *P. vulgaris* G04017, a cultivated salt-sensitive type (CS); and *P. acutifolius* G40142, a cultivated salt-tolerant type (CT). Plants were grown in nutrient solution under greenhouse conditions between April and July 2007. Seedlings were allowed to grow with no salinity stress until the emergence of the first trifoliate leaf, when several NaCl treatments were added to the solutions (0, 60 and 90 mM). A randomized complete block design with a split-plot arrangement of salt treatments and six replications was used. The rate of ion uptake (mg g^{-1} d^{-1}), an index of the elemental uptake efficiency of roots was calculated as the rate of mineral accumulation in the plant per unit root dry weight (Hunt 1990). The specific utilization rate on a leaf basis SUR_L (g g^{-1} d^{-1}), an index of the efficiency of the element in producing biomass, was calculated as the rate of plant biomass production per unit of element in the leaves (Hunt 1990). Leaf and root mineral nutrient concentration, and SAR and SUR_L values are only presented for the period between Days 10 and 20. Data were analyzed using GLM procedure (SAS, 2002).

RESULTS AND DISCUSSION

The specific absorption rates (SARs) of salt ions (Na^+ and Cl^-) were very low in non-saline conditions (<1.4 mg element g^{-1} root d^{-1}), but salinity increased the absorption rates of Na^+ and Cl^- to about 6 to 100 mg M g^{-1} root d^{-1} (where M represents the mineral element under consideration) at the 20 d harvest (Fig 1). Differences in uptake rate among species were evident at 90 mM NaCl treatment, *P. acutifolius* CT showed the lowest uptake rate of Cl^- and *P. vulgaris* CS the highest rate. Potassium SARs during the vegetative phase were relatively high in non-saline conditions; with a maximum (about 26-40 mg M g^{-1} root d^{-1}) at the 20 d. Salinity reduced the SAR of potassium in this period but did not significantly affect the SAR among species. Similar trends and values were obtained for the utilization rates of the salt ions Na^+ and Cl^- on a leaf basis (SUR_L). SUR_L values for these salt ions in control plants attained maximum values at day 20 (about 120-250 g^{-1} mg M d^{-1}). In salt-stressed plants, much lower SUR_L values for both salt ions (Na^+ and Cl^-) were obtained. The SUR_Ls of potassium (up to about 6 g^{-1} mg M d^{-1}) was not significantly reduced by increased salinity during vegetative stage and, among species; the highest SUR_L was for *P. vulgaris* WT at 90 mM NaCl. Salinity affected the pot assium SAR in the vegetative phase, probably by significantly reducing the absorption of K^+ in roots. The K^+ concentration fell continuously in roots and stems of

salt-stressed *Phaseolus* species, whilst the leaves attained similar concentrations to those in unsalinized plants at the medium salt stress, suggesting a compensation over time, probably by translocation of K^+ from roots and stems to leaves, a sustained acquisition despite appreciable overall Na^+ uptake and/or a high K^+ selectivity and/or K^+/Na^+ exchange across the plasmalemma of the root epidermis (Maathius and Amtmann 1999). The ability to withdraw sodium and to retranslocate potassium seems crucial for salt tolerance (Chen et al. 2007). Therefore, the maintenance of higher leaf K^+ concentrations in salt-tolerant *Phaseolus* species could be, by far, one of the most important mechanisms underlying superior salt tolerance as reported in *P. filiformis* (Bayuelo-Jiménez et al. 2003).

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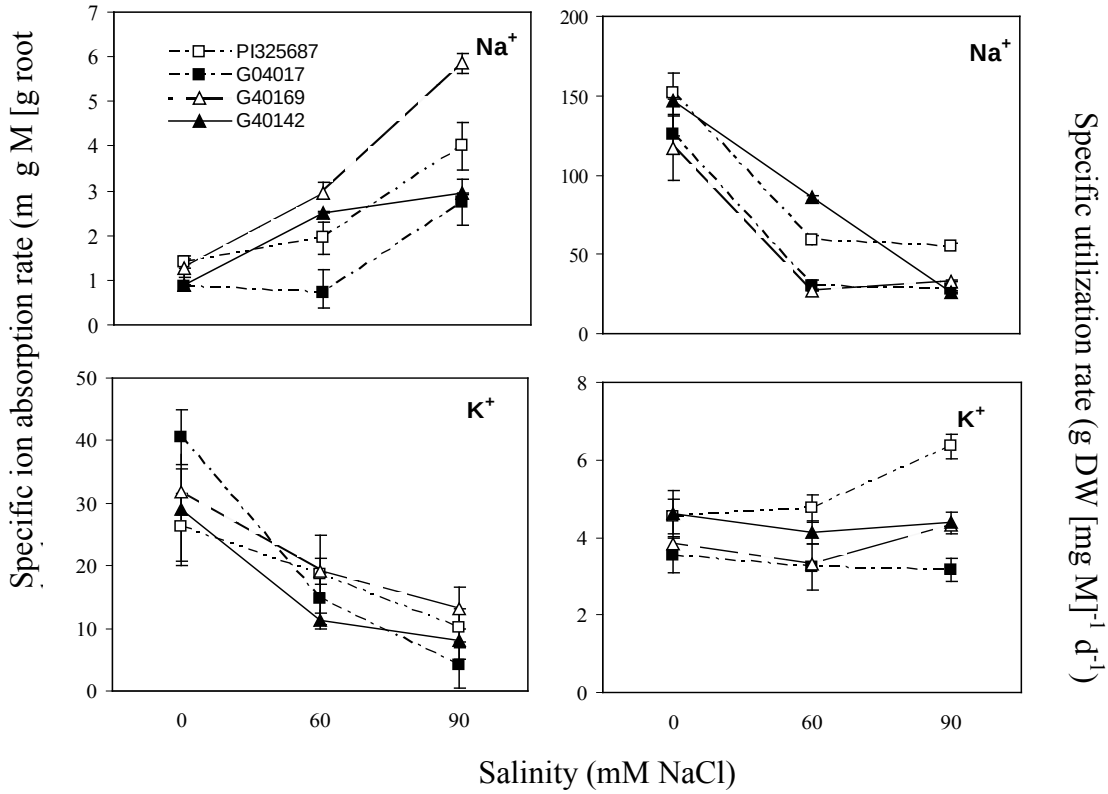

Figure 1. Changes in the specific absorption rate (SAR) and specific utilization rate (SUR) for Na^+ and K^+ in *Phaseolus* species between 10 and 20 days. Each value represents the mean ± SE of six replicates.

LEAF AREA DURATION, NET ASSIMILATION RATE AND YIELD OF *P. COCCINEUS* L. AND *L. P. VULGARIS* L. IN ALKALINE SOILS

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INTRODUCTION

The production of dry matter (biomass) of a crop is mainly determined by the magnitude and leaf area duration (LAD) and net assimilation rate (NAR) (Escalante and Kohashi, 1993). Thus, the study of the relationship between LAD, NAR, biomass and yield would lead to the search for strategies to achieve a higher yield in alkaline soils. Moreover, a relationship between NAR and seed production is not always the case due to other processes such as translocation are involved in generating the yield of seed. Previously reported in beans (Escalante and Rodriguez, 2008) indicate a relationship between the seed yield and biomass, leaf area, the absolute growth rate and specific leaf area. They also reported that although the cultivar Canario 107 showed higher NAR than Ayocote, Bayomex and Criollo, which was not reflected in higher biomass and seed yield. The aim of this study was to determine the relationship between the leaf area duration and net assimilation with biomass production, seed yield and its components in *Phaseolus* cultivars in alkaline soil.

MATERIALS AND METHODS

The study was conducted during rainy season in Montecillo Mex. (19º N, 98º W and 2250 m of altitude) of semiarid climate. Three cultivars of bush bean *Phaseolus vulgaris* L. Bayomex, Criollo Tequexquinahuac (Criollo) of indeterminate type, and Canario 107 (Canario) of determinate type and one cultivar of *P.coccineus* L. "Ayocote" of indeterminate type were sown at population density of 6.25 (80*25 cm) plants m^{-2} on June 10, 2005 in a dry clay soil with pH 8. The experimental design was a randomized block with 4 replications. All experiments were fertilized with 100-100-00 NPK. The phenology was registered and harvest of two plants realized to 24, 53 and 83 days after sowing (das) and maturity physiological (MF) by treatment-replication for calculated the: biomass duration ($BD=\sum(W2+W1)(T2-T1)/2$);, leaf area duration ($LAD=\sum(AF1+AF2)(T2-T1)/2$) and net assimilation rate ($NAR=BD/LAD$) according with Hunt (1978) and Escalante and Kohashi (1993). The final harvest the biomass (BIO; dry weight g $plant^{-1}$ and m^2), seed yield (SY, g $plant^{-1}$ and m^2), pod and seed number per plant, seed per pod and seed size were evaluated.

RESULTS AND DISCUSSION

Genotypes emerged 10 days after sowing (das). Flowering occurred to 45 das for Ayocote and Canario, and 51 and 70 das for Bayomex and Criollo. Physiological maturity was reached 92 and 110 das by Canario and Bayomex, and 130 das by Ayocote and Criollo. In alkaline soil, Ayocote showed higher biomass (319 g m^{-2}) and yield (156 g m^{-2}) than Bayomex, Criollo and Canario which showed the lowest seed yield (37 g m^{-2}). These changes were related to the seed and pod number and seed size (Table 1) and with a longest biomass duration, leaf area duration and net assimilation rate (Table 2). Similar to previous studies (Escalante and Rodriguez, 2008), Canario showed a higher NAR than Bayomex but a lower LAD that was decisive for biomass and seed yield was the lowest (Table 1). This suggests that to achieve greatest production of biomass and seed yield in beans grown in alkaline soils, is required to generate management strategies that lead to highest leaf area duration and net assimilation rate.

Table 1 - Biomass (dry matter,g), seed yield (g) and its components in *Phaseolus* cultivars. Data per plant to physiological maturity. Summer 2005.Montecillo Méx. México.

Cultivar	Biomass (g)	Seed yield(g)	Pod number	Seed number	Seed size (mg)	Seeds/pod
Ayocote	51 a	25 a	18 ab	44 b	568 a	2.4 b
Bayomex	42 b	22 a	20 a	75 a	293 b	3.7 a
Criollo	23 c	10 b	15 b	43 b	232 c	2.8 b
Canario	12 c	6 b	8 c	23 c	261 b	2.8 b
Mean	25.8	12.5	12.8	12.5	338	2.9
F prob.	**	***	**	**	**	*

*,**,*** P<0.05,0.01,0.001,respectively. Similar letters between columns indicate no significant differences

Table 2 - Biomass duration (g days),Leaf area duration (dm^2 days) ,net assimilation rate (g dm^{-2} day^{-1}),Biomass and seed yield (g m^{-2}) in *Phaseolus* cultivars. Summer 2005.Montecillo Méx. México.

Cultivar	BD (g days)	LAD (dm^2 days)	NAR (g dm^{-2} day^{-1})	Biomass (g m^{-2})	SY (g m^{-2})
Ayocote	942 a	685 a	1.37 a	319 a	156 a
Bayomex	447 c	522 b	0.86 b	262 b	137 a
Criollo	665 b	504 b	1.32 a	144 c	62 b
Canario	290 d	221 c	1.31 a	75 c	37 b
Mean	586	483	1.21	200	97
F prob.	**	**	**	**	***

*,**,*** P <0.05,0.01,0.001,respectively. S imilar l etters between c olumns in dicate no s ignificant d ifferences. SY=Seed yield.

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GERMINATION OF SNAP BEAN (*PHASEOLUS VULGARIS* L.) UNDER CONDITIONS OF SALINITY

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INTRODUCTION

Salinity is a limiting crop yields due to low population density generated by the reduction in germination and seedling survival (Gama *et al*., 2007). One strategy for achieving higher production in these areas is the search for cultivars tolerant to salinity. In snap bean that is highly sensitive to salinity (De Pascale *et al*., 1997). The search for tolerant cultivars from germination is an alternative to achieve highest yield in these conditions. The aim of this study was to determine the salinity tolerance of different of snap beans cultivars assessing germination.

MATERIALS AND METHODS

The study was conducted in the laboratories of the Postgraduate College in Montecillo, Mexico State. Snap bean cultivars were: "Hav-14", "Strike," "Black Valentine", "Opus" and "Japonés" sowing seeds of five, was done in petri dish of diameter 15 cm under five saline and three levels of osmotic potential (OP, MPa): Sodium chloride -0.44, -0.71, -1.09, -0.49; calcium chloride, -0.62, -0.94, -0.35; magnesium chloride, -0.73, -0.98; Magnesium sulphate -0.14, -0.33, -0.38, -0.28; sodium bicarbonate, -0.48, -0.73, and control (distilled water). The experiment was a factorial with a design completely randomized and three replications. After sowing, the petri dish was covered to keep the seeds in conditions of darkness. The first evaluation of germination was to 10 days after sowing (DAS) and thereafter every 2 days for 8 days. In the analysis of results, was considered a control as the reference.

RESULTS AND DISCUSSION

Analysis of variance showed significant differences between cultivars, type of salt, salt * cultivar, PO and PO * cultivar (Table 1). The highest germination was found in cv. "Japonés" (83%) followed by "Black Valentine" (64%), "Strike" (61%), "Opus" (60%) and "Hav-14 (54%). This is also indicative of the degree of sensitivity to salinity ("Hav-14"> "Opus"> "Strike"> "Black Valentine"> "Japonés"). In order of salt type to reduce the germination rate was calcium chloride (47%)> magnesium chloride (40%)> sodium chloride (39%)> sodium bicarbonate (36%)> magnesium sulfate (16%). As shown in Table 2, the germination was reduced from 83.6% to 45.0% with increasing PO. On average the levels of PO, the cultivar "Japones" showed a higher germination, than Black Valantine, Strike, and Hav-Opus 14, which showed the lowest. In *Vigna ungiculata* (L.) Walp., similar trends were observed. This response may be due to that a high PO decrease the imbibition and reserves mobilization rate and consequently the germination (Murillo and Troy, 2000).

Table 1. Germination (G,%) in relation to cultivar and saline solution.

Sol. salina	Hav-14 G %	Strike G %	B.V G %	Opus G %	Japonés G %	Mean saline solution
Sodium chloride	33	73	40	80	80	**61 b**
Calcium chloride	40	60	53	46	66	**53 b**
Magnesium chloride	46	53	53	60	86	**60 b**
Magnesium sulfate	86	66	93	73	100	**84 a**
Sodium bicarbonate	66	53	80	40	80	**64 b**
Control	100	100	100	100	100	**100 a**
Mean-cultivar	**54 b**	**61 b**	**64 b**	**60 b**	**83 a**	

Means in rows and columns with similar letter indicated that the treatments were statistically equal. . B.V = Black Valantine.

Table 2. Germination (G,%) in relation to cultivar and Osmotic Potential (PO;Mpa).

Osmótic Potential Mpa		Hav-14 G %	Strike G %	B.V G %	Opus G %	Japonés G %	Mean (PO)
Low	-0.14 -0.49	77 ab	79 ab	92 a	77 ab	93 a	**83.6 a**
Medium	-0.33 -0.73	67 bc	72 ab	78 ab	62 bc	90 a	**73.8 b**
High	-0.38 -1.09	37 d	37 d	37 d	49 cd	65 bc	**45.0 c**
Mean-cultivar		**60.3 b**	**62.6 b**	**69.0 b**	**62.6 b**	**82.6 a**	

Means in rows and columns with similar letter indicated that the treatments were statistically equal. B.V = Black Valantine.

CONCLUSIONS

Cultivars show differences in germination as response to the type and concentration of salinity. The increase in osmotic pressure also reduces germination. The cultivar "Japones" shows more tolerance to salinity, followed by "Black Valentine", "Strike," "Opus" and "Hav-14" which is the most sensitive.

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SEEDLING SYSTEM, FERTILIZATION, AND TILLAGE EFFECTS ON GRAIN YIELD OF DRY BEANS UNDER RAINFED CONDITIONS IN AGUASCALIENTES, MEXICO

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INTRODUCTION

Traditionally, producers of dry beans under rainfed conditions in the semiarid North Central region of Mexico sow beans in 0.76 m width furrows; and till the soil with a pass of the plow and one or two passes of disking. It is also very common that the bean crop is not fertilized under rainfed conditions, and that fertilizers are applied in inappropriate quantities, or are applied in periods where plants do not totally uptake the fertilizer. Plant density ranges between 85,000 and 90,000 plants per hectare, and there are few farmers who practice rain harvest and conservation practices. With rain harvesting, the crop will have more soil moisture than if this practice is not performed (Padilla *et al*., 2008), so that there will be more water for higher plant densities, however plants will need more nutrients.

MATERIALS AND METHODS

In 2008, an experiment was established under rainfed condition in Sandovales, Aguascalientes, Mexico, elevation of 2000 m.a.s.l. Recorded rainfall during the growing season was 653 mm, average temperature was 16.3˚C, and the length of the growing season was 110 days. Soil has 0.5 m depth, 0.9% of organic matter, sandy clay loam texture, 1% slope, and pH 6.8. Factors studied were: a) vertical tillage (root cutter or Multiarado) and conventional tillage (plow and disk), b) sowing methods in single row (HS) with 0.76 width furrows with 94,000 plants per hectare and double rows with different plant population (DH_{20}=132,000; DH_{14}=188,000, and DH_{10}=262,000 plants per hectare), using the variety Pinto Saltillo, c) fertilization in rates of N-P_2O_5-K_2O 40-40-40 and 12-06-00 was applied to soil (FS) and leaves (FF) respectively, compared with no fertilization(SF). A split plot with randomized block design and four replicates was used. Sowing date was July 31. Cultivation was made 28 days after planting. Pests were controlled with one chemical treatment. In each plot, the following information was recorded: number of pods per plant (average of 10 plants random selected inside the central rows in each plot), number of pods per square meter (number of pods per plant times the number of plants per square meter), and grain and straw yield (two center rows three meter length were harvested in each treatment).

RESULTS AND DISCUSSION

Rainfall of 440 mm was recorded during the crop cycle, and most of the rain fell before bloom. In Table 1, the average yield, number of pods per plant and square meter in each one of 24 treatments is shown. There was not statistical difference due to the effect of tillage. The highest grain yield was obtained with vertical tillage followed by conventional tillage with an average grain yield of 1.91 and 1.22 t ha^{-1}, respectively. A significant response was observed under different plant populations using different sowing methods with average grain yield of 1.86, 1.67, 1.54 and 1.20 t ha^{-1} for DH_{10}, DH_{14},

DH_{20}, and HS respectively. Multiarado, combined with the highest plant population and soil fertilization produced the highest grain yield and obtained the highest number of pods per square meter (Table1).

Table1. Grain yield average (t ha^{-1}), number of pods per plant and square meter for tillage method, plant density, and fertilization. Sandovales, Aguascalientes, Mexico. 2008.

Tillage method	Plant density per hectare	Fertilization NPK (kg ha^{-1})	Grain yield (t ha^{-1})	Pods per plant	Pods per square meter
Traditional	94,000	40-40-30	1.11	12	81
Traditional	94,000	12-06-00	1.10	11	74
Traditional	94,000	00-00-00	0.73	8	51
Traditional	132,000	40-40-30	1.05	10	100
Traditional	132,000	12-06-00	1.08	9	90
Traditional	132,000	00-00-00	0.81	8	80
Traditional	188,000	40-40-30	1.59	9	126
Traditional	188,000	12-06-00	1.45	8	109
Traditional	188,000	00-00-00	1.05	6	88
Traditional	264,000	40-40-30	1.89	9	180
Traditional	264,000	12-06-00	1.62	8	160
Traditional	264,000	00-00-00	1.24	7	140
Multiarado	94,000	40-40-30	1.46	14	96
Multiarado	94,000	12-06-00	1.61	14	98
Multiarado	94,000	00-00-00	1.21	11	81
Multiarado	132,000	40-40-30	2.33	16	158
Multiarado	132,000	12-06-00	2.12	14	143
Multiarado	132,000	00-00-00	1.86	13	125
Multiarado	188,000	40-40-30	2.28	11	154
Multiarado	188,000	12-06-00	1.96	11	147
Multiarado	188,000	00-00-00	1.68	11	147
Multiarado	264,000	40-40-30	2.40	12	235
Multiarado	264,000	12-06-00	2.14	11	220
Multiarado	264,000	00-00-00	1.86	9	185

In general terms, the production of dry bean under rainfed conditions with a high productivity system (tillage conservation, rain harvesting *in* situ, narrow furrows, high plant population, and adequate fertilization) is more profitable than traditional systems, at least in years with adequate precipitation.

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GRAIN YIELD OF DRY BEAN GENOTYPES SEVERELY AFFECTED BY HAILSTORM

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INTRODUCTION

Common bean is the second most important crop in Mexico, after corn. During last five years, average of cultivated dry beans has been about 2.1 millions hectares, with a total production volume of 1.16 millions tons (1). This grain has played an important role in the diet of high percentage of people in our country. Nevertheless, average consumption has declined from almost 20 to 10 kg per person per year in a period of three decades. The main producing region of dry beans in México is known as the Highlands, which includes the states of Zacatecas, Durango, Chihuahua, Guanajuato, San Luis Potosi and Aguascalientes, where most of the cultivated dry beans is under rainfall conditions. Predominate climate at the Highlands is semiarid, with an average precipitation of 300 to 350 mm during the growth cycle. Thus, drought stress is one of the main limiting factors affecting grain yield. Another constraint that may reduce dry bean production is the occurrence of a hailstorm, which can cause severely damages in dry bean plants. Under this situation, farmers have to make a decision to maintain or eliminate the crop to sow again, mainly depending on the growth stage when hailstorm may occur. In this work, a group of 15 dry bean genotypes of different growth cycle was studied after being severely affected by hail to evaluate its recovery capability.

MATERIALS AND METHODS

The study was conducted at the Experimental Station of Pabellón (22º 09' North Latitude; 102º 17' West Longitude; and an altitude of 1912 masl) located in Aguascalientes state, during the summer of 2008. Some soil characteristics of the experimental site are: Texture: sandy loam; pH: 7.5; Organic matter: less than 1.0%; Field Capacity: 20%; Permanent Wilting Point: 11%; Apparent Density: 1.4. Sowing date was June 19th and the following dry bean genotypes were included: Azufrado Tapatio, Flor de Mayo Bajío, Pinto Saltillo, P. Villa, P. Colobri, P. Ventura (early genotypes), Bayo Madero, B. Victoria, Blanco Español, Flor de Mayo Sol, Flor de Junio Victoria, Negro Altplano (intermediate genotypes), Bayo Criollo del Llano, Flor de Mayo M-38 and Tlaxcala-62 (late genotypes). Most of the cultivars were obtained at the dry bean genetic improvement program of INIFAP and have Type III habit (2). Experimental unit consisted of eight rows of 30 m long and 0.76 m apart. Precipitation was recorded at daily bases from a near meteorological during the growing season. At the end of the growth cycle the following traits were estimated in each genotype from four samples of two rows and 4.0 m long: aerial biomass, grain yield, harvest index (HI=grain yield/aerial biomass) and weight of 100 seeds.

RESULTS AND DISCUSSION

After 43 days of sowing date, a hailstorm occurred and caused severe damages to the bean plants, specially those early genotypes, because of its growth stage. Early genotypes were at preflowering and damages included broken branches and lost of flower bottoms. Plants of intermediate and late genotypes had not yet to initiated flowering, thus damages were only on the broken branches. This situation was used to evaluate the recovery capacity of the plants and to produce grain. Figure 1, shows the aspect of plants after the hailstorm and one month later, demonstrating that dry bean plants

were able to produce new branches and to produce (Table 1). However, a delay of about 30 days was observed in the maturity of all genotypes.

Figure 1. Dry bean plants damaged by the hailstorm at 43 days after sowing (A) and plants recovered a month later (73 days after sowing) (B).

Table 1. Growth cycle, aerial biomass, grain yield, harvest index and weight of 100 seeds observed in 15 dry bean genotypes severely affected by hail. Pabellón, Ags., México.

Genotype	Growth cycle[1]	Aerial biomass Kg/ha	Grain yield Kg/ha	Harvest Index (%)	Weight of 100 seeds (g)
Pinto Saltillo	E	5740	3640	63.5	35.9
Azufrado Tapatio	E	4355	2820	64.6	31.4
Negro Altiplano	I	4003	2360	59.1	24.1
Flor de Mayo M-38	L	3950	2290	57.9	28.7
Flor de Junio Victoria	I	4172	2250	54.0	31.5
Bayo Victoria	I	3081	1840	59.8	41.8
Bayo Madero	I	4508	1620	35.7	25.0
Tlaxcala-62	L	2611	1520	58.1	37.9
Pinto Villa	E	2821	1460	51.5	31.5
Flor de Mayo Bajío	E	2295	1440	52.4	24.3
Pinto Ventura	E	2617	1410	53.6	27.6
Pinto Colibri	E	2491	1340	54.0	29.3
Flor de Mayo Sol	I	2335	1300	55.7	25.3
Bayo Criollo del Llano	L	2706	1060	43.9	27.5
Blanco Español	I	1643	860	52.0	32.9

[1]E = Early (40-45 day to flowering; 85-90 days to maturity); I = Intermediate (45-50 day to flowering; 90 -95 d ays t o m aturity); L = Late (50-55 da ys t o f lowering; 95 -100 da y t o maturity).

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ENVIRONMENTAL STRATIFICATION IN TRIALS OF COMMON BEANS IN THE RAINY GROWING SEASON, IN THE STATES OF PARANÁ AND SANTA CATARINA, BRAZIL

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INTRODUCTION

The common bean is submitted to different environmental conditions, since it is grown in several Brazilian States, in different growing seasons (rainy, dry, and winter), under different tillage systems. In this condition it is expected an accentuated interaction among genotypes and environments. This way, the evaluation of common bean lines should be conducted in environmental conditions that better represent the real conditions of the culture and for that it is necessary the implantation of a network of evaluation of trials, involving the main producer states. In Brazil, the States of Santa Catarina and Paraná distinguish in production, together are responsible for about 35% of dry bean national production (IBGE, 2008). The implantation of this network is a procedure quite arduous and expense. The implantation should verify if the sites of evaluation represent the diversity of environments where beans are grow, a specific region, and if specific sites can produce additional information since any redundancy among sites should be eliminated. Therefore, the objective of this work was to apply the procedures of environmental stratification to identify little informative sites when evaluating bean genotypes during the rainy growing season in the States of Paraná and Santa Catarina.

MATERIALS AND METHODS

The trials were conducted in the years of 2003 e 2004 in 12 environments (six sites in each year) in the States of Paraná and Santa Catarina, during rainy growing season (October to December), in randomized complete blocks with three replications and plots with four rows of three meter. Each combination of date of seeding/year was considered a yield. Each trial was composed of 16 genotypes belonging to carioca type (CNFC 9435, CNFC 9458, CNFC 9461, CNFC 9471, CNFC 9484, CNFC 9494, CNFC 9500, CNFC 9504, CNFC 9506, CNFC 9518, CNFE 8009, Carioca 11, Pérola, Iapar 81, Carioca Pitoco and FTS Magnífico). The data of yield of each trial were submitted to analyze of variance and joint analyzes by year. Analyzes of environmental stratification were done following the traditional method by Lin (1982) and by Ecovalence method (Wricke, 1965), using the Genes statistic program (Cruz, 2001). For identification of little informative sites in each year, it were considered the results obtained from the joint methods, that is, sites identified as little informative according to the two methodologies. For identification of the sites to be discharged, it were observed the sites where the results were little informative in two years.

RESULTS AND DISCUSSION

Joint analyzes showed good experimental precision (CV=16% e CV=12%, for 2003 and 2004, respectively) and showed clearly significant differences among sites and genotypes x sites interaction, showing the possibility of using analysis of environmental stratification.

Analyze of stratification by Lin (1982) method in 2003 grouped sites named Campos Novos, Major Vieria and Roncador, indicating that those are similar. Ecovalence analyses showed that sites

with small contribution to interaction were Campos Novos e Roncador (Table 1). Considering the methods in conjunct it can see that the site Campos Novos was grouped by Lin (1982) method and showed small ecovalence estimate. So, Campos Novos was considered the site less informative in this year.

In 2004 the Lin (1982) method grouped the sites Abelardo Luz and Laranjeiras do Sul as similar. Estimates of ecovalence for the sites Laranjeiras do Sul (7%) and Abelardo Luz (8%) indicated that those were the sites less informative (Table 1). Considering the results of both methods, it can be concluded that in this year the lesS informative sites was Laranjeiras do Sul, since it was grouped to Abelardo Luz by Lin (1982) method and presented small ecovalence estimate.

In order to ensure the identification of the sites little informative, it is recommended to identify those in different growing seasons, throughout the time. This did not occurred, since each year of evaluation it was identified a different site considered low informative: Campos Novos in 2003 and Laranjeiras do Sul in 2004. So, the results suggest that among the sites evaluated in the States of Paraná and Santa Catarina does not exist any site passive of being eliminated from the network of evaluation trials of common beans carioca type. However, it is worth to mention that not all sites were replicated in each year. This decreases the chance of identification of low informative sites in several years. Among the sites evaluated, Ponta Grossa, Abelardo Luz and Major Vieira, were always between the informative sites, confirming that they should stay in the nework of evaluation.

Table 1. Estimates of Ecovalences (W) for sites where trials with common beans were conducted, during rainy season in 2003 and 2004, in the States of Paraná and Santa Catarina, Brazil.

Site	Growing Season			
	2003		2004	
	W[1]	%	W	%
Ponta Grossa	4,448	12	8,982	36
Campos Novos	1,203	3	3,581	16
Abelardo Luz	8,165	23	2,048	8
Major Vieira	4,708	13	3,902	16
Concórdia	15,981	44	-	-
Roncador	1,748	5	-	-
Laranjeiras do Sul	-	-	1,711	7
Londrina	-	-	4,215	17
Total	36,255	100	24,441	100

[1]($x10^3$)

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ACCUMULATION OF MACRONUTRIENTS BY DIFFERENT COMMON BEAN CULTIVARS GROWN IN DIFFERENT PLANT DENSITIES IN CONVENTIONAL CROP SYSTEM

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INTRODUCTION:

The aim of the current study was to follow the accumulation of macronutrients throughout the crop cycle in different cultivars of common bean with carioca seed type, grown under different plant densities in field experiments using a conventional cropping system.

MATERIALS AND METHODS:

The experimental design was randomized blocks with three replications and a 4x5 factorial scheme, involving four bean cultivars (Table 1) and five plant densities (75, 145, 215, 285 and 355 thousand plants.ha^{-1}). The soil preparation was carried out by one operation with plow and two operations with harrow. The experiment had not been irrigated. Each plot had four rows with 5.0 m length and spacing of 0.5 m between rows. At sowing (November, 2006), all the plots had received identical fertilization, determined by the soil analysis interpretation. The N fertilization at covering (at 21 days after emergency-DAE) was 30 kg.ha^{-1} of N, urea source. Every 10 days, samples of 10 plants were collected and dried under air circulation to 65-70^{o}C, until constant mass, soon after they had been triturated and sent to the Laboratory of Leaf Analysis of the Soil Science Department (UFLA) for determination of the macronutrients content. The N content was evaluated by Kjedahls method while P, K, Ca, Mg, S were extracted by digestion by nitric and percloric acid and quantified in the extract (P colorimetrically, K- flame photometry; S -turbidimetry; Ca, Mg spectrophotometry of atomic absorption).

Table 1. Principal characteristics of the studied cultivars.

Characteristics	BRS Radiante*	Bolinha**	Ouro Vermelho*	Jalo EEP 558*
Commercial group	Others	Others	Others	Jalo
Seed color	cream / beige	yellow	red	yellow
Growth habit	I	II	II/III	III
100 grain's weight	44-45 g	32-33g	25 g	30-40 g
Stem	erect	erect	semi-erect	semiclimber
Cultural cycle	early	middle	normal	middle

* Ramalho & Abreu (2006), ** Alves (2008)

RESULTS AND DISCUSSION:

At flowering, large proportion of the each macronutrient is reached by the bean cultivars. The maximum accumulations of N, P, K, Mg and S are registered at the end of the crop cycle, wile the maximum Ca accumulation occurring around 50-60 DAE; the cv. Bolinha showed signifficative interation between DAE and plant population (Table 2). The cv. Ouro Vermelho accumulates more K, Ca, Mg and S (Table 3). The general decreasing order of accumulation is N>K>Ca>Mg>P>S.

Table 2. Accumulation of macronutrients (kg ha^{-1}) by four bean cultivars, in function of DAE.

Cultivar	Nutrient	Regression	R^2 (%)
Jalo EEP 558	N	$Y= 2,552751 + 0,270073\ x + 0,005232\ x^2$	95,77
	P	$Y= 1,546980 - 0,057874\ x + 0,000156\ x^2$	91,98
	K	$Y= 0,480305 + 0,151709\ x + 0,003715\ x^2$	97,15
	Ca	$Y= -6,354678 + 0,697968\ x - 0,005085\ x^2$	81,13
	Mg	$Y= -0,484072 + 0,077824\ x - 0,000020\ x^2$	93,37
	S	$Y= -0,110939 + 0,037889\ x + 0,000058\ x^2$	95,69
BRS Radiante	N	$Y= -2,862843 + 0,631350\ x + 0,000290\ x^2$	79,68
	P	$Y= -0,446296 + 0,057872\ x + 0,000210\ x^2$	92,10
	K	$Y= -3,511032 + 0,484458\ x - 0,001608\ x^2$	89,27
	Ca	$Y=-6,726046 + 0,760801\ x - 0,007753\ x^2$	81,39
	Mg	$Y=-0,729224 + 0,106695\ x\ -0,000681\ x^2$	90,97
	S	$Y=-0,335356 + 0,058732\ x - 0,000338\ x^2$	92,99
Ouro Vermelho	N	$Y= -12,324369 + 1,327124\ x - 0,006238\ x^2$	82,68
	P	$Y= -0,296778 + 0,106324\ x - 0,0000237\ x^2$	86,32
	K	$Y= -3,204919 + 0,331758\ x + 0,003843\ x^2$	91,63
	Ca	$Y= -12,966067 + 1,218299\ x - 0,008724\ x^2$	92,86
	Mg	$Y= -1,044143 + 0,117457\ x - 0,000174\ x^2$	86,23
	S	$Y=-1,414437 + 0,132443\ x - 0,000220\ x^2$	92,83
Bolinha	N at 215*	$Y= -1,751540 + 0,373600\ x + 0,000941\ x^2$	84,23
	N at 285	$Y= -20,113270 + 2,220640\ x - 0,018531\ x^2$	90,98
	N at 355	$Y= -14,578836 + 2,132255\ x - 0,012888\ x^2$	92,30
	P at 215	$Y= -0,597096 + 0,052913\ x + 0,106578\ x^2$	84,32
	P at 285	$Y= -2,783199 + 0,248119 - 0,001970\ x^2$	85,38
	P at 355	$Y= -3,738039 + 0,327908 - 0,002166\ x^2$	77,59
	K at 215	$Y= 1,496467 - 0,029352\ x + 0,004518\ x^2$	92,98
	K at 285	$Y=-9,523853 + 0,982414\ x - 0,006271\ x^2$	94,98
	K at 355	$Y=-10,401975 + 1,186585\ x - 0,005212\ x^2$	87,81
	Ca at 285	$Y=-17,346703 + 1,618399\ x - 0,014868\ x^2$	76,26
	Ca at 355	$Y=-16,936917 + 1,755528\ x - 0,015393\ x^2$	76,39
	Mg at 215	$Y=0,201817 + 0,011150\ x + 0,000588\ x^2$	87,06
	Mg at 285	$Y=-2,682902 + 0,276498\ x - 0,002126\ x^2$	88,47
	Mg at 355	$Y=-2,729931 + 0,302017\ x - 0,001953\ x^2$	84,65
	S at 215	$Y=-0,222967 + 0,031707\ x + 0,000014\ x^2$	88,97
	S at 285	$Y=-1,654399 + 0,164519\ x - 0,001299\ x^2$	90,34
	S at 355	$Y=-1,922884 + 0,211422\ x - 0,001389\ x^2$	73,79

*At considered significant plant populations.

Table 3. Macronutrients accumulation (kg ha^{-1}) in the aerial part of the plant of four bean cultivars.

	N	P	K	Ca	Mg	S
Bolinha	31,70 a	3,50 a	24,74 b	14,24 b	4,93 b	2,46 b
Jalo EEP 558	44,03 a	4,60 a	27,83 b	13,89 b	4,39 b	2,66 b
BRS Radiante	34,50 a	4,11 a	19,51 b	7,23 b	3,05 b	1,96 b
Ouro Vermelho	42,16 a	4,65 a	41,52 a	30,11 a	8,18 a	6,75 a
Means	38,09	4,21	28,40	16,36	5,13	3,45

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ALVES, A.F. Densidades populacionais para cultivares alternativas de feijoeiro em Minas Gerais. 2008. 50 p. Dissertação(Mestrado em Fitotecnia) – Universidade Federal de Lavras, Lavras, 2008.

ACCUMULATION OF MICRONUTRIENTS BY DIFFERENT COMMON BEAN CULTIVARS GROWN IN DIFFERENT PLANT DENSITIES IN CONVENTIONAL CROP SYSTEM

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INTRODUCTION: The aim of the current study was to follow the accumulation of micronutrients throughout the crop cycle in different cultivars of common bean with carioca seed type, grown under different plant densities in field experiments using a conventional cropping system.

MATERIALS AND METHODS: The experimental design was randomized blocks with three replications and a 4x5 factorial scheme, involving four bean cultivars (Table 1) and five plant densities (75, 145, 215, 285 and 355 thousand plants.ha^{-1}). The soil preparation was carried out by one operation with plow and two operations with harrow. The experiment had not been irrigated. Each plot had four rows with 5.0 m length and spacing of 0.5 m between rows. At sowing (November, 2006), all the plots had received identical fertilization, determined by the soil analysis interpretation. The N fertilization at covering (at 21 days after emergency-DAE) was 30 kg.ha^{-1} of N, urea source. Every 10 days, samples of 10 plants were collected and dried under air circulation to 65-70^{o}C, until constant mass, soon after they had been triturated and sent to the Laboratory of Leaf Analysis of the Soil Science Department (UFLA) for determination of the micronutrients content. The Cu, Fe, Mn e Zn contents were evaluated by digestion by nitric and percloric acid and quantified in the extract (espectrophotometry of atomic absorption) and B by incineration and colorimetric determination by the curcumin method.

Table 1. Principal characteristics of the studied cultivars.

Characteristics	BRS Radiante*	Bolinha**	Ouro Vermelho*	Jalo EEP 558*
Commercial group	Others	Others	Others	Jalo
Seed color	cream / beige	yellow	red	yellow
Growth habit	I	II	II/III	III
100 grain's weight	44-45 g	32-33g	25 g	30-40 g
Stem	erect	erect	semi-erect	semiclimber
Cultural cycle	early	middle	normal	middle

* Ramalho & Abreu (2006), ** Alves (2008)

RESULTS AND DISCUSSION: At flowering, large proportion of the each micronutrient is reached by the bean cultivars. B and Cu showed low initial accumulation, what is even developed from 40-50 DAE up to maturing. The others micronutrients are accumulated in the significant form from the beginning of the cycle; in the cases cv. Bolinha-B and cv. Ouro Vermelho-Fe there was signifficative interation between DAE and plant population (Table 2). The bean cultivars do not differ in relation to the B and Zn accumulations, but the cvs. Jalo EEP 558 and BRS Radiante accumulates more Fe and the cv. Ouro Vermelho accumulates more Mn and Cu (Table 3). The general decreasing order of accumulation is Fe>B>Mn>Zn>Cu.

Table 2. Accumulation of micronutrients (g ha^{-1}) by four bean cultivars, in function of DAE.

Cultivar	Nutrient	Regression	R^2 (%)
Bolinha	B at 215*	$Y= 7,654401 - 0,970793 x + 0,035184 x^2$	91,14
	B at 285	$Y= -4,134850 + 0,484578 x + 0,014964 x^2$	94,27
	B at 355	$Y= 25,855394 – 1,435278 x + 0,054272 x^2$	91,54
	Cu	$Y= 3,780875 - 0,291062 x + 0,009606 x^2$	96,20
	Fe	Y=338,195160 + 30,677495 x - 0,159501 x2	78,46
	Mn	$Y= -33,616847 + 3,469581x - 0,028700 x^2$	81,76
	Zn	$Y= -18,188573 + 1,796735 x - 0,009419 x^2$	93,43
Jalo EEP 558	B	$Y= 70,842289 - 6,221562 x + 0,121331 x^2$	95,33
	Cu	$Y= 3,430436 - 0,153673 x + 0,006931 x^2$	96,68
	Fe	Y=574,245040 + 14,765826 x + 0,208370 x2	79,76
	Mn	$Y= -16,214513 + 2,227783 x - 0,013614 x^2$	96,53
	Zn	$Y= -1,848392 + 0,515472 x + 0,008154 x^2$	99,59
BRS Radiante	B	$Y= 101,004786 - 8,835937 x + 0,166445 x^2$	94,50
	Cu	$Y= -0,794424 + 0,260449 x + 0,000655 x^2$	83,25
	Fe	Y=43,641668 + 64,753179 - 0,634523 x2	83,13
	Mn	$Y= -24,017412 + 2,859347 x - 0,027997 x^2$	84,06
	Zn	$Y= -10,457097 + 1,320357 x - 0,004796 x^2$	94,84
Ouro Vermelho	B	$Y= 21,234398 - 1,861711 x + 0,054747 x^2$	95,39
	Cu	$Y= -5,275257 + 0,455941 x + 0,001429 x^2$	95,29
	Fe at 75*	$Y= 26,432282 - 1,276536 x + 0,179910 x^2$	76,07
	Fe at 145	$Y= -94,064832 + 5,103501 x + 0,130694 x^2$	79,15
	Fe at 215	$Y= -18,615219 + 2,872428 x + 0,196607 x^2$	89,64
	Fe at 285	$Y= -189,034036 + 15,583867 x + 0,011961x^2$	81,04
	Fe at 355	$Y= -122,765127 + 13,021096 x + 0,076238 x^2$	91,53
	Mn	$Y= -36,998363 + 3,769834 x - 0,022769 x^2$	95,48
	Zn	$Y= -26,654019 + 2,624665 x - 0,013706 x^2$	94,97

*At considered significant plant populations.

Table 3. Micronutrients accumulation (g ha^{-1}) in the aerial part of the plant of four bean cultivars.

	Cu	Mn	Zn	B	Fe
Bolinha	49 a	61 b	65 a	126 a	1221 b
Jalo EEP 558	25 b	68 b	73 a	145 a	1626 a
BRS Radiante	20 b	38 c	53 a	153 a	1479 a
Ouro Vermelho	39 a	105 a	79 a	159 a	1039 b
Means	33	68	68	146	1341

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PERFORMANCE OF DRY BEAN LINES IN A LOW N SOIL IN PUERTO RICO

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Low nitrogen (N) availability is an important constraint for crop production in highly weathered soils of the Tropics (Graham and Vance, 2000). Selection of dry beans (*Phaseolus vulgaris* L.) for improved adaptation to low N soils may help to improve seed yield on farms where N fertilization is limited or unavailable. Field experiments were conducted at Isabela, Puerto Rico to identify N efficient dry bean lines in an Oxisol (very-fine, kaolinitic, isohyperthermic Typic Eutrustox). An initial screening of 228 lines from the bean breeding programs of the Escuela Agrícola Panamericana, Honduras and the University of Puerto Rico was performed in February 2007 using a moderate level (50 kg ha^{-1}) of N fertilization. Thirty-four lines were selected based on seed yield, adaptation and rust resistance. These lines were evaluated for agronomic performance in field experiments planted in June 2007 and January 2008 using a split plot arrangement of a RCB design with five replications. Two fertilization regimes, 50 kg N ha^{-1} (+N), 57 kg P_2O_5 ha^{-1} (+P), 54 kg K_2O ha^{-1} (+K), and –N, +P, +K, were the whole plots and the bean breeding lines were the sub-plots. The experimental units were single rows, 4 m in length that were spaced 0.6 m apart. Another field experiment was planted in January 2008 using an intermediate level of N fertilization (25 kg N ha^{-1}) to study nodulation and the partitioning of N of 13 lines that performed well in 2007. The experimental design was a RCB with 5 replications. The experimental unit was three rows, 3 m in length that were spaced 0.6 m between rows. The seed in all experiments was treated at planting with inoculant containing 2.9 x 10^6 viable cells g^{-1} of *Rhizobium leguminosarum* bv. *phaseoli*. The mean seed yield of the trial in 2008 was lower than 2007. Because the experiment was conducted on the same site, this seed yield reduction may have been the result of a depletion of the availability of N in the soil. The black bean line PR0443-151 had the best overall performance. At both levels of N, the mean seed yield of PR0443-151 was ranked no lower than third in the 2007 and 2008 field experiments (Tables 1 and 2). In the – N treatment, the small red line VAX 3 produced the greatest seed yield in 2007 and was ranked 2nd in 2008. The performance of PR0340-3-3-1 in 2007 was inconsistent. However, this line did produce the greatest seed yield at all levels of N fertilization in 2008 (Tables 1 and 2). In 2007 and 2008, PR0443-151 and VAX 3 had the greatest efficiency of N use (kg of seed yield in the – N plots/ kg N in the soil) (Table 1). PR0443-151, IBC 309-23 and MER 2226-28 had the greatest agronomic efficiency means (kg of seed yield / kg of N applied) which suggested that these lines were most responsive to N fertilization in 2007 and 2008 (Table 1). In the experiment using an N fertilization rate of 25 kg ha^{-1}, PR 0340-3-3-1 and A 774 accumulated the greatest amount of N in the aerial biomass (Table 2). PR0443-151 accumulated the greatest amount of N in the seed resulting in the highest % of total N in the seed. Mean nodulation scores were intermediate to poor (> 6.5). Cardenal and PR0443-151 had the lowest nodulation scores. In a related study, VAX 3 was identified as having a shallow root system and PR 0443-151 had an intermediate root system in a low N soil.

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Table 1. Performance of bean lines in + N (50 kg/ha) and – N (0 kg/ha) plots planted at Isabela, Puerto Rico in June 2007 and January 2008.

Line	Seed yield (kg/ha)		Rank in the trial		Efficiency of N use (kg of seed yield in – N / kg N in the soil)	Agronomic efficency (kg of seed yield / kg of N applied)
	+ N	- N	+ N	- N		
June 2007 planting						
PR0443-151	2544	1707	2	2	55.0	18.8
IBC 309-23	2184	1258	6	4	47.2	25.8
A 774	2162	1167	8	6	44.2	21.5
MER 2226-28	2205	1102	15	7	40.8	15.2
VAX 3	1929	1880	16	1	62.5	1.2
PR0340-3-3-1	2270	820	3	25	27.6	31.0
Mean	1874	941			34.0	20.9
LSD (0.05)	748				39.8	34.0
January 2008 planting						
PR0443-151	1918	1461	2	3	72.3	15.9
IBC 309-23	1460	950	3	9	44.5	13.7
A 774	1359	1343	8	4	60.6	0.5
MER 2226-28	1192	805	16	15	37.3	12.0
VAX 3	1467	1479	3	2	66.1	0.5
PR0340-3-3-1	2300	2065	1	1	89.8	7.6
Mean	1199	837			35.6	10.4
LSD (0.05)	509				30.4	20.0

Table 2. Mean seed yield, N accumulation of the aerial biomass, seed N accumulation, and % of total N in the seed of bean lines in low N (25 kg ha^{-1}) plots planted at Isabela Puerto Rico in January 2008.

Line	Seed yield (kg ha^{-1})	Total N accumulation (kg ha^{-1})	Seed N accumulation (kg ha^{-1})	% of total N in the seed	Mean nodulation score[1]
PR 0340-3-3-1	997	81.5	29.6	47.0	7.0
PR 0443-151	876	69.3	33.8	53.9	6.0
VAX 3	809	74.7	21.1	31.1	6.5
RAB 655	808	62.6	22.9	29.6	6.7
A 774	724	87.6	26.1	28.6	9.0
Arroyo Loro Negro	548	62.3	25.1	41.6	8.0
Cardenal	512	44.5	13.5	32.7	5.0
Mean of the trial	554	56.2	18.1	34.9	6.4
LSD (0.05)	205	37.3	8.3	24.6	2.6
CV (%)	29.2	39.6	26.0	42.0	24.4

[1] Evaluated 67 days after planting using the CIAT 1-9 scale where 1 = > 80 nodules/plant, 3 = 41-80 nodules/plant, 5 = 21-40 nodules/plant, 7 = 10-20 nodules/plant and 9 = < 10 nodules/plant.

ROW SPACING AND NITROGEN FERTILIZATION EFFECT ON ARCHITECTURAL TRAITS AND YIELD LOSS OF DRY BEAN VARIETIES UNDER DIRECT HARVEST

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INTRODUCTION

Higher levels of plant available nitrogen (N) have resulted in increases in the number of dry bean pods per plant, number of seeds per pod, seed weight, and consequently, a yield increase (Fageria and Santos, 2008). However, in order to achieve optimum seed yield at a low cost, N fertilization should be appropriately managed. For a yield goal of 1,700 kg ha^{-1} (North Dakota's mean yield) it is recommended that 84 kg ha^{-1} total nitrogen (soil N plus fertilizer) be applied (NDSU, 2003). However, in ND, MN, and other regions, farmers usually correct the available N to a level of 112 kg ha^{-1} and some even go beyond. Row spacing is another important factor affecting plant architecture and consequently, seed yield (Grafton et al., 1998). The effect of row spacing on dry bean yield and plant architecture appears to be different for the variable amount of applied N (MAFRI, 2007). Given the recent increases in fertilizer costs, it is important to find the optimum growing conditions that maximizes yield and reduce production costs. The objective of this study was to evaluate the effect of N fertilization and row distances on yield performance and yield loss due to direct harvesting of new upright pinto varieties.

MATERIALS AND METHODS

This study was conducted at Carrington and Prosper, ND, in 2008. The experimental design was a RCBD in a split-plot arrangement with three replicates where row spacings were the main plot and a factorial of N levels and genotypes were the subplots. The genotypes tested were the new Lariat and Stampede pinto beans (Type II), and Maverick pinto bean as a control (Type III). The study had three row spacings: solid seeded, narrow rows, and wide rows (30, 46, and 76 cm row spacings, respectively). Two nitrogen availability levels: 56 kg ha^{-1} N (soil N) and 112 kg ha^{-1} N (soil N + fertilizer N) were used with all row spacings and varieties. Characteristics evaluated included plant height, pod distribution, seed yield, harvest loss, and seed weight. The varieties were planted in plots 7.62 m long at recommended seeding rates. A Hege 125B plot combine was used to direct harvest. Harvest losses, in each plot, were estimated by counting the seeds on the ground, in two samples, within an area bounded by a square metal hoop (0.21 m^2), and then converted to seed weight to calculate yield loss.

RESULTS AND DISCUSSION

Analysis across the two locations showed a significant genotype x environment interaction. Both locations have similar average rainfall, but different soil type which makes the environments very contrasting. Therefore, results are shown by location. Pod distribution of the three genotypes was significantly different, especially for Maverick which had the greatest number of pods in the lower third of the plants (Tables 1 and 2). Pods in the medium third did no vary across genotypes, and in Prosper, Lariat and Stampede tended to have greater number of pods in the upper third compared to Maverick. When pods are located closer to the ground, there is a higher chance of being cut and lost during the direct harvesting. Nitrogen did not affect how pods were distributed on the plants in

most cases, or over other architectural traits evaluated. In the same way, increases in N did not show a direct effect in seed yield across varieties, although Lariat showed to be more responsive than the others. Row spacing had different effects on the architecture of the plants. In Carrington for example, pod numbers in the lower third were greater in the wider row spacing (due to less competition among plants). In Prosper, differences were found for pods on the medium and on the upper thirds of the plants, with greatest number of pods in the intermediate row spacing. Plant height was not affected by N levels or row spacing, but was significantly different across genotypes. Preliminary conclusions show that Lariat was the highest yielding when direct harvested and also had the lowest seed loss. However, yield potential of Lariat and Stampede were similar. There was no significant difference in yield and yield loss between N levels. Yield was increased with narrower to intermediate row spacing in Prosper (30 and 46 cm apart), whereas intermediate to wider row spacing appears to be the best in Carrington (46 and 76c m a part). This study will be repeated in 2009 to obtain more accurate information across more environments.

Table 1: Means of the main effects (Genotypes, N level, and row spacing) of agronomic and architectural traits evaluated in Prosper, ND, in the 2008 season.

Location: Prosper	**Genotypes**			**Nitrogen Levels**		**Row Spacing**		
Trait	Lariat	Stampede	Maverick	50	100	76	46	30
Yield (kg ha^{-1})	2,312	2,025 B	1,249 C	1,830	1,894	1,640	2,082	1,865
Yield Loss (kg ha^{-1})	172 C	243 B	246 A	225 A	215 A	240 A	208 A	213 A
Yield Potential (Yield + Yield	2,484	2,269 B	1,496 C	2,056	2,109	1,880	2,290	2,078 A
Hundred seeds weight (g)	40.23	35.91 B	34.52 C	36.53	37.24	36.36	36.87	37.44 A
Plant Height (cm)	40.16	44.27 A	33.20 C	39.07	39.36	39.58	38.37	39.69 A
Number of pods on the lowest third	3.37 B	4.44 B	5.95 A	4.58 A	4.60 A	4.23 A	4.56 A	4.97 A
Number of pods on the medium	6.88 A	7.65 A	6.97 A	7.29 A	7.04 A	6.22 B	8.01 A	7.27 AB
Number of pods on the upper third	11.93	12.52 A	9.75 B	11.37	11.42	9.23 B	13.12	11.84

Table 2: Means of the main effects (Genotypes, N level, and row spacing) of agronomic and architectural traits evaluated in Carrington, ND, in the 2008 season.

Location: Carrington	**Genotypes**			**Nitrogen Levels**		**Row Spacing**		
Trait	Lariat	Stampede	Maverick	50	100	76	46	30
Yield (kg ha^{-1})	1,080	888 B	678 C	855 A	909 A	970 A	928 A	747 B
Yield Loss (kg ha^{-1})	196 B	294 A	301 A	263 A	264 A	252 A	260 A	279 A
Yield Potential (Yield + Yield	1,276	1,182 A	979 B	1,119	1,173	1,223	1,188	1,027
Hundred seeds weight (g)	33.97	31.97 B	29.83 C	31.44	32.41	31.32	31.80	32.65
Plant Height (cm)	45.70	41.09 B	40.00 C	42.09	42.44	43.38	42.37	41.04
Number of pods on the lowest third	2.05 C	2.98 B	4.05 A	3.01 A	3.04 A	3.06	3.38 A	2.63 B
Number of pods on the medium	7.02 A	7.25 A	7.86 A	7.44 A	7.31 A	7.27 A	7.75 A	7.11 A
Number of pods on the upper third	7.88 A	6.70 A	7.13 A	6.91 A	7.57 A	7.94 A	7.62 A	6.16 A

Only letters in the same row within genotypes, nitrogen level or row spacing should be compared. If letter behind number is similar the numbers are not significantly different at $p<0.05$.

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COMPARATIVE RESPONSES OF COMMON BEAN TO DIFFERENT SOWING DATES OF GREEN MANURES

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INTRODUCTION

Little emphasis has been given to the effectiveness of the N supply by green manures (Muraoka, 1984), despite the use of green manure may represent a cheap alternative to supply N requirements for the crops. However, many of the plants used as green manure are sensible to the day length (photoperiod), which can result in different amount of dry mass production according to the cropping season. Consequently, the quantity of N provided by these plants for the crops may vary among cropping seasons.

MATERIALS AND METHODS

Aiming to evaluate the effect of different sowing dates of green manures on the common bean (*Phaseolus vulgaris* L.) crop, a field experiment was carried out at the Embrapa Arroz e Feijão. The green manures used were *Crotalaria juncea*, *Crotalaria ochroleuca*, *Cajanus cajan*, *Canavalia ensiformis* and *Mucuna aterrima*, sowed in three different dates (November 28th and December 12th, 2007 and February 13th, 2008). At the flowering, green manures were managed using a disc plough, followed by the common bean sowing. The experiment was performed in a randomized block design with three replicates. For each date of green manure sowing it were evaluated the stand, number of pods per plant, number of grains per pod, 100 grain weight and the productivity of common bean crop.

RESULTS AND DISCUSSION

On the first and third sowing dates it was not observed significant differences on the dry mass production of the green manures (Table 1). On the second sowing date, *Cajanus cajan* showed the greatest dry mass production (Table 1). The regression analysis revealed a significant effect of the sowing dates of the green manures on the stand, number of pods per plant and productivity of common bean (Figure 1), as an effect of a greater dry mass production of the green manures on the second sowing date, as related above. This effect was only observed on the second sowing date, in which were observed the greater stand and number of pods per plant after *Crotolaria ochroleuca* cropping and greater productivity after *Crotolaria juncea* and *C. ochroleuca* cropping (Table 1). Although *Cajanus cajan* had shown a greater dry mass production on the second sowing date, it was not observed a direct effect of the dry mass production on the common bean productivity (Table 1). This fact may be related with the content of N present in the plant tissues and, consequently on their capacity of N supplying.

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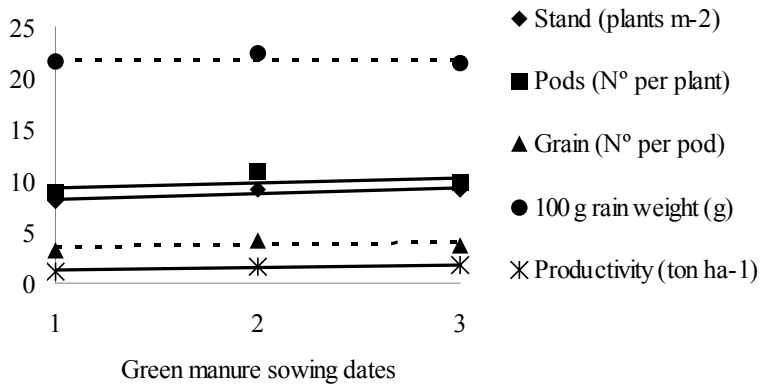

Figure 1. Regression analysis of agronomic parameters of production of common bean in response to different sowing dates of green manures. Dotted lines means non significant regression.

Table 1. Dry mass production of green manures and production components of common bean in response to sowing date of green manures.

Green manure			Common bean				
Sowing date	Specie	Dry mass (ton ha^{-1})	Stand (plants m^{-2})	Pods (Nº plant^{-1})	Grain (Nº pod^{-1})	100 grain weight (g)	Productivity (ton ha^{-1})
1st	*C. juncea*	6.41 a	8.33 a	8.93 a	3.29 a	21.94 a	1.32 a
	C. ochroleuca	6.71 a	8.33 a	8.67 a	2.97 a	22.07 a	1.29 a
	C. cajan	7.38 a	8.33 a	9.17 a	3.42 a	21.04 a	1.35 a
	C. ensiformis	5.78 a	7.00 a	7.73 a	2.93 a	22.44 a	1.00 a
	M. aterrima	6.13 a	7.67 a	9.23 a	3.53 a	20.30 a	0.73 a
2nd	*C. juncea*	6.62 b	8.67 b	11.70 ab	4.73 a	23.56 a	1.88 a
	C. ochroleuca	6.27 b	9.67 a	14.87 a	5.00 a	20.60 a	1.91 a
	C. cajan	9.73 a	8.67 b	8.80 b	3.43 a	23.40 a	1.06 b
	C. ensiformis	4.22 b	9.33 ab	9.67 ab	3.74 a	21.93 a	1.55 ab
	M. aterrima	5.70 b	9.00 b	9.13 b	3.89 a	22.61 a	1.69 ab
3rd	*C. juncea*	5.72 a	9.00 a	10.07 a	3.20 a	21.34 a	1.24 a
	C. ochroleuca	4.62 a	9,67 a	9.63 a	3.97 a	21.12 a	2.09 a
	C. cajan	3.82 a	8.33 a	10.27 a	3.76 a	20.47 a	1.78 a
	C. ensiformis	3.91 a	9.00 a	8.40 a	3.31 a	22.46 a	1.47 a
	M. aterrima	5.38 a	9.33 a	10.23 a	4.52 a	22.04 a	1.94 a
CV (%)		18.94	11.05	22.65	21.38	7.23	30.69

Means followed by the same letter in the column, within green manure sowing time, are not different by Tukey's test ($p<0.05$).

NATURAL SELECTION FOR NITROGEN USE EFFICIENCY IN COMMON BEAN POPULATIONS

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INTRODUCTION

The nitrogen (N) biological fixation in the common bean does not supply all the demand of the culture, by this, most studies that evaluate N levels presents positive response (Vieira, 2006). Whereas in Brazil there is huge diversity of farmers, ranging those of family agriculture that employ no fertilizers, to the big farmers that use high levels, it is necessary to obtain lines that have good yield under conditions of low N availability and/or also responsive to the nutrient application. However, a question that arises is whether natural selection in environments with and without stress of N would increase the chance of select specific lines for such environments. Of the above, the objective of this study was to verify if natural selection in populations obtained from crosses between lines differing in the nitrogen use efficiency is acting to get specific populations for environments with and without stress of N.

MATERIALS AND METHODS

In previous stage of the breeding program of the common bean in the Federal University of Lavras - UFLA (South of Minas Gerais, Brazil, 21º14' S, 44º59' W and average altitude of 919 m) were identified two tolerant lines to low nitrogen availability (Ouro Negro and VC-5) and also two responsive lines to the nitrogen application (CI-107 and IAPAR-81). It was obtained the F_1 generation of crosses involving the lines Ouro Negro x CI-107, VC-5 x IAPAR-81 and VC-5 x CI-107. From the F_2 those populations were advanced by the "bulk" in two environments. The first one received 100 kg ha^{-1} of N, being 1/3 applied at sowing and the remainder in cover, and the ammonium sulfate as source of N. In the second, no nitrogen fertilizer was used. In both environments the crop fertilization was the same, ie, 80 kg ha^{-1} of P_2O_5 and K_2O.

Each bulk was constituted by 2,000 plants. After harvested, the seeds from each environment were mixed and used for seeding the next generation. This procedure was repeated until the F_5 generation. The six populations, three from the environment with N and three without N, were evaluated in randomized blocks design in F_6, F_7 and F_8 generations. The plots were constituted by four lines of four meters in length. Data for grain yield were submitted to variance analysis.

RESULTS AND DISCUSSION

It was detected significant difference ($P \leq 0,01$) between the generations (seasons). The F_8 generation was the most yield and there was no difference between the other two (Table 1). The generations x levels interaction significant ($P \leq 0,05$), indicates that the effect of N stress was not coincident in different generations. It was observed that the nutrient response occurred in two of the three

generations evaluated. It was found that the generations x populations x origins x levels interaction was significant, indicating that the behavior of populations was not coincident in different origins, levels and generations. Because of this fact, and considering that the biggest interest is in the interactions involving levels and origins, the results will be discussed considering the two generations of greater response to N, ie, F_6 and F_7.

In the average of the two generations, grain yield with N was 22% higher than with no N (Table 2). The population with higher average yield, regardless of origin and level of N, was the IAPAR-81 x VC-5, and there was no difference between the other two. Regarding the origin, if the population was advanced with or without N, was detected significant difference ($P \leq 0,08$). The same occurred with populations x origins, populations x levels and populations x levels x origins interactions.

The most significant result was that populations advanced in the environment without N presented higher yield in this environment, except the population CI-107 x Ouro Negro. The same occurred with the populations advanced in the environment with N. This fact shows, in principle, that during the progress of populations, the natural selection has acted to select more adapted individuals to that particular environment.

Table 1. Grain yield (kg ha^{-1}) of common bean populations, obtained with and without nitrogen application, in the different generations of evaluation.

Generations	Levels		with N/without N	mean
	with N	without N		
F_6	2363	1938	1,22	2151
F_7	2365	1938	1,22	2152
F_8	2463	2495	0,99	2479

Table 2. Grain yield (kg ha^{-1}) of common bean populations, in the different origins, evaluated with and without nitrogen application.

Segregating Population	Environment of evaluation						with N/ without N	mean
	with N			without N				
	Origin of the bulk			Origin of the bulk				
	with N	without N	mean	with N	without N	mean		
Ouro Negro x CI-107	2416	2208	2312	2141	1488	1815	1,27	2064
VC-5 x IAPAR-81	2483	2286	2385	2139	2251	2195	1,08	2290
VC-5 x CI-107	2461	2326	2394	1610	1997	1804	1,33	2099
Mean	2453	2273	2363	1963	1912	1938	1,22	2151

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ACCUMULATION OF MACRONUTRIENTS BY DIFFERENT COMMON BEAN CULTIVARS GROWN IN DIFFERENT PLANT DENSITIES IN NO-TILLAGE CROP SYSTEM

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INTRODUCTION

The aim of the current study was to follow the accumulation of macronutrients throughout the crop cycle in different cultivars of common bean with carioca seed type, grown under different plant densities in field experiments using a no-tillage cropping system.

MATERIALS AND METHODS

The experimental design was randomized blocks with three replications and a 4x5 factorial scheme, involving four bean cultivars (Table 1) and five plant densities (75, 145, 215, 285 and 355 thousand plants.ha^{-1}). The sowing was carried out under *Brachiaria* grass dried with Roundup® (2,5 L.ha^{-1}, 30 days before the sowing) and Gramoxone® (2,0 L ha^{-1}, 8 days before the sowing). The experiment had not been irrigated. Each plot had four rows with 5.0 m length and spacing of 0.5 m between rows. At sowing (November, 2006), all the plots had received identical fertilization, determined by the soil analysis interpretation. The N fertilization at covering (at 30 days after emergency-DAE) was 30 kg.ha^{-1} of N, urea source. Every 10 days, samples of 10 plants were collected and dried under air circulation to 65 -70^{o}C, until constant mass, soon after they had been triturated and sent to the Laboratory of Leaf Analysis of the Soil Science Department (UFLA) for determination of the macronutrients content. The N content was evaluated by Kjedahls method while P, K, Ca, Mg, S were extracted by digestion by nitric and perchloric acid and quantified in the extract (P colorimetrically, K- flame photometry; S -turbidimetry; Ca, Mg spectrophotometry of atomic absorption).

Table 1. Principal characteristics of the studied cultivars.

Characteristics	BRS Radiante*	Bolinha**	Ouro Vermelho*	Jalo EEP 558*
Commercial group	Others	Others	Others	Jalo
Seed color	cream / beige	yellow	red	yellow
Growth habit	I	II	II/III	III
100 grain's weight	44-45 g	32-33g	25 g	30-40 g
Stem	erect	erect	semi-erect	semiclimber
Cultural cycle	early	middle	normal	middle

* Ramalho & Abreu (2006), ** Alves (2008)

RESULTS AND DISCUSSION

At flowering, large proportion of the each macronutrient is needed by the bean cultivars. The maximum accumulation of N, P, K, Mg and S are registered at the end of the crop cycle, while the maximum Ca accumulation occurring around 50-60 DAE; in the cases of the cv. BRS Radiante-Mg and the cv. Ouro Vermelho-N there was significant interation between DAE and plant population (Table 2). The cv. Ouro Vermelho accumulates more S (Table 3). The general decreasing order of accumulation is N>K>Ca>P>Mg>S.

Table 2. Accumulation of macronutrients (kg ha^{-1}) by four bean cultivars, in function of DAE.

Cultivar	Nutrient	Regression	R^2 (%)
Bolinha	N	$Y= -17,242786 + 1,729470 x - 0,009548 x^2$	95,93
	P	$Y= -3,137164 + 0,253465 x - 0,001402 x^2$	91,68
	K	$Y= -10,842384 + 1,113746 x - 0,006118 x^2$	98,78
	Ca	$Y= -12,906267 + 1,110607 x - 0,009873 x^2$	85,48
	Mg	$Y= -2,757450 + 0,245389 x - 0,001625 x^2$	92,37
	S	$Y= -1,275420 + 0,119268 x - 0,000896 x^2$	87,53
Jalo EEP 558	N	$Y= -23,881558 + 2,546946 x - 0,019602 x^2$	89,85
	P	$Y= -1,389023 + 0,162554 x + 0,000019 x^2$	92,20
	K	$Y= -24,662699 + 2,215676 x - 0,020268 x^2$	88,23
	Ca	$Y= -10,836283 + 1,160066 x - 0,011047 x^2$	94,84
	Mg	$Y= -2,993595 + 0,302583 x - 0,002275 x^2$	89,65
	S	$Y= -0,833826+ 0,122483 x - 0,001039 x^2$	86,84
BRS Radiante	N	$Y= -30,268660 + 2,977720 x - 0,025923 x^2$	87,14
	P	$Y= -4,249551 + 0,383262 x - 0,003434 x^2$	83,76
	K	$Y= -26,721263 + 2,400184 x - 0,023948 x^2$	93,60
	Ca	$Y= -9,550083 + 1,052842 x - 0,010530 x^2$	90,30
	Mg at 75*	$Y= -2,167784 + 0,196681 x - 0,001601 x^2$	88,97
	Mg at 145	$Y= -3,428203 + 0,328345 x - 0,003281 x^2$	81,97
	Mg at 215	$Y= -3,583281 + 0,365128 x - 0,003315 x^2$	89,67
	Mg at 285	$Y= -4,006907 + 0,410473 x - 0,003928 x^2$	81,48
	Mg at 355	$Y= 0,030401 + 0,058133 x + 0,000962 x^2$	93,48
	S	$Y= -1,148438 + 0,139843 x - 0,001374 x^2$	86,03
Ouro Vermelho	N at 75*	$Y= -22,858209 + 1,663721 x - 0,005415 x^2$	95,97
	N at 145	$Y= -33,347804 + 2,606274 x + 0,019163 x^2$	81,09
	N at 215	$Y= -40,301943 + 3,429931 x - 0,029679 x^2$	88,35
	N at 285	$Y= -54,887495 + 4,837341 x - 0,040262 x^2$	88,32
	N at 355	$Y= -52,377400 + 4,297711x - 0,035221 x^2$	88,88
	P	$Y= -5,821291+ 0,441218 x – 0,003379 x^2$	86,17
	K	$Y= -25,838459 + 2,050326 x - 0,015526 x^2$	92,12
	Ca	$Y= -20,052668 + 1,588502 x - 0,013082 x^2$	77,56
	Mg	$Y= -5,942437 + 0,464979 x - 0,003554 x^2$	87,32
	S	$Y= -2,346744 + 0,223357 x - 0,001780 x^2$	91,32

*At considered significant plant populations.

Table 3. Macronutrients accumulation (kg ha^{-1}) in the aerial part of the plant of four bean cultivars.

	N	P	K	Ca	Mg	S
Bolinha	54,06 a	7,25 a	36,55 a	12,96 a	6,17 a	2,49 b
Jalo EEP 558	58,80 a	6,11 a	31,13 a	15,21 a	7,10 a	2,73 b
BRS Radiante	47,19 a	7,09 a	26,89 a	13,37 a	5,05 a	1,96 b
Ouro Vermelho	49,80 a	7,25 a	32,73 a	18,81 a	6,98 a	3,43 a

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ACCUMULATION OF MICRONUTRIENTS BY DIFFERENT COMMON BEAN CULTIVARS GROWN IN DIFFERENT PLANT DENSITIES IN NO-TILLAGE CROP SYSTEM

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INTRODUCTION

The aim of the current study was to follow the accumulation of micronutrients throughout the crop cycle in different cultivars of common bean with carioca seed type, grown under different plant densities in field experiments using a no-tillage cropping system.

MATERIALS AND METHODS

The experimental design was randomized blocks with three replications and a 4x5 factorial scheme, involving four bean cultivars (Table 1) and five plant densities (75, 145, 215, 285 and 355 thousand plants.ha^{-1}). The sowing was carried out under *Brachiaria* grass dried with Roundup® (2,5 L.ha^{-1}, 30 days before the sowing) and Gramoxone® (2,0 L ha^{-1}, 8 days before the sowing). The experiment had not been irrigated. Each plot had four rows with 5.0 m length and spacing of 0.5 m between rows. At sowing (November, 2006), all the plots had received identical fertilization, determined by the soil analysis interpretation. The N fertilization at covering (at 30 days after emergency-DAE) was 30 kg.ha^{-1} of N, urea source. Every 10 days, samples of 10 plants were collected and dried under air circulation to 65 -70^{o}C, until constant mass, soon after they had been triturated and sent to the Laboratory of Leaf Analysis of the Soil Science Department (UFLA) for determination of the micronutrients content. The Cu, Fe, Mn e Zn content was evaluated by digestion by nitric and percloric acid and quantified in the extract (espectrophotometry of atomic absorption) and B by incineration and colorimetric determination by the curcumin method.

Table 1. Principal characteristics of the studied cultivars.

Characteristics	BRS Radiante*	Bolinha**	Ouro Vermelho*	Jalo EEP 558*
Commercial group	Others	Others	Others	Jalo
Seed color	cream / beige	yellow	red	yellow
Growth habit	I	II	II/III	III
100 grain's weight	44-45 g	32-33g	25 g	30-40 g
Stem	erect	erect	semi-erect	semiclimber
Cultural cycle	early	middle	normal	middle

* Ramalho & Abreu (2006), ** Alves (2008)

RESULTS AND DISCUSSION

At flowering, large proportion of the each micronutrient is reached by the bean cultivars. B is gradually accumulated along the whole cycle and Fe is accumulated in the quicker form in relation to the others micronutrients; in the case cv. Bolinha-B there was significant interation between DAE and plant population (Table 2). The bean cultivars do not differ in relation to the B, Mn and Zn accumulations, but the cv. Jalo accumulates more Cu, while the cvs. Bolinha and Radiant accumulates more Fe (Table 3). The general decreasing order of accumulation is Fe>B>Mn>Zn>Cu.

Table 2. Accumulation of micronutrients (g ha^{-1}) by four bean cultivars in function of DAE.

Cultivar	Nutrient	Regression	R^2 (%)
Bolinha	B at 75	$Y= -4{,}155611 + 0{,}441273\ x + 0{,}003228\ x^2$	94,88
	B at 145	$Y= -21{,}718631 + 1{,}662916\ x - 0{,}002350\ x^2$	92,35
	B at 215	$Y= -19{,}741923 + 1{,}867050\ x - 0{,}008535\ x^2$	97,48
	B at 285	$Y= -4{,}975721 + 0{,}834361\ x + 0{,}007499\ x^2$	98,28
	B at 355	$Y= -28{,}216113 + 2{,}712476\ x - 0{,}008321\ x^2$	90,61
	Cu	$Y= -9{,}436216 + 0{,}930805\ x - 0{,}006817\ x^2$	88,11
	Fe	$Y= -1003{,}677262 + 113{,}937586\ x - 1{,}172568\ x^2$	92,43
	Mn	$Y= -25{,}977993 + 3{,}741935\ x - 0{,}029216\ x^2$	95,29
	Zn	$Y= -27{,}074703 + 2{,}264467\ x - 0{,}013827\ x^2$	93,21
Jalo EEP 558	B	$Y= -18{,}756220 + 2{,}039851\ x - 0{,}008537\ x^2$	95,02
	Cu	$Y= -5{,}506187 + 0{,}590396\ x - 0{,}001624\ x^2$	88,87
	Fe	$Y= -769{,}060555 + 93{,}986887\ x - 1{,}822413\ x^2$	83,17
	Mn	$Y= -24{,}884055 + 4{,}219734\ x - 0{,}040569\ x^2$	91,51
	Zn	$Y= -29{,}846043 + 2{,}793384\ x - 0{,}021157\ x^2$	89,45
BRS Radiante	B	$Y= -17{,}625115 + 1{,}733246\ x - 0{,}000899\ x^2$	99,09
	Cu	$Y= -19{,}423468 + 1{,}666065\ x - 0{,}014755\ x^2$	87,29
	Fe	$Y= 1472{,}733119 + 178{,}060250\ x - 2{,}143283\ x^2$	85,63
	Mn	$Y= -32{,}832960 + 4{,}650177\ x - 0{,}047058\ x^2$	97,78
	Zn	$Y= -28{,}793130 + 2{,}784689\ x - 0{,}021323\ x^2$	82,14
Ouro Vermelho	B	$Y= -39{,}746584 + 3{,}172639\ x - 0{,}018709\ x^2$	86,27
	Cu	$Y= -18{,}000999 + 1{,}429942\ x - 0{,}010922\ x^2$	88,16
	Fe	$Y= -1166{,}673254 + 117{,}814557\ x - 1{,}140581\ x^2$	83,52
	Mn	$Y= -82{,}790714 + 7{,}388865\ x - 0{,}062482\ x^2$	87,85
	Zn	$Y= -62{,}642615 + 4{,}492009\ x - 0{,}031991\ x^2$	88,07

*At considered significant plant populations.

Table 3. Micronutrients accumulation (g ha^{-1}) in the aerial part of plant of four bean cultivars.

	B	Cu	Fe	Mn	Zn
Bolinha	80a	21b	959a	89a	60a
Jalo EEP 558	91a	32a	756b	72a	55a
BRS Radiante	85a	22b	1139a	70a	53a
OuroVermelho	77a	21b	553b	80a	53a

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VARIABILITY AMONG COMMON BEAN GENOTYPES ON MOLYBDENUM CONCENTRATION AND CONTENT IN SEED

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INTRODUCTION

At Zona da Mata region of the Minas Gerais State, Brazil, application of molybdenum (Mo) on foliage has been used instead of nitrogen (N) as topdressing to achieve common bean yield as high as 3000 kg ha^{-1} (Amane et al., 1999; Vieira et al., 2005). The recommended rate of Mo for foliar application in this region is between 70 and 100 g ha^{-1} (Amane et al., 1999). However, for significantly improvement of Mo content in seed, rates of Mo applied on foliage should be over 500 g ha^{-1} (Vieira et al., 2005). The objective with this research was to evaluate accumulation Mo in seed of common bean genotypes in response to Mo applied as solution on foliage.

MATERIALS AND METHODS

Two trials were conducted, one during summer-fall season (soil pH in H_20 = 4.8) and other during winter-spring season (pH = 5.2), in Coimbra, at Zona da Mata region, in a Mo-deficient Alfissol. Twelve genotypes (Table 1) were sprayed with both 300 g ha^{-1} of Mo at pre-flowering stage and 300 g ha^{-1} of Mo at pod development stage. Fifteen seeds per meter were sown in rows 0.5 m apart. Plants received a basal fertilization of 28 kg N ha^{-1}, 43 kg P ha^{-1}, and 46 kg K ha^{-1}. Urea was applied as topdressing 10 or 16 DAE at 100 kg ha^{-1}. Applications of Mo were done with a hand-held CO_2 sprayer delivering 450 L ha^{-1}. An overhead sprinkler irrigation was used. Treatments were replicated six times in a randomized complete block design. Each plot had one 2 m-long row.

RESULTS AND DISCUSSION

Yields were high in both years (Tables 1 and 2). There was no difference among genotypes in Mo concentration in seed, but Mo content in seed varied from 1.47 (Table 2) to 4.28 µg Mo seed^{-1} (Table 1) owing to differences in seed weight. Mo content in seed of cv. Pérola obtained by Vieira et al. (2005), also in Coimbra, raised from plants sprayed with 720 g ha^{-1} of Mo was lower (0.74 µg Mo seed^{-1}) than Mo contents in seed verified in this study with 600 g ha^{-1}.

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Table 1 – Mean results of summer-fall season trial.

Genotype[1]	Yield (kg ha^{-1})	100-seed weight (g)	Mo in seed (µg g^{-1})	Mo content[2] (µg seed^{-1})
Ouro Negro	4279 a[3]	28.3 d	7.38 a	2.09 c
VC-3	4073 a	25.8 e	8.05 a	2.07 c
Pérola	3698 b	26.7 d	7.83 a	2.09 c
Ouro Vermelho	3636 b	28.7 c	8.27 a	2.37 b
Majestoso	3415 b	27.7 d	7.92 a	2.19 b
Talismã	3371 b	27.9 d	8.52 a	2.38 b
VC-8	3305 b	29.9 c	8.02 a	2.40 b
Pioneiro	3228 b	21.8 g	8.30 a	1.81 c
Carnaval MG	2903 c	54.4 a	7.88 a	4.28 a
Jalo MG-65	2858 c	48.0 b	8.40 a	4.03 a
Valente	2751 c	23.6 e	7.43 a	1.73 c
Horizonte	2254 c	24.7 e	7.83 a	1.94 c
CV (%)	17.1	4.9	10.9	11.4

[1]VC-3, Pérola, Majestoso, Talismã, VC-8, Pioneiro, and Horizonte are carioca beans; Ouro Negro and Valente are black beans; Carnaval MG is a cranberry bean; Ouro Vermelho is a red bean; and Jalo MG 65 is a yellow bean. Genotypes were sprayed with 600 g ha^{-1} of Mo.

[2] Dry seed weight of each plot was used in the calculation of Mo content in seed.

[3]Treatments followed by different letters come from different groups ($P \leq 0.05$) according to the Scott-Knott cluster analysis method.

Table 2 – Mean results of winter-spring season trial.

Genotype	Yield (kg ha^{-1})	100-seed weight (g)	Mo in seed (µg g^{-1})	Mo content (µg seed^{-1})
Ouro Negro	3689 a	28.0 c	7.37 a	1.89 b
VC-3	3625 a	24.2 d	8.37 a	1.89 b
VC-8	3536 a	28.1 c	7.84 a	2.00 b
Ouro Vermelho	3451 a	23.7 d	8.04 a	1.72 c
Pioneiro	3167 a	20.2 e	7.91 a	1.47 c
Pérola	3039 a	26.1 c	7.59 a	1.82 b
Valente	2700 b	22.3 e	7.17 a	1.47 c
Talismã	2628 b	24.7 d	8.20 a	1.85 b
Horizonte	2528 b	24.2 d	7.78 a	1.71 c
Majestoso	2428 b	25.0 d	7.88 a	1.79 b
Carnaval MG	2038 c	41.3 a	7.72 a	2.97 a
Jalo MG-65	1922 c	30.5 b	7.82 a	2.16 b
CV (%)	16.3	9.6	7.9	12.6

See Table 1.

SEED QUALITY AND MOLYBDENUM IN SEED IN RESPONSE TO THE MICRONUTRIENT SPRAYED ON FOLIAGE AT DIFFERENT GROWTH STAGES OF COMMON BEAN

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INTRODUCTION

At Zona da Mata region of the Minas Gerais State, Brazil, application of molybdenum (Mo) on foliage (20 to 100 g ha^{-1}) has been used instead of nitrogen (N) as topdressing to achieve common bean yield as high as 3000 kg ha^{-1} (Amane et al., 1999; Vieira et al., 2005). Despite advantages of using Mo on common bean, many Brazilian farmers have no access to this technology because they are unaware of it and/or there is no Mo fertilizer available locally. A feasible strategy would be providing seeds of high Mo content to the farmers. Vieira et al. (2005) showed that it is possible to produce enriched seeds by foliar application of high Mo rates without seed yield reduction, and that plants raised from these seeds have higher yield when used in soil with deficiency of Mo and/or N. They applied Mo on foliage during the vegetative stage in common bean. However, later application of most part of Mo fertilizer (during the reproductive stage) could improve Mo content in seed. The objective with this research was to evaluate concentrations and contents of Mo in seed and seed quality in response to Mo application in different stages of common bean.

MATERIALS AND METHODS

Trial was conducted during winter-spring (soil pH in H_20 = 5.2), in Coimbra, at Zona da Mata region, in a Mo-deficient Alfissol. Twelve treatments of Mo were tested (Table 1). Fifteen seeds per meter were sown in rows 0.6 m apart. Plants received a basal fertilization of 28 kg N ha^{-1}, 43 kg P ha^{-1}, and 46 kg K ha^{-1}. Urea was applied as topdressing 16 DAE at 100 kg ha^{-1}. Applications of Mo were done with a hand-held CO_2 sprayer delivering 450 L ha^{-1}. An overhead sprinkler irrigation was used. Treatments were replicated six times in a randomized complete block design. Each plot had four 2 m-long rows. The two central rows were harvested. Seed vigor classes were determined by accelerated aging and conductivity test.

RESULTS AND DISCUSSION

Yield varied from 1427 (untreated control) to 2224 kg ha^{-1} (90 g Mo ha^{-1} at V4 + 510 g Mo ha^{-1} at late R8). Except for treatment 4 (90 g Mo ha^{-1} at V4 + 510 g Mo ha^{-1} at R6), all treatments that differed significantly from untreated control had part of Mo applied at R8 growth stage (Table 1). Seeds of five treatments were heavier than those of untreated control. There was no significant difference between treatments 1 and 2 in both Mo concentrations and Mo contents in seed. In general, there were higher concentrations and contents of Mo in seeds when most of the Mo was applied at R7 or R8 growth stages (Table 1). One explanation for that may be more soil coverage by common bean foliage at those stages, which permitted more interception of the Mo applied on the plants. In general, Mo application improved seed vigor.

Table 1 – Mean results of the trial, winter-spring season, Coimbra, Minas Gerais State, Brazil.

Treatment[1]	Yield (kg ha^{-1})	100-seed weight (g)	Mo in seed (μg g^{-1})	Mo content[2] (μg seed^{-1})	Conductivity (μS cm^{-1} g^{-1})	Seed germination[3] (%)
1	1427 b[4]	26.0 b	0.59 d	0.14 d	99.4 a	39.0 b
2	1758 ab	27.5 ab	1.52 d	0.38 d	79.4 b	73.7 a
3	1889 ab	28.2 a	7.05 bc	1.81 bc	87.2 ab	76.8 a
4	2162 a	27.5 ab	6.75 c	1.70 c	82.2 b	70.5 a
5	1982 ab	28.1 a	7.77 abc	2.00 abc	83.8 b	80.0 a
6	1931 ab	28.6 a	8.55 abc	2.23 ab	82.9 b	64.0 ab
7	2016 a	27.8 ab	7.88 abc	2.00 abc	79.8 b	69.2 a
8	2224 a	26.9 ab	8.30 abc	2.03 abc	83.6 b	65.2 ab
9	1903 ab	28.4 a	8.88 ab	2.31 ab	81.0 b	82.0 a
10	2134 a	28.0 a	9.58 a	2.45 a	84.7 b	77.2 a
11	1951 ab	27.1 ab	7.60 bc	1.87 bc	87.8 ab	76.3 a
12	2144 a	28.0 ab	8.40 abc	2.15 abc	85.0 b	64.8 ab
CV	15.0	3.8	13.7	14.7	7.8	20.8

[1]Mo applied, in g ha^{-1} (growth stage in which Mo was applied): **1** = untreated control, **2** = 90 (R5), **3** = 600 (R5), **4** = 90 (V4) + 510 (R6), **5** = 90 (V4) + 510 (R7), **6** = 90 (V4) + 510 (early R8), **7** = 90 (V4) + 510 (in the middle of R8), **8** = 90 (V4) + 510 (late R8), **9** = 90 (V4) + 255 (R5) + 255 (R7), **10** = 90 (V4) + 255 (R7) + 255 (early R8), **11** = 90 (V4) + 255 (R5) + 255 (in the middle of R8), **12** = 90 (V4) + 255 (early R8) + 255 (late R8). V4 = third trifoliate leaf, R5 = pre-flowering, R6 = flowering, R7 = pod development, R8 = pod filling.
[2] Dry seed weight of each plot was used in the calculation of Mo content in seed.
[3]After stress at 41 ± 0.5 °C and 100% relative humidity for 48 hours.
[4]Means, in a column, followed by different letters differ ($P < 0.05$) by Tukey test.

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EVALUATION OF ELITE DRY BEAN GERMPLASM FOR RESISTANCE TO POTATO LEAFHOPPER, *EMPOASCA FABAE*, IN MICHIGAN

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INTRODUCTION: *Empoasca fabae,* also known as potato leafhopper (PLH), is currently the most abundant insect pest of dry beans in Michigan. In the 1980's PLH replaced tarnished plant bugs and aphids as the major insect pest of dry beans and they continue to be an annual threat that reduce bean seed yield and quality. By evaluating PLH incidence in a range of both commercial varieties and breeding lines, we can assess PLH preferences and determine possible sources of resistance to the pest. By monitoring the differences in the number of PLH nymph numbers present, inferences can be made as to oviposition (egg-laying) and feeding preferences of the insects (antixenosis interactions) (Schaafsma et al., 1998). In addition, the number of nymphs present can indicate antibiosis interactions where the bean genotype causes direct deleterious effects to the pest. Another method for assessing pest resistance is to evaluate plant damage caused by the pest. PLH causes a specific set of symptoms known collectively as "hopperburn" that are evaluated on a 1-5 scale for leaf curl and leaf burn (Murray et al., 2001).The objective of this study was to evaluate potato leafhopper feeding preference and/or avoidance in a diverse range of dry bean varieties and breeding lines under field conditions in Michigan.

MATERIALS AND METHODS: 32 genotypes were selected to include released commercial varieties and elite lines in eight commercial classes as well as two tropical unadapted lines (EMP507; EMP509), previously selected for resistance to *E. kraemeri*. These genotypes encompass the range of growth habits and seed types currently available (Table 1).Two nurseries were established at the Montcalm Research Farm near Entrican, MI and the MSU Plant Pathology Research Farm in East Lansing, MI. Tests were planted as two-row plots, 80 seeds per 20-foot row, spaced 4 inches apart. Neither trial was treated with insecticide. Both trial locations were planted near alfalfa fields which are a consistent source of PLH. Insect population incidence was recorded by counting the number of PLH nymphs per 3 randomly selected trifoliates per plant. 5 plants were sampled per plot. Hopperburn damage scores were recorded. Data was collected at 42 and 49 days after planting. The trials were taken to harvest. Seed yield and size were recorded. Data was analyzed using the PROC MIXED procedure of SAS (SAS Institute, 2001).

RESULTS: PLH nymph counts differed among genotypes. However, genotypic effects were only found to be significant for a few entries. Using a significance level of $\alpha = 0.05$, the following lines were found to have significant effects on leafhopper incidence: K03240, K74002, K90101, K90902, C03157, C99833, and I81010. Obvious trends became clear between the different genotypes. Overall, large-seeded Andean genotypes had consistently higher PLH counts than small-seeded genotypes in each trial location. Specifically, the kidney and cranberry bean entries had the highest numbers of leafhopper nymphs present across field trial locations indicating susceptibility to the pest. I81010 (Bunsi), an avy bean, was one exception which also indicates PLH susceptibility. While not statistically significantly, EMP507 and EMP509 both had higher than expected PLH counts, which raises the question whether resistance to *E. kraemeri* is transferable to *E. fabae.* B90222 (Raven), N05311, R02002, I03388 (Hime tebo) and P86299 (Sierra pinto) had the lowest PLH counts and may indicate resistance. Overall, the average PLH count for large-seeded entries was 78% higher than for small-seeded entries. Damage ratings were not included in this analysis as insect pressure was too low to detect significant differential damage between the entries.

Table 1. Dry Bean Germplasm Screening for Incidence to Potato Leafhopper (PLH; *Empoasca fabae*) in Michigan

Genotype	Variety Name	Seed Type	Seed Size	Mean 100 Seed Weight (g)	Mean Plot Yield (g)	Mean PLH Count (individuals)	p-value (α<0.05)
B00101	Condor	Black	Small	18.6	898	2.2	0.1460
B04316		Black	Small	19.1	1105	1.0	0.4204
B04554	Zorro	Black	Small	19.8	1721	1.4	0.2233
B05055		Black	Small	18.5	1601	1.7	0.4007
B05040		Black	Small	20.0	1784	1.1	0.2795
B90222	Raven	Black	Small	17.2	1554	0.4	0.8327
B95556	Jaguar	Black	Small	17.3	1325	1.0	0.4615
I01892	115M	Black	Small	20.4	1688	1.9	0.2365
I81066	T-39	Black	Small	18.7	1475	2.8	0.0744
I03388	Hime	Tebo	Small	27.0	1309	0.7	0.5741
N05311		Navy	Small	17.6	1672	0.5	0.6988
N97774	Seahawk	Navy	Small	21.1	1431	2.2	0.1052
I81010	Bunsi	Navy	Small	18.7	1397	2.8	0.0381
I92002	Vista	Navy	Small	17.6	1554	2.1	0.0983
G93414	Matterhorn	Gr. Northern	Medium	31.5	1397	1.3	0.3364
I07152	EMP 507	Carioca	Medium	26.6	845	2.9	0.0645
I07153	EMP 509	Carioca	Medium	24.5	1189	3.3	0.0645
I99117	Buster	Pinto	Medium	35.1	1694	1.0	0.4722
P04205	Sante Fe	Pinto	Medium	38.2	1609	1.6	0.1815
P04207		Pinto	Medium	40.4	1602	2.4	0.0744
P86299	Sierra	Pinto	Medium	32.6	1671	0.8	0.6226
P89430	Aztec	Pinto	Medium	35.7	1562	1.5	0.3111
R02002		Small Red	Medium	33.1	1623	0.6	0.6603
R98026	Merlot	Small Red	Medium	34.7	1759	1.5	0.2720
S00809	Sedona	Small Red	Medium	34.4	1501	1.4	0.2107
C03157		Cranberry	Large	49.2	1386	3.8	0.0353
C99833	Capri	Cranberry	Large	52.5	1537	3.2	0.0178
K03240		Kidney	Large	45.3	1310	5.8	<0.0001
K74002	Montcalm	Kidney	Large	49.9	1208	3.9	0.0029
K90101	Red Hawk	Kidney	Large	47.9	1329	3.3	0.0498
K90902	Beluga	Kidney	Large	47.8	1260	3.3	0.0237
K94601	Chinook2000	Kidney	Large	48.3	1162	2.8	0.0557

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MANAGEMENT OF SEED CORN MAGGOT FOR ORGANIC SNAP BEAN PRODUCTION

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INTRODUCTION

Organic snap bean production for processing currently meets only one-third of current demand. In spite of price incentives, it is difficult for processors to contract sufficient acres to meet demand due to the higher risk associated with plant disease and insect pests in large-scale organic production.

Currently, most organic vegetable producers tend to be small entrepreneurs who spread the risk of disease, pests, weeds, and weather patterns among many different crops and cultivars. The crops are intensively managed on small plots. In contrast, large-scale production of processing vegetables cannot spread risk among crops; it is a contractual agreement with a grower, often for a specified cultivar to be harvested and delivered on a specific date. Irrigation, fertilizer, and pesticides have, in conventional agriculture, been applied when necessary to reduce risk to the grower on a large acreage monoculture. To achieve large-scale production that is compatible with organic standards, technology must be developed to reduce the risk and costs associated with organic production.

An important insect pest of snap bean is seed corn maggot (*Delia platura*). Entrust organic seed treatments have previously been demonstrated as effective means of controlling seed corn maggot in snap beans and is currently under review with the Inter-Regional Research Project 4 (IR-4) (http://ir4.rutgers.edu/FoodUse/PerformanceDMP1.cfm?Prnum=X0251) and approval could be anticipated in 2010-2011.

RESEARCH OBJECTIVE

The objective of this research was to determine the efficacy of varying spinosad (Entrust: an OMRI approved formulation) seed treatment rates for management of seed corn maggot in snap beans.

MATERIALS AND METHODS

A trial consisting of the snap bean cultivar 'Hystyle' with six seed treatment rates was planted on June 4, 2008 to coincide seedling emergence and development with 2nd generation adult seed corn maggot (*Delia platura*) emergence at 600 DD_{50}. The trial was planted at Hancock Agricultural Research Station (ARS), Hancock, WI using a randomized complete block, factorial design including 4 replications of each seed treatment. Plots consisted of 2-rows, each 20 ft in length. Rows were spaced 36" apart and seeded with a single row cone planter at a rate of 8 seeds/ft for a total of 320 seeds/2-row plot. Seed treatments (performed by Dr. Alan Taylor, Cornell University) included three levels of Entrust (low, 0.25 mg ai/seed; medium, 0.50 mg ai/seed; high 0.75 mg ai/seed), Captan 400 fungicide (3.0 fl oz/cwt), Cruiser insecticide (0.136 mg ai/seed) as an insecticide standard, and no seed treatment as an untreated control. Immediately after planting, a narrow band of blood meal (12-0-0) was applied over each row at a rate of 320 g per 2-row plot. The trial was machine harvested and graded for sieve size on July 30.

RESULTS AND CONCLUSIONS

The data suggests that there were no significant differences among the Captan fungicide treatment, the Cruiser insecticide treatment, and all three rates of Entrust for percent stand loss. All treatments were equally effective in terms of preventing stand reductions compared to the no seed treatment. The effectiveness of Captan suggests that fungal pathogens may have contributed to reductions in yield more so than seed corn maggot. No significant differences were observed in maturity of Hystyle (percent of 5 sieves) regardless of treatment. Entrust rates, when graphed, appear to have a quadratic increase in both stand and yield (Fig. 1 and 2). The highest yield was achieved with the lowest rate (0.25 mg ai/seed) of Entrust. Preliminary yield data suggest that the medium (0.5 mg ai/seed) and high (0.75 mg ai/seed) rates of Entrust may have negatively influenced yield, however, this phenomenon will need to be investigated further.

Source		Seed germination		Yield (ton/acre)		Pct 5's	
	df	Mean square	Prob>F				
Block	3	351.15	0.043	1.831	0.183	6.62	0.599
Treatment	5	696.24	0.001	3.020	0.043	18.11	0.923
Error	15	45.08		0.099		4.25	

Means by Treatment

	Pct. stand		Yield (tons/A)		Pct. 5's	
Cruiser 5F (50g)	A	62.4	3.72	AB	48.33	A
fungicide only	A	62.4	4.19	A	49.41	A
Entrust Low rate	A	61.6	4.12	A	49.72	A
Entrust High rate	A	59.1	3.64	AB	48.56	A
Entrust Medium rate	A	57.7	3.58	B	51.01	A
no seed trtmt	B	49.5	2.98	B	49.42	A

Means with the same letter are not significantly different (Students t test).

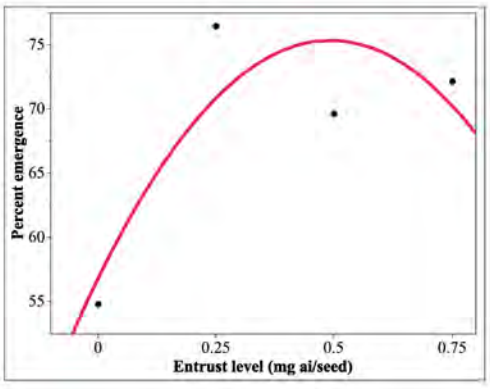

Fig. 1. Quadratic increase in percent emergence with increased level of Entrust.

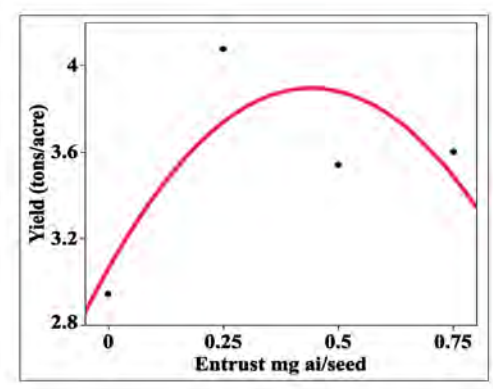

Fig. 2. Quadratic increase in yield with increased levels of Entrust.

ACKNOWLEDGEMENTS

The authors would like to thank the Wisconsin Department of Agriculture, Trade & Consumer Protection for funding this research.

STUDYING OF BEAN VARIETIES (*PHASEOLUS VULGARIS* L) REACTION TO BEAN WEEVIL INFESTATION (*ACANTHOSCELIDES OBTECTUS* SAY)

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Bean is a main leguminous culture in the vegetable crops production. It is attacked by a great number of pests. Bean weevil (*Acanthoscelides obtectus* Say) makes serious problems in grain storage. Eating the inner content and contaminating the production with excrements the larva of bean weevil makes seed flavour and sowing seed properties worse, as well as seeds decrease their weights from 2.1% to 23.8% (2). Variety specification in *Phaseolus vulgaris* L. is observed by the index of infestation of bean weevil under field and laboratory conditions (1, 3).Alternative methods for control such as genetic and breeding one have bean carried out in Maritsa- Vegetable Crops Research Institute in Plovdiv during the last years with aim to respond to ecological agriculture requirements. Reaction of different bean accessions, RILs and varieties for establishment of resistant sources and their enclosure in the breeding programs has bean studied in this direction (4, 5, 6). The object of this work was to study the reaction of 14 common and snap bean varieties towards bean weevil infestation and the establishment of their seed damages.

MATERIALS AND METHODS: Bean weevil has bean under investigation for the period 2003-2005. Fourteen common bean (*Phaseolus vulgaris* L.) varieties and bean weevil populations with origin from different bean varieties were used. Trails were studied under controlled laboratory and field conditions. Laboratory experiment was under an artificial infestation ground using "no choice" host conditions. Test from 50 seeds was prepared from each standard and was infested with 5♀ and 5♂ weevils, temperature of 27±1°C. The seed damage is recorded after 50 days and index of infestation (%) is calculated by McKinney formula. Field experiment was carried out under natural infestation ground using "free choice" host conditions. An average test of 100 pods in physiological maturity was used from each variety. Laboratory experiment for seed sowing property determination were carried out under the temperature of 25±1°C.

RESULTS AND DISCUSSION: High index of infestation over 20% was determined in 'Starozagorski cher' (22.97%), 'Dobroudjanski ran' (21.77%), 'Trakiiski' (21.56%) and 'Oreol' (20.27%) varieties in testing under no choice digestive source conditions (fig.1). Low index of defeat was recorded in the seeds of 'Abritus' (8.81%), 'Zarya' (9.97%), and 'Perun' (10.00%) varieties. Differences in the index of damage in common bean varieties included in the study were recorded in the field trails carried out in natural infestation ground from bean weevil under free choice of host conditions (fig.2). Seeds of 'Starozagorski cher' and 'Nikos' varieties demonstrated the highest index of infestation - (11.31%) and (9.27), respectively. Low index of infestation is recorded for 'Obraztsov chiflik 24' (2.06%) and 'Dodroudjanski 7' (2.46%) varieties. Fall in these indexes was recorded after seed infestation with bean weevil in the trails for determination of germination energy and seed germination (tab.1). It was clearly from the obtained results that the highest per cent of damaged seeds and index of infestation was recorded in 'Nikos' variety and the highest decreasing of the germination energy (35.27%) and the seed germination (35.46%), respectively. The decreasing of sowing properties varied in the other varieties. The loss of germination energy was from 3.83% ('Oreol' and 'Fiesta') to 25.53% ('Veritsa'). The decreasing of seed germination compared to the healthy seeds was from 4.54% ('Abritus') to 24.13% ('Starozagorski cher'). High per cent of infested seeds and index of infestation was recorded in 'Nikos', 'Trakiiski' and 'Starozagorski cher' varieties. The lowest decreasing of seed germination was observed in the same varieties. That was a result from damages caused during larva's eating. Weight loss also varied within the different varieties from 2.46% ('Oreol') to 7.13% ('Trakiiski').

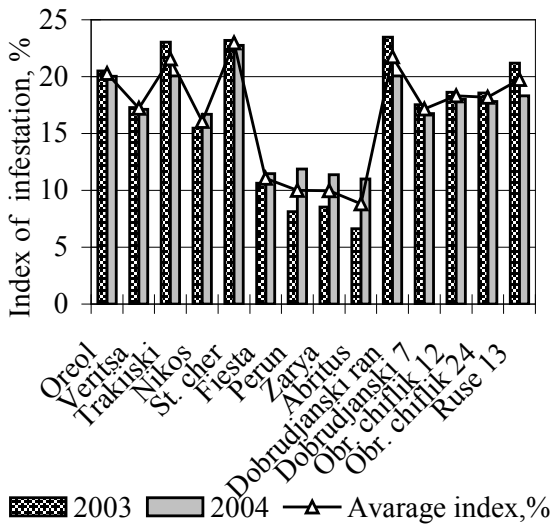

Figure 1. Index of infestation of bean weevil in common bean varieties under artificial infestation ground ("no choice")

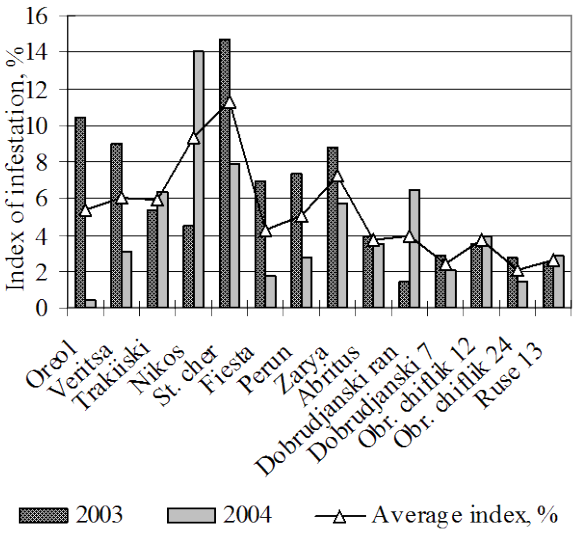

Figure 2. Index of infestation of bean weevil in common bean varieties under natural infestation ground ("free choice")

Table 1. Bean weevils (*Acanthoscelides obtectus* Say) damages in common and snap bean varieties (*Ph. vulgaris* L.)

Variety	Damaged seeds (%)	Index of infestation (%)	Weight loss (%)	Decreasing of germination energy compared to healthy seeds (%)	Decreasing of seed germination compared to healthy seeds (%)
Oreol	21.00	8.00	2.46	3.83	4.89
Veritsa	15.67	4.17	4.79	25.53	17.73
Trakiiski	30.33	14.67	7.13	17.86	19.49
Nikos	33.33	16.17	4.28	35.27	35.46
Starozagorski cher	22.67	9.00	3.81	24.57	24.13
Fiesta	4.67	1.83	2.59	3.83	4.89
Perun	3.67	1.08	2.81	10.71	12.35
Zarya	10.00	4.42	3.16	12.81	12.49
Abritus	3.67	1.58	3.12	4.34	4.54
Dobrudjanski ran	18.33	7.67	5.61	19.92	19.50
Dobrudjanski 7	15.00	5.25	4.79	20.24	15.84
Obraztsov chiflik 12	6.00	2.58	4.19	23.17	14.70
Obraztsov chiflik 24	7.33	2.92	3.08	7.35	8.57
Ruse 13	14.33	4.75	3.12	9.70	13.11

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EVALUATION OF BLACK BEAN LINES IN THE NORTH OF MATO GROSSO DO SUL STATE, BRAZIL

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INTRODUCTION

In Brazil, the bean plant (*Phaseolus vulgaris*, L.) cultivation is done in all regions and in different periods: " waters time ", " droughts time " and " autumn-winter". The culture occupied, in the agricultural crop 2004/2005 an area of 3.8 million of hectares, with a medium productivity of 798 kg ha^{-1} (AGRIANUAL, 2006). The introduction of new cultivars with higher genetic potential of production and resistance to the diseases and pests gave conditions to increments in culture productivity. This work was led out with the objective to evaluate the grains production of different lines of black bean cultivated in the droughts period in north of Mato Grosso do Sul state, Brazil.

MATERIALS AND METHODS

This experiment was led out during the agricultural crop 2007/2008, at São Gabriel do Oeste city, MS, Brazil (latitude: 19º23'S; longitude: 54th 23'W). The following commercial lines of black bean were used: CNFP 10025, C NFP 10214, C NFP 10221, CNFP 10793, C NFP 10794, C NFP 10799, CNFP 10800, CNFP 10805, CNFP 10806, CNFP 10807, besides the control cultivars, such as, BRS Valente, BRS Grafite, BRS Supremo and IPR Uirapuru. The experimental design was in randomized blocks with 14 treatments and three replications. It was used the conventional tillage and the sowing was done in 16/03/2007 (the droughts time) with 0,45 m of row spacing and 15 seeds m^{-1}. For soil fertilization was applied 250 and 150 kg ha^{-1} of the 08-20-20 (0 day) and 20-00-20 (30 days after sowing), respectively. It was necessary the use of complementary irrigations due to the low precipitations. The experimental plots was constituted by a group of four lines with four meters length, but only the two central lines were considered in the evaluation. The following variables were evaluated: mass of 100 grains; crop productivity and grain quality (notes: 1 = very good to 5=bad). The obtained data were submitted to the variance analysis and the averages compared by the Scott-Knot test at 5%.

RESULTS AND DISCUSSION

The irregular precipitations influenced negatively in the flowering period and the grains filling. The mass of 100 grains that depends directly of size and grains filling, presented significant variations among the lines, forming three different groups, and CNFP 10793 lines was superior when compared to the others. Interactions among genotypes and environmental conditions affect the bean productivity (Carbonell et al., 2007, Melo et al., 2007 and Ribeiro et al., 2008). However, in this study were not observed significant differences of productivity among the lines (Table 1).

Visual aspects (format, size and shine) and grain quality (time of cooking, sensorial aspects and nutrient contents) of beans are important factors to define the acceptance by consumers and the success of news lines selection program. In spite of was not observed grains of very bad quality, any

material of high quality was not observed. The low precipitations and the bad distribution must have contributed to obtaining of these results.

Table 1. Mass of 100 grains, productivity and grain quality of different black bean lines in São Gabriel do Oeste, MS, Brazil, cultivated in the period of droughts, 2007.

Lines	Mass of 100 grains (g)	Productivity (kg ha^{-1})	Grain quality (note)
* BRS Valente	18.03 c	779	3.0
* BRS Grafite	22.57 b	914	2.0
* BRS Supremo	20.10 b	1,191	2.0
* IPR Uirapuru	19.33 c	721	2.0
CNFP 10025	14.87 d	953	2.0
CNFP 10214	22.30 b	1,025	2.0
CNFP 10221	15.43 d	852	2.5
CNFP 10793	25.60 a	992	2.5
CNFP 10794	20.63 b	1,007	2.0
CNFP 10799	18.30 c	694	2.0
CNFP 10800	19.07 c	865	2.0
CNFP 10805	18.47 c	943	2.0
CNFP 10806	18.13 c	813	2.0
CNFP 10807	21.07 b	1,004	2.0
C.V. (%)	**9.62**	**19.96 ns**	**-**

Averages followed by same letter in the columns do not differ by Scott-Knot test at 5%.
* Control cultivars. **ns**= not significant. Grain quality (note: 1 = very good to 5 = bad)

CONCLUSIONS

. The line CNFC 10214 presented the largest value for the mass of 100 grains.
. There is no significant difference of productivity among the different lines.
. The line CNFC 10214 was more close to the control variety BRS Supremo in productivity and grains quality.

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EVALUATION OF CARIOCA BEAN LINES IN A SAVANNAH AREA OF BRAZIL

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INTRODUCTION

The bean plant (*Phaseolus vulgaris L.*) presents great importance in Brazilian's diet because contributed about 70% of the daily protein ingestion (Machado et al, 2008). The low productivity of Brazilian bean crop results from the low agrosystems technology. The introduction of new breeding lines with high productivity rates is the current tendency (Costa et al, 2008). The objective of this work was to evaluate the performance and the productivity of different carioca bean lines during the drought period, in a savannah area of Mato Grosso do Sul, Brazil.

MATERIALS AND METHODS

The experiment was conducted in a typical savannah area (oxisoil) at São Gabriel do Oeste city, MS, Brazil (latitude: 19º23'S; longitude: 54th 23'W). The commercial Carioca bean lines: CNFC 10703, CNFC 10713, CNFC 10716, CNFC 10721, CNFC 10729, CNFC 10733, CNFC 10742, CNFC 10753, CNFC 10757, CNFC 10758, CNFC 10762, CNFC 10763, CNFC 10813 (derived from the genetic improvement program of EMBRAPA), were used, besides the control cultivars such as Pérola, BRS Cometa, BRS Pontal and IPR Juriti. The experimental design was in randomized blocks with 17 treatments and three replications. It was used the conventional tillage and the sowing was done at 16/03/2007 (the droughts period) with 0.45 m of row spacing and 15 seeds m^{-1}. For soil fertilization was applied 250 and 150 kg ha^{-1} of the 08-20-20 (0 day) and 20-00-20 (30 days after sowing), respectively. The experimental plots were constituted by a group of four lines with four meters length, but only the two central lines were considered in the evaluation. Complementary irrigation was used. The following variables were evaluated: flowering period; crop cycle; height of plants; mass of 100 grains; crop productivity and grains quality (notes: 1 = very good to 5= bad). The obtained data were submitted to the variance analysis and the averages compared by the of Scott-Knot test at 5%.

RESULTS AND DISCUSSION

Considering the flowering period, it was observed that there was a variation of six days among the shortest period (41 days) to the slower period (47 days), which demonstrated a certain similarity among the materials (Table 1). The cycle of the culture had an equal tendency, which the most precocious presented physiologic maturation with 88 days and the latest with 97 days. The height of the plants is an important characteristic for mechanical harvest. In this study were not observed significant differences among the tested lines (Table 1).

The irregular precipitations influenced negatively the flowering period and the grains filling. The mass of 100 grains that depends directly of size and grains filling, presented significant variations among the tested lines. The largest values were obtained with the CNFC 10813 (28,43g), followed by Pérola (25,73g) that differed significantly to the others lineages (Table 1). With relationship to the productivity of the cultures was observed the formation of two different groups. Only the lineages CNFC 10762, CNFC 10763, CNFC 10721 and CNFC 10813 presented productivities above 1.000 kg ha^{-1}. This result agrees with Melo et al. (2007) who observed that

interactions among genotypes and environmental conditions affect the productivity of the bean plant. Visual aspects and grains quality are important factors to define the beans acceptance for the consumers and the grains quality varied among the materials (Table 1). According Vieira et al. (2006), the environmental conditions contribute to seed coat integument darkening which means that the consumer confuses it with old bean, which is difficult to cook. The lines CNFC 10763, CNFC 10762, CNFC 10753 and CNFC 10757 have the best scores for grain quality (Table 1).

Table 1. Flowering time (FT), maturity, height of plant, mass of 100 grains, productivity and grains quality of different bean plant lineages of the group Carioca, at São Gabriel do Oeste, MS, Brazil. 2007.

Lineages	FT (days)	Maturity (days)	Height (cm)	Mass of 100 grains (g)	Productivity (kg ha^{-1})	Grain quality (note)
*Pérola	45	93	45,0	25,73 b	1.177,62 a	2,0
*BRS Cometa	44	88	43,3	19,83 d	843,65 b	3,0
*BRS Pontal	46	94	41,7	21,13 d	1.062,34 a	2, 0
*IPR Juriti	44	93	41,0	21,60 d	931,44 b	3,0
CNFC 10703	45	89	41,3	20,30 d	1.042,73 b	2,5
CNFC 10713	47	95	41,3	20,00 d	950,21 b	2,5
CNFC 10716	47	96	40,3	20,53 d	954,10 b	2,5
CNFC 10721	47	92	38,3	20,40 d	1.150,03 a	2,0
CNFC 10729	45	93	46,0	24,17 c	1.004,68 b	2,5
CNFC 10733	46	93	35,3	21,23 d	909,13 b	2,0
CNFC 10742	45	89	37,7	21,73 d	875,47 b	4,0
CNFC 10753	46	94	44,0	23,97 c	1.028,90 b	1,5
CNFC 10757	46	96	43,3	20,93 d	843,90 b	1,5
CNFC 10758	45	97	42,7	23,23 c	830,27 b	3,5
CNFC 10762	45	96	39,3	23,20 c	1.310,59 a	1,5
CNFC10763	45	88	39,3	23,50 c	1.245,32 a	1,0
CNFC 10813	41	91	41,0	28,43 a	1.118,26 a	2,0
CV (%)	--	--	**9,55 ns**	**4,11**	**16,38**	--

* Control cultivars. ns: not significant, for Scott-Knot test at 5%. Averages followed by same letter in the columns do not differ to each other by Scott-Knot test at 5%. Grain quality: 1 = very good and 5 = bad.

CONCLUSIONS

- The appraised lines present homogeneity for maturity, flowering period and height of plants.
- There is great variation among the productivity of different lines.
- The lines CNFC 10762 and CNFC 10763 produced the largest values for productivity and grain quality.

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PERFORMANCE OF BLACK BEAN BRAZILIAN GENOTYPES IN 2005 AND 2006

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INTRODUCTION

In the last years, Brazil has ranked first in production and consumption of common beans, *Phaseolus vulgaris* L., which is a basic food and one of the main sources of proteins in the diet of Brazilian population. Among the different common beans types of grain, the black one occupies 20% of the consumption market (Del Peloso & Melo, 2005). The breeding programs have supplied the demand of Brazilian market with new cultivars with desirable characteristics, such as yield stability and also contributed to increase yield. The common bean breeding program of Embrapa Rice and Beans Research Center systemize the evaluations of lines developed in the program in a national network in regions responsible for 90% of the national production, estimated in 2,8 t (IBGE, 2008). The final evaluation of the lines is done in a network of evaluation trials, in several environments, which should represent the diverse environmental conditions that bean cultivars can be grown.

The indication of new cultivars has contributed for increase of yield in 40% in the last ten years. So, the program looks for new lines with better phenotypes that can be indicated as new cultivars.

MATERIALS AND METHODS

In 2005 and 2006, a network of trials were conducted during rainy, dry and winter growing seasons, in 57 environments in 11 states: Goiás, Distrito Federal, Mato Grosso, Mato Grosso do Sul, Paraná, Santa Catarina, Rio Grande do Sul, São Paulo, Sergipe, Bahia, and Alagoas. The experimental design was randomized complete blocks, with three replications and plots of four rows measuring four meters. The yield data were collected in the two center rows. Each trial was constituted of 12 genotypes of common beans with black grain type (Table 1). Evaluations of plant architecture, resistance to disease and lodging tolerance, were made using a grate scale where the note 1 indicates a desirable genotype and note 9 the undesirable genotype. Yield data were submitted to analyze of variance and joint analyze grouping all trials. The Duncan test (0.05) was used to compare treatment means.

RESULTS

The joint analyze showed a good experimental precision (CV=14.1 %) and it were detected significant difference ($P<0.01$) among genotypes, environments and interaction genotypes x environments. It was identified one line CNFP 10104 that can be indicated as a new cultivar. This line was the most promissory line for yield (Table 1), with erect plant architecture, high insertion of the first pod, closed ramifications and few vines like the control BRS Valente. CNFP 10104 was more resistant to causal agent of anthracnose when compared to BRS Valente (control).

The line CNFP 10104 will be first indicated for Goiás State and Distrito Federal for rainy and winter growing seasons (with irrigation) and for Santa Catarina State for the rainy season. For the other States, new trials will be conducted to complete the minimum number of trials required to register the cultivars.

Table 1. Yield (kg ha^{-1}), average grades[(1)] and e highest grades[(2)] for evaluations of plant architecture (ARQ), lodging (ACA), and reaction to common bacterial blight (CBC), angular leaf spot (MA), mildew (OI), rust (FE) and anthracnose (AN), of 12 genotypes of black common beans, evaluated in 57 environments in Brazil, in 2005 and 2006.

Genotype	Yield	ARQ	ACA	CBC	MA	OI	FE	AN
CNFP 10104	2,584 a	4[(1)]/5[(2)]	4/7	5/8	5/8	4/7	1/2	1/1
BRS Valente	2,454 b	4/5	4/6	5/9	5/8	4/7	4/4	6/7
CNFP 10103	2,452 b	5/5	3/5	4/7	6/8	7/8	2/3	1/1
IPR Uirapuru	2,415 bc	4/6	3/7	3/6	5/8	4/6	2/3	3/6
CNFP 10035	2,381 bc	4/5	3/7	5/8	7/8	4/6	2/3	5/7
CNFP 10206	2,365 c	4/5	4/7	3/7	7/9	6/8	2/3	2/5
CNFP 10093	2,362 c	4/6	4/7	4/8	6/8	7/8	3/4	1/1
CNFP 10109	2,285 d	5/6	4/7	3/6	6/9	6/8	1/2	1/1
BRS Grafite	2,257 d	5/6	4/7	4/6	5/8	4/6	2/3	3/4
CNFP 10076	2,246 d	4/6	4/8	4/7	6/8	5/7	2/4	2/6
CNFP 10120	2,227 d	5/6	3/5	3/7	5/8	7/8	1/1	1/1
FTS Soberano	2,136 e	4/6	4/7	6/9	6/8	3/6	2/3	1/1

[1]Means followed by the same letter do not differ by Duncan test 0.05 of probability.

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GENOTYPES OF COMMON BEANS WITH GRAIN TYPE CARIOCA IN THE CENTRAL REGION OF BRAZIL

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INTRODUCTION

In the last years, Brazil has ranked first in production and consumption of common beans, *Phaseolus vulgaris* L., which is a basic food and one of the main sources of proteins in the diet of Brazilian population. Among of the different common beans types of grain, the carioca occupies 70% of the market (Del Peloso & Melo, 2005). The breeding programs have supplied the demand of Brazilian market with new cultivars with desirable characteristics, such as yield stability and also contributed to increase yield. The common bean breeding program of Embrapa Rice and Beans Research Center systemize the evaluations of lines developed in the program in a national network which includes a central region of the country (Goiás, Distrito Federal, Mato Grosso and Mato Grosso do Sul), responsible for 13,5% of the national bean production (IBGE, 2008) (383.856 t), in 206.235 ha. The final evaluation of the lines is done in a network of evaluation trials, in several environments, which should represent the diverse environmental conditions that bean cultivars can be grown.
The indication of new cultivars has contributed for increase of yield in this region, from 1.324 kg ha^{-1} in 1997, to 1.861 kg ha^{-1} in 2007 (IBGE, 2008). So, the program looks for new lines with better phenotypes that can be indicated as new cultivars.

MATERIALS AND METHODS

In 2005 and 2006, a network of trials were conducted during rainy, dry and winter growing seasons, in 29 environments in the Central Region of Brazil in the States of Goiás, Distrito Federal, Mato Grosso and Mato Grosso do Sul. The experimental design was randomized complete blocks, with three replications and plots of four rows measuring four meters. The yield data were collected in the two center rows. Each trial was constituted of 14 genotypes of common beans carioca grain type (Table 1). Evaluations of plant architecture, resistance to disease and lodging tolerance, were made using a grate scale. Yield data were submitted to analyze of variance and joint analyze grouping all trials. The Duncan test (0.05) was used to compare treatment means.

RESULTS AND DISCUSSION

The joint analyze showed a good experimental precision (CV=15.3 %) and it were detected significant difference ($P<0.01$) among genotypes, environments and interaction genotypes x environments. It was identified some lines that can be indicated as new cultivars. The lines CNFC 10429 e CNFC 10432 were most productive with mean yield equal to obtained by BRS Pontal, used as control (Table 1). Those lines presented plant architecture upright, with high insertion of pod, closed ramifications and few vines, and low grade of lodging, when compared to BRS Pontal, which presents plants ramified, lodged, with many vines. Besides, these lines were better than cultivar Pérola in yield, plant architecture and lodging. The cultivar Perola is the most grown in the country.

The line CNFC 10408 presented yield inferior to obtained by BRS Pontal (control) and superior to that obtained by Pérola (control). This genotype also presented better plant architecture and higher tolerance to lodging than the two control cultivars. Besides, it showed reaction to common bacterial blight similar to showed by BRS Pontal, which is a bean cultivar with grain type carioca with higher level or resistance to that disease. Other advantage of the line CNFC 10408 the median-short cycle (75 to 85 days), when compared to BRS Pontal and Pérola which has a normal cycle (85 a 95 days). The line CNFC 10467, which has a retardation of darkening in the seed coat had the worst performance in yield, compared to the control cultivars.

The superior lines will be indicated for growing, initially for the State of Goiás during the rainy and winter growing seasons. For the other States, new trials will be conducted to complete the minimum number of trials required to register the cultivars. Those trials started to be conducted in 2008 and are being conducted in these States and in the seasons in which the minimum number of trials has not been satisfactory.

Table 1. Yield (kg ha^{-1}), average grades(1) and e highest grades(2) for evaluations of plant architecture (ARQ), lodging (ACA), and reaction to common bacterial blight (CBC), angular leaf spot (MA) and mildew (OI) and rust (FE), of 14 genotypes of common beans with grain type Carioca, evaluated in 29 environments in Central Region of Brazil, in 2005 and 2006.

GENOTYPE	YIELD	ARQ	ACA	CBC	MA	OI	FE
CNFC 10429	2,404 a	4(1)/5(2)	4/6	4/7	3/8	1/2	2/3
BRS Pontal	2,350 ab	6/8	6/8	3/4	5/8	4/6	1/1
CNFC 10432	2,304 abc	4/6	4/6	4/8	4/7	2/4	1/1
CNFC 10431	2,257 bc	4/6	3/6	4/8	4/7	3/7	1/2
CNFC 10410	2,221 cd	4/7	4/6	4/8	5/8	1/2	1/1
CNFC 10408	2,214 cd	4/6	4/6	3/5	7/9	5/7	2/4
CNFC 10470	2,131 de	5/7	5/7	5/7	6/9	2/3	1/1
CNFC 10438	2,120 def	5/6	4/7	3/6	4/8	3/6	2/3
Pérola	2,092 efg	6/8	6/9	4/7	6/8	3/4	3/5
CNFC 10455	2,064 efg	4/5	4/6	5/9	5/7	3/6	5/7
Iapar 81	2,018 fg	5/7	5/8	4/6	7/8	3/7	3/6
FTS Magnífico	2,004 g	5/8	5/7	3/5	6/9	5/7	2/4
CNFC 10444	1,898 h	4/7	3/6	4/7	4/7	2/3	2/3
CNFC 10467	1,896 h	6/7	5/7	4/7	5/9	2/4	2/3

[1]Means followed by the same letter do not differ by Duncan test 0.05 of probability.

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'PINTO SALTILLO' ADOPTION EFFECT ON DRY BEAN YIELD AND DIVERSITY CONSERVATION IN DURANGO, MÉXICO

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INTRODUCTION. Since 2006, when the government seed distribution program was implemented in Durango (Pajarito and Rosales, 2007), massive adoption of Pinto Saltillo bred cultivar has been occurring. Higher seed yield, grain market acceptance, seed coat darkening tolerance, and preferred prices had a significant influence in Pinto Saltillo adoption. The objective of the study was to evaluate the effect of Pinto Saltillo adoption on the grain yield average and the dry bean genetic diversity level in the State of Durango, México.

MATERIALS AND METHODS. A total of 100 random samples were taken from 2006 to 2008 in the main dry bean production areas in Durango, México. Sampled areas included Los Llanos, Valle del Guadiana, Canatlán, and Poanas. For each year, sampled plots were randomly located across dry bean production areas as georeferenciated points. Plots were visited after sowing and contact was established with the farmers for data collection about cultivars planted. Field trips were performed trough the crop season and morphological and agronomic traits were registered to corroborate cultivar identity under field conditions. Four sub-samples, consisting in two rows 5 m long and 76 cm apart, were randomly harvested at maturity in each plot for yield determination. Field sub-samples were sun dried, threshed and then the grain was weighed. Cultivar characterization was made considering seed traits recommended in *Phaseolus vulgaris* guidelines for the conduct of tests for distinctness, uniformity and stability (SNICS, 2001). Cultivar identification was made comparing plant and seed traits with those observed in main cultivars grown in Durango, such as: Pinto Saltillo, Negro San Luis, Pinto Villa, Flor de Mayo Media Oreja, and Canario. When difficulties were observed for cultivar identification, the seed´s commercial class was used as a grouping trait. The frequency and seed yield for each commercial class and cultivar were then computed.

RESULTS AND DISCUSSION. Increments were observed in Pinto Saltillo frequency from 1 in 2006 to 21 in 2008, in contrast to results observed for Pinto Villa and black seeded cultivars. Other cultivars and seed classes observed during sampling period were Canario (small, yellow seeds), Pinto Nacional, Flor de Mayo (pink seeds), and Flor de Junio (pink striped seeds). Pinto Saltillo showed high yield variation among locations from 89 kg ha^{-1} (Amado Nervo, Dgo.) during 2008 to 2 062 kg ha^{-1} (Pánuco de Coronado, Dgo.) in 2007. Variation was also observed for average seed yield registered by Pinto Saltillo from 582 kg ha^{-1} in 2008 to 1 086 kg ha^{-1} in 2007 (Table 1). Variations registered in seed yield resulted from marginal lands sowings, hail damage and low density plantings observed in sampled plots. Other high yielding cultivar was Negro San Luis, mainly planted in sites with higher annual rain records (450-500 mm), such as southern Cuencamé and Guadalupe Victoria. Flor de Mayo and Canario registered high yields during 2006 due to good distribution of the rains and long-duration of the growing season. Yield average observed in 2008 was lower than those observed in 2006 and 2007 due to heavy rains, flooding and maturity delay observed in several dry bean plots.

In spite of abovementioned problems, in some locations Pinto Saltillo showed higher yields than the average and maintained commercial acceptance in Durango and other domestic markets. Considering

market class as a grouping criterion, Pinto was the most popular seed type planted in Durango during 2006, 2007 and 2008. Three main cultivars were found in this class: Pinto Saltillo, Pinto Villa and Pinto Nacional. Other cultivars and commercial classes found were Negro San Luis (rounded shiny black), Canario, and Flor de Mayo. Some commercial classes planted traditionally in Durango (such as Bayo, Río Grande, Bayo Rata) were not found in the samples. A reduction in frequency was also observed for Pinto Villa due to its accelerated seed coat darkening related to low grain prices. Rapid cultivar adoption registered for Pinto Saltillo was promoted by the government seed distribution program, higher seed yield, seed coat darkening tolerance, grain market acceptance, and preferred prices.

CONCLUSIONS. The rapid adoption of Pinto Saltillo has contributed to increase seed yield, in some areas, and grain quality but it has reduced the genetic diversity in Durango, México.

Table 1. Yield observed for dry bean seed classes and cultivars planted in Durango, México, 2006-2008.

Seed Class/Cultivar	(n)	Yield (kg ha^{-1}) Minimum	Maximum	Average
	2006			
Pinto Villa	12	372	1761	878
Negro	13	309	2304	1309
Flor de Mayo	3	1059	1923	1404
Canario	3	945	1582	1216
Flor de Junio	1	483	483	483
Pinto Saltillo	1	993	993	993
Average	Sum = 33	694	1508	1047
	2007			
Pinto Saltillo	11	214	2062	1086
Negro	10	476	1982	1120
Pinto Villa	5	806	1474	1081
Flor de Mayo	4	170	830	580
Pinto Nacional	3	1044	1428	1177
Canario	2	555	1126	841
Flor de Junio	1	399	399	399
Average	Sum= 36	523	1329	898
	2008			
Pinto Saltillo	21	89	1599	582
Negro San Luis	4	399	855	569
Pinto Nacional	3	107	582	350
Canario	2	90	211	151
Flor de Mayo	1	547	547	547
Average	Sum= 31	246	759	440

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ADOPTION OF 'PINTO SALTILLO' BEAN CULTIVAR IN DURANGO, MÉXICO

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INTRODUCTION

Dry beans (*Phaseolus vulgaris* L.) are grown under rainfed conditions in 272 000 hectares in Durango State—northern México—where specialization was observed for pinto market class production. The increment in total area planted with pinto class was caused by the higher yields obtained with Pinto Villa and preferred prices observed for Pinto Saltillo and Pinto Mestizo bred cultivars. However, a reduction in grain prices was observed for Pinto Villa cultivar during high production years, due to seed coat darkening. Therefore, Pinto Saltillo was developed using high yield and seed coat darkening tolerance as selection criteria. In 2006, a massive seed distribution program was implemented by the state government promoting cultivar conversion and grain quality improvement for marketing purposes (Pajarito and Rosales, 2007). A significant increment was observed for Pinto Saltillo planted area due to market acceptance and preferred prices. The aim of the study was to estimate the adoption level under rainfed conditions for Pinto Saltillo bred cultivar in Durango, México.

MATERIALS AND METHODS

A survey was conducted for data collection using a questionnaire completed in a face-to-face interview. Sample size was determined with the equation: $n=Z^2pq/E^2$ (Rojas, 2005), where: n= sample size; Z= confidence interval (1.96 for 95 %); pq=variability (p=q=0.5); and E=precision level (0.08). Estimated sample size was n= 150 [$n=(1.96)^2 (0.5)(0.5)/(0.08)^2=150$]. A random sample was drawn from the government register of farmers planting beans in 2008 (SAGARPA, 2008) and the field work was carried out from October to November 2008. The most important counties producing dry beans in Durango were included for the study, such as: Guadalupe Victoria, Cuencamé, Pánuco de Coronado, and Poanas. In Guadalupe Victoria selected localities were Guadalupe Victoria, Ignacio Allende, Antonio Amaro, Calixto Contreras, Felipe Carrillo Puerto, and Ignacio Ramírez. In Cuencamé: Cuauhtémoc, Emiliano Zapata, and Ramón Corona. In Pánuco de Coronado: Francisco I. Madero and Pánuco de Coronado. In Poanas: Cieneguilla, La Joya, Plutarco Elías Calles, Villa Unión, and Villita de San Aténogenes.

RESULTS AND DISCUSSION

Pinto Saltillo was planted by 92 % of the farmers along with other dry bean bred cultivars such as: Pinto Villa (20 %) and other cultivars (4 %) (Table 1). Farmers also planted landraces such as Negro San Luis or Negro Bola (10 %), Canario (small yellow seeds) (8 %), Pinto Nacional (5 %), and Flor de Mayo (3 %). These bred cultivars and landraces are the most well known by farmers and they also have been planted in previous years, including Pinto Mestizo. A significant reduction was observed for the area planted with Pinto Villa cultivar, in spite of its adaptation and drought tolerance, mainly due to rapid seed coat darkening. Currently, Pinto Saltillo represents a productive and commercial success in Durango and Chihuahua, where menonites described it as "The Champion" based on its high seed yield and market acceptance. However, most of the farmers recommended to develop cultivars with larger seed size, early maturity and grain similar traits to those observed in Pinto Saltillo (seed coat darkening tolerance and short cooking time), in order to improve productivity and market seed quality.

The main reason for adopting Pinto Saltillo, for 46 % of the farmers was a better price, for 28 % it was a high seed yield, and for 26 % it was market preference. The source of the seed was the previous harvest for 83 % of the farmers and 17 % got it from the government seed distribution program. Only 3 % of the farmers planted certified seed, 4 % received direct technical assistance, 5% received a short-term loan, and 9 % belonged to an organization.

The yields expected by farmers with Pinto Saltillo on commercial plots were: 270 kg ha^{-1} in bad years (less than 350 mm of rain from July-October, with bad distribution), 606 kg ha^{-1} in typical years (350-450 mm of rain, with irregular distribution), and 1 086 kg ha^{-1} in good years (350-450 mm of rain, with good distribution).

Farmers stated that their bean production was distributed according to the following percentages: 86 % for sale in the market (mainly to intermediaries), 8 % for self-consumption, and 6 % is stored as seed for the next planting season. On average, the wholesale prices by market class, were: Pinto Saltillo MXN $8.00 per kilo, Pinto Villa MXN $5.00 per kilo, Negro MXN $5.50 per kilo, Canario MXN $6.00 per kilo, Flor de Mayo MXN $8.00 per kilo, and Querétaro (cream) MXN $5.00 per kilo.

Increasing average for farmer age (55.7 years) has been observed in dry bean production areas (Table 2), due to young people migration to USA and Mexican big cities in search of employment and better standard of living (Talamantes, 2006). Remittances of migrants from the USA are frequently used to cover the production costs for dry bean in México. The schooling for the individuals in the sample was 6.5 years on average, showing a low education level. The average plot area was 44.2 ha from which 82 % was planted with dry beans. The most frequent kind of tenure among farmers was the 'ejido' or common land (87 %), followed by private property (26 %), shared land (19 %) and rented land (2 %).

CONCLUSIONS

Pinto Saltillo has been successfully adopted among farmers in Durango and currently represents a productive and marketable option. Pinto Saltillo adoption has been influenced mainly by the government seed distribution program, higher seed yields, better grain prices and slow seed coat darkening.

Table 1. Cultivars planted under rainfed conditions in Durango, México, 2008. (percentage of farmers)*

Improved cultivars (%)			Landraces (%)			
Pinto Saltillo	Pinto Villa	Other	Negro	Canario	Pinto Nacional	Flor de Mayo
92	20	4	10	8	5	3

*Sum can be more than 100 % given that some farmers planted more than one cultivar.

Table 2. Characteristics of farmers and land tenure observed in Durango, México, 2008.

				Land tenure (% of farmers)*			
Age (years)	Schooling (years)	Agricultural area (ha)	Bean area (ha)	Ejido	Private property	Shared	Rented
55.7	6.5	44.2	36.5	87	26	19	2

*Sum can be more than 100 %, given that some farmers are under more than one kind of tenure.

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RELEASE OF 'COYNE' GREAT NORTHERN BEAN

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The Agricultural Research Division, University of Nebraska announces the release of the great northern common bean (*Phaseolus vulgaris* L.) cultivar 'Coyne'. Coyne was developed by the dry bean breeding program at the University of Nebraska Agricultural Research Division and tested as NE1-06-12. It is named in honor of Dermot P. Coyne who was the bean breeder at the University of Nebraska for about 30 years before retiring in 2001. He died in 2002. During his career at the University of Nebraska, Dr. Coyne and his cooperators developed 22 dry bean releases. Dr. Coyne was noted for his work on the genetics of resistance to bacterial diseases and several of the lines he developed are included in the pedigree of this cultivar.

Coyne was bred specifically for enhanced resistance to common bacterial blight (CBB), a major seed borne disease of common bean caused by the bacteria *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye [Syn. *X. axonapodis* pv. *phaseoli* (Smith) Vauretin et al.] (*Xcp*), bean common rust *Uromyces appendiculatus* (Pers.:Pers) Unger, and for adaptation to Nebraska common bean growing conditions.

Coyne is a $F_{7:8}$ line derived from a three-way cross (G95023/Weihing//BMN-RMR-11). G95023 is a great northern line developed by the Michigan Agricultural Experiment Station derived from G 91213/7/G90123/P86295/3/WM1-85-45/Sierra//P86241/4/90MS-36/G2913. BMN-RMR-11 is a great northern line developed by the USDA-ARS, Beltsville, MD, Michigan Agricultural Experiment Station and the University of Nebraska Agricultural Research Division that combines four genes for resistance to the bean common rust pathogen (*Ur3*, *Ur4*, *Ur-6* and *Ur-11*), with two genes for resistance to the viruses BCMV and BCMNV. BMN-RMR-11 was derived from bulked F_5 generation from Kodiak/9/P94232*2/8/92 BR-3-10-1084B/7/BR3-1006B/6/88-011-03*2/5/Aztec/4/87-039-34*2/3/POX10//Fiesta/PI 19007 8. 'Weihing' is a great northern cultivar derived from a cross between two great northern breeding lines (NE6-91-115 and NE6-91-73) from the University of Nebraska dry bean breeding program. Weihing has the *Ur-3* and *Ur-6* rust-resistance genes and resistance to the halo blight pathogen [*Pseudomonas syringae* pv. *phaseolicola* (Burkholder) Young et al.] in Nebraska combined with partial avoidance to white mold due to its upright and porous plant architecture. Weihing also has excellent seed quality and possesses the *I* gene for resistance to BCMV. Coyne has high yield potential, broad adaptation to Nebraska, and good seed quality while maintaining rust and bean common mosaic virus resistance.

Disease Resistance: Reaction of Coyne to *Xcp* was consistent across three years at the WCREC, North Platte, NE, where field disease ratings of 3.62, 3.5, and 4.4 were recorded in 2005, 2006 and 2007, respectively. This was similar to reaction of Marquis (2.5, 4.1 in 2006 and 2007, respectively) and Beryl-R (1.7, 4.5, and 5.4 in 2005, 2006, and 2007, respectively). Conversely, the susceptible great northern, Orion, scored 6.0, 8.5 ad 9.0 in 2005, 2006 and 2007, respectively.

Difference in CBB reaction among sources of CBB resistance (USPT-CBB-1, ABCP-8, ABC-Weihing, and Neb#1-Sel-27) and Coyne was not significant. Coyne carries the SAP 6 SCAR marker linked with major QTL for CBB resistance derived from Montana No. 5 (via Weihing).

Inoculation of Coyne with races 41, 44, 47, 49, 53, 67, 73, and 108 under greenhouse conditions at Beltsville, MD from 2005-2007, provided evidence for the presence of *Ur-3* and *Ur-6* genes for resistance to common bean rust. Coyne also carries the SK14 SCAR marker linked to the QTL for *UR-3* common bean rust resistance gene.

Based on top necrosis reaction to *NL-3* strain of BCMNV, it was determined that Coyne carries the single dominant hypersensitive *I* gene that provides resistance to all non-necrotic strains of BCMV, but is hypersensitive to the temperature-dependent necrosis-inducing strains of BCMV and to the temperature-independent necrosis inducing strains of BCMNV. Coyne has the same partial avoidance to white mold [*Sclerotinia sclerotiorum* (Lib.) de Bary] as Weihing due to its semi-upright and porous plant architecture in field nurseries.

Agronomic Characteristics: Coyne exhibits a semi-upright Type 2b indeterminate growth habit. Plants averaged 57 cm in height during 2007 with excellent lodging resistance. Coyne has white flowers and blooms 44 d after planting. Coyne is a midseason bean maturing 91 d after planting and ranging in maturity from 90-92 days. The seed coat of Coyne is bright white.

Yield Performance: Average seed size for Coyne (36.2 g 100 seeds^{-1}) was slight larger than Orion (34.9 g 100 seeds^{-1}) and Beryl-R (29.3 g 100 seeds^{-1}) in the intermediate, advanced, and growers' field trials grown from 2005-2007. For the same trials, Coyne (2,819 kg ha^{-1}) had a slightly higher yield than Marquis (2,758 kg ha^{-1}).

Availability: Husker Genetics Foundation Seed Program, University of Nebraska-Lincoln (UNL), will maintain a small quantity of Breeder Seed of Coyne. An application will be filed for cultivar protection under Title V of the U.S. Plant Variety Protection Act. A small quantity of seed of NE1-06-12 for research purposes will be available from the corresponding author for the first five years. UNL approval will be required to market a new cultivar that is 25% or more Coyne. This will include a negotiated license agreement and fee structure. We ask that appropriate recognition of source be given when this cultivar contributes to the development of a new cultivar.

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RELEASE OF 'ECLIPSE' BLACK BEAN

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ABSTRACT

Eclipse is an early maturing, high-yielding black bean, with very good size, shape and visual appearance (dull black seed coat). This cultivar has purple flowers and dark glossy green leaves. Growth habit is Type II upright with excellent lodging resistance. Eclipse is and early maturing cultivar with very good synchronous drydown prior to harvest (both plant and pods mature concurrently). The improved plant structure, combined with its synchronous drydown, suggests that these lines may be suitable for direct harvest, given appropriate equipment, field conditions, and operator care. Yield testing across more than 25 environments have shown that Eclipse is one of the best cultivars adapted to the Northern Great Plains. Over the last four to five years, it has almost replaced T-39, the most common black bean cultivar grown in the region.

PEDIGREE AND BREEDING HISTORY

Eclipse black bean (previously coded as ND 9902621-2) is a selection resulting from a hybridization series that started in 1996 and was released in 2004: 'Tacaragua'/ 'Nighthawk'// 'Navigator'. A modified pedigree was used as the main breeding method that allowed selecting this line. This is the first black bean variety to be released by the North Dakota Agricultural Experiment Station. It was an attempt to combine tolerance to white mold with early maturity and erect plant architecture. 'Tacaragua' is a black bean landrace from Venezuela with a Type IIIa growth habit which offers some resistance to white mold, posses the *I* gene and immune resistance to rust (Coyne et al., 1991; Fuller et al., 1984). In some field and green houses test, 'Tacaragua' expressed greater resistance to white mold than 'ICA-Bunsi'. 'Nighthawk' is an early maturing black bean cultivar from the University of Saskatchewan. 'Navigator' navy bean (Rogers® Brothers Seed Company) was used in this cross because it has excellent plant architecture, white mold avoidance, and BCMV resistance.

Eclipse is resistant to rust (*Ur-3* gene), BCMV (*I* gene), and shows white mold avoidance. The upright plant growth of Eclipse, together with excellent lodging resistance, early maturity and synchronous dry down of plant and pods at harvest, may provide a benefit in harvest efficiency for dry bean producers, given appropriate equipment, field conditions, and operator care. Black bean is the third most important class in North Dakota, accounting for 10% of the total dry bean production in the state (USDA-NASS, 2008). Over the last six years, Eclipse was tested at more than 30 locations environments across North Dakota (Kandel, 2009), as well as other states. Eclipse has showed excellent performance across most of the environments, with yields superior to other black bean commercial varieties (Table 1). Eclipse has exhibit a yield potential advantage over T-39 (the most popular black bean cultivar in the region) and also canned product appearance scores similar to T-39.

Additional information about Eclipse black bean can be obtained directly from the breeder. Breeder and Foundation seed of Eclipse will be maintained by the NDSU Foundation Seedstocks Program. Eclipse is protected under Title V of the Plant Variety Protection Act (Cert. # 200500293).

Small quantities of seed of Eclipse for research purposes are available from the corresponding author for the first five years. If Eclipse is used for research or contribute to germplasm enhancement or development of breeding line or cultivar, appropriate acknowledgment of the researchers and institutions responsible for development of Eclipse will be highly appreciated.

We would like to express our gratitude to the following persons and institutions: Robin Lamppa (NDSU – Plant Pathology) for inoculum preparation, James Kelly (MSU) for canning tests, Northarvest Bean Growers Assoc. for their long-term economic support, and Angela Linares (Ph.D. student from the NDSU breeding program), for compiling and editing information for this release note.

Table 1. Comparison of Eclipse with commercial check cultivars for agronomic and disease reactions summarized from several locations in North Dakota.

Trait	Eclipse	T-39	Raven	Jaguar
Yield (kg ha^{-1})[1]	2632	2297	-	2367
Maturity (d)	99	102	100	98
Seed Size (seeds/lb)	2,248	2,226	2,522	2,467
Growth Habit[2]	II	IIb	II	II
Plant Height (cm)	60	54	61	62
Lodging (0-9)[3]	2	7	1	2
Canning Score	3	2.7	2	4.5
Rust[4]	R	R	S	R
BCMV[4]	R	R	R	R
Anthracnose (7)[4]	R	R	R	R
(73)	S	S	S	R

[1]Average seed yield across 25 environments.
[2]Growth Habit = CIAT scale where I = determine bush; II = upright, shorth vine (IIb tendency toward floppines); III = prostate vine (IIIa will be erect in certain environmental conditions); IV = indeterminate climber.
[3]Lodging scores 0 = 100% erect, 9 = no erect plants
[4]Rust, BCMV and Anthracnose: R= Resistant, MR= Moderately Resistant, S=Susceptible

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'NUA35' AND 'NUA56', HIGH MINERAL RED MOTTLED BEANS

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Two common bean (*Phaseolus vulgaris* L.) germplasm lines with red mottled grain type, NUA35 and NUA56, were bred for high seed mineral content at the International Center for Tropical Agriculture (CIAT). These genotypes are products of an initiative to improve the nutritional quality of common beans through the enhancement of micronutrient content and the reduction in anti-nutritional factors found in bean seeds that is part of the a novel breeding program at CIAT and other CGIAR centers meant to combat micronutrient deficiencies in human populations. The germplasm lines *per se* belong to a series of genotypes bred for Nutritional Improvement of Andean beans useful for production zones in the tropics and subtropics, hence the code NUA. The two germplasm lines were developed to be high in seed iron and zinc content through selection for these two minerals in early generations of a backcross population created by crossing a commercial, red mottled variety as the recurrent parent with a high mineral donor parent. Both lines produce large grain (above 45 g/ 100 seed) and are bush beans. NUA35 has determinate type I growth habit while NUA56 is indeterminate and of type II growth habit.

Origin: NUA35 and NUA56 are both advanced generation $F_{3:5}$ derived breeding lines from the backcross [CAL96 x (CAL96 x G14519)], where 'CAL96' is a type I growth habit, released variety in Uganda (known as 'K132'), that was originally bred at CIAT and that is widely adapted for Eastern and Southern Africa as well as for the Andean region of South America. CAL96 also has red mottled seed type which is appreciated for its dark burgundy color combined with medium red and light cream mottled pattern and is in consideration for variety release in Colombia as well. 'G14519' (Hickman pole bean from the CIAT germplasm bank) is a type IV growth habit, climbing bean germplasm accession with brown seed that is originally from the United States and which was selected from the CIAT core collection due to it high seed mineral content. Both CAL96 and G14519 are adapted to tropical environments and produce well at 1000 to 1800 masl altitude testing sites in Colombia. The development of the NUA35 and NUA56 was carried out at CIAT headquarters (Palmira, Colombia) using hand emasculation to produce the simple cross and subsequent backcross. Pedigree selection was used from the BC_1F_1 to the BC_1F_3 generation when families were derived based on single plant selection and similarity in seed type and seed color to the recurrent parent. The $BC_1F_{3:4}$ seed from individual families was tested for iron and zinc mineral content using atomic absorption spectrophotometry (AAS) and the highest mineral entries with red mottled grain type were selected for further testing in the $BC_1F_{3:5}$ generation. Seed of this generation was tested in Colombia (Darién and Popayán) from 2004 onward, with subsequent generations tested more widely in other locations within Colombia as well as in Bolivia in South America, Guatemala and Mexico in Central and South America and Kenya, Malawi and Zimbabwe in Eastern and Southern Africa. The seed color of the two NUA lines likewise is very acceptable as they have the same deep red color that is noteworthy of CAL96, the recurrent parent.

Description: Nutrition quality evaluations for seed iron and zinc show that the NUA lines produces more than the average amount of iron for common bean which is 55 ppm; and close to the average amount of zinc for the crop which is 35 ppm. NUA56 has somewhat higher average seed iron content (83.3 ppm) than NUA35 (77.9 ppm) across 15 sites (Table 1) where the genotypes have been tested; while the opposite occurs for average seed zinc content where NUA35 (34.1 ppm) and NUA56 (32.5 ppm). Considering that Andean beans usually have lower than average seed zinc content, this amount of zinc can be considered moderate within the gene pool.

NUA35 and NUA56 are being registered as germplasm lines. They are currently being evaluated in regional trials in the Andean region (Bolivia and Colombia) as well as in Central America (Costa Rica and Guatemala) with additional trials in Eastern and Southern Africa (Kenya, Malawi and Zimbabwe) that are ongoing. NUA lines have proven adaptable in several countries and are being considered for variety releases in Bolivia (NUA35 and NUA56), Colombia (NUA35), and Malawi (NUA56). In addition they have been widely tested on-farm in Colombia and in several additional Eastern and Southern African countries. Analysis with the software program Homologue™ predicts adaptation range in regions such as inter-Andean valleys of Colombia and Ecuador, lowlands of Bolivia, and parts of Kenya, Uganda, Malawi and Mozambique.

Table 1. Seed iron and zinc concentration[1] (in ppm) and seed yield (in kg/ha) for NUA35 and NUA56 grown over 15 sites in Latin America.

Location (Deparment, Country)	Seasons	NUA 35				NUA 56			
		Iron (ppm)		Zinc (ppm)		Iron (ppm)		Zinc (ppm)	
		Avg.	CV	Avg.	CV	Avg.	CV	Avg.	CV
A. Ibañez (Santa Cruz,Bolivia)	1	99	-	41	-	95	-	34	-
San Juan (Santa Cruz, Bolivia)	1	101	-	34	-	100	-	30	-
Darién (Valle, Colombia)	6	69	0.16	28	0.12	61	0.18	25	0.15
Palmira (Valle, Colombia)	4	70	0.16	29	0.11	75	0.22	27	0.12
Yotoco (Valle, Colombia)	1	71	-	43	-	-	-	-	-
Vijes (Valle, Colombia)	1	53	-	31	-	-	-	-	-
Sandoná (Nariño, Colombia)	1	83	0.02	33	0	82	0.09	36	0.16
Yacuanquer (Nariño, Colombia)	2	83	0.17	37	0.12	112	0.03	43	0.03
Consacá (Nariño, Colombia)	3	67	0.23	30	0.23	81	0.11	36	0.03
Popayán (Cauca, Colombia)	5	73	0.11	30	0.14	67	0.13	25	0.19
Quilichao (Cauca, Colombia)	3	64	0.23	29	0.18	78	0.12	29	0.19
Caldono (Cauca, Colombia)	2	79	0.14	35	0.04	-	-	-	-
Puriscal (San Jose, Costa Rica)	1	70	-	34	-	84	-	34	-
Quesada (Jutiapa, Guatemala)	1	95	0.08	38	0.13	89	0.09	39	0.14
Chinantenango (Guatemala)	1	92	0.16	39	0.19	75	0.11	32	0.18
Average across sites		76	0.14	34	0.12	81	0.13	33	0.15

1/ Iron and zinc content were determined by atomic absorption spectrophotometry by the CIAT analytical services lab. To avoid variability seed for each experiment was hand harvested and processed to avoid contamination and then shipped to CIAT for analysis where seed mineral content was evaluated by grinding 4 g of grain from each sample into a fine powder using a modified Retsch mill with a teflon capsule chamber and zirconium grinding balls. Powder was transferred to 25 ml plastic tubes and analyzed for both iron and zinc concentration measured in parts per million (ppm) with a wet digestion method.

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2008 FINANCIAL STATEMENT
BEAN IMPROVEMENT COOPERATIVE

BALANCE ON HAND January 1, 2008		**$11,722.36**
INCOME		
2008 Dues	2,952.00	
2008 Dues CD	565.00	
Back Issues	30.00	
Bic Meeting	6,817.79	
Bank Interest/Setup Deposits	316.95	
TOTAL INCOME	$10,681.74	
EXPENSE		
Labor Charges	1,170.00	
Postage, Copy Charges and Office Supplies	4.239.09	
Printing – Volume 51	2,033.71	
Bank Charges	119.00	
TOTAL EXPENSE	$ 7,571.80	
BALANCE ON HAND December 31, 2008		**$ 14,832.30**