*ANNUAL REPORT OF THE*

# BEAN IMPROVEMENT COOPERATIVE

BIC

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

**VOLUME 54**
**2011**


# THE LIV

Report of The

## BEAN IMPROVEMENT COOPERATIVE

No. 54 March 2011

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## Coordinating Committee



Please address correspondence about BIC membership and BIC annual reports to:

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

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

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## RESEARCH PAPERS OF 2011

**Cover**: Climbing bean trial in Arusha, Tanzania. Photo courtesy of Jim Myers.

# THE 54th 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-sixth Biennial Meeting from October 30 through November 4, 2011 at the Verdanza Hotel in San Juan, Puerto Rico, near the Airport (SJU). The local organizers are Jim Beaver, Tim Porch, and Mildred Zapata. In addition, the associated meetings with our colleagues in the North American Pulse Improvement Association (NAPIA), Phaseolus Crop Germplasm Committee, BIC Genetics Committee and the Regional W-2150 Committee are scheduled. A field trip to visit dry bean trials is planned for November 3rd. Please refer to the information provided by the local organizing committee in the current report, and look for additional information and updates posted on the BIC web site www.css.msu.edu/bic. A call for abstracts will be posted on line. Please review the call for nominations for the **BIC Meritorious Service Award** and the **BIC Achievement Award**, and forward your nominations to the Awards Committee Chairperson, Howard Schwartz by May 1, 2011. We will continue to recognize our founding members through the **Frazier-Zaumeyer Distinguished Lectureship**. The Lectureship will be awarded at the meeting in Puerto Rico 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.

The BIC website continues to be maintained at Michigan State University under the direction of Dr. James Kelly. A smooth transition for movement of the location for the BIC website from MSU to USDA-ARS in Prosser, WA, has not yet occurred, in part, because of a general lack of IT support at the new location. The BIC recognizes this continued support of Dr. Kelly for maintaining the website, and for his mentorship of the new BIC President. Note that some Research Technology sections on the website have been updated while others await new contributions. The goal for this Research section is to provide an overview of appropriate techniques for breeding common beans for a particular trait, identify cultivars and breeding lines that can be used as sources of resistance for a particular stress, and to provide references where researchers can obtain more detailed information. The traits include angular leaf spot, anthracnose, BCMV and BCMNV, trait scales, BGYMV, root rots, bean processing, Brazilian beans, color scales, common bacterial blight, cooperative dry bean nursery (CDBN), drought, halo bacterial blight, rust, snap beans, web blight, and white mold. Please feel free to contact Dr. Kelly or myself with any new ideas, contributions, or updates for the BIC website.

To reduce mailing costs and expedite communications, the BIC continues to conduct business by email and through postings on the web page. Members are asked to ensure that email addresses are current and to periodically review the web page for information on meetings, deadlines and critical dates. We are always open to new ideas and suggestions to make the BIC a more versatile and effective organization and any thoughts can be shared with members of the coordinating committee. See you in San Juan, Puerto Rico in October…….

**Dr. Phillip Miklas, BIC President**

# BIC COMMITTEE MEMBERSHIP - 1957 to 2011

## Coordinating Committee (approximate year of appointment):

1957 Dean, Enzie, **Frazier* (BIC Coordinator/President)**, McCabe, Zaumeyer
1960 Anderson, Atkin, Dean, Enzie, **Frazier**, McCabe, Zaumeyer
1962 Anderson, Atkin, Dean, **Frazier**, Pierce, Polzak, Zaumeyer
1968 Anderson, **Coyne**, Dean, Jorgensen, Polzak, Zaumeyer
1971 Briggs, **Coyne**, Dean, Jorgensen, Polzak, Zaumeyer
1972 Burke, **Coyne**, Dean, Jorgensen, Kiely, Polzak, Zaumeyer
1974 Ballantyne, Bravo, Burke, **Coyne**, Dickson, Emery, Evans, Kiely, Saettler, Zaumeyer
1977 Ballantyne, Bliss, Coyne, **Dickson**, Emery, Evans, Graham, Meiners, Morris, Saettler, Zaumeyer
1978 Atkin, Ballantyne, Bliss, Coyne, **Dickson**, Graham, Meiners, Morris, Saettler, Sprague
1979 Atkin, Bliss, **Dickson**, Graham, Hagedorn, Meiners, Morris, Sprague, Wallace
1980 Atkin, Bliss, **Dickson**, Hagedorn, Morris, Sprague, Steadman, Temple, Wallace
1982 Atkin, Coyne, **Dickson**, Hagedorn, Sprague, Steadman, Temple, Wallace, Wyatt
1983 Coyne, **Dickson**, Hagedorn, Saettler, Silbernagel, Steadman, Temple, Wallace, Wyatt
1985 Coyne, **Dickson**, Mok, Saettler, Silbernagel, Steadman, Temple, Wallace, Wyatt
1986 Coyne, **Dickson**, Mok, Saettler, Schoonhoven, Schwartz, Silbernagel, Steadman, Wallace
1988 Brick, Dickson, Emery, Magnuson, Roos, **Schwartz**, Singh, Steadman, Uebersax
1992 Dickson, Emery, Grafton, Magnuson, **Schwartz**, Singh, Stavely, Steadman, Uebersax
1994 Antonius, Dickson, Grafton, Magnuson, Park, **Schwartz**, Singh, Stavely, Uebersax
1996 Antonius, Grafton, Park, **Schwartz**, Singh, Stavely, Myers, Kotch, Miklas, Riley
1998 Antonius, Beaver, **Kelly**, Kotch, Miklas, Myers, Park, Riley, Schwartz (ex officio), Singh, Vandenberg
2001 Antonius, Beaver, **Kelly**, Kotch, Miklas, Myers, Park, Riley, de Ron, Schwartz (ex officio), Vandenberg
2003 Beaver, **Kelly**, Kmiecik, Kurowski, Miklas, Myers, Park, Riley, de Ron, Schwartz (ex officio), Vandenberg
2006 Beaver, **Kelly**, Kmiecik, Miklas, Myers, Park, Riley, de Ron, Schwartz (ex officio), Shellenberger, Vandenberg
2008 Beaver, **Kelly**, Kmiecik, Miklas, Myers, Pauls, Riley, de Ron, Schwartz (ex officio), Shellenberger, Vandenberg
2010 Beaver, Kelly (ex officio), Kmiecik, **Miklas**, Myers, Pauls, Riley, de Ron, Schwartz, Shellenberger, Vandenberg

## 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
2010 Hosfield, Schwartz, Singh

## Genetics Committee

**2008**: Tim Porch (Chair), Kirstin Bett, Matthew Blair, Paul Gepts, Phil McClean, Phil Miklas, Carlos Urrea, Molly Welsh (ex officio).
**2010**: Tim Porch (Chair), Kirstin Bett, Matthew Blair, Paul Gepts, Phil McClean, Phil Miklas, Carlos Urrea, Molly Welsh (ex officio).
**2011**: Tim Porch (Chair), Kirstin Bett, Matthew Blair, Paul Gepts, James Kelly, Phil McClean, Carlos Urrea, Molly Welsh (ex officio).

**Genetics Committee Minutes**
2011 BIC Meeting, Arizona

| | |
|---|---|
| Meeting location: | Westward Look Hotel, Tucson, Arizona |
| Date: | Feb. 17, 2011 |
| Time: | 1:30 PM to 3:30 PM |

Attendance (not complete)

| | | |
|---|---|---|
| Kirstin Bett | University of Saskatchewan, Saskatoon (by Skype) | Committee member |
| Paul Gepts | University of California, Davis | Committee member |
| Phillip Miklas | USDA/ARS, WA | Committee member |
| Tim Porch | USDA/ARS, PR, Chairperson | Committee member |
| Carlos Urrea | University of Nebraska | Committee member |
| Molly Welsh | USDA/ARS, WA (ex officio) | Committee member |
| Mark Brick | Colorado State University | |
| Phillip Griffiths | Cornell University | |
| James Kelly | Michigan State University | |
| Jim Myers | Oregon State University | |
| Steve Noffsinger | Seneca Foods Corp. | |
| Juan Osorno | North Dakota State University | |
| Talo Pastor-Corrales | USDA/ARS, Beltsville | |
| Jim Steadman | U. of Nebraska | |

## Old Business

1. **Approval of the Genetics Committee meeting minutes from the W1150 Meeting in Ft. Collins, Colorado on Oct 28, 2009**

**Decision**: Minutes approved by those attending.

2. **Recently accepted gene symbols**

For general information of the committee, the gene symbol and QTL symbol accepted by email since the last meeting, *Ur-14* and SY, respectively, were presented. No decision was made.

## New Business

1. **Approval of *Phg-1* gene symbol**
   Additional marker work was completed on the manuscript submitted for review (Colorado meeting 2009) has now been published (Goncalves-Vidigal et al., 2010). The group has provided the evidence requested by the Committee for the map location for *Phg-1* using two additional markers and showed linkage to *Co-1*$^4$. **Decision:** The gene symbol, *Phg-1*, was accepted and will be added to the List of Genes and to the bean map.

2. **New gene symbol for slow-darkening locus** (Kirstin Bett, by Skype)
   The investigators found that there are two genes controling slow darkening with *J* epistatic to *sd*. The Genetics Committee approved the recessive, *sd*, gene symbol based on the evidence in the manuscript (Elsadr et al., in preparation).

3. **Update of the Fusarium wilt gene symbols** (Jim Kelly)
   There are two gene symbols for Fusarium wilt, *Fop-1* and *Fop-2*. Ribeiro and Hagedorn (1979) first described the two genes, *Fop-1* conditioning resistance to a race from Brazil that has since been named race 2 (Woo et al., 1996), while *Fop-2* confers resistance to a race from South Carolina that has since been named race 1. In addition to other races, Mark Brick and Howard Schwartz (Cross et al., 2000) have additional research results to include in this Fusarium wilt story. **Decision**: Leave gene symbols and race designations as they currently are. Request that Jim Kelly work with Mark Brick and Howard Schwartz on clarifying the Fusarium wilt gene/race symbols.

4. **New gene symbols for Angular Leaf Spot (ALS) resistance**
   There are new gene symbols published for ALS (Mahuku et al., 2009) on B4 and B9 and include $Phg_{G5686A}$, $Phg_{G5686B}$, and $Phg_{G5686C}$. **Decision:** These gene symbols, in addition to other gene symbols published by a Brazilian group, will be reviewed by Tim Porch, Jim Kelly, and Talo Pastor-Corrales. A more complete review of the current status of ALS symbols will be presented at the 2011 BIC meeting in Puerto Rico.

5. **Discussion on QTL symbols**
   There are several cases of use of different QTL symbols for the same trait:
   e.g. plant height; HT (core map), PH (recent manuscript).
   **Decision**: A list of QTL symbols will be assembled with the assistance of Paul Gepts. Previously published QTL symbols will be accepted, but new symbols will need to be reviewed by the Genetics Committee before publication.

6. **Membership and Next meeting**
   Jim Kelly will join the Genetics Committee.
   Phil Miklas (current BIC President) will step down as a member of the Genetics Committee.

   The next meeting will be held at the Verdanza Hotel, San Juan, Puerto Rico on Nov 2, 2011.

# RECIPIENTS of BIC MERITORIOUS SERVICE & ACHIEVEMENT AWARDS

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

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

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

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

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

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

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

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

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

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

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

**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 54-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 sixth **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-sixth Anniversary of BIC Biennial Meeting in San Juan, Puerto Rico from October 30 to November, 4, 2011. Please help us select worthy recipients.

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# BIC AWARD NOMINATION

Return by May 1, 2011 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]

# BIC – Bean Improvement Cooperative 2011 Biennial Meeting

## San Juan, Puerto Rico (October 30 – November 4, 2011)

Meeting Dates:

| Date | Day | BIC Events | NAPIA Events |
|---|---|---|---|
| 10/30/2011 | Sunday | Arrival | |
| 10/31/2011 | Monday | BIC Meeting | |
| 11/1/2011 | Tuesday | BIC Meeting and Banquet | |
| 11/2/2011 | Wednesday | W2150, Genetics & Germplasm Committee meetings | Arrival |
| 11/3/2011 | Thursday | Visit to Latin American/Caribbean Regional Bean Nursery at Isabela, PR | NAPIA Meeting |
| 11/4/2011 | Friday | Departure | NAPIA Meeting and Awards Lunch |
| 11/5/2011 | Saturday | | Departure |

**Hotel Information:**

The BIC/NAPIA Meetings and accommodations have been arranged at the Verdanza Hotel.

The Standard room rate is $110 + taxes (~$130/night)
Hotel location and contact information:
Verdanza Hotel, Isla Verde
www.verdanzahotel.com
8020 Tartak Street
Isla Verde Carolina, PR 00979
Telephone: +1 787.253.9000
Toll Free Reservations: +1 800.625.031

Deadline for hotel reservations is: Sept 15, 2011
A credit card is required at time of reservation, but it will not be charged
Reservations can be made through the phone number above or through the Verdanza group website using the following steps:

1. Go to www.verdanzahotel.com
2. Go to Reservation, then Group
3. Enter B10 in “Attendee Login”
4. The reservation information can then be entered.

**Meeting Registration Fees:** Approximately $150 (full) and $100 (for students).

**Customs and Immigration Information:**
**US Citizens**: There are no passports or visas necessary for United States citizens, which mean that US citizens can travel freely in and out of the island without going through immigration or customs. US citizens only need to have some form of official government issued picture identification to enter Puerto Rico such as a current driver's license or a photo-identification card issued to non-drivers by a state's motor vehicles department. For additional information, contact your local U.S. embassy or call the Puerto Rico State Department at (787) 722-2121.

**Citizens of other countries**: Citizens of other countries have the same requirements for entering Puerto Rico as for entering the USA. Please see your local US Embassy for additional information or the US State Department website: http://travel.state.gov/visa/temp/temp_1305.html

**Local Transportation Information:**
The Verdanza Hotel is located a mile from the San Juan airport (SJU) and accessible by taxi (there are no hotel shuttles). The fixed rate from the airport to the hotel is $11.50 in taxi. The luggage rate is $1.00 per piece, excluding hand pieces.

**Contacts:**
Have questions? Please contact local arrangement committee members Dr. James Beaver (787-832-4040 ext 2492; j_beaver@hotmail.com) and Dr. Tim Porch (787-831-3435 ext 254; timothy.porch@ars.usda.gov), or Dr. Phil Miklas – BIC President (509-786-8492; phil.miklas@ars.usda.gov).

# IN MEMORY OF HOMERO AIDAR

We lost a longtime colleague and dear friend, Dr. Homero Aidar. He died suddenly on June 7, 2010 during a meeting soon after he presented the research program and future plan of the newly formed EMBRAPA Research Center for Fishery, Aquiculture and Agriculture Systems in Palmas, Tocantins, Brazil. He was the first Director and founder of the Research Center. Through his exceptional ability he laid the groundwork and created a joint venture between the State Agriculture Secretary, University, and Extension Service in Tocantins. His absence is a great and irreparable loss for us all.

Homero was the second child of Abrahão Bou Aidar and Aydéa de Carvalho Aidar, born on November 9, 1943 in Nova Granada, São Paulo. He graduated from Federal Rural University, Rio de Janeiro in 1965 and earned M.S. in 1975 and Ph.D. in Agronomy in 1978 from University of Viçosa, Minas Gerais.

He joined EMBRAPA Research Center for Rice and Bean Research (CNPAF) in Goiania, Goias in 1978 and was the first Bean Program Leader until 1981. He dedicated his beginning career to intercropping systems with bean as the main crop, commonly practiced by small farmers in Latin America and the Caribbean. As Research Director, he modernized the Research Department of EMGOPA, the agricultural enterprise for the state of Goias from 1983 to 1987. He launched the most successful and venerable cultivar EMGOPA 201 Ouro resistant to six major bean diseases in 1984. He spearheaded the release of large seeded beans, BRS Embaixador and BRS Executivo, new market classes for Brazil. During this period he also initiated research in sub-surface irrigation (i.e., supplying water by raising the water table) in the Formoso region of Goias, far in the North and beyond the traditional bean growing areas of that time. A joint venture between the research centers and the farm equipment industry was promoted, which to this day has been an integrated activity of the National Research Center. From 1989 to 1997 he was Director of CNPAF, where he incessantly pursued research in bean production systems and sub-surface irrigation. His persistence to develop a successful crop production technology in the remote low latitude areas of the North made possible commercial seed production of bean and other crops. For this milestone achievement he was awarded the Honorary Citizenship of the State of Tocantins. He also promoted collaboration with universities and state, national, and international research and development institutions while keeping the coordination of bean research in Brazil at CNPAF. He insisted the free flow of germplasm of national and international origins. This resulted in release of many bean cultivars by CNPAF and state institutions in Brazil with superior yield, disease resistance, and broad adaptation benefiting small and large farmers. He also was a steamroller of integration of annual crops, animal production, and forestry programs. His idealism and conviction for an integrated research and development strategy greatly influenced many scientific communities, students, and business entities, especially in the Center-West region of Brazil.

Homero, the idealist and naturally articulated leader, was a very skilled administrator, research promoter, and developer thus benefiting mankind. He was well ahead of his time. Homero is survived by his wife Cristina and four sons, Abrahão, Severino, Rafael and Alexandre.

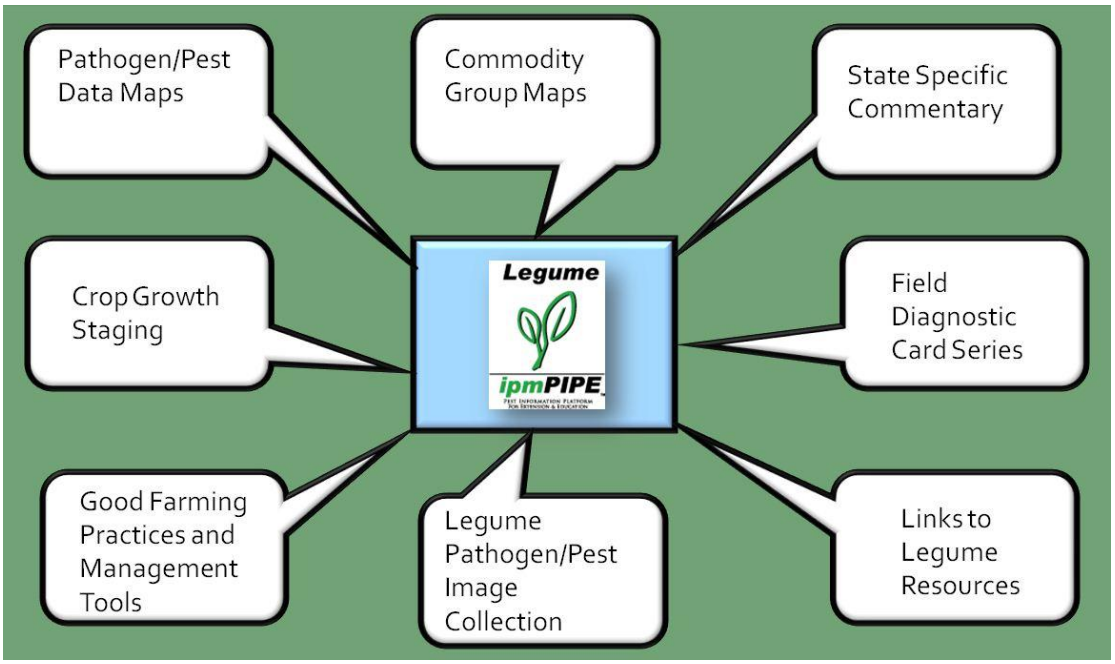
# LEGUME IPMPIPE — UPDATE AND FUTURE PLANS


## H.F. Schwartz[1] and M.A.C. Langham[2]

Regional Project Coordinators: [1]Colorado State University, Dept. of Bioagr. Sci. & Pest Mgmt., Fort Collins, CO; and [2]South Dakota State University, Plant Science Dept., Brookings, SD


**Legume ipmPIPE - Project Update**

The Legume ipmPIPE (Integrated Pest Management Pest Information Platform for Extension and Education) at http://legume.ipmpipe.org provides a dynamic system that combines pathogen/pest information provided by state coordinators into an Information Technology (IT) platform facilitated by ZedX (Penn State) to promote efficient and coordinated IPM decisions through the information and its products (e.g., 20 diagnostic cards) provided to extension educators and stakeholders (Schwartz et al., 2009). This system developed a network of extension educators, researchers and stakeholders who establish sentinel or mobile plots in target legumes to monitor pathogen/pests at research facilities and commercial fields in the U.S. and limited areas of Canada and Mexico. Three groups of legume crops were organized with stakeholder's input. These are: Common bean: fresh and dry beans; Cool-season legumes: fresh and dry peas, lentils, and chickpeas; and Warm-season legumes: cowpeas and lima beans. Four groups of legume diseases and insect pests were selected by legume specialists and industry stakeholders. These include: soybean rust and common rust (Group 1); regionally prevalent diseases such as white mold or common bacterial blight (Group 2); viral diseases such as those caused by *Alfalfa mosaic virus, Bean pod mottle virus, Bean common mosaic virus, Bean yellow mosaic virus, Beet curly top virus, or Cucumber mosaic virus* (Group 3); and soybean aphid and other insects (Group 4).



Specialists enter and update commentary on legume crops, pests, and diseases, scouting and management tools, and provide links to other resources such as crop and pest/disease models, pathogen vector population densities, pesticide recommendations, and other IPM products. Descriptive growth stages for legume crops or other resource links are available for the user as they devise a specific pest management strategy. As specialists update files and displays, ZedX populates the public web site with constantly changing information. Public maps are customizable to the user by legume crop group and/or pathogen/pest group. Users query the map by positioning the cursor over a state/county/site for highlights. The user can access state-specific information by selecting the state, which is then displayed with county boundaries. Specific reporting information and commentary are provided by the specialist for that state.

The frequency of hits to the ipmPIPE web sites (including the Legume ipmPIPE site) by users throughout 2010 was summarized by J. Golod at ZedX. The ipmPIPE site had a monthly average greater than 69,000 hits. As expected, interest climbs during the growing season with above 100,000 hits recorded during July and August. Thus, the ipmPIPE is a well-utilized site, and we anticipate that interest in the Legume ipmPIPE will continue to increase as additional components such as legume marketing are added to the suite of resources available at this one-stop site for stakeholders and specialists across the country.

**Assets and Future Plans**

The greatest asset of the Legume ipmPIPE has always been and remains the outstanding extension specialists, researchers, coordinators, diagnosticians, stakeholders, field workers and others who each year provide "the eyes and feet on the ground" to make this project happen. They are dedicated to the service of the Legume industry, producers, and general public and have been unflagging in their time and devotion. It has forged new linkages with Legume stakeholders and industry enabling the Legume ipmPIPE to be more responsive to their needs. For example recently, weather damage led Legume stake holders to request information on this, and the Legume ipmPIPE published a diagnostic card on Environmental Damage to aid in assessing this effect. Information flow in the ipmPIPE depends on people collecting information, posting data, reporting to others, listening to new information, writing new materials, and educating others in a living bridge between data and stakeholder.

The Legume ipmPIPE projects continued service to the Legume stakeholders and industry through its future planning. In the upcoming months, the project plans to continue its 2010-2011 activities as in recent years and will focus on monitoring and reporting priority diseases and insect pests with timely recommendations on effective IPM strategies. An additional set of 4 diagnostic cards will be produced, and a real-time price discovery tool will be added to the web site. This scalable commodity component will enhance the overall utility and economic value of the ipmPIPE to legume crop stakeholders, and the sustainability of their production and pest management system throughout the United States.

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# DIETARY FIBER CONTENT OF DRY EDIBLE BEANS BASED ON THE 2009.01 CODEX METHOD

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## INTRODUCTION

Dietary fiber has been shown to be an important component in the human diet and related to reducing incidence of the four major chronic diseases, namely heart disease, cancer, obesity, and type 2 diabetes. A comprehensive nine-year study by Park et al. (2011) reported that increased dietary fiber intake lowered the risk of death from cardiovascular, infectious, and respiratory diseases by 24% to 56% in men and by 34% to 59% in women. Fiber intake also showed an inverse association with cancer death in men but not in women. Dry edible beans are known to be an excellent source of dietary fiber; however the definition of dietary fiber has been the subject of debate for decades. Originally the term dietary fiber, in contrast to crude fiber, was used to describe a component of human diet containing "lignin, cellulose, and the hemicelluloses" (Hipsley, 1953). In the early 1970's dietary fiber was defined in greater detail due to emerging interest in the physiologic relationship between fiber and the relationship to health benefits. More recently, a method that was modified and validated by 43 collaborative laboratories was adopted as the first Official Method of Analysis of Dietary Fiber by the Association of Analytical Chemists (AOAC), Method 985.29. This method utilized an enzymatic-gravimetric digestion method to separate digestion-resistant portions in food. Despite broad acceptance of this method, the definition of dietary fiber remained in flux. In 2005, the CODEX Alimentarius Commission met to begin an effort to develop a new definition of dietary fiber that would support nutritional claims. In 2009, the CODEX Committee on Nutrition and Foods for Special Dietary Uses officially adopted a new, more specific method of analysis to support the new definition (McCleary, 2010). Thus, method AOAC 2009.01 was designed to complement the new definition and provide up-to-date and accurate measurements. This method consists of similar enzymatic-gravimetric procedures to the previous AOAC methods, but includes the determination of low molecular weight soluble dietary fiber through use of high performance liquid chromatography (HPLC). HPLC analysis allows quantification of low molecular weight molecules that are present but are too small to be detected by filtration. These molecules can be as small as 3-10 monomeric unites in length, such as the oligosaccharides raffinose, stachyose, and verbascose.

## MATERIALS AND METHODS

Seed from 33 varieties of common dry bean (*phaseolus vulgaris L.*) were obtained through the Common Bean Coordinated Agricultural Project (Bean CAP). These seeds were grown under greenhouse conditions during the winter 2009-2010. Thirty three entries grown in Michigan were evaluated for modified total fiber content (MTFC), and 16 common entries grown in Michigan and Idaho, were evaluated to compare environmental effects. Modified fiber content as used in this report includes total dietary fiber according to AOAC 2009.01 except the oligosaccharides raffinose, stachyose, and verbascose that are present in the final filtrate. Oligosaccharides were not measured in this study because the HPLC analysis on the final filtrate has not been conducted to date. Consequently, the MTFC reported herein will be approximately 3 to 4 % lower than total dietary fiber expected using the entire CODEX 2009.01 AOAC method. This research was supported by USDA-NIFA Bean CAP.

## RESULTS

Modified total fiber content varied from 23.2 to 15.5% (SE ± 0.7%) among the 33 entries grown in one location. When MTFC was analyzed among the 16 entries grown at two locations, mean content varied from 21.6 to 16.6 % (Table 1). Mean MTFC was higher for seed produced in Michigan compared to Idaho (19.7 *vs* 17.8%; P<0.05), suggesting that the environment had an influence on MTFC. Bean market classes differed for MTDF. In general, Andean beans were higher than Mesoamerican navy or black beans. These results suggest that the genetic diversity exists for MTFC and may be related to domestication events. Furthermore, the levels of genetic diversity seen in these results suggest that plant breeders should be able to modify fiber content by selection.

**Table 1.** Mean modified total fiber (MTDF) content among 16 dry bean lines for seed produced in Michigan and Idaho.

| **Bean Class** | **Variety** | **% MTDF[1]** |
|---|---|---|
| Pink | CDC Rosalee | 21.6[a] |
| Pink | PK915 | 21.0[ab] |
| Carioca | A801 | 20.6[ab] |
| Black | Eclipse | 19.8[bc] |
| Black | Blackjack | 19.8[bc] |
| Pink | UCD 9634 | 19.7[bc] |
| Flor de Mayo | UCD 9623 | 19.4[bc] |
| Black | Midnight | 18.7[cd] |
| Black | Black Knight | 18.6[cd] |
| Navy | Crestwood | 17.8[de] |
| Navy | Vista | 17.8[de] |
| Navy | Seabiskit | 17.7[de] |
| Navy | Avalanche | 17.3[de] |
| Navy | Midland | 17.3[de] |
| Small Red | CENTA Pupil | 16.9[e] |
| Great Northern | HY4181 | 16.6[e] |

[1]Values in the column followed with the same letter are not significantly different with REGWQ multiple range test at P < 0.05. n=4.

**Table 2.** Mean percentage of modified total dietary fiber (MTDF) among common bean market class.

| Bean Class | No. Genotypes[1] | % MTDF[2] |
|---|---|---|
| Andean | 6 | 20.9 ± 0.7 |
| Pink | 7 | 20.8 ± 0.9 |
| Pinto | 4 | 20.2 ± 0.3 |
| Small Red | 5 | 20.1 ± 3.1 |
| Black | 8 | 19.2 ± 2.0 |
| Great Northern | 5 | 19.0 ± 2.2 |
| Navy | 10 | 17.6 ± 1.6 |

[1]Number of genotypes by bean class
[2]Mean ± standard deviation

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# COMPARISON OF BREEDING METHODS FOR FIBER CONTENT IN COMMON BEANS

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The breeding program of common beans in Brazil is focused on the development of cultivars more adapted to weather variations, maintaining crop yield aggregated to other characteristics of interest such as fiber content, an important component in human diet, providing beneficial effects on health. Investigation of the available genetic variability as well as the quantification of fiber content in bean genotypes in Brazil are important and necessary, considering the low availability of information on this subject (Londero, 2008). The objective of this work was to compare raw fiber content of families obtained by different breeding methods.

Families were obtained from crosses between genitors CNFC 7812 and CNFC 7829, which are contrasting for fiber content, and conducted by three breeding methods: *bulk* ($F_{5:8}$), *bulk* within families $F_2$ ($F_{2:8}$) and Single Seed Descent - SSD ($F_{5:8}$) up to generation $F_8$. The experimental design was a completely randomized block with three replicates arranged in plots with four meter long rows spaced by 0.5 m and 15 seed/meter. The treatments were 45 families (F), 15 by method, 2 controls (C) and 2 genitors (G). Trials were conducted in four locations: Anapolis-GO (wet season 2009); Ponta Grossa-PR (wet season 2009 and dry season 2010); and Lavras-MG (dry season 2010). Methodology used to determine raw fiber content was the acid-base digestion, using the fiber determinator Tecnal® model TE-149. Individual and joint analyses of variance were performed using family mean values for raw fiber content through program Genes (Cruz 2006).

Family x Environment interaction (F x E) was observed in the joint analysis (Table 1), indicating different family behavior according to the four evaluated locations. Heritability ($h^2$) for this trait was 47. 61%, considered satisfactory to obtain selection gains. The largest $h^2$ estimate (64.47%) was obtained for the *bulk* method followed by *bulk* method within families (41.80%). In the SSD method, $h^2$ was equal to zero, indicating that this method presents difficulties in keeping genetic gain in time, as opposed by the bulk method the assures higher genetic gains over time. Families conducted by *bulk* ($P \leq 0.01$) and *bulk* within families ($P \leq 0.055$) showed significant differences for raw fiber content. SSD method did not show significant differences ($P \leq 0.05$). There were no significant differences between genitors, constrasting with values previously obtained, showing the contrast between them, this may indicate the existence of interaction Genitors x Environment. Also there were no significant differences between controls and methods, as well as controls *vs* families and genitors *vs* families. The SSD method provided the largest number of familes (eight) among the 20 best and the smaller number among the 20 worst families obtained. Looking at the general mean value obtained for raw fiber content among families (4.61%), the SSD method, undoubtedly, was the most efficient to generate superior families as well as the largest number of families surpassing the best genitor mean (4.46%) (Table 2). However, the low $h_2$ values obtained indicate its difficulty to maintain selection gains over time.

**Table 1.** Summary of the joint analysis of variance and analysis of variance of the three method evaluated for raw fiber content (%) of families $F_8$ evaluated in Anápolis/GO (wet season 2009), Ponta Grossa/PR (wet season 2009 and dry season 2010), and Lavras/MG (dry season 2010).

| Source of variation | Degree of freedom | Mean square | |
|---|---|---|---|
| | | Raw fiber content | P-value |
| Environment (E) | 3 | 0.674* | _ |
| Treatment (T) | 48 | 0.169* | _ |
| *Bulk d.$F_2$ Families* | 14 | 0.153 | 0.055 |
| *Bulk Families ($F_{5:8}$)* | 14 | 0.250 | 0.001 |
| SSD Families *($F_{5:8}$)* | 14 | 0.084 | 0.509 |
| Controls (C) | 1 | 0.245 | 0.099 |
| Methods (M) | 2 | 0.007 | 0.923 |
| Genitors (G) | 1 | 0.020 | 0.639 |
| C *vs* F | 1 | 0.066 | 0.390 |
| G *vs* F | 1 | 0.150 | 0.196 |
| T x E | 144 | 0.178* | _ |
| Efetive error | 192 | 0.089 | _ |
| Mean | | 4.61 | |
| $h^2$ (%) | | 47.61(Bd.$F_2$: 41.80; *Bulk*: 64.47; SSD: 0) | |
| $CV_g$ (%) | | 2.17 | |
| $CV_g/CV_e$ | | 0.33 | |

Where: $h^2$: heritability; $CV_e$: weather coefficient of variation; $CV_g$: genetic coefficient of variation; *F test at 5% probability.

**Table 2.** Number of families in each method overcame the overall mean and the best genitor mean for raw fiber content.

| Method | Number of superior families | |
|---|---|---|
| | Overall mean | Best Genitor mean |
| *Bulk* $F_{2:8}$ | 8 | 12 |
| *Bulk* | 8 | 11 |
| SSD | 11 | 14 |

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# PROTEIN CONTENT AND CANNING QUALITY OF HISTORICALLY IMPORTANT NAVY BEAN VARIETIES IN MICHIGAN

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## INTRODUCTION

Navy beans are one of the most important dry bean market classes in the U.S, and from the late 1800's until 2005 they have been the most planted market class in Michigan (USDA-ERS., 2011; Bingen and Siyengo, 2002). The Michigan State College Agricultural Experiment Station established the first dry bean breeding program in the country in 1907 (Anderson, 1982). In 1915 Robust was the first navy bean to be released. This variety was selected in 1907 "from a miscellaneous group of bean plants" and was the most popular variety in MI from 1915 to1935 (Down and Thayer, 1938; Hedrick, 1931). Following the release of Robust, a crossing program was begun to improve agronomic traits and as a result, in 1938 Michelite was the first bred variety to be released (Down and Thayer, 1938). Since the early days of bean breeding in the US, disease resistance, yield, and seed coat color/integrity have been described as essential traits for breeders to consider (Hedrick, 1931). Canning quality has also been an important consideration for success of a variety, as approximately 90% of the navy beans in the U.S. are sold as canned product (USDA-ERS, 2011). Plant architecture and days to maturity have also received high priority by breeders, however the preferred architectural type has changed over the years as cropping systems and harvest methods have changed (Kelly et al., 1998). The change in plant architecture of navy bean varieties is reflected in some of the most popular varieties in the US from 1915 to present day (Table 1). One trait that is not a major focus of bean breeding programs is seed protein content in spite of the fact that beans are largely considered a good source of protein (Leterme, 2002). The objective of this research was to determine if seed protein levels and canning quality are similar in select navy beans varieties of commercial importance released between 1915 and 2008.

## MATERIALS AND METHODS

Five varieties of navy beans were planted at the Saginaw Valley Research and Extension Center near Frankenmuth, MI in June 2010. Four replications of each variety were planted in 4-row plots 6.4 m in length with 0.5 m row spacing in a randomized complete block design. Following harvest, seed from each variety was cleaned, freeze dried, and ground into a fine powder. Ground samples were sent to A & L Great Lakes Laboratories in Fort Wayne, IN for nitrogen content. Crude protein (%) was estimated by multiplying % total N by a conversion factor (6.25). Three replications of each variety were canned according to Hosfield et al. 1984.Visual appeal of the canned beans were subjectively rated on a scale of 1 to 7 where one is least desirable and 7 is most desirable and takes into account whole bean integrity, uniformity of size and brine color (Wright and Kelly, 2011). Color of canned bean samples was measured with a Hunter Lab Colorimeter Lab scan XE. The L value measures white/black level of a sample with 0 being black and 100 white. A measure of canned bean texture was measured with a Kramer Shear Press where higher values indicate firmer beans.

## RESULTS AND DISCUSSION

There was significant variability for percent protein between the five navy bean varieties of historical commercial importance, but there was not a clear relationship with plant architecture or release date (Table 1). Canning quality, measured by overall appearance was similar among these

varieties. The color of the canned bean was also similar. This uniformity in canning quality is probably an indication of the importance of acceptable canning quality for a commercially successful navy bean variety. . There was some variability for canned bean texture (Table 1), though each of the varieties fall within in the range of soft texture.

**Table 1.** Characterization of plant growth habit and seed characteristics of 5 navy bean varieties grown in Frankenmuth, MI in 2010.

| Variety | Release date | Plant architecture type | Maturity range (days) | Protein % | Canning score (1-7) | Color (L value) | Texture (kg force/100g) |
|---|---|---|---|---|---|---|---|
| **Robust** | 1915 | III | 99 | 20.6 | 3.1 | 53.1 | 44 |
| **Michelite** | 1938 | III | 88-95 | 21.8 | 3.3 | 54.9 | 50 |
| **Sanilac** | 1956 | I | 84-88 | 20.2 | 3.5 | 55.2 | 54 |
| **Vista** | 1990 | II | 87-100 | 21.2 | 3.1 | 54.1 | 44 |
| **Medalist** | 2008 | II | 86-102 | 18.8 | 3.4 | 54.5 | 53 |
| **Mean** | | | | 20.5 | 3.3 | 54.4 | 49 |
| **CV** | | | | 5.6 | 15.5 | 1.4 | 11.1 |
| **LSD** | | | | 1.7 | 0.93 | 1.4 | 10 |

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# PROTEIN CONTENT IN GENOTYPES OF COMMON BLACK BEANS

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Common beans commercial type black are the second most consumed in Brazil, with 20% market share (Del Peloso & Melo, 2005). Brazilian breeding programs have been supplying farmers with improved cultivars to increase yield. Besides agronomic characteristics, other traits related to nutritional quality are becoming important. Among them, bean protein content assumes prominent importance considering that common beans are the main vegetable protein source in the Brazilian diet. Therefore it is highly desirable to determine bean protein content, comparing it to adopted standards, during genotype evaluation to aggregate value to new cultivars. Taking into account that the final evaluation of common beans commercial type black, developed by the breeding program of the Embrapa Arroz e Feijão is conducted in a large number of locations and environments, there is a possibility of measuring the protein content and to verify the existence of interaction genotypes x environments for that characteristic. Based on the above, the objective of this work was to evaluate the protein content of common black beans genotypes and to verify the presence of genotypes x environments for that characteristic.

In 2009, trials were conducted in four environments: Inhumas-GO, dry season; Ponta Grossa-PR, dry season; Santo Antônio de Goiás-GO, winter; and Porangatu-GO, winter. The experimental design was a completely randomized block arranged in four meter long four row plots, with two replicates. Each trial was composed of 14 genotypes of black common beans (Table 1). The protein content was measured in bean samples collected from the two central rows. Raw protein content (PC %) was calculated multiplying nitrogen content by factor 6.25. Total N content was determined by the sulfuric digestion method, according to Sarruge and Haag (1974). Data were submitted to the analysis of variance, and the Scott Knott test at 10% probability was applied for mean comparison.

Joint analysis showed adequate experimental precision (CV=5.9%) and significant differences ($P<0.01$) among environments. There were not significant differences observed for treatments as well as for genotypes x environments interaction, suggesting that genotypes evaluated in those environments had the similar protein contents. The average protein content was 22.3%, varying from 17.9% to 24.4%, depending upon environment (Table 1). The significant differences observed among environment means may be related to environmental conditions (soil; temperature; moisture; and rain fall) during trial conduction. Those differences indicate that bean protein content is highly affected by weather conditions. The environment producing beans with the highest protein content were Santo Antônio de Goiás/winter (24.4%) and Ponta Grossa, dry season (23.9%), while the lowest values were observed in Porangatu/winter (17.9%).

Protein content of the genotypes varied from 21.3 to 22.9%, absolute values; representing a relative difference of approximately 8% between the highest and the lowest values, showing small genetic variability for that trait among the genotypes evaluated. It is important to mention that all genotypes evaluated came from 12 distinct crosses, suggesting that the difference observed in the protein content is due to the small variability found for that characteristic. Farinelli & Lemos (2010) also found low variability for protein content in common beans when working with a number of various commercial genotypes (black and carioca). Results obtained in this work indicate that the highest protein content found in black beans was obtained especially due to the environmental conditions where the genotypes were cultivated. However, further evaluations should be carried out to confirm these results.

**Table 1.** Protein contents (PC) of 14 genotypes of common black beans evaluated in four environments in Brazil, in 2009.

| Genotypes | PC % | Inhumas/Dry | Ponta Grossa/Dry | Santo A. de Goiás/Winter | Porangatu/ Winter |
|---|---|---|---|---|---|
| CNFP 11978 | 22.0 | 21.5 | 24.0 | 24.5 | 18.0 |
| CNFP 11991 | 22.0 | 23.0 | 22.0 | 25.5 | 17.5 |
| BRS Esplendor | 22.1 | 22.0 | 24.0 | 25.0 | 17.5 |
| IPR Uirapuru | 21.3 | 22.5 | 23.5 | 23.0 | 16.0 |
| CNFP 11995 | 22.3 | 22.5 | 24.5 | 24.5 | 17.5 |
| CNFP 11983 | 22.4 | 23.0 | 24.0 | 23.5 | 19.0 |
| BRS 7762 Supremo | 22.5 | 22.0 | 23.5 | 26.0 | 18.5 |
| CNFP 11985 | 22.6 | 24.5 | 23.0 | 25.0 | 18.0 |
| CNFP 11973 | 21.8 | 22.0 | 24.0 | 23.0 | 18.0 |
| CNFP 11976 | 21.8 | 22.0 | 23.5 | 23.5 | 18.0 |
| BRS Campeiro | 22.8 | 24.0 | 25.0 | 24.0 | 18.0 |
| CNFP 11979 | 22.8 | 22.5 | 24.5 | 25.0 | 19.0 |
| CNFP 11984 | 22.9 | 24.5 | 24.0 | 25.0 | 18.0 |
| CNFP 11994 | 22.9 | 24.0 | 25.0 | 24.5 | 18.0 |
| Mean | 22.3 | 22.9 b | 23.9 a | 24.4 a | 17.9 c |

[1]Means followed by the same letter do not differ among them by the Scott Knott test at 10% probability.

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SARRUGE, J.R.; HAAG, H.P. **Análises químicas em plantas**. Piracicaba: ESALQ, Departamento de Química, 1974. 56p.

# PROTEIN CONTENT IN COMMON BEAN CARIOCA TYPE GENOTYPES EVALUATED IN VARIOUS ENVIRONMENTS

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Common bean commercial type carioca stands out among the many beans consumed in Brazil, with 70% market share (Del Peloso & Melo, 2005). Brazilian breeding programs have been supplying farmers with improved cultivars to increase yield. Besides agronomic characteristics, other traits related to nutritional quality are becoming important. Among them, bean protein content assumes prominent importance considering that common beans are the main vegetable protein source in the Brazilian diet. Therefore it is highly desirable to determine bean protein content, comparing it to adopted standards, during genotype evaluation to aggregate value to new cultivars. Taking into account that the final evaluation of common beans commercial type carioca, developed by the breeding program of the Embrapa Arroz e Feijão is conducted in a large number of locations and environments, there is a possibility of measuring the protein content and to verify the existence of interaction genotypes x environments for that characteristic. Based on the above, the objective of this work was to determine the protein content of common beans carioca type and to verify the presence of interaction genotypes x environments for those traits.

In 2009 four trials were conducted in the state of Goiás: Inhumas, dry season (AMB1); Santo Antônio de Goiás, winter (AMB2); Porangatu, winter (AMB3); and Senador Canedo, winter (AMB4). The experimental design was a completely randomized block arranged in four meter long four row plots, with two replicates. Each trial was composed of 17 genotypes of common beans carioca commercial type (Table 1). Protein content was determined in samples collected from the two central rows. Analyses were performed in grinded beans, using the sulfuric digestion method, where the nitrogen determined was converted to raw protein multiplied by factor 6.25. Data were submitted to the analysis of variance, and the Scott Knott test at 10% probability applied for mean comparison.

Joint analysis showed adequate experimental precision (CV=4.9%) and significant differences ($P<0.01$) among genotypes and among environments. Genotypes x environments interaction was not significant, showing no change in the relative performance of the genotypes. Therefore, genotypes with the highest protein content were the same in the various environments tested. The general average was 21.1%, varying from 18.3 to 23.7%, depending upon ambient (Table 1). The significant variability observed in protein content of beans from different environments may be related to location specific characteristics (soil; climate; moisture; rainfall). The environment producing beans with the highest protein content was Santo Antônio de Goiás/ winter (23.7%), while the lowest values were observed in Porangatu/winter (18.3%).

Although the analysis of variance was significant among genotypes, differences among them were not very impressive, since the difference between the lowest and the medium average value genotypes (respectively 20.3% and 22.1%) was only 1.8%, representing a relative difference around 9%.

The control genotypes, BRS 9435 Cometa and Pérola, showed the highest protein content values, respectively 22.1% and 22.0% (Table 1). Other six lines were grouped by the mean comparison test with those controls, suggesting they present similar protein content of cultivars already released. None of the lines evaluated surpassed the best controls. BRS Estilo and IPR Jurití were grouped with those of lower protein content, together with other seven lines.

**Table 1.** Protein content means (PC) of 17 genotypes of common beans carioca type evaluated in four environments in Brazil, in 2009.

| Genotype | PC % | AMB1 | AMB2 | AMB3 | AMB4 |
|---|---|---|---|---|---|
| BRS 9435 COMETA | 22.1 a | 22.5 | 26.0 | 19.5 | 20.5 |
| PEROLA | 22.0 a | 23.5 | 24.0 | 19.5 | 21.0 |
| CNFC 11951 | 21.9 a | 24.0 | 24.0 | 19.0 | 20.5 |
| CNFC 11948 | 21.6 a | 23.5 | 23.0 | 20.0 | 20.0 |
| CNFC 11962 | 21.5 a | 23.5 | 25.0 | 18.0 | 19.5 |
| CNFC 11952 | 21.4 a | 24.0 | 23.5 | 18.0 | 20.0 |
| CNFC 10429 | 21.3 a | 22.0 | 25.5 | 18.5 | 19.0 |
| CNFC 11946 | 21.3 a | 21.5 | 24.0 | 18.5 | 21.0 |
| CNFC 11956 | 21.0 b | 23.0 | 24.0 | 17.5 | 19.5 |
| CNFC 11959 | 21.0 b | 22.5 | 23.5 | 18.5 | 19.5 |
| CNFC 11945 | 20.8 b | 22.5 | 23.0 | 18.0 | 19.5 |
| BRS ESTILO | 20.6 b | 22.5 | 23.0 | 16.5 | 20.5 |
| CNFC 11944 | 20.6 b | 22.0 | 24.0 | 17.5 | 19.0 |
| CNFC 11953 | 20.6 b | 23.5 | 22.5 | 18.5 | 18.0 |
| CNFC 11966 | 20.5 b | 22.0 | 22.0 | 18.5 | 19.5 |
| CNFC 11954 | 20.4 b | 21.5 | 22.5 | 18.0 | 19.5 |
| IPR JURITI | 20.3 b | 22.5 | 23.0 | 17.5 | 18.0 |
| MÉDIA | 21.1 | 22.7 b | 23.7 a | 18.3 c | 19.7 d |

[1]Means followed by the same letter do not differ among them by the Scott Knott test at 10% probability.

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# UTILIZATION OF NEAR INFRARED SPECTROPHOTOMETRY (NIRS) ANALYSIS FOR EVALUATION OF MINERAL CONTENT IN ANDEAN BEAN SAMPLES

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**INTRODUCTION:** Currently in biofortification activities, the Andean bean breeding component relies heavily on atomic absorption spectrophotometry (AAS) for mineral analysis. As these analyses are time consuming and expensive we are interested in applying near infrared reflectance scanning (NIRS) as a replacement method. We have been using the same ground samples of bean flour for NIRS as we have used in AAS to validate whether this technique can work across a range of seed types (from medium to large sized seed, with various seed coat colors and from various commercial classes) and produce reliable results equivalent to AAS. Both bush and climbing bean samples were used.

**MATERIALS AND METHODS:** Seed tissue was ground in Teflon chambers with zirconium grinding balls. Scans were read in the monochrome NIRS6500, FOSS-NIRS equipment found in the Forages lab. Iron and zinc values were estimated based on six equations or formulae (Beanmeal, Beanmeal st, Bmeal 508 Bmeal 508 st, Bmealand, Bmealand st) of which three formulae were standardized and three were not-standardized. In each case humidity was also calculated. The control AAS values were obtained from the CIAT analytical services lab.

**RESULTS AND DISCUSSION:** A total of 3,429 samples were evaluated with Bean meal of which more analysis was applied with the other five formulae for 328 bush bean samples from Darien 2008[a] and 284 bush and climbing bean samples from 2007[a]. The overall correlation coefficients among AAS and the different NIRS formulae results for the 612 samples is shown in table 1. Given that correlation coefficients between AAS and NIRS were generally low (below r=0.2) or negative, subsamples from the different ranges of AAS results were evaluated for correlation coefficients. These subsegments of the range were from 20 to 50 ppm, 50 to 70 ppm and 70 to 104 ppm iron (Figure 1 a) and had n=305, n=239 and n=68 sample numbers, respectively. Correlations and their significance improved slightly with the first subsegment and the first three formulae (Beanmeal, Beanmeal st and Bmeal 508). Prediction rates for the formulae versus AAS however were low. An analysis through Principal Components was used to evaluate what influenced the low correlations considering the variables of 1) type of source material was used by location and growth habit (bush Darien, climber Darien, bush Palmira, climber Popayan); 2) what type of cross it was derived from (simple cross, triple cross, double cross or backcross) and 3) the humidity level of the sample. In addition new formulae were evaluated using Winisi III project manager v 1.60. software for evaluating different parts of the NIRS spectra and interpreting the data through additional statistical methods such as PCA, partial regression, SNV, Detrent, MSC and first or second derivatives (Figure 1 b).

**CONCLUSIONS:** NIRS results was influenced by many factors but predominant among these was the humidity level in the sample. Overall the predictive value of iron concentration by NIRS in the Andean samples was low. The noise in the analysis could be reduced by several mathematical treatments to the NIRS spectra using PCA in Winisi where correlations of up to r=0.6 were obtained.

However there was still some GxE influence that affected the value of NIRS for each season's harvest. Expanding on these results another set of 1394 samples were evaluated from the climbing bean breeding program and analysis of prediction rates and correlations are pending. It is obvious that NIRS will remain experimental in Andeans until better formulae can be obtained for each seed class and for the major experimental sites in use for the breeding program.

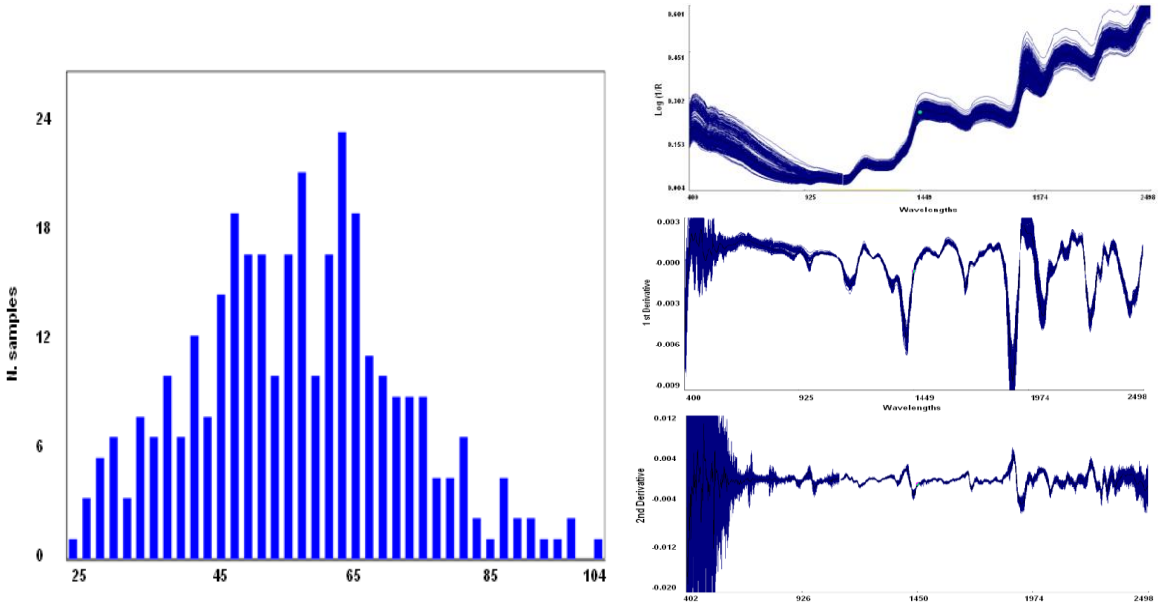


**Figure 1.** Distribution of AAS iron values for the samples analyzed (a, to left) and NIRS sample spectra under standard and derived formulae (b, to right).

**Table 1.** Correlation coefficients between AAS and NIRS results for iron.

| | Fe A.A | beanmeal | beanmeal st | bmeal 508 | bmeal 508 st | bmealand | bmealand st |
|---|---|---|---|---|---|---|---|
| Fe A.A<br>Fe A.A | 100.000 | -0.05865<br>0.1473 | 0.05599<br>0.1666 | 0.22686<br><.0001 | -0.25293<br><.0001 | -0.21967<br><.0001 | -0.18519<br><.0001 |
| beanmeal<br>beanmeal | -0.05865<br>0.1473 | 100.000 | 0.91034<br><.0001 | -0.49694<br><.0001 | 0.56971<br><.0001 | 0.63891<br><.0001 | 0.74345<br><.0001 |
| beanmeal st<br>beanmeal st | 0.05599<br>0.1666 | 0.91034<br><.0001 | 100.000 | -0.31037<br><.0001 | 0.38148<br><.0001 | 0.58190<br><.0001 | 0.67755<br><.0001 |
| bmeal 508<br>bmeal 508 | 0.22686<br><.0001 | -0.49694<br><.0001 | -0.31037<br><.0001 | 100.000 | -0.93142<br><.0001 | -0.58552<br><.0001 | -0.60925<br><.0001 |
| bmeal 508 st<br>bmeal 508 st | -0.25293<br><.0001 | 0.56971<br><.0001 | 0.38148<br><.0001 | -0.93142<br><.0001 | 100.000 | 0.60051<br><.0001 | 0.69865<br><.0001 |
| bmealand<br>bmealand | -0.21967<br><.0001 | 0.63891<br><.0001 | 0.58190<br><.0001 | -0.58552<br><.0001 | 0.60051<br><.0001 | 100.000 | 0.82748<br><.0001 |
| bmealand st<br>bmealand st | -0.18519<br><.0001 | 0.74345<br><.0001 | 0.67755<br><.0001 | -0.60925<br><.0001 | 0.69865<br><.0001 | 0.82748<br><.0001 | 100.000 |

# CHARACTERIZATION AND EXPRESSION ANALYSIS OF A DEHYDRIN GENE IN *PHASEOLUS VULGARIS*

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Zinc is an essential micronutrient for human health and is often found in insufficient quantities in the diet. Increasing the zinc density in staple crops such as dry bean is a strategy to address human zinc deficiency. Characterization of genes involved in zinc transport and remobilization to the seed is a useful step to effectively increase seed zinc content. Recent molecular studies have identified a number of gene families involved in metal transport and homeostasis within the plant. One such gene is dehydrin, which codes for certain late embryogenesis abundant (LEA) proteins (Close, 1996). The exact function of these proteins is still unclear but numerous studies have demonstrated the induction of dehydrins by drought stress. Dehydrins were also recently shown to facilitate phloem-mediated long distance transport of micronutrients (Zhang et al. 2006). The objective of this research was to characterize this gene and determine its expression level in two navy bean lines, Voyager and Albion.

## MATERIALS AND METHODS

**RNA isolation and cDNA synthesis**: total RNA was extracted from pod tissue of two navy bean varieties with contrasting seed zinc concentration, Voyager (high Zn) and Albion (low Zn). The pods were harvested 15 days after flowering using the RNA isolation NucleoSpin$^{R}$ Macherey Nagel. The concentration was measure by Quan-IT$^{TM}$ Ribogreen RNA (Invitrogen). The cDNA synthesis was done on 2 µg total RNA per reaction using Oligo dT and SuperScript$^{TM}$ III First-Strand Synthesis System. The RT-qPCR was performed using SYBR$^{R}$ Green qPCR SuperMix-UDG with ROX Invitrogen in a Real Time Instrument from Applied Biosystems. The PCR conditions:denaturation at 50$^{o}$C for 2 min, 95°C for 10 min, 40 amplification cycles at 95°C for 15 sec, and a final elongation step at 60°C for 60 sec. Relative quantification by comparative Ct was used to determine expression levels, where an internal reference gene, B-Actin was used. The data was analyzed with 2-(Delta Delta $C_t$) Method (Pfaffl 2001).

## RESULTS AND DISCUSSION

The sequence for the *P. vulgaris* dehydrin gene was obtained from EST database and this sequence contained an SSR $(AAG)_6$ which was named Pvm073 and mapped to linkage group B9 (Hanai et al. 2010).. Based on sequence alignment with the soybean genome, this gene is composed of one intron and two exons. Amino acid alignment with *Arabidopsis thalina, Glycine. max, Pisum sativum* and poplar showed that the protein is highly conserved in three domains Y, S and K segments and the K-segment representing a highly conserved 15 amino acid motif (EKKGIMDKIKE KLPG) (Fig 1).

The primers of the EST-SSR Pvm 073 were screened on two navy bean lines, Voyager and Albion and found to be polymorphic. Previous mapping studies have shown that there is a QTL on B9 for seed Zn concentration in the Albion x Voyager RIL population close to SSR marker BM 154. The DNA sequence of BM154 and dehydrin from P. vulgaris showed synteny with Gm04, and Gm06 chromosomes in soybean located (http://www.phytozome.net/soybean). The synteny analysis with soybean also placed dehydrin and BM154 in close proximity to each other on B09, suggesting dehydrin as a candidate gene for the seed Zn QTL identified by Gelin et al (2007) (Figure 2).

The level of expression of the dehydrin gene in Voyager and Albion pods was determined using the Pvm073 primers which the span exon-exon junction of the gene. The analysis revealed that the dehydrin gene is highly expressed in pod tissue. There were differences in expression between Albion and Voyager, where Voyager (high seed zinc) showed 1.5 fold higher expressing than Albion (low zinc).

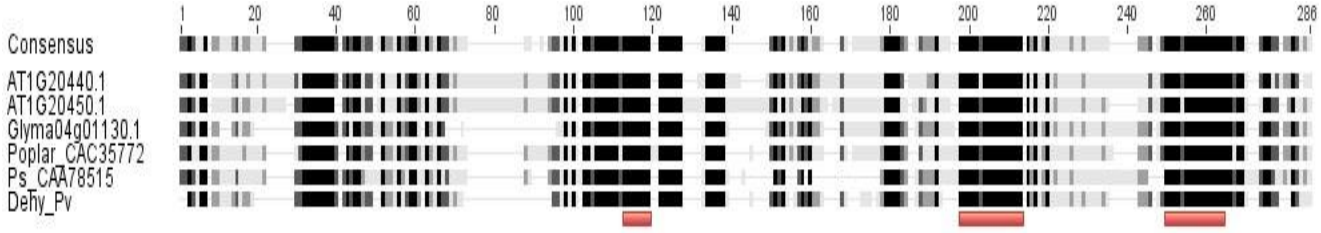


**Figure 1**. Alignment of dehydrin genes reported from different species. The red bar below the sequences indicated the domain that are highly conserved. Abrev:AT: Arabidopsis thaliana; Glyma: Glycine max; Ps: Pisum sativum; Dehy_Pv

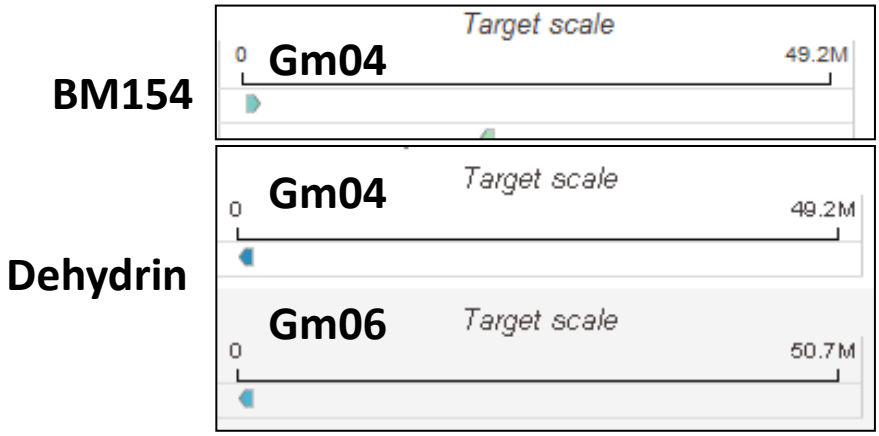


**Figure 2**. Synteny analysis and position of Dehydrin and BM154 in the soybean genome.

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# DEVELOPMENT OF NEW CLIMBING BEAN LINES WITH ENHANCED NUTRITIONAL QUALITY

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**INTRODUCTION:** Biofortification uses plant breeding for higher micronutrient content in staple food as an approach to address the problem of iron and zinc deficiencies in human populations. Legumes are a good source of iron and zinc and when consumed daily can substantially contribute to dietary intake. An ongoing project has shown that bean seeds are variable in the amount of minerals (iron, zinc and other elements) that they contain and that these traits are likely to be inherited quantitatively. Progress has been made in breeding bush beans with high mineral traits (Blair et al. 2009), however climbing beans have higher potential yield than bush beans and would be a good delivery mechanism for biofortification especially where they are likely to be widely adopted in the Great Lakes region. With this in mind we have initiated crossing of the high mineral trait into mid-latitude climbing beans (MAC) and especially into those with BCMNV resistance (MBC lines). Most of the breeding effort has been with three high mineral parents, G14519, G21242 and G23823E and the approach has been largely one of backcrossing the trait into MAC, MBC and VRM lines as well as East African beans. This report summarizes the creation of close to 500 advanced lines from these crosses all of which have been named in the new NUC (Nutrition Climber) series and selected for heat tolerance.

**MATERIALS AND METHODS:** A range of MAC and MBC or VRM lines were used in backcrosses and simple crosses with the high mineral parents, G14519 and G23823E. In addition the following East African genotypes were used in the same types of crosses: AFR708, K20, KALIMA, LYAMUNGO85 (all red mottled), SCAM80CM/15 (black seeded), SUA135, UYOLE90, UYOLE94 (cream striped). After initial crosses and generation advance, single plant selections were made in the $F_2$ and $F_4$ generations, all in CIAT headquarters at 956 masl elevation. Mass selections and seed multiplication were done for two generations (2008B and 2009A) to the $F_{4:7}$ so as to purify seed type and to obtain seed for iron and zinc analysis. Mineral analysis was carried out with atomic absorption spectroscopy (AAS) in CIAT analytical lab. Advanced lines were also selected for yield potential.

**RESULTS AND DISCUSSION**: A total of 498 lines were selected (315 from the backcrosses and 183 from the single crosses) showing that backcrossing tended to be more successful (especially with MBC and VRM lines) than simple crosses. This was despite the fact that the selections made in the backcrosses represented fewer parental combinations than the single crosses. Specifically, the selections made in the backcrosses represented 13 recipient parents including MBC36, MBC40, MBC46, VRM8, as advanced line parents and SUA135, AFR708, K20, KALIMA, LYAMUNGO85, SEL1475, UYOLE94, VCB81010, SCAM80CM/15 as East African selection parents. The simple cross selections meanwhile represented a total of 16 parents including MBC29, MBC36, MBC37, MBC40, MBC46, SEL1483, SEL1484 and VRM8 as well as AFR708, K20, KALIMA, LYAMUNGO85, SCAM80CM/15, SUA135, UYOLE90 and UYOLE94. The seed colors of the selected lines were predominantly red mottled (305 out of the 498 lines) or purple mottled (24) with some cream mottled (28), cream striped (27), yellow (36), red (27) or white (11) lines also segregating, in addition to non-commercial but productive other seed types such as cream (31), purple (6), pink (2) and black (1). The selected genotypes were numbered in the NUC series with these listed as NUC1 through NUC498. The summary of the NUC lines is given in table 1 with their pedigree and range of iron and zinc values (measured in ppm) as well as the seed color of F4 lines from each cross.

**CONCLUSIONS:** The range in iron concentration went up to 97 ppm and it was notable that both G14519 and G23823E produced high iron segregants in combination with MBC36, MBC40 and MBC46. Some very high zinc values (up to 73 ppm) were found for segregants from G23823E. The results will be confirmed by repeating AAS analysis across the second generations of seed multiplication for the lines as part of the shipment to Harvest Plus partner countries. A second set of climbing beans selected in Popayán and Darién with less heat tolerance perhaps but more ideal seed colors will also be evaluated in the same manner as part of the project. Overall the results are promising in terms of combining the high productivity of climbing beans with the high iron and zinc traits.

**Table 1.** NUC lines developed from backcrosses and simple crosses with climbing beans and high iron parents.

| NUC number | Genotype | Total of lines | Iron range F2.4 (ppm) | Zinc range F2.4 (ppm) | Color type of beans |
|---|---|---|---|---|---|
| 1-63 | MBC36 x (MBC36 x G23823E) | 63 | 97-27 | 50-26 | Red mottled, Cream Mottled |
| 64-117 | MBC46 x (MBC46 x G23823E) | 54 | 82-20 | 53-16 | Red mottled, Cream Mottled and Pink Mottled |
| 118-167 | MBC36 x (G14519 x MBC36) | 50 | 75-28 | 56-24 | Red mottled,Cream and Pink Mottled |
| 168-203 | MBC46 x (MBC46 x G14519) | 36 | 82-31 | 45-25 | Red mottled, Cream Mottled and Yellow |
| 204-235 | MBC40 x (G14519 x MBC40) | 32 | 96-31 | 50-26 | Red mottled,Cream and Pink Mottled |
| 236-246 | MBC29 x (G14519 x MBC29) | 11 | 60-32 | 49-26 | Red mottled,Cream and Red |
| 247-257 | VRM8 x (G14519 x VRM8) | 11 | 75-37 | 52-20 | Red mottled,Purple mottled |
| 258-267 | SUA135 x (G14519 x SUA135) | 10 | 72-48 | 58-39 | Cream mottled,Cream and Cream striped |
| 268-277 | AFR708 x (G14519 x AFR708) | 10 | 70-39 | 60-32 | Red mottled,Purple mottled and Yellow |
| 278-285 | KALIMA x (G14519 x KALIMA) | 8 | 73-58 | 50-43 | Red mottled,Red |
| 286-293 | VRM8 x (VRM8 x G23823E) | 8 | 66-43 | 46-30 | Red mottled,Purple mottled |
| 294-300 | K20 x (G14519 x K20) | 7 | 68-47 | 49-39 | Red mottled,Purple mottled |
| 301-306 | LYAMUNGO85 x (G14519 x LYAMUNGO85) | 6 | 71-64 | 51-43 | Red mottled, Red and Yellow |
| 307-310 | SEL1475 x (G14519 x SEL1475) | 4 | 56-53 | 33-33 | Red |
| 311-312 | UYOLE94 x (G14519 x UYOLE94) | 2 | 63-63 | 42-42 | Cream mottled |
| 313 | VCB81010 x (VCB81010 x G23823E) | 1 | 52 | 41 | Black |
| 314 | SCAM80CM/15 x (G14519 x SCAM80CM/15) | 1 | . | . | Red mottled |
| 315 | MBC29 x (MBC29 x G23823E) | 1 | . | . | Purple |
| 316-339 | G14519 x UYOLE90 | 24 | 72-39 | 52-33 | Cream,Yellow |
| 340-355 | MBC46 x G23823E | 16 | 77-52 | 62-41 | Red mottled, Pink and Red |
| 356-369 | MBC36 x G23823E | 14 | 47-43 | 35-34 | Red mottled, Red and Pink Mottled |
| 370-381 | MBC46 x G14519 | 12 | 82-58 | 55-32 | Cream,Red Mottled and Yellow |
| 382-392 | G14519 x MBC40 | 11 | 70-42 | 53-37 | Red mottled,Yellow |
| 393-402 | G23823E x MBC36 | 10 | 48-44 | 34-33 | Red,Red Mottled |
| 403-411 | G14519 x KALIMA | 9 | 60-45 | 44-34 | Red mottled,Cream |
| 412-420 | MBC37 x G23823E | 9 | 67-38 | 69-32 | Red mottled,Red |
| 421-429 | G14519 x K20 | 9 | 65-63 | 46-43 | Red mottled,Purple mottled and Yellow |
| 430-437 | MBC29 x G23823E | 8 | 84-68 | 73-62 | Red mottled,Purple mottled and Pink |
| 438-444 | MBC29 x G14519 | 7 | 50-40 | 41-31 | White, Red and Yellow |
| 445-451 | SEL1483 x G23823E | 7 | 56-51 | 38-37 | Yellow, Red |
| 452-457 | G14519 x MBC36 | 6 | 65-58 | 43-41 | Red mottled,Cream |
| 458-463 | SEL1484 x G14519 | 6 | 43-43 | 55-55 | White,Red Mottled |
| 464-467 | G14519 x LYAMUNGO85 | 4 | . | . | Red mottled, Red and Yellow |
| 468-471 | G14519 x UYOLE94 | 4 | 65-65 | 47-47 | Cream striped,Cream |
| 472-475 | G14519 x SUA135 | 4 | . | . | Yellow,Red Mottled |
| 476-479 | SEL1483 x G14519 | 4 | 68-53 | 42-36 | Red,Red Mottled and Pink |
| 480-483 | MBC37 x G14519 | 4 | 49-45 | 43-41 | Yellow |
| 484-487 | G14519 x SEL1476 | 4 | 58-47 | 45-36 | Cream mottled,Yellow |
| 488-490 | G14519 x SCAM80CM/15 | 3 | 60-54 | 44-41 | Cream,Red Mottled and Yellow |
| 491-493 | G14519 x VRM8 | 3 | . | . | Red mottled,Purple mottled |
| 494,496 | G14519 x AFR708 | 2 | 73-47 | 48-35 | Red mottled,Yellow |
| 495,497 | G14519 x MBC29 | 2 | . | . | Red mottled |
| 498 | G14519 x SEL1484 | 1 | . | . | White |

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# DEVELOPMENT OF NEW ADVANCED LINES FROM THE PROGRAM FOR THE NUTRITIONAL ENHANCEMENT OF ANDEAN BUSH BEANS

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International Center for Tropical Agriculture

**INTRODUCTION:** Iron deficiency anemia and other micronutrient deficiencies affect large number of people worldwide and biofortification is an approach to address this problem by breeding for higher micronutrient content in staple food crops. Legumes are a good source of iron and other essential micronutrients that are found only in low amounts in the cereals or root crops. Also beans and other grain legumes are usually consumed whole, thus conserving their nutritional content. An ongoing project has shown that bean seeds are variable in the amount of minerals (iron, zinc and other elements) that they contain and that these traits are likely to be inherited quantitatively. Over the past 4 years we have developed an initial set of Andean bush beans that have higher mineral content and red mottled seed type and named this set the NUA (Nutritional Andean) lines, two lines of which have been registered with the USDA (Blair et al. 2010). The original NUA lines, especially NUA35, NUA 45 and NUA56 have now been widely distributed and have been or are near varietal release in various contries; their one drawback being a limited number of genetic parents used to develop the lines (predominantly CAL96 as recurrent parent and G14519 as high mineral parent). The objective of this research and varietal development program has been to create a new generation of NUA lines based on triple, double and multiple crosses using the original NUA lines as well as additional sources in various breeding combinations. Most of the new genotypes aim to combine the high iron trait into red mottled background but we also derive some large red and cream mottled genotypes.

**MATERIALS AND METHODS:** Advanced lines were developed from the following donor parents for the high mineral trait: G14519, G23823E, G21242, NUA35, NUA56, BID29, BID115, through a series of backcrosses, simple crosses, triple crosses, double crosses and multiple crosses with the commercial parents: CAL96, CAL143, CAL144, PVA773, AND277 and AFR612 (all red mottled); as well as AFR298, RADICAL SAN GIL, RADICAL CERINZA, RED CANADIAN WONDER (G6592) (all large red seeded); SUG131 (cream mottled), KABLANKETI (G22454) (purple stippled), and DORE DE KIRUNDO (G21715) (large yellow). Some combinations contained the angular leaf spot resistance sources G5686 and MEX54 to complement those crosses with AND277 and CAL143 which also provide some resistance to the disease. After initial crosses and generation advance, single plant selections were made in the $F_3$ and $F_6$ generations. Seed multiplication for one generation was used to obtain seed for iron and zinc analysis which was carried out with atomic absorption spectroscopy (AAS) in CIAT analytical lab. Advanced lines were also selected for highly acceptable architecture, growth habit, yield potential and seed types.

**RESULTS AND DISCUSSION:** The selected genotypes were numbered consecutively beginning with the numbering of the previous NUA lines developed from the initial crosses for the biofortification program. A total of 488 advanced lines were developed with these listed as NUA101 through NUA589. The new NUA lines were organized based on pedigree and information on seed type was used to create separate nurseries for red mottled, large red and cream mottled genotypes for planting in Darien 2008B (Andic Dystrudept, pH 5.5, 1500 masl, Average temperature 19°C, station rainfall 5000 mm) season. A total of 76 pedigrees were represented by the 488 selected NUA lines and these were divided into Backcrosses, triple, double and simple cross derived genotypes.

Table 1 shows the summary of genotypes per pedigree, cross number, NUA codes and range of iron and zinc content (measured as parts per million or ppm) within each pedigree from backcrosses as well as the seed colors of the $F_{6:7}$ advanced lines, although more lines were derived from simple, triple and double

crosses (not shown). In terms of iron and zinc content the genotypes varied between 90 and 40 ppm iron and 35 to <10 ppm zinc in backcrosses, 98 to 43 ppm iron and 28 to <10 ppm zinc in triple crosses, 105 to 49 ppm iron and 34 to <10 ppm zinc in double crosses and 90 to 37 ppm iron and 36 to 10 ppm zinc in simple crosses. In each case some lower zinc advanced lines were kept because the corresponding iron levels were high or vice versa and in for all the lines there are plans to confirm iron and zinc content either through NIRS or through a repetition of AAS analyses. These results show that there is large variability for iron and zinc content in the germplasm pool for Andean bush beans after improvement for mineral levels through the nutritional breeding. We were also pleased to find that high iron and zinc levels seem to be combining well with very commercial seed type and with better plant architecture and yield potential which was somewhat deficient in the first set of NUA lines. In terms of seed colors, the commercial types could be classed into 145 large red, 127 dark red mottled, 125 light red mottled, 44 cream mottled, 19 purple speckled, 13 yellow, 7 pink mottled, 4 large white and 4 bayo types. This set of NUA lines will provide a basis for additional breeding in each of these seed classes as the previous NUA lines were all of the red mottled seed class. Within the red mottled seed class, variability for seed brightness and tone will also be useful for ensuring that future combinations of breeding crosses have variability for this important commercial class which is preferred as a predominant type in Eastern and Southern Africa as well as parts of the Andean region.

**Table 1.** Development of a NUA lines from the program for the nutritional enhancement of Andean bush beans.

| NUA number | Genotype | Total lines | Iron range | Zinc range | Color type of beans |
|---|---|---|---|---|---|
| | **Backcross** | | **(ppm)** | **(ppm)** | |
| 101 - 140 | AFR612 X (AFR612 X G14519) | 40 | 73 - 41 | 31 - 18 | Red mottled and red |
| 141 - 161 | CAL144 X (CAL144 X G14519) | 21 | 84 - 53 | 49 - 23 | Purple mottled, red mottled, red and purple |
| 162 - 181 | AFR612 X (AFR612 X G21242) | 20 | 66 - 44 | 33 - 20 | Red, red mottled and purple mottled |
| 182 - 198 | SUG131 X (SUG131 X G21242) | 17 | 88 - 64 | 33 - 25 | Cream mottled and red mottled |
| 199 - 209 | G21715 X (G21715 X G21242) | 11 | 69 - 53 | 31 - 25 | Yellow |
| 210 - 220 | AFR298 X (AFR298 X G14519) | 11 | 66 - 45 | 37 - 27 | Red |
| 221 - 231 | CAL144 X (CAL144 X G21242) | 11 | 90 - 59 | 45 - 32 | Red mottled, purple mottled and cream mottled |
| 232 - 241 | PVA773 X (PVA773 X G14519) | 10 | 75 - 63 | 37 - 25 | Red, red mottled |
| 242 - 248 | SUG131 X (G14519 X SUG131) | 7 | 81 - 67 | 36 - 26 | Red mottled and pink mottled |
| 249- 250 | SUG131 X (SUG131 X G14519) | 2 | 65 | 25 | Cream mottled and red mottled |
| 251 - 255 | AFR298 X (AFR298 X G21242) | 5 | 64 - 52 | 28 - 25 | Red |
| 256 - 258 | G6592 X (G14519 X G6592) | 3 | 60 - 48 | 35 - 27 | Red |
| 258 - 260 | G21715 X (G14519 X G21715) | 2 | 61 | 25 | Yellow |
| 261 - 262 | G22454 X (G14519 X G22454) | 2 | 65 - 64 | 39 - 27 | Purple mottled |
| 263 - 264 | G22454 X (G22454 X G21242) | 2 | 56 - 53 | 25 | Purple mottled |
| 265 | G22454 X (G22454 X G14519) | 1 | 67 | 29 | Purple mottled |
| | **Triple Cross** | | | | |
| 266 - 310 | NUA35 X (AFR612 X BID115) | 45 | 92 - 54 | 32 - 22 | Purple mottled, red mottled and purple |
| 311 - 328 | NUA56 X (BID29 X SUG131) | 18 | 75 - 48 | 30 - 24 | Cream mottled, purple mottled, red, red mottled |
| 329 - 340 | NUA35 X (PVA773 X BID29) | 12 | 77 - 63 | 30 - 25 | Red, red mottled and purple mottled |
| 341 - 351 | NUA56 X (CAL143 X G23823E) | 11 | 84 - 51 | 34 - 22 | Purple mottled and red mottled |
| 352 - 363 | NUA35 X (AFR612 X G23823E) | 12 | 81 - 67 | 30 - 25 | Purple mottled and red mottled |
| 364 - 371 | NUA35 X (CAL96 X G23823E) | 8 | 86 - 65 | 31 - 25 | Red mottled and purple mottled |
| 372 - 379 | NUA56 X (AFR612 X BID115) | 7 | 71 - 49 | 29 - 23 | Red mottled and purple mottled |
| 380 - 385 | NUA35 X (BID29 X CAL144) | 6 | 83 - 61 | 31 - 23 | Purple mottled and red mottled |
| 386 - 391 | NUA56 X (PVA773 X BID29) | 6 | 83 - 54 | 26 - 22 | Red mottled |
| 392 - 396 | NUA56 X (AFR612 X G23823E) | 5 | 86 - 62 | 31 - 22 | Red and red mottled |
| 397 - 400 | NUA35 X (SUG131 X G23823E) | 4 | 105 - 79 | 33 - 30 | Purple mottled and red mottled |
| 401 - 403 | NUA35 X (CAL143 X G23823E) | 3 | 98 - 62 | 27 - 24 | Purple and red mottled |
| 404 - 405 | NUA35 X (CAL144 X G23823E) | 2 | 84 - 83 | 29 - 27 | Purple mottled |
| 406 - 407 | NUA56 X (CAL144 X G23823E) | 2 | 80 - 60 | 24 - 21 | Red mottled and purple mottled |
| 408 - 409 | NUA56 X (SUG131 X G23823E) | 2 | 66 | 27 - 21 | Purple mottled |
| 410 | NUA56 X (PVA773 X G23823E) | 1 | 71 | 28 | Purple mottled |
| 411 | NUA56 X (BID115 X PVA773) | 1 | 72 | 28 | Purple mottled |

## REFERENCE


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# YIELD TESTING OF NEW ANDEAN COMMON BEAN ADVANCED LINES FOR NUTRITIONAL BREEDING

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**INTRODUCTION:** We have recently developed 488 new NUA (Andean nutrition) bush beans all of which have higher than average iron content (55 ppm or above) and some also with high levels of zinc. These lines complement the first set of NUA lines which were from a limited set of two pedigrees using CAL breeding lines. In the new set of NUA lines the pedigrees are more complex with simple, double, triple and backcross derived lines. We hope to increase the agronomic adaptation of the high mineral varieties using these various crossing strategies. The goal of this experiment was to evaluate the yield potential of the new NUA lines for mid-elevation production.

**MATERIALS AND METHODS:** The experiment was planted in Darién (Andic Dystrudept, pH 5.5, 1500 masl, Average temperature 19°C, station rainfall 500 mm) during the short dry season (December 2008 – February 2009). The planting included all the new NUA lines (NUA101 - NUA589) for a total of 488 test genotypes plus 13 check / parental genotypes based on the parents used in the development of the NUA lines. The lines were organized into 165 lines from 16 backcrosses, 145 lines from 17 triple crosses, 31 lines from 9 double crosses and 147 lines from 33 simple crosses. Each experiment was planted with three repetititions. Mean comparisons were carried out to compare each group of lines for mineral levels and yield by the type of cross, the parental source or recipient parent used. Mineral levels were estimated on the seed used for the planting stock which was the same sent to Central Africa. In addition, NIRS analysis was attempted on the same ground seed samples that had gone for AAS analysis as a way to calibrate NIRS analysis for Andean beans.

**RESULTS:**

***Effect of Cross type:*** Grouping of NUA lines based on cross type (Table 1) found that simple crosses tended to give higher values of iron content (71 ppm average) and yield potential (1627 kg/ha) than backcrosses (65 ppm and 1455 kg/ha) although the tendency in zinc was the opposite although no-significant differences were found for this mineral. Double crosses meanwhile, had averages that were statistically similar to triple crosses and both of these were intermediate in both iron and zinc content as well as yield potential between the simple crosses and the backcrosses. The results suggest that the number of genotypes included in the crosses can influence the productivity of the breeding process and shows the promise of certain simple crosses.

***Effect of mineral donor parent on iron content:*** Grouping by high iron donor parents (Table 2) showed the effect on the resulting progeny in the F5:6 and F6:8 generations to be significant (F6:7 generation was not tested as widely and is therefore not reported). Differences were found between the lines derived from two high mineral sources (for example the 29 lines derived from the combination of NUA35 and G23823E versus the 51 lines derived from NUA56, respectively). One observation from this analysis was the utility of G14519 which had been used in the development of the early NUA lines (series 1-100).

***Effect of mineral donor parent on yield potential:*** The average yields of each pedigree derived from the high mineral parents for the yield trial conducted in the F6:8 generation was also compared and we observed that those lines with NUA 35, BID115, and NUA56 in their pedigrees out-yielded those lines with BID29, G14519, G23823E and G21242 in their pedigríes but that the range in yield potential was still reasonable for Andean bush beans ranking from 1309.9 kg/ha up to 1795 kg/ha averages. G23823E

in combination with BID29 produced higher yielding lines than the other sources with a G lines alone or in combination with a NUA line.

**CONCLUSIONS:** A low number of selections from double crosses suggests the difficulty of combining multiple iron sources in a single cross, while the value of triple (even with two commercial types) is also questionable since simple crosses tended to out-yield these and higher mineral content was more likely. However seed quality tended to be better in triple crosses as well as in the backcrosses. Certain parents had better combining ability which may be due to specific adaptation. Certainly in terms of mineral content, a major new discovery has been the value of G23823E compared to G14519 which was used in the original series of NUA lines. G21242 was found to be an intermediate donor parent which is difficult to work with for bush beans, while some of the early NUA lines and BID lines can contribute useful genes for high mineral content. This indicates that the search for and incorporation of new sources of the high mineral trait continues to be a priority that merits careful analysis to guide proper breeding strategies. One further observation is that the new NUA lines have achieved a higher yield potential of 2,000 kg/ha than was available previously. Combining ability for yield is best with those lines that have been selected for better mineral content and good adaptation like the NUA and BID lines and therefore transfer of the high mineral trait from the G14518, G21242 and G23823E would benefit from careful introgression. This may be possible in the future with marker assisted selection as QTL for high iron have been identified in the first two sources (Blair et al. 2010, 2011). In summary, biofortification requires patience and careful pyramiding of various sources of high mineral traits.

**Table 1.** Comparisons of means by cross type for iron (Fe) and zinc (Zn) concentation measured in ppm (parts per million) and yield (kg/Ha) for NUA lines in Darién 2009A.

| Fe (ppm) | | | | Zn (ppm) | | | | Yield (Kg/Ha) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cross type (lines) | Average ± S.E | α | | Cross type | Average ± S.E | β | | Cross type | Average ± S.E | χ | |
| Simple (147) | 71.1 ± 0.7 | A | | Backcross | 37.0 ± 0.3 | A | | Simple | 1627.8 ± 19.8 | A | |
| Double (31) | 70.1 ± 1.2 | A | B | Double | 35.9 ± 0.6 | A | B | Triple | 1528.9 ± 21.3 | B | |
| Triple (145) | 68.9 ± 0.7 | | B | Simple | 35.9 ± 0.3 | A | B | Double | 1466.0 ± 50.7 | B | C |
| Backcross (165) | 65.8 ± 0.6 | | C | Triple | 35.0 ± 0.3 | | B | Backcross | 1455.2 ± 19.2 | | C |

**Table 2.** Mean comparisons for NUA lines by high mineral donor parent for the AAS data from the F6 generation (Darién 2007B) and from the F6.8 generation (Darién 2009A)

| Donor parent[s] for high minerals (lines) | Fe (ppm)-F6 | α | | | | | Donor parent(s) for high minerals | Fe (ppm) - F6.8 | β | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| NUA35 and G23823E (29) | 79.0 | A | | | | | G23823E | 79.8 | A | | |
| NUA35 and BID29 (18) | 71.6 | B | | | | | NUA35 and G23823E | 78.1 | A | | |
| NUA56 and G23823E (21) | 71.3 | B | | | | | G23823E and BID29 | 72.8 | A | B | |
| G23823E (29) | 70.8 | B | | | | | BID29 | 72.7 | | B | |
| G23823E and BID115 (25) | 67.6 | B | C | | | | BID115 | 70.8 | | B | |
| NUA35 and BID115 (45) | 66.9 | B | C | D | | | NUA56 and G23823E | 70.7 | | B | |
| NUA56 and BID115 (8) | 65.0 | B | C | D | E | | G23823E and BID115 | 69.6 | | B | |
| G21242 (75) | 64.8 | | C | D | E | | NUA56 and BID29 | 68.9 | | B | |
| NUA56 and BID29 (24) | 62.7 | | C | D | E | | G21242 | 67.8 | | B | |
| **G14519 (97)** | 61.7 | | | | E | | NUA35 | 66.2 | | B | |
| NUA35 (4) | 61.7 | B | C | D | E | | NUA35 and BID29 | 65.8 | | B | C |
| BID29 (22) | 61.5 | | | D | E | | NUA56 and BID115 | 65.1 | | B | C |
| G23823E and BID29 (6) | 61.1 | | C | D | E | F | **G14519** | 64.6 | | | C |
| BID115 (50) | 55.1 | | | | | F | NUA35 and BID115 | 64.2 | | | C |
| NUA56 (31) | 54.9 | | | | | F | NUA56 | 64.0 | | | C |

**α, β, χ:** Comparison of means for unequal samples sizes, GT-2 method (Averages with the same letters are not statistically different, $p < 0.05$)

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# FARMER PARTICIPATORY PLANT BREEDING OF BIOFORTIFIED CLIMBING BEANS

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## INTRODUCTION

Participatory plant breeding (PPB), which involves the selection by farmers of genotypes from a breeding program, is a complement to plant breeder-led selections. Farmer evaluations are used as a way to incorporate their criteria into the breeding process and to complement evaluations by plant breeders. With this in mind we have begun a participatory evaluation process for biofortified (high mineral) beans as part of the breeding for high seed iron in climbing beans. The initial evaluation has been in an on-station trial at CIAT. The farmers participating in the evaluations were interested in climbing beans as an alternative in vegetable crop rotations especially with tomato plantings and coffee plantations. All the farmers have been long-term bean producers with expert opinions on marketability and agronomic traits. The material evaluated has been the new climbing beans from the NUC series which is being produced for Central African production as part of Harvest Plus.

## MATERIALS AND METHODS

The participatory evaluation (PE) was conducted with 10 producers from Pescador and Morales (Cauca Department) who have expertise in the evaluation of red mottled climbing beans. The evaluation was conducted as part of the breeding nursery which consisted in a randomized complete block experiment with two repetitions of NUC (Nutritional Climbing bean) lines with red mottled seed color. This was part of a larger set of trials with red mottled, cream mottled, large red, yellow and white NUC lines each in a separate experiment. All the trials were planted as randomized complete block experiments (RCBD) yield trials in the F4:7 generation with three repetitions in the 2009[a] season (March-June) on station at CIAT headquarters. Instead of evaluating all the lines in each block only the 46 lines with highest iron content were included in the evaluation based on early generation AAS evaluations which were to be repeated after the participatory evaluation. The farmers' visit was timed for late-pod development to evaluate yield potential, diseases and fresh seed size. Evaluation consisted in a decision of whether the genotype was good, intermediate or poor with a subjective evaluation of the reason for the ranking.

## RESULTS AND DISCUSSION

From a total of 488 $F_{4.7}$ Andean seed type NUC lines grown in the preliminary yield trial in Palmira planted in March 2009, 46 red mottled NUC lines were selected for the PPB selection. The criteria for selection of these NUC were acceptable seed color, to be similar to the commercial type " Cargamanto Rojo" and to have higher than average iron content above 75 ppm in the AAS evaluation conducted in the $F_{2.4}$ generation. The selected rows were tagged with red tape and the NUC number but were not distinguished by any other factor. During the PE exercise, the 10 farmers evaluated characteristics typical of harvest maturity during the visit in May 2009. The main criteria were based on varietal acceptability to the farmers and these were compared to the yield potential and iron content of each line. Table 1 compiles the favorable and unfavorable traits described by the farmers for each of the genotypes evaluated at the moment of evaluation. The top 10 favorable or unfavorable criteria are given with the frequency of their appearance among the 46 lines evaluated.

Interestingly the green shelled bean quality was important to the farmers as was pod size and number of seed per pod which would be related traits. Seed color characteristics determine marketability to a large

extent and were also important traits with pale or dark purple mottled seed unacceptable and bright or shiny red mottled seed desirable. Overall, the number of unfavorable and favorable observations were about even showing that many of the 46 lines could be used for commercial trials. A biplot analysis (Figure 1) of yield (x axis) versus mineral content (y axis) with the level of acceptability by the farmers evaluated was generated with reference points being the yield and mineral content of the check varieties G14519 (high mineral donor parent) and MBC37 (commercial check, improved heat tolerance and good seed color) are indicated with vertical and horizontal lines. The lines with highest acceptability were NUC 76, NUC78, NUC412 and NUC 433, the latter of which surpassed the yield for the commercial check MBC37 (1900 kg/ha). It was surprising that the most acceptable had the same level of iron as the MBC37 check (60 ppm) which is higher than average. We found that differences in the pedigree of the NUC lines were related to their acceptability to the farmers from Caldono (Cauca) and also to the mineral content of the progeny lines. For example the backcrosses with MBC46, MBC 37 and MBC29 were the genotypes with the high to intermediate level of acceptance, good yield potential and moderate to high iron content. This validates results from other areas of Colombia where MBC46 has been acceptable to a large number of local farmers as a quick maturing, high yielding climbing bean with wide adaptation, something that was needed for the tomato-bean rotation systems these farmers practice. In summary, the PPB experiences were very valuable for helping the breeding program to decide which genotypes to advance and which are most apt for varietal release. In the case of the NUC climbing bean lines, the PPB evaluation was done in an earlier stage of the breeding process to help eliminate genotypes. This was a learning experience for both the breeders and the farmers/PPB trainers since it was more genotypes than would normally be evaluated in a typical PPB experiment.

**Table 1.** Criteria used by producers to rank the NUC lines and the frequency with which they were observed by 10 farmers evaluating 46 genotypes.

| Favorable Criteria | Frequency | Unfavorable Criteria | Frequency |
|---|---|---|---|
| 1 –Resistance to disease and insects | 61 | 1 – Low yield potential (1 pod per raceme) | 123 |
| 2 – Pod load (more than 3 pods per raceme) | 57 | 2 – Small grain | 47 |
| 3 – Red mottled grain type (Calima) | 52 | 3 – Pale red color | 47 |
| 4 – Large seed size when dry | 50 | 4 – Small pod (shorter than <5cm) | 41 |
| 5 – Long pods (longer than 10 cm) | 42 | 5 – Uneven pod load | 31 |
| 6 - Earliness | 26 | 6 – Non-uniform maturity | 26 |
| 7 – Green shelled bean (marketable size) | 17 | 7 – Empty pods | 23 |
| 8 – Overall marketability | 15 | 8 – Dark purple mottling | 17 |
| 9 – Plant height (>2m) | 14 | 9 - Lateness | 16 |
| 10 – Seed brightness (red tone) | 14 | 10 - Segregation (non-uniform seed type ) | 16 |
| *Total observations* | *415* | *Total observations* | *501* |

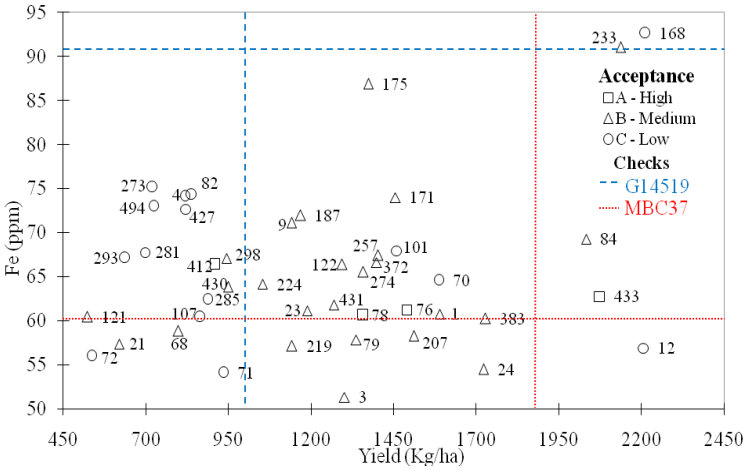


**Figure 1.** Comparison of farmer acceptance of NUC lines with yield (kg/ha) and iron conent in the Palmira (2009[a]) season.

# PARTICIPATORY VARIETAL SELECTION OF BIOFORTIFIED BUSH BEANS FOR THE ANDEAN REGION

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## INTRODUCTION

Participatory varietal selection (PVS) has been a long-established method for selecting genotypes that are apt for release as varieties and complements formal processes of variety release. Farmer evaluations are used as a way to incorporate their criteria into the breeding process and to complement evaluations by plant breeders of advanced lines and agronomic trial information. Early (pre-release) incorporation of the farmers' criteria are critical to obtain desirable new crop varieties; and furthermore, can increase the chances for rapid adoption of the new advanced lines as varieties (Ashby 1991; IPRA, CIAT, Cali, Colombia). With this in mind we began a participatory evaluation process for biofortified (high mineral) beans as part of a project focusing on the Andean region of South America called Cambio Andino. The initial evaluations have been in two on-station CIAT sites in Colombia with extension to on-farm evaluations and national program sites in other countries of the region. The farmers participating in the evaluations have been from two departments of Colombia, Cauca and Valle de Cauca and have been long-term bean producers with expert opinions on marketability and agronomic traits. The material evaluated has was a set of NUA (Andean Nutrition) advanced lines for variety release in Colombia and Bolivia.

## MATERIALS AND METHODS

Two trial sites were considered. In Palmira, ten farmers evaluated a group of 10 bush beans in vegetative and reproductive maturity. The genotypes included AFR298, CAL96, CAL143, NUA30, NUA35, NUA45, PVA773, RAA30 and SAB625. In Darién, eight producers from Yotoco, Darien, Vijes and La Cumbre evaluated a set of similar lines twice, once during vegetative stages and once during seed-filling stage of the bush bean crop between May and August 2009. The evaluation was conducted with an especially designed randomized block experiment of 9 pre-release genotypes including those from the NUA (Nutritional Andean bean) series and CIAT checks; namely NUA30, NUA35, NUA45, NUA56, NUA59, CAL96 (recipient parent of NUA lines), AFR612, CAL143, CAL144 (Calima type checks). Evaluations consisted in both a good, intermediate or poor decision plus a ranking of the 9 genotype in order of preference.

## RESULTS AND DISCUSSION

*NUA series evaluation (bush beans – heat tolerant):* Ten farmers performed an evaluation of bush beans which were near harvest. Logistic regression was used to simulate the probability of acceptability of each of the varieties based on the ranking of the varieties during the two visits. Graphs were drawn showing the cumulative probability of acceptability and the order of varietal preference for each genotype as shown in Figure 1. Among the genotypes those with higher or faster adoption would be those genotypes at the top of the graph while those genotypes at the bottom of the graph would be of lower or slower adoption. The notable winner in terms of preference was NUA35 which beat even CAL96 (the red mottled recurrent parent), AFR298 (a dark red kidney drought tolerant genotype) and PVA773 (ICA Caucaya a well established variety). NUA45 was intermediate in acceptability. The other non-preferred lines inorder were CAL143, NUA30, RAA30 and SAB625.

*NUA series evaluation (bush beans – mid-elevation):* A second evaluation was conducted in Darién (Valle del Cauca) during the dry season (May to August 2009) with two visits to a replicated randomized complete block design experiment. The visits coincided with early pod filling to observe disease and abiotic stress pressure and near harvest/ full maturity to observe yield potential and grain quality. The farmers came from four local towns: Darién (1), Yotoco (3), Vijes (2) and la Cumbre (2) and had worked with the Participatory Research program before in CIALs established in each of those communities. In the logistic regression shown in Figure 1, it was notable that NUA45, CAL96, NUA35 and NUA30 (in that order) would be preferrable to the check genotypes AFR612, CAL144 or CAL143 (in that order) with NUA56 being the only nutritionally improved genotype that would be in the lower half of the acceptability ranking. These results show that the NUA lines (NUA45, NUA35 and NUA30) along with CAL96 have favorable grain quality characteristics of dark burgundy red mottling which are very important for marketability as well as the long grain typical of Calima types. AFR612 was productive but its grain type does not match up with the NUA lines or with CAL96. All of this shows that the biofortified lines could be readily accepted by farmers based on these participatory evaluations. It must be said also that the farmers selected these grain types without knowing about the nutritional differences rather considering normal agronomic and market characteristics since the test was done with a random numbering system so as not to bias the farmers.

Overall, the PPB experiences were very valuable for helping the breeding program to decide which genotypes to advance and which are most apt for varietal release. This was especially the case for the NUA lines which are finished material that has already been seed multiplied from basic and breeders seed resulting from the breeding program and which have been entering the pre-varietal release process in Colombia through NARS and NGO involvement.

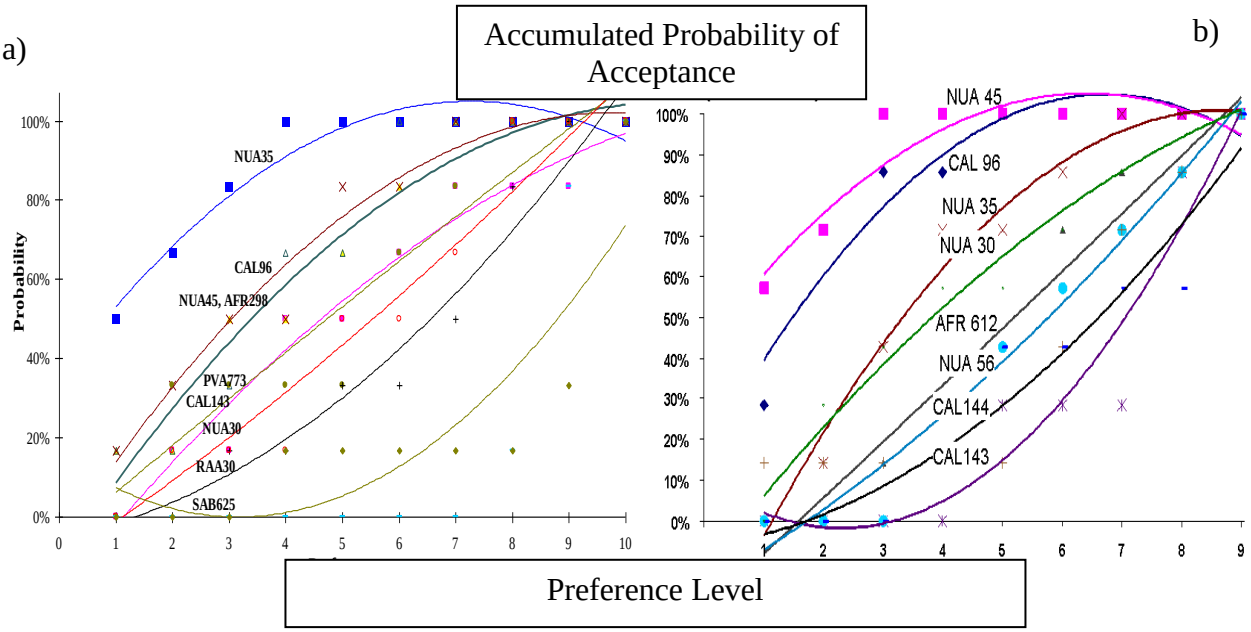


**Figure 1.** Order of preference of farmers from a) Caldono (Cauca) for Calima type biofortified (NUA) and non-biofortified (AFR, CAL, PVA, SAB) lines determined by logistical regression during the Palmira 2009A season and b) from Darién, Yotoco, Vijes and la Cumbre (Valle del Cauca) for Calima type biofortified (NUA) and non-biofortified (AFR, CAL, PVA, SAB) lines determined by logistical regression during the Darién 2009B season.

# OLIGOELEMENT LEVELS IN COMMON BEAN BREEDING LINES AND LANDRACES FROM RIO GRANDE DO SUL, BRAZIL

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## INTRODUCTION

Food quality and economical viability have been common goals in breeding programs worldwide. Embrapa Temperate Climate common bean breeding program is seeking bean cultivars that have different characteristics from a nutritional standpoint, since it is known that inadequate intake of micronutrients leads to numerous disorders and metabolic abnormalities (FRANCO, 1999). This study focused on the variability of oligoelements concentrations among landraces from Rio Grande do Sul State, as compared to those from different breeding programs.

## METHODS

Twenty six cultivars were studied, being nine landraces and seventeen derived from breeding programs. Seed multiplication was performed in 2009.The samples were dried in a mill and packed in glass jars with plastic lid. The elements analyzed were copper (Cu) - using a method of Perkin-Elmer (1982), Miyazawa et al. (1992b); Malavolta et al. (1989), iron (Fe)-through methods Ohlweiler (1974); Malavolta et al. (1989), manganese (Mn) - the methodology of Perkin-Elmer (1982), Miyazawa et al. (1992b) and zinc (Zn) - the method of Perkin-Elmer (1982); Malavolta et al. (1989), by atomic absorption spectrophotometry (AAS), as quoted by SILVA (1999).

## RESULTS AND DISCUSSION

Results show that 16 of the cultivars had above-average results for at least one of the oligoelements examined and from these, three are landraces and the others from breeding programs. None of the cultivars was higher than the average for all oligoelements examined (Table 1).

Iron was the most abundant oligoelement showing an average of 30 mg $kg^{-1}$ followed by Zn, Mn and Cu, which is confirmed by Ribeiro et al. (2008). The cultivars BRS Expedito, Uirapuru, Fepagro-26, Minuano, and the lines TB 02-04 and TB 02-01, had higher concentrations of Fe.

Cultivars Guateian 6662 and FT Soberano showed higher concentrations of Cu, Fe and Zn, demonstrating its effectiveness in absorption and accumulation of micronutrients. Ribeiro et al. (2008) confirmed this characteristic for Guateian 6662, though this fact was not confirmed for FT Soberano. Cultivar FT Bonito had higher concentrations of Fe and Zn. The cultivars Macanudo and Macotaço had higher content of Cu and Fe and Rio Tibagi and FT Bionobre showed elevated levels of Cu and Zn, all of them from breeding program.

Landraces Cubano Cerrito, Preto Comprido and the line TB 02-24 showed high of Cu, Zn and Mn, respectively.

## CONCLUSIONS

Cultivars that had higher levels of oligoelements are mostly part of breeding programs. Among the analyzed elements, iron has the highest mean concentration, followed by zinc, manganese and copper.

**Table 1**: Oligoelement levels in common bean breeding lines and landraces, from Rio Grande do Sul State. Pelotas, RS, Brazil, 2009.

| **Cultivar** | **Cu** | **Fe** | **Mn** | **Zn** |
|---|---|---|---|---|
| | **.....mg kg$^{-1}$ .....** | | | |
| Preto Comprido | 10.01 | 26.92 | 16.81 | 33.04 * |
| Guabiju | 10.90 | 22.84 | 14.37 | 19.63 |
| Chocolate | 8.48 | 19.74 | 15.92 | 20.88 |
| Balim Grosso | 11.06 | 27.94 | 15.05 | 21.09 |
| Cubano Cerrito | 12.30 * | 21.05 | 16.10 | 19.34 |
| Vermelho Escuro | 7.36 | 32.18 | 14.86 | 24.72 |
| Felipe | 8.24 | 31.50 | 11.31 | 21.67 |
| Grosso Amarelo | 7.67 | 35.86 | 11.24 | 21.94 |
| Amarelinho | 7.47 | 33.97 | 12.04 | 23.16 |
| TB 02-23 | 8.79 | 19.76 | 19.41 | 23.61 |
| TB 02-24 | 8.76 | 32.08 | 25.85 ** | 29.44 |
| TB 02-25 | 8.00 | 36.88 | 17.08 | 26.17 |
| TB 02-26 | 9.96 | 19.02 | 17.30 | 19.82 |
| TB 02-01 | 10.63 | 41.66 * | 18.35 | 28.94 |
| TB 02-04 | 10,53 | 17.46 | 21.46 * | 23.51 |
| Macanudo | 11.85 * | 38.97 * | 15.27 | 29.50 |
| FT Bonito | 10.40 | 37.94 * | 17.13 | 30.58 * |
| Guateian 6662 | 11.82 * | 39.07 * | 16.55 | 32.50 * |
| Macotaço | 12.30 * | 40.23 * | 16.50 | 27.64 |
| Fepagro-26 | 10.09 | 38.69 * | 15.78 | 27.39 |
| FT Soberano | 12.22 * | 38.33 * | 16.45 | 33.93 * |
| Minuano | 11.30 | 37.91 * | 13.87 | 28.04 |
| Rio Tibagi | 12.90 * | 33.69 | 17.11 | 32.27 * |
| FT Bionobre | 12.51 * | 35.12 | 16.38 | 32.42 * |
| BRS Expedito | 9.36 | 29.00 | 23.83 * | 24.63 |
| Uirapuru | 9.21 | 28.33 | 31.47 ** | 19.49 |
| **Standard Deviation** | **1.57** | **7.63** | **3.57** | **4.64** |
| **Mean** | **10.13** | **30.24** | **16.76** | **25.19** |

*indicates a value above mean plus one standard deviation and ** indicates a value above mean plus two standard deviations.

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# CHEMICAL COMPOSITION OF BEAN GRAINS OF DIFFERENT CULTIVARS STORED UNDER TWO CONDITIONS

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## INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is a rich source of essential nutrients such as proteins, iron, calcium, vitamins, carbohydrates and fibers (RIOS et al., 2002), its being able to be used as an alternative in the place of meats or other protein products.

Bean cultivars with alternative types of grains can stand for novel options of income for farmers, with the possibility of marketing a distinct product in order to use new niches of market, with a higher pay of his product (ALVES et al., 2009). Nevertheless, to make the use of those cultivars feasible, there is the need, among other studies, of characterizing chemically their grains de forma to know better their nutritional value as compared with the predominant type on market, the carioca type.

The objective of his work was the chemical characterization of grains of five cultivars of common bean plant and the comparison of four alternative groups with the grains of the most commercialized type, the carioca, employing lots of beans stored for twelve months' time under room or refrigerated condition.

## MATERIAL AND METODOS

The investigated cultivars were BRS Radiante (Pinto group), Ouro Vermelho (Roxo), BRS Talismã (Carioca), Supremo (Black) and Bolinha (Yellow), which were multiplied in the field in the town of Lavras, MG, Brazil, in the rainy crop of 2008/2009.

The grains of each one of the cultivars were stored and packed in braided plastic bags (raffia), under room temperature (covered concrete block room on wooden slats) and in cold room (with constant temperature of 10°C and relative humidity of 50%).

After twelve months' storage, the centesimal composition was determined according to the AOAC (with some modifications), by utilizing the completely randomized design and factorial scheme 5x2, with three replicates.

## RESULTS AND DISCUSSION

For the values of ether extract, crude protein, crude fiber and glucides, there was a significant interaction between cultivars and storage condition (Table 1). By comparing cultivar Talismã with the four alternative cultivars, the Carioca type stood out only for ether extract when stored under refrigerated condition.

The values of protein, ether extract and glucides lay close to those determined by some authors, even after months' storage. For protein, cultivar Bolinha stood out, outyielding inclusive the cultivar of the Carioca group. In relation to crude fiber, cultivar Bolinha stood out, but only under the refrigerated condition, the values found were superior to those of the literature, which may have occurred due to the loss of the selectivity of the cell membranes with storage time.

The values of moisture and ashes were not influenced by the interaction between cultivars and storage condition (Table 2). As to ash contents, cultivars Talismã (Carioca), Ouro Vermelho and Supremo overcome the others.

**Table 1** Grain contents of ether extract, crude protein, crude fiber and glucides by five bean cultivar under two conditions

| | Content in % (g/100g)* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Ether extract | | Crude protein | | Crude fiber | | Glucides | |
| Cultivars | Amb.** | Ref.*** | Amb. | Ref. | Amb. | Ref. | Amb. | Ref. |
| Radiante | 1.82Bb | 1.15Aa | 18.93Ba | 19.71Aa | 6.53Aa | 6.11Aa | 72.45Ba | 73.51Da |
| O.vermelho | 1.80Ba | 1.67Ba | 20.05Ba | 20.50Ba | 7.06Aa | 7.23Ba | 70.10Aa | 70.82Ba |
| Talismã | 1.52Aa | 2.38Cb | 19.50Ba | 19.59Aa | 6.92Aa | 6.38Aa | 71.40Aa | 71.87Ca |
| Supremo | 1.83Ba | 2.38Cb | 17.69Aa | 19.43Ab | 6.48Aa | 7.19Ba | 73.40Bb | 71.13Ba |
| Bolinha | 1.17Aa | 0.98Aa | 22.04Cb | 20.68Ba | 6.38Aa | 10.12Cb | 70.41Ab | 68.90Aa |
| Mean | 1.63 | 1.71 | 19.64 | 19.98 | 6.67 | 7.41 | 71.55 | 71.25 |
| CV(%) | 14.10 | | 2.39 | | 6.67 | | 0.99 | |

Means followed by the same small letter in the row and capital in the column belong to the same group according to the Scott-Knott test at 5 % of probability. *Values in dry matter. ** Under room temperature. *** In cold room

**Table 2** Grain contents of moisture and ash by five bean cultivars

| | Content in % (g/100g) | |
|---|---|---|
| Cultivars | Moisture | Ash* |
| Radiante | 11.85 | 3.87 A |
| O. Vermelho | 12.56 | 4.34 B |
| Talismã | 12.59 | 4.30 B |
| Supremo | 12.61 | 4.21 B |
| Bolinha | 12.89 | 4.03 A |
| Ambiente | 11.70 a | 4.25 |
| Refrigerada | 13.70 b | 4.05 |
| CV (%) | 5.82 | 6.76 |

Means followed by the same letter belong to a same group by the Scott-Knott test at 5 % level of probability. *Values in dry matter. CV: Coefficient of Variation

## CONCLUSIONS

The values of nutrients presented by the alternative cultivars do not differ, to a great extent, from the values presented by cultivar Talismã, of the Carioca group.

The bean cultivars present protein contents comparable to those of the literature and Bolinha cultivar presenting the highest content.


## ACKNOWLEDGEMENTS

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# DRY BEAN SEED FLOUR USED AS A PROTEIN SOURCE FOR FEEDING BEEF CATTLE

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**INTRODUCTION:** In Durango, México, dry bean (*Phaseolus vulgaris* L.) is grown in 224,000 hectares, where near to 125,000 MT, on average, are produced per year (SAGARPA, 2011). Residues obtained after the grain cleaning process, locally known as granza, reaches near to 36,000 MT. Dry bean seed residues have been used in ovine feeding (Esqueda, 2007) and bovine supplements (Rodrigues *et al*., 2008). Dry bean is a drought tolerant crop and could be used to replace alfalfa production and reduce water consume. Dry bean seeds contain lectins and trypsin inhibitors, considered as important antinutritional factors (Reynoso *et al*., 2005). Thus, feeding growing calves with diets containing raw beans as the main source of protein could produce undesirable physiological and biochemical effects. These effects are reflected by growth reduction, poor nitrogen balance, reduced intestinal absorption of sugars and amino acids, and impaired immune response (Marzo *et al*., 2002). In Durango, bovine production showed recurrent deficit in food supply and bean wastes could be used to enrich bovine supplementary diets. The objective was to evaluate the effect of dry bean seed flour as a component of supplements used in calves feeding.

**MATERIALS AND METHODS:** A feeding trial was carried out from October 30$^{th}$ 2010 to January 2011 at INIFAP-Durango Experiment Station. Eight $F_1$ calves were used in the experiment, which showed a mean of 170 kg for individual starting weight and belonging to Charolais (5) and Limousin (3) crossbreds. Calves couples were separated in four stall under a Latin Square (4 x 4) design, organized according to feeding treatments. Four experimental diets were evaluated, which contained silage, cotton seed meal, steam rolled corn, distillers dried grains, Microfos 12-10™ and Corral Elite™. Sanitary management was applied to calves and two protein sources were considered: alfalfa hay and mixed granza flour. Diets were supplied daily at 9 am in a single ration offered *ad libitum*. The daily rations were supplied according to 3 % of the body weight and orts were also weighted in order to estimate the voluntary feed intake. The experiment lasted 68 days and was divided into four periods of 17 days each. Calves were individually weighted every 17 days on an electronic scale (Gallagher, SmartScale, 500). Data were used to calculate daily weight gains and feed efficiency. The analysis of variance was performed using the Latin Square (4 x 4) design, using the PROC GLM of SAS®. Means comparison was performed by the LSD test, with alpha level of 0.05.

**RESULTS AND DISCUSSION**: Highly significant differences were observed ($P<0.01$) among diets, for daily weight gains and similar effects were observed for feed efficiency (Table 1). Diets including alfalfa as the main protein source showed highest daily increments in weight with mean values of 2.03 kg day$^{-1}$ for corn:apple silage+alfalfa and 1.97 kg day$^{-1}$ for corn silage+alfalfa (Table 2). In contrast, diets including bean flour as the main protein source showed lower values for daily increments in weight averaging 1.51 kg day$^{-1}$ for corn silage+bean and 1.28 kg day$^{-1}$ for corn:apple silage+bean. Values observed for feed efficiency showed that diets containing bean flour required

higher amounts of forage and supplements to obtain similar increments in weight. Results showed that alfalfa is an important protein source which enhances nutritional quality in bovine forages. The use of flour obtained from dry bean grains requires further study to increase its usefulness in calves feeding, by reducing antinutritional factors (Reynoso *et al*., 2005) and negative effects caused in digestive tract, metabolic processes and growth (Marzo *et al*., 2002).

**Table 1.** Mean squares of the analysis of variance performed for experiment including four diets used for feeding growing calves. Durango, 2010-2011.

| Source of variation | Degrees of freedom | Weight Increment (kg day$^{-1}$) | Feed efficiency (kg kg$^{-1}$) |
|---|---|---|---|
| Rows | 3 | 0.225* | 7.1n.s. |
| Columns | 3 | 0.064n.s. | 3.4n.s. |
| Diets | 3 | 0.520** | 1.5n.s. |
| Error | 6 | 0.048 | 0.6 |
| Mean | | 1.7 | 6.1 |
| *CV (%) | | 12.9 | 12.9 |
| $LSD_{0.05}$ | | 0.11 | -- |

*CV = Variation Coefficient; Least Significant Difference.

**Table 2.** Means observed in feeding trial performed by using four diets for growing calves. Durango, 2010-2011.

| Diet | Weight Increment (kg day$^{-1}$) | Feed efficiency (kg kg$^{-1}$) |
|---|---|---|
| Corn:Apple silage +Alfalfa | 2.03a | 5.58 |
| Corn :Apple silage +Bean | 1.28b | 6.99 |
| Corn Silage+Alfalfa | 1.97a | 5.82 |
| Corn Silage+Bean | 1.51ab | 6.13 |

**CONCLUSIONS:** Further research is needed in order to increment efficiency in using dry bean residues for animal feeding. Main research topics are methods for reducing antinutricional factors and ecological impacts of using dry bean instead alfalfa hay for cattle feeding.

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# CHARACTERIZATION OF BLACK BEAN CULTIVARS FOR PROCESSING

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Common beans are widely consumed in Brazil, being considered the ingredient symbol of Brazilian gastronomy. Their acceptability is connected with various characteristics such as color, size, appearance, cooking time and flavor. Delayed cooking time has always been a limiting factor for purchase and preparation, especially for long time stored old beans; resulting in sensory and nutritional losses. The objective of this work was to evaluate some physicochemical characteristics of black bean cultivars required by the bean processing industry and related to quality. Eight cultivars of black beans (BRS 7762 Supremo – "SUP", Xamego – "XAM", Diamante Negro – "DN", BRS Campeiro – "CAMP", BRS Esplendor – "ESP", Ônix, BRS Grafite – "GRAF", BRS Valente – "VAL") from the 2010 cropping season (Santo Antônio de Goiás-GO) were donated by Embrapa Common Bean National Breeding Program and tested in the Grain and Byproducts Laboratory at Embrapa Rice and Beans Research Center for: minimum cooking time in Mattson Cooker Apparatus [1]; % of water absorption before (WABC) and after (WAAC) cooking [2,3]; determination of color parameters $L^*$, $a^*$ and $b^*$ in raw and cooked beans in colorimeter (Color Quest XE; HunterLab; USA); instrument texture evaluation of the cooked bean by texture analyzer (TA.Xtplus, Stable Micro Systems, Surrey, United Kingdom; probe P/2 (2 mm Cylinder Stainless), using charge cell of 50 kg) [4]; grain moisture content after oven drying at 105ºC [5]. Samples conditioning for the texture test followed two cooking methods: in oven (water soaking (1:3) for 16 hours in glass flasks; taken to oven at 105ºC for two hours, and resting for 30 min. at room temperature); in autoclave (30 g samples were placed in flasks with 100 mL of distilled hot water and taken to the autoclave for 15 min. at 121 ºC, and further resting at room temperature for 30 min.) [6]. Data was submitted to the analysis of variance and Tukey test was applied for mean comparison at 5% probability using SAS program [7].

Significant differences were observed among samples evaluated (Table 1). For moisture, only SUP and ESP were superior, but all samples were within a uniform moisture content range. Regarding color, ESP had beans less dark than SUP and VAL (lower $L^*$ values, Table 1). After cooking, all samples showed a more intense color, especially XAM and DN, with the lowest $L^*$ values, and ESP showed the lightest color, being more sensible to discoloration. Cooked beans presented a tendency to change to purple (values of $a^*>0$) and yellow (values of $b^*>0$), and values higher than $a^*$ and $b^*$ were observed in CAMP and ESP. For cooking time, SUP was considered resistant, being associated to the highest hardness values after autoclaving. All samples had broth with dark chocolate color, except SUP, CAMP and ESP, which had light chocolate broth color. It was observed that cooking in autoclave, usual in the industry, generated the lowest hardness values when compared to oven cooking; demonstrating to be a process that strongly affects grain structure. Besides, there were differences in performance according to the cooking process applied. DN presented the lowest cooking time and the lowest hardness values, regarding cooking method. GRAF had the cooking time similar to DN, and the lowest hardness value after autoclaving, but the highest in oven. WABC was normal, without significant differences among varieties, but with significant differences after cooking (WAAC), where variety GRAF showed the highest value and ESP the lowest. These results are linked to the yield of cooked beans. After visual evaluation, it was observed that all cooked samples had good appearance, with small amount of cracked beans.

There is variability in the performance of the cultivars tested for the attributes evaluated, especially for DN with good grain color stability after cooking, among other characteristics, followed

by CAMP, with good texture. The majority of samples showed good industry processing and commercialization potential as pre cooked food, without expressive loss of technological quality.

**Table 1**. Characterization of color[i], moisture[ii], cooking time[ii] and instrumental texture of black bean cultivars tested (means ± standard deviation).

| | | Color – L* | | | Texture after autoclave[iii] | | Texture after oven[iv] | |
|---|---|---|---|---|---|---|---|---|
| **Black Beans** | **Moisture (%)** | **Raw** | **Cooked** | **Cooking time (min)** | **Hardness (N)** | **Stickiness (N)** | **Hardness (N)** | **Stickiness (N)** |
| SUP | 9.03±0.27a | 32.19±0.51b | 21.25±1.68cd | 37.22±1.03a | 1.03±0.43a | -0.21±0.06c | 16.50±1.09b | -0.11±0.03c |
| XAM | 7.44±0.08b | 32.54±0.63ab | 17.25±0.31e | 29.87±0.11b | 0.95±0.27ab | -0.19±0.04bc | 15.52±1.14bc | -0.11±0.03bc |
| DN | 7.71±0.07b | 32.43±0.41ab | 17.55±0.62e | 28.84±0.72b | 0.80±0.23abc | -0.15±0.04ab | 14.65±1.46c | -0.08±0.03ab |
| CAMP | 7.49±0.09b | 32.44±0.51ab | 24.15±2.14ab | 33.95±2.23ab | 0.93±0.21abc | -0.13±0.03a | 15.56±2.25bc | -0.08±0.03ab |
| ESP | 9.15±0.25a | 32.99±0.50a | 26.32±1.56a | 34.05±2.99ab | 0.71±0.19abc | -0.18±0.03abc | 16.91±1.76b | -0.07±0.03a |
| ÔNIX | 6.89±0.03b | 32.61±0.56ab | 20.08±1.38d | 31.87±0.08ab | 0.62±0.14bc | -0.13±0.03a | 16.27±1.69bc | -0.09±0.02abc |
| GRAF | 7.84±0.14b | 32.57±0.45ab | 22.81±0.98cd | 28.07±2.91b | 0.55±0.21c | -0.14±0.03a | 19.34±1.79a | -0.09±0.03abc |
| VAL | 7.32±0.46b | 32.23±0.40b | 21.68±2.32cd | 30.75±0.39b | 0.78±0.29abc | -0.14±0.03ab | 15.35±2.17bc | -0.09±0.03abc |

[i](n = 10); [ii](n = 3); [iii](n = 10); [iv](n = 20). Means followed by the same letters in rows do not differ according to Tukey ($p < 0.05$).

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# COOKING QUALITY AND CHANGES IN COLOR BY EFFECT OF AGEING IN A YELLOW DRY BEAN POPULATION

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## INTRODUCTION

Post harvest darkening of the seed coat is a problem in clear dry beans. Yellow seed color is a preferred market class for the northern and central area of México; however this type of beans tends to darkening, which decreases its commercial value.

Usually darkening is accompanied by an increase in cooking time for dry beans, and this affects culinary quality, making it undesirable for consumers. In many fruit and vegetables, post-harvest enzymatic browning is catalyzed by the enzyme polyphenol oxidase

In México, yellow dry bean varieties are only developed for the northwest area of the country, in which they are grown under irrigation conditions. Recently, the dry bean breeding program at the Valle de Mexico Experimental Station is working on yellow bean varieties for the central plateau of México. The objective of this study was assessing the tendency of a group of yellow seed coat inbreed lines to postharvest darkening.

## MATERIALS AND METHODS

During PV 2009, a recombinant inbreed line population was sown at Santa Lucía de Prías, Texcoco, Estado de México. The experimental plot was one 4 m- long row. All the plants of each plot were hand threshed and grain production was estimated. A group of fifty agronomical outstanding RILs was taken for characterizing aging.

Seed samples were kept at 5°C until finishing the analyses. Grain moisture content was adjusted to 12%, separated into two lots; one was stored at 40ºC, 75% Relative Humidity, (in a saturated NaCl solution) during 28 days. The other lot of each variety was kept at 5°C until being analyzed. Coat color was measured using a CM-5 spectrophotometer (Konica Minolta, Inc., Osaka, Japan). Color reflectance was recorded in the CIE Lab color coordinate system, with D65 Illuminant and 10º observer. The aged RILs were also ordered from one to fifty according to visual appreciation of darkness. Seed coats were obtained from dry grains using a scalpel.

Activity of polyphenol oxidase (PPO), enzyme associated to darkening, was also estimated. Its activity was determined at room temperature according to the method described by Anderson and Morris (2001). One unit of PPO was defined as the amount of protein, producing a change of 0.001 in absorption at 540 nm. The weight and volume of one hundred seeds wt. were measured, and cooking time was evaluated using a sensorial method (Guzmán *et al.,* 1995). Data were processed through an analysis of variance. Weight and volume, of one hundred grains as well as water absorption capacity, solids in broth, and protein content were determined in replicated samples. Cooking time was measured according to sensorial method in two samples of 25 grains, previously soaked in water for 18 hours.

## RESULTS AND DISCUSSION

RILs exhibited significant differences in color variables L*, a*, and b* ($P \leq 0.01$) in response to ageing. Darkening of grains produced a decrease in L* value compared to its control up to [- 9.56] units. Genotypes increased in different magnitude of reddish tones (Δa* from 2.38 to 10.68) while the b* variable had a tendency to decrease in 75% of RILs, which means lessening of yellow tones (Table 1). In aged dry beans the darker seed coat (classified by visual appreciation) was associated to higher a* value (0.47**), lower b* value (0.79**), and lower Hue (-0.60**). The RILS with smaller grain size tend to show darker seed coat (-0.49**). Cooking time of the RILs varied from 59 to 107 minutes, some of which presented hard shell defect, thus water absorption capacity was from 17 to 110 %. The amount of solids in broth varied from 0.16 to 0.41 %. RILs with higher water absorption capacity tend to faster cooking (-0.24*).

The RILs showed different activity of polyphenol oxidase, varying from 0.740 to 3.76 units of PPO per mg of seed coat. However the activity level of this enzyme apparently was not associated with the degree of darkening in the RILs, as detected in a previous study (Jacinto *et al*. 2007) with clear coat dry beans that did not include yellow dry beans.

Approximately seven out of the 50 RILs, that is 14%, seem to show resistance to darkening in response to aging. Among them there were three RILs with short cooking time (less than 70 minutes). Those characteristics will be confirmed in different environments.

**Table 1.** Color changes in 50 yellow dry bean RILs under accelerated ageing

| | **Variable** | **Minimun** | **Maximun** | **Average** |
|---|---|---|---|---|
| control | L* | 58.61 | 68.37 | 64.42 |
| Aged | L* | 55.32 | 65.37 | 59.95 |
| Differences | Δ L* | 0.9 | 9.43 | -4.81 |
| control | a* | -1.73 | 2.28 | 0.71 |
| Aged | a* | 3.85 | 10.30 | 6.89 |
| Differences | Δ a* | 2.38 | 10.68 | 6.18 |
| control | b* | 22.06 | 43.02 | 33.80 |
| Aged | b* | 21.94 | 38.57 | 30.93 |
| Differences | Δ b* | 3.31 | 9.43 | -3.01 |

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# COOKING TIME OF GRAINS OF BEAN CULTIVARS ON THE BASIS OF THE DURATION AND STORAGE CONDITIONS

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## INTRODUCTION

Out of the technological characteristics of the bean grain, cooking time is, without a doubt, that of greatest importance. Short cooking time of grains is important from the nutritional standpoint, for decreasing the loss of solids and advisable in the reducing of energy spent. Grains which demand increased cooking time are past by on market and storage can influence directly such a characteristic. The objective of the work was studying the average cooking time of grains (TMC) of bean cultivars of different commercial groups, submitted to storage for different periods and under distinct conditions.

## MATERIAL AND METHODS

The statistical design was randomized blocks with five replicates and experimental scheme of split plots in time. The plots were made up of five cultivars (BRS Radiante, Ouro Vermelho, BRS MG Talismã, BRS Supremo and Bolinha, harvested in the rainy season of 2008/09, in Lavras-MG, Brazil), packed under two storage conditions (room temperature and under refrigeration) and the subplots for four storage times (3, 6, 9 and 12 months).

The TMC was determined by the Mattson cooker, according to the method modified by Proctor and Watts (1987). The data were submitted to the variance analysis, the significant effect of cultivar and storage condition being evaluated by clustering of means by using the Scott-Knott test, the effect of storage time was studied by regression analysis.

## RESULTS AND DISCUSSION

The analysis of variance revealed that there was a good experimental precision (CV1=10.87% and CV2=10.34%) and significance of the triple interaction.

In Table 1, it is found that, in general, the storage under refrigeration provided shorter cooking time in all the storage periods. Small variations were found in the individual behavior of the cultivars at 3 and 6 months of storage, but a thenceforth all the cultivars presented smaller TMC under refrigeration (Table 1), confirming other authors. Sievwright and Shipe (1986), for example, found that the increased storage temperature prolongs cooking time. Brackmann et al. (2002), working with refrigerated air, room air and controlled atmosphere and cultivars Carioca, Pérola and M90-012, observed longer time for cooking when room storage was used.

On the average, both cultivars Talismã and Radiante presented the lowest values of TMC, overcoming the others. Cultivar Talismã had also stood out as to TMC on the occasion of harvest and this performance, in a given way, does not startle, for being a cultivar with carioca type grains, the one of greatest national acceptance. Cultivar Bolinha, in turn, was the one which presented longest cooking time, confirming the reputation of the yellow group as the one which possesses the worst cooking quality.

TMC grew with increasing storage period under both the conditions. In some cultivars, mainly under refrigeration, there was a trend toward slight reduction of TMC from the 9th months of storage on (Figure 1). These results support earlier ones such as by Brackmann et al. (2002), who found increase in TMC from 9 to 19 months' storage in some cultivars of the carioca type.

**Table 1.** Average values of average cooking time (TMC), in minutes, on the basis of cultivar, storage condition and period*

| | TMC | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Storage time (months) | | | | | | | |
| C[1] | 3 | | 6 | | 9 | | 12 | |
| | Amb. | Refr. | Amb. | Refr. | Amb. | Refr. | Amb. | Refr. |
| **R** | 31.43bA | 23.87aA | 30.70bA | 23.28aA | 74.41bA | 40.21aB | 76.61bB | 30.41Ab |
| **OV** | 31.40aA | 31.39aA | 57.83bC | 46.40aB | 95.21bB | 45.81aB | 91.41bC | 33.41aB |
| **T** | 30.02aA | 24.12aA | 51.33bC | 26.00aA | 77.61bA | 31.41aA | 67.41bA | 24.61aA |
| **S** | 29.84aA | 24.60aA | 52.06bC | 29.56aA | 100.81bB | 41.00aB | 74.61bB | 31.10aB |
| **B** | 34.08bA | 25.20aA | 37.75aB | 44.42bB | 96.01bB | 38.01aB | 85.21bC | 40.61aC |
| **M*** | 31.35b | 25.84a | 45.93b | 33.93a | 88.81b | 39.29a | 79.05b | 32.03a |

* Means followed by the same small letter in the row and capital in the column belong to the same group according to the Scott-Knott test at the 5% level of probability.
[1] C= Cultivar; R= BRS-Radiante; OV= Ouro Vermelho; T= BRS-Talismã; S= BRS-Supremo; B= Bolinha

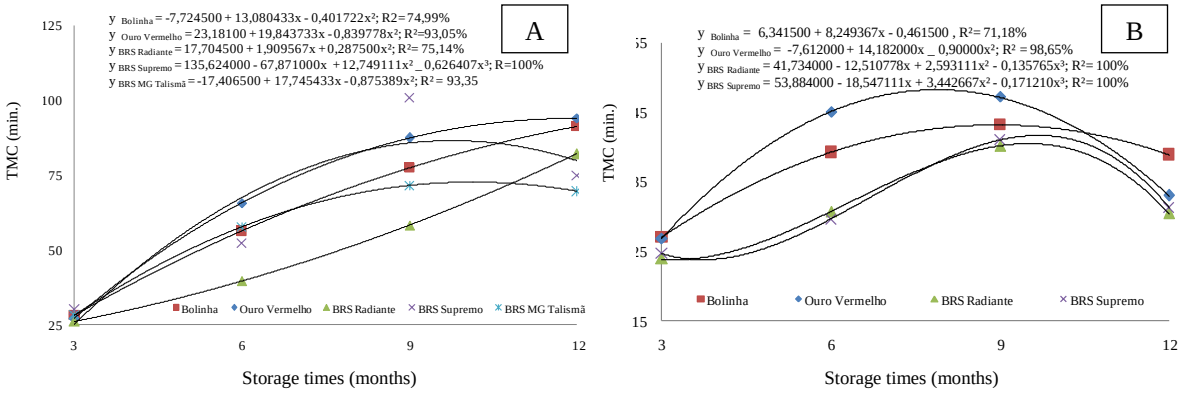


**Figure 1** Average cooking time (TMC), in minutes, of bean cultivars base upon cooking time under room (A) and refrigerated temperature (B)

## CONCLUSIONS

Storage time raises TMC, mainly when under packing conditions.
Cultivars Radiante and Talismã present the shortest TMC along the storage.


## ACKNOWLEDGEMENTS

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# COOKING TIME OF COMMON BEANS CARIOCA TYPE EVALUATED IN DIFFERENT ENVIRONMENTS

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Brazil is the world largest producer and consumer of common beans. Among the many types cropped, carioca stands out as the most consumed, sharing more than 70% of the market (Del Peloso & Melo,2005) and corresponding to near two million tons per year (FEIJÃO, 2011). Given the great importance of the bean crop, breeding programs are conducted by agricultural research institutions to supply the market with new improved cultivars. These cultivars bring together suitable characteristics, such as disease resistance, modern plant architecture, besides high yielding potential, contributing to increase crop yields from 729 kg/ha in 1997 to 1160 kg/ha in 2009 (FEIJÃO, 2001). In addition to those important agronomic traits, new enhanced lines should also provide good culinary characteristics such as short cooking time. This may contribute to increase bean consumption, mainly by reducing the time spent in bean meal preparation, since this activity has led Brazilians to seek ready to eat foods. Cooking time is affected by various environmental factors and by the genotype. Considering that the final evaluation of carioca lines is performed in several environments, there is the possibility of measuring the cooking time of these lines, and also, to verify the existence of genotypes x environments interaction for that characteristic.

Trials were conducted in eleven environments, each one consisting of 17 genotypes of carioca common bean type (Table 1). The experimental design was a randomized complete block with two replications, and plots with four rows four meters long. Samples to perform the cooking time tests were drawn from the central rows and stored at room temperature from 30 to 90 days. Tests were performed using a method similar to that proposed by Proctor and Watts (1978) described by Torga et al. (2010) with two replicates for each sample. Data were submitted to the analysis of variance, using the Scott Knott test at 10% for mean comparison.

The coefficient of variation of the joint analysis was 15.7%, suggesting a good experimental precision. Significant differences ($P<0.01$) were detected among genotypes, environments, and for the interaction genotype x environment. The average cooking time (CT) was 28 minutes, varying from 25 min. for line CNFC 11945 to 33 min. for cultivar BRS 9435-Cometa (Table 1). This variability among genotypes allows selection of those with reduced cooking time. There was variability from 20.9 to 41.2 min. among environments. Data presented in Table 1 demonstrate that environment was more important than genotype in affecting cooking time of beans. The large variability in the environment means is related to differences in the environmental conditions during experiment conduction and harvest as well as to differences in the storage period. The environment showing the largest cooking time was Santo Antônio de Goiás, in the wet season 2009. The shortest were also observed for that same location but in different seasons, in the 2009 winter season and 2010 dry season. This fact emphasizes the importance of the environment to determine cooking time, once the shortest and longest cooking times were observed in the same location, but in different cropping seasons.

Among the controls, BRS Estilo showed the shortest cooking time (27.3 min.); Perola and IPR Juriti were grouped in the second group, while BRS 9435 Cometa remained in the third group (Table 1). None of the lines showed CT lower than the best control. Torga et al. (2010) found a line superior to the control, when working with black beans; however eight lines showed cooking time similar to cultivar BRS Estilo, with cooking time shorter than cultivar Perola (30 min), the main cultivar cropped in Brazil. Seven other genotypes showed CT similar to Perola, which could be considered satisfactory. The short cycle cultivar BRS 9435 Cometa showed the largest CT. Results indicated that CT presented by the evaluated lines were within standards adopted in Brazil.

**Table 1.** Average cooking time (CT) (minutes) of 17 genotypes of common beans carioca type, and of 11 environments evaluated in Brazil, 2009/2010.

| Genotype | CT | Environment | CT |
|---|---|---|---|
| CNFC 11945 | **25,0 a** | Sto. Ant. Goiás/GO/Winter 2009 | **20,9 a** |
| CNFC 10429 | **25,8 a** | Sto. Antônio Goiás/GO/Dry 2010 | **21,4 a** |
| CNFC 11951 | **25,8 a** | Sem. Canedo/GO/Winter 2009 | 23,9 b |
| CNFC 11944 | **25,8 a** | Porangatu/GO/Winter 2009 | 24,0 b |
| CNFC 11962 | **26,0 a** | Arco Verde/PE/Wet 2010 | 25,3 b |
| BRS Estilo | **27,3 a** | Coronel João Sá/BA/Wet 2010 | 25,7 b |
| CNFC 11946 | **27,3 a** | Brasília/DF/Winter 2010 | 28,5 c |
| CNFC 11966 | **27,4 a** | Rio Verde/GO/Wet 2009 | 31,7 d |
| CNFC 11952 | **28,0 a** | Inhumas/GO/Dry 2009 | 33,9 e |
| IPR Juriti | 29,2 b | Carira/SE/Wet 2010 | 34,4 e |
| CNFC 11953 | 29,7 b | Sto. Ant. Goiás/GO/Wet 2009 | 42,1 f |
| CNFC 11954 | 29,9 b | | |
| CNFC 11959 | 30,0 b | | |
| Pérola | 30,0 b | | |
| CNFC 11956 | 30,4 b | | |
| CNFC 11948 | 31,0 b | | |
| BRS9435 Cometa | 33,4 c | | |

[1]Means followed by the same letter do not differ by the Scott Knott test at 10% probability.

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# SELECTION OF SEGREGATING POPULATIONS WITH DELAYED DARKENING IN CARIOCA TYPE COMMON BEANS

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Carioca common beans type is cropped throughout Brazil, representing 70% of the total cultivated area (Del Peloso and Melo, 2005). During storage chemical changes take place, modifying tegument color, leading to depreciation of quality and market value. Darkened tegument is associated to old beans, with extended cooking time, rejected by consumers. Study reports had pointed out the possibility of selecting breeding lines with delayed darkening (Junk-Knievel et al., 2008; Silva et al., 2008). Elite genotypes with delayed darkening trait are already available, such as BRS MG Madrepérola, BRS Requinte and CNFC 10467. However there are no reports comparing the delayed darkening level of each of those genotypes neither the way these genotypes combine with others without desired trait.

Crosses were performed in a partial diallel scheme with: three genotypes having tegument delaying characteristics (Group I: BRS Requinte, BRS MG Madrepérola and CNFC 10467) and ten cultivars/elite lines, with usual tegument darkening (Group II: BRS Estilo, Pérola, BRS Cometa, BRS Pontal, BRS MG Majestoso, IAC Alvorada, IPR Saracura, IPR Siriri, CNFC 10429 and CNFC 10408). The segregating populations were evaluated in 2010 in Santo Antônio de Goiás in a randomized block design with three replicates in four line four meter long plots. Seeds of thirty individual plants were collected in each replicate in each treatment. Collected beans were stored in transparent polyethylene bags and kept at room temperature for further evaluation of tegument darkening. Tests were performed 106 days after harvest, assigning grades in a scale ranging from 1 (light beans) to 5 (beans very dark). Data were submitted to the analysis of variance, with further partial diallel analysis according to procedures described by Kempthorne, to estimate the effect of the general combination capability (GCC) and specific combination capability (SCC).

Significant differences were observed ($p<0.01$) among GCC ($g_1$) in the two groups of genitors analyzed, showing the existence of variability in the general combination capability of the genitors of the two groups (Table 1). GCCs of all genotypes were significantly different from zero (standard error of group 1=0.05 and standard error of group 2=0.11). In group I, BRS MG Madrepérola was the genitor with the best GCC, for delayed darkening (-0.51). Other genotypes in this group were BRS Requinte (0.22) and CNFC 10467 (0.23). In group II there was also variability for darkening, and the genitors that contributed the most for lesser darkening were CNFC 10429 (-0.31); IAC Alvorada (-0.28); BRS Estilo (-0.24); IPR Siri (-0.18); and IPR Saracura (-0.14). Genotypes CNFC 10408 (0.49) and BRS Cometa (0.25) showed the worst estimates to form new populations with darkening time delaying. It was not observed significance in SCC.

Populations with the best averages for increased darkening delay were originated from crosses between: BRS MG Madrepérola (group I) and IAC Alvorada (2.5); and BRS Estilo (2.6) and CNFC 10429 (2.7) (Table 2); these populations are promising genotypes for obtaining breeding lines.

**Table 1.** Summary of partial diallel analysis for delayed darkening in populations of common beans carioca type. Santo Antônio de Goiás-GO, 2011.

| Source of variability | GL | SS | MS | Probability |
|---|---|---|---|---|
| Treatments | 42 | 41.83 | 0.99 | 0.00000 |
| Genitors | 12 | 22.13 | 1.84 | 0.00000 |
| Group I | 2 | 3.02 | 1.51 | 0.00003 |
| Group II | 9 | 11.95 | 1.33 | 0.00000 |
| Group I x II | 1 | 7.15 | 7.15 | 0.00000 |
| Parents x Crossing | 1 | 0.30 | 0.30 | 0.13090 |
| Crossing | 29 | 19.40 | 0.70 | 0.00000 |
| GCC I | 2 | 11.78 | 5.89 | 0.00000 |
| GCC II | 9 | 5.80 | 0.64 | 0.00040 |
| GCC I x GCC II | 18 | 1.82 | 0.10 | 1.00000 |
| Residue | 84 | 10.92 | 0.13 | |

**Table 2**. Darkening means of genitors and populations of common bean carioca type. Santo Antônio de Goiás-GO, 2011.

| Genotype | BRS MG Madrepérola | CNFC 10467 | BRS Requinte | Genitors means |
|---|---|---|---|---|
| BRS Cometa | 3.2 | 3.7 | 4.1 | 4.7 |
| BRS Estilo | 2.6 | 3.6 | 3.5 | 3.4 |
| BRS Pontal | 2.9 | 3.8 | 4.0 | 3.3 |
| IAC Alvorada | 2.5 | 3.9 | 3.2 | 3.1 |
| IPR Saracura | 2.8 | 3.5 | 3.5 | 4.0 |
| IPR Siriri | 2.8 | 3.5 | 3.4 | 2.8 |
| Pérola | 3.2 | 3.9 | 3.5 | 3.9 |
| BRS MG Majestoso | 3.0 | 3.9 | 3.7 | 4.1 |
| CNFC 10408 | 3.5 | 4.2 | 4.0 | 4.8 |
| CNFC 10429 | 2.7 | 3.2 | 3.4 | 3.4 |
| Genitors means | 2.1 | 2.4 | 3.5 | - |

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SILVA, G.S.; RAMALHO, M.; ABREU, A.F.; BOTELHO, F.B. Genetic control of early grain darkening of carioca. **Crop Breeding and Applied Biotechnology,** 8: 299-304, 2008.

# ELECTRIC CONDUTIVITY OF GRAINS OF BEAN CULTIVARS ON THE BASIS OF DURATION AND STORAGE CONDITIONS

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## INTRODUCTION

A number of tests have been proposed for evaluation of the technological quality of bean grains. Out of them, the electric conductivity test (CE) is cited as a good indicator of the solute leaching, which can be related to grain aging. The present paper was intended to evaluate the electric conductivity of grains of bean cultivars of different commercial groups, submitted to storage for different time periods and under distinct conditions.

## MATERIAL AND METHODS

The statistical design was randomized blocks with five replicates and experimental scheme of split plot in time. The plots were made up of five cultivars (BRS Radiante, Ouro Vermelho, BRS MG Talismã, BRS Supremo and Bolinha, harvested in the rainy season of 2008/09 in Lavras-MG, Brazil), packed under two storage conditions (room temperature and under refrigeration) and the subplots, for four storage times (3, 6, 9 and 12 months).

The CE was obtained according to Brasil (2009) and Lopes et al. (2010), with results expressed in $\mu S\ cm^{-1}\ g^{-1}$ of grains. The data were submitted to the variance analysis, the significant effects being of cultivar and storage condition evaluated by grouping of means by the Scott-Knott test; the effect of storage time was studied by regression analysis.

## RESULTS AND DISCUSSION

The variance analysis revealed that there was a good experimental precision (CV1=10.60% and CV2=11.91%) and significance of the double interactions condition x cultivar and time x condition.

Refrigeration reduced CE in relation to room condition and this occurred in a significant manner in all the cultivars (Table 1) and studied times (Table 2).

In Table 1, it is still found that in both the conditions, the cultivars differed as to the CE. Under room conditions, cultivar Talismã is stood out with the lowest conductivity (58.01 $\mu S\ cm^{-1}\ g^{-1}$), followed by cultivars Radiante and Supremo; high conductivity (values above 90 $\mu S\ cm^{-1}\ g^{-1}$ ) was shown by cultivars Bolinha and Ouro Vermelho. Under refrigeration, cultivars Talismã (group carioca) and Radiante () stood out, followed by Supremo (black), Ouro Vermelho and Bolinha. These results point out that in non-refrigerated storage, utilize don a large scale, in Brazil, the Carioca grain seems to keep its membrane structures better, being less subject to exudate drainage when immersed in water. The black type grain, several times the second in the Brazilian popular preference (ANDRADE; RAMALHO, 1999), also showed good quality, evaluated by the electric conductivity test. Similar result was obtained with the pinto grains of cultivar Radiante, a type which has a great chance in the international market.

The effect of the storage period on the CE of grains was significant under room condition, where storage time increased the CE of the grains linearly (Figure 1).

**Table 1** Average values of electric conductivity (CE) of bean cultivars on the basis of storage condition

| Cultivar[1] | CE (µS cm$^{-1}$ g$^{-1}$) | | Mean |
|---|---|---|---|
| | *Amb.* | *Ref.* | |
| **R** | 79.19bB | 52.08aB | 65.63B |
| **OV** | 101.18bD | 72.85aC | 87.01D |
| **T** | 58.01bA | 45.99aA | 52.00A |
| **S** | 82.83bB | 76.34aC | 79.59C |
| **B** | 91.33bC | 70.95aC | 81.14C |
| **Mean** | 82.51 | 63.64 | 73.07 |

Means followed by the same small letter in the row and capital in the column belong to a same group according to the test of Scott-Knott at the level of 5% of probability. [1]R= BRS-Radiante; OV= Ouro Vermelho; T= BRS-Talismã; S= BRS-Supremo; B= Bolinha

**Table 2** Average values of electric conductivity (CE) on the basis of storage conditions and time

| Time (months) | CE (µS cm$^{-1}$ g$^{-1}$) | | Mean |
|---|---|---|---|
| | *Amb.* | *Ref.* | |
| **3** | 71.61b | 63.99[a] | 67.80 |
| **6** | 78.32b | 63.11[a] | 70.71 |
| **9** | 86.60b | 63.99[a] | 75.29 |
| **12** | 93.49b | 65.07[a] | 79.28 |
| **Mean** | 82.50[a] | 64.04b | 73.27 |

Means followed by the same small letter in the row and capital in the column belong to a same group according to the test of Scott-Knott at the level of 5% of probability

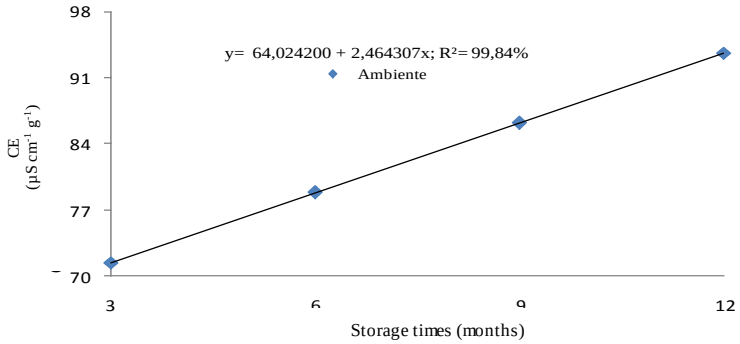


**Figure 1** Electric Conductivity (CE), in µS cm$^{-1}$ g-$^{1}$, of five cultivars stored under room condition on the basis of the storage period.

## CONCLUSIONS

Electric conductivity of grains is greater when storage takes place under room conditions. Under this condition, electric conductivity of grins rises with cooking time.

Cultivar Talismã (carioca group) stands out from the others with lower values of electric conductivity.


## ACKNOWLEDGEMENTS

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# CONSUMER PREFERENCES FOR FIVE NEW PINTO BEAN CULTIVARS IN DURANGO, MÉXICO

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**INTRODUCTION:** Common bean (*Phaseolus vulgaris* L.) is grown in 224,000 hectares in the state of Durango, México, where, on average, 125,000 MT per year are produced (SAGARPA, 2011). In recent years, Pinto Saltillo became the most popular bred cultivar due to its higher yield, better market acceptance, and higher price, compared to traditional pintos (Ávila *et al*., 2010). However, farmers are demanding cultivars with precocious and larger seeds, keeping the slow-darkening coat trait. Compared to P. Saltillo, the five new pinto cultivars (P. Bravo, P. Centauro, P. Centenario, P. Coloso, and P. Libertad) showed either similitude for slow-darkening coat, larger seeds, earlier maturity or darker broth color, and all registered longer cooking time. In order to increase the possibilities of adoption of the new cultivars, consumer preferences need to be considered as one the main factors driving the breeding programs. The objective of this study was to identify the preferences of the consumer regarding the new pinto bean cultivars.

**MATERIALS AND METHODS:** For raw dry beans, a survey (n=149) was conducted for data collection using a questionnaire completed in a face-to-face interview and numerical codes instead of the name of the varieties. Data were taken for seed coat color, brightness, seed size, seed shape and hilum color. Transparent bags, with 100 seeds for each variety, were showed to the persons interviewed and, for each trait, they were asked which seed was preferred. Field work was carried out from March to April 2010 in Francisco I. Madero, Guadalupe Victoria, and Durango. For cooked dry beans, another survey (n=80) was performed with housewives of families consuming beans regularly. Samples of 500g of grain of each cultivar were given to the housewives and they were asked to evaluate: smell of the cooked beans, color of the broth, appearance of the beans, softness of the grain, and flavor of the beans. A scale from 1 to 5 was used for the evaluation (1= very bad; 2= bad; 3= regular; 4= good; 5= very good). Cooking time was determined using a Mattson apparatus with 25 plungers (91 ± 1 g), heat resistant glasses of 2,000 mL, distilled water, and an electric stove.

**RESULTS AND DISCUSSION**: For raw dry beans, P. Libertad showed the highest preference according to seed color (22.2%), which was related to its white-cream seed coat background color and brown strips pattern. Other preferred cultivars were P. Coloso and P. Bravo based on its seed coat brightness (20.0%), P. Coloso for seed size (22.4%), P. Saltillo for seed shape (23.0%) and P. Centenario for hilum color (19.4%) (Table 1). These preferences observed for the raw seed traits need to be considered as selection criteria in the dry bean breeding programs. The results suggest that almost white background, light brown-dispersed strips, shining seed coat, larger seed size, kidney shaped seeds and light-yellow hilum are preferred in pinto cultivars. Recently developed cultivars (P. Libertad, P. Coloso and P. Centenario) showed high percentage of preference for most of the seed traits, except for seed shape. For cooked dry beans, P. Bravo registered the highest scores for all the traits evaluated (Table 2) indicating it was preferred to the checks but also to other new pintos (Coloso, Centenario, Centauro, and Libertad). P. Coloso was the variety in the second place and P. Centenario was in the third place of preferences. P. Centauro and P. Libertad were in the next level of preferences along with the traditional variety P. Saltillo, with most of the scores between 4.0 and 4.2. In the lowest level of preference, were the varieties P. Americano and P. Villa, with most of

the scores less or equal to 4.0. The cooking times determined in the laboratory were: P. Centauro 158 min, P. Bravo 136 min, P. Villa 113 min, P. Americano 70 min, P. Coloso 67 min, P. Libertad 61 min, P. Centenario 58 min, and P. Saltillo 56 min. In spite of its long cooking time, P. Bravo was preferred by the consumers over the other pintos.

**Table 1.** Consumer preference for raw grain of pinto bean varieties by trait. Durango, Méx., 2010.

| Cultivar | Color | Brightness | Seed Size | Seed Shape | Hilum Color |
|---|---|---|---|---|---|
| | (percentage)** | | | | |
| Pinto Coloso | 19.4 | 20.0 | 22.4 | 12.8 | 16.7 |
| Pinto Libertad | 22.2 | 17.2 | 19.6 | 14.2 | 17.4 |
| Pinto Centenario | 18.1 | 17.2 | 16.1 | 18.2 | 19.4 |
| Pinto Bravo | 18.1 | 20.0 | 16.8 | 8.1 | 12.5 |
| Pinto Americano* | 5.6 | 10.3 | 9.1 | 10.8 | 18.1 |
| Pinto Saltillo* | 6.9 | 6.9 | 8.4 | 23.0 | 6.9 |
| Pinto Centauro | 6.3 | 4.1 | 7.0 | 8.8 | 1.4 |
| Pinto Villa* | 3.5 | 4.1 | 0.7 | 4.1 | 7.6 |

*Commercial check ** Consumers that, for each trait, selected one variety as the preferred one.

**Table 2.** Consumer evaluation of cooked pinto bean varieties by trait in Durango, Méx., 2010.

| Cultivar | Traits evaluated** | | | | |
|---|---|---|---|---|---|
| | Smell | Broth color | Beans appearance | Grain Softness | Flavor |
| Pinto Coloso | 4.2 | 4.4 | 4.7 | 4.5 | 4.6 |
| Pinto Libertad | 4.0 | 4.1 | 4.2 | 4.1 | 4.3 |
| Pinto Centenario | 4.1 | 4.4 | 4.1 | 4.3 | 4.6 |
| Pinto Bravo | 4.6 | 4.8 | 4.8 | 4.7 | 4.8 |
| Pinto Americano* | 3.8 | 3.6 | 3.8 | 4.1 | 4.2 |
| Pinto Saltillo* | 3.8 | 4.1 | 4.4 | 4.1 | 4.1 |
| Pinto Centauro | 4.1 | 4.0 | 4.3 | 4.0 | 4.3 |
| Pinto Villa* | 3.7 | 3.3 | 3.7 | 4.0 | 4.0 |

*C= Commercial check **Scale: 1= Very bad; 2= Bad; 3= Regular; 4= Good; 5= Very good

**CONCLUSIONS:** Consumer preferential traits observed in raw pinto bean seeds were white-shining coat background, dispersed brown strips, larger size, kidney shape and light-yellow hilum. In cooked beans, preferred traits among consumers were intermediate brown broth color, appearance of the beans (low proportion of shattered grains), and softer grain. There was not a clear relationship between preferences and cooking time.

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# SYSTEMATIC PRESENTATION OF A GENETIC NOTATION CONVENTION FOR THE COMPLEX *C* LOCUS: *C* AND *R* GENES ONLY

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The genetics of seed coat color and pattern in common bean was recently reviewed by Bassett (2007). In the presence of the "ground factor" gene *P* and the two basic color genes *C* and *J*, a series of color modifying genes (*G*, *B*, *V*, and *Rk*) express a wide range of seed coat colors. The interactions among the seven genes are actually very complex, but presentation of a brief overview requires over simplification. With *C J*, the colors expressed by *G* (yellow), *B* (brown), and *V* (blue to black) are all expressed by the dominant allele at the respective color modifying genes, whereas the various red colors expressed at *Rk* (the red kidney gene locus) are usually expressed by the recessive alleles *rk*, $rk^{d}$, $rk^{cd}$, and $rk^{p}$ (Bassett and Miklas 2003), with the exception of $Rk^{r}$ (Bassett et al. 2010). The $c^{u}$ gene (u = unchangeable) expresses cartridge buff seed coats, where the color is *not* modified by the genes *G*, *B*, and *V*, but *is* modified by the recessive alleles at *Rk*.

There is a dominant gene *R*, tightly linked to *C* that expresses oxblood red seed coats. The heterozygous genotype *Rr* expresses mottled seed coat colors, the actual hue depending on the allelic constitution of the other genes in the system. The interactions of *R* with the other genes controlling seed coat colors were reviewed and summarized by Prakken (1972) and Bassett (2007). The principal gene for seed coat pattern in common bean is *C*. For striped seed coat pattern, the color of the stripes is controlled by the seed coat genotype, but the remainder of the seed is nearly always cartridge buff. A rare exception to this rule is expressed in the Liborino market class, which has thin red stripes on a largely yellow brown seed coat. The genetics of this rare seed color pattern has not been investigated.

Although the genes *C* and *R* are tightly linked, most authors give only the genotype at *C* or *R* for any particular seed coat color expression (Bassett 2007). When the genotypes of the two parents in a cross are homozygous for one of the genes, specifying only the differences at the other gene is adequate, but when the genotype of the two parents differs at both loci, dual specification is needed for clarity. Bassett (1991) proposed using brackets to enclose the gene symbols at *C* and *R* to indicate the very tight linkage, i.e., the bracket convention. The proposed application of the bracket convention to the Liborino class genotype was given by Bassett (2007) as [*C* $R^{st}$], which indicates that although the red color is restricted to stripes, the remainder of the seed coat expressed *C* normally. To demonstrate the power of the bracket convention to express complex situations at *C* and *R* with symbolic simplicity, a systematic presentation of its possibilities is presented for the first time (Table 1). To simplify the presentation, the possible interactions with red colors controlled at *Rk* are ignored. For the same reason, the consideration of the great variability in pattern types found at the *C* locus in common bean is restricted to stripes only.

**Table 1.** Systematic application of the bracket convention for all known combinations of patterned or unpatterned expression of the tightly linked genes *C* and *R* (dominant red color) for seed coat color in common bean.[z]

| Gene combinations | Seed coat expression for color and pattern |
|---|---|
| [*C r*] | Non-red seed coat colors without pattern |
| [*? R*] | Red[y] seed coat without pattern[x] |
| [$C^{st}$ *r*] | Stripes in various non-red colors on an otherwise cartridge buff seed coat |
| [$C^{st}$ *R*] | Red[y] stripes on an otherwise cartridge buff seed coat. |
| [*C* $R^{st}$] | Red[y] stripes on various colors |
| [$c^{u}$ *?*] | Cartridge buff seed coat[x] |

[z]Only striped pattern is illustrated for simplicity, ignoring many other pattern types. For simplicity, this presentation ignores all interactions possible with genes for red color expressed at the *Rk* locus.
[y]The actual hue may be red or other colors depending on genotype at *G*, *B*, *V*, and *Rk*.
[x]The question mark indicates that the status of the indicated gene cannot be determined in this case.

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# THE GENETICS OF THE COLOR PATTERN OF SEED COATS IN G19833, A LIBORINO CLASS COMMON BEAN

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## INTRODUCTION

In common bean, seed coat pattern can be expressed by several genes: *P*, *T*, *C*, and *J* (Bassett, 2007), but in this paper only the *C* locus will be considered. There is also a dominant gene *R* for (oxblood) red seed coats, which is very tightly linked to *C*, forming a 'complex *C* locus.' The $c^u$ gene expresses cartridge buff (pale beige) seed coat color that cannot be modified (u = unchangeable) by the three principal color modifying genes: *G* for yellow brown, *B* for mineral brown, and *V* for blue to black color. A fourth color modifying gene *Rk* can express with $c^u$, but most of the color expressing alleles at *Rk* are recessive, except for $Rk^r$ (Bassett et al. 2010). *Rk* itself has no color expression. In genotypes with pattern expressed at *C*, the type of pattern is typically indicated by a superscript. For example, $C^{st}$ indicates a seed that expresses cartridge buff over all the seed coat except for *stripes*, which express the color coded by the full seed coat genotype when free of $c^u$ effects. If the color were yellow brown, the full seed coat color genotype would be *P T C J G b v Rk*. In this paper where the materials presented do not differ or segregate genetically at certain genes, those genes will not be indicated in the seed coat genotypes presented. Specifically, the gene symbols *P*, *T*, and *J* will always be omitted because both parents carry the dominant gene at those loci. A brief summary of seed coat genetics is given in the previous companion paper.

CIAT accession G19833 has a seed coat pattern of thin red stripes on yellow brown background color. This is a novel pattern with respect to all previous work published on seed coat genetics. We propose that the patterning effect in G19833 is in the *R* gene rather than at *C*. Thus, for red stripes on cartridge buff the genotypes would be [$C^{st}$ *R*], where the brackets indicate very tight linkage. In G19833, we propose that the genotype in the 'complex *C* locus' is [*C* $R^{st}$]. In Bassett et al. (2010), the full seed coat genotype of G19833 was demonstrated to be *P T* [*C* $R^{st}$] *G b v Rk*, but this is based on the *assumption* that the red color in the stripes is controlled (as well as the stripes themselves) at *R*. In this paper we will make a formal allelism test with a genetic tester stock to test that hypothesis rigorously.

## MATERIALS AND METHODS

Matt Blair performed an allelism test at CIAT during the winter of 2007-2008 in a greenhouse experiment. He crossed the genetic tester stock PI 632730, which is known to carry *R* in the genotype [*? R*] *g b v Rk*, with G19833. The $F_2$ progeny had 100 plants grown in greenhouse conditions. Seed samples from the $F_1$ parent and each $F_2$ progeny plant were scanned, and the images were sent to the senior author for genetic analysis and interpretation.

## RESULTS AND DISCUSSION

The seed coats of the $F_2$ seeds had the typical heterozygous mottling of genotype *Rr*, viz., oxblood red/yellow brown mottling with highly variable red stripes superimposed on that pattern. Combining the segregation classes at *G*, the remaining $F_2$ classes at *R* fell into three types in a 1:2:1 ratio for the PI-type, the $F_1$ pattern, and the Liborino-type seed coats, respectively (Table 1). The observed single-factor segregation data supports the hypothesis of allelism at *R*, i.e., the red stripes in G19833 are expressed by [*C* $R^{st}$]. On the basis of data presented in Table 1, we can also infer that the full genotype for seed coat color of G19833 is [*C* $R^{st}$] *G b v Rk*.

**Table 1.** Segregation for color and pattern of seed coats in the $F_2$ from the cross PI 632730 ([*? R*] *g b v Rk*) x G19833 ([*C* $R^{st}$] *G b v Rk*).

| Seed coat color and pattern | Genetic hypothesis | Number of observations[z] |
|---|---|---|
| Oxblood red without stripes | [*? R*] *G*/- and [*? R*] *g* | 20 |
| *Rr* mottling in oxblood red/yellow brown (*G*/-) or chamois (*g*) with highly variable red stripes | [*C* $R^{st}$]/[*? R*] *G*/- or [*C* $R^{st}$]/[*? R*] *g* | 40<br>12 |
| Red stripes (broader and paler than G19833) on either yellow brown (*G*/-) or chamois (*g*) | [*C* $R^{st}$] *G*/- or [*C* $R^{st}$] *g* | 20<br>4 |

[z]With [*? R*] , segregation at *G* had no effect on color expression of seed coats. For the data 20, 40, 12, 20, 4, the $\chi^2$ (4:6:2:3:1) = 2.000, *P* = 0.74. Combining the *G* locus segregation classes within each *R* locus class, the data were 20, 52, 24, and the $\chi^2$ (1:2:1) = 1.000, *P* = 0.61.

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# RECREATING AND ANALYZING AN $F_2$ POPULATION SIMILAR TOTHE ONE RESULTING FROM THE CROSS DORADO X G19833, BUT WITHOUT SEGREGATION AT *B*

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## INTRODUCTION

In common bean, seed coat pattern can be expressed by several genes: *P*, *T*, *C*, and *J* (Bassett, 2007), but in this paper only the *C* locus will be considered. There is also a dominant gene *R* for (oxblood) red seed coats, which is very tightly linked to *C*, forming a 'complex *C* locus.' The $c^u$ gene expresses cartridge buff (pale beige) seed coat color that cannot be modified (u = unchangeable) by the three principal color modifying genes: *G* for yellow brown, *B* for mineral brown, and *V* for blue to black color. A fourth color modifying gene *Rk* can express with $c^u$, but most of the color expressing alleles at *Rk* are recessive and *Rk* itself has no color expression. In genotypes with pattern expressed at *C*, the type of pattern is typically indicated by a superscript. For example, $C^{st}$ indicates a seed that expresses cartridge buff over all the seed coat except for *stripes*, which express the color coded by the full seed coat genotype when free of $c^u$ effects. If the color were yellow brown, the full seed coat color genotype would be *P T C J G b v Rk*. In this paper where the materials presented do not differ or segregate genetically at certain genes, those genes will not be indicated in the seed coat genotypes presented. Specifically, the gene symbols *P*, *T*, and *J* will always be omitted because both parents carry the dominant gene at those loci. A brief summary of seed coat genetics is given in the first of two previous companion papers.

Bassett et al. (2010) investigated the genetics of 'Dorado' seed coat color and demonstrated that 'Dorado' carries a new gene, $Rk^r$, expressing the seed coat color of the Dark Red Kidney class as a *dominant* gene. The color expression of $Rk^r$ is identical to the well-known $rk^d$ gene, viz., garnet brown. The full genotype for seed coat color in 'Dorado' is [*C r*] *G B v* $Rk^r$ (Bassett et al., 2010). CIAT accession G19833 has a seed coat pattern of thin red stripes on yellow brown background color. In the previous companion paper we demonstrate that the patterning effect in G19833 is in the *R* gene rather than at *C*, giving genotype [*C* $R^{st}$] at the 'complex *C* locus.' If the stripe pattern of red stripes on cartridge buff were controlled at *C*, the genotype at the complex locus would be [$C^{st}$ *R*]. In Bassett et al. (2010), the full seed coat genotype of G19833 was demonstrated to be *P T* [*C* $R^{st}$] *G b v Rk*. In this paper, we investigate the phenotypes expressed by complex interactions resulting from heterozygosity at the *Rk* gene, the 'complex *C* locus,' and the *G* gene.

## METHODS AND MATERIALS

The derivation of line 2-140 from the cross 'Dorado' x PI 608685, a genetic tester stock with genotype [*? R*] *g b v* $BC_3$ 5-593, was described in Bassett et al. (2010). The garnet brown seed coat color of line 2-140 was demonstrated to be [*C r*] *g b v* $Rk^r$ (Bassett et al., 2010). Matt Blair crossed 2-140 x G19833 at CIAT and grew 100 $F_2$ progeny plants in greenhouse conditions in 2007-2008. A seed sample of the $F_1$ parent and each $F_2$ progeny plant was scanned, and the images were sent to the senior author for genetic analysis.

## RESULTS AND DISCUSSION

The genotype [*C* $R^{st}$]/- $Rk^r$/- expressed red stripes of highly variable hues, varying from garnet brown to oxblood red, but the variation was too great too permit unambiguous classification of $Rk^r$/- vs. *Rk* classes (Table 1). With the genotype [*C* $R^{st}$] *Rk*, no significant difference in the color of the red stripes segregating for *G*/- vs. *gg* was observed, i.e., there was no sense of two classes being observed, but too close for unambiguous classification. The genotype [*C r*] $Rk^r$/- expressed highly variable red haze on yellow brown (with *G*/-) or chamois (with *g*). The haze was too variable to permit unambiguous classification of $Rk^r$/- vs. *Rk* classes (Table 1). Clearly, this is a two factor segregation: one for red stripes ($R^{st}$) and another for red hazes ($Rk^r$).

**Table 1.** Segregation for color and pattern of seed coats in the $F_2$ from the cross 2-140 [*C r*] *g b v* $Rk^r$ (garnet brown) x G19833 [*C* $R^{st}$] *G b v Rk* (oxblood red stripes on yellow brown).

| Seed coat color and pattern | Genetic hypothesis | Number of observations[z] |
|---|---|---|
| Highly variable red stripes on yellow brown (*G*/-) either with ($Rk^r$/-) or without (*Rk*) red haze | [*C* $R^{st}$]/- $Rk^r$/- (and *Rk*) *G*/- | 53 |
| Highly variable red stripes on chamois (*gg*) either with ($Rk^r$/-) or without (*Rk*) red haze | [*C* $R^{st}$]/- $Rk^r$/- (and *Rk*) *g* | 19 |
| Highly variable red haze over yellow brown (*G*/-) or chamois (*g*) | [*C r*] $Rk^r$/- *G*/- (and *g*) | 19 |
| Yellow brown | [*C r*] *Rk G*/- | 8 |
| Chamois | [*C r*] *Rk g* | 1 |

[z]Classes with or without red haze were combined because the high variability of the haze made unambiguous classification impossible. For the data 53, 19, 19, 8, 1, the $\chi^2$ (36:12:12:3:1) = 2.738, $P = 0.60$.

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# POTENTIAL PRODUCTIVITY OF SEED COLOR CLASSES OF COMMON BEAN LANDRACE

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## INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is the primary protein source in the Brazilian diet (Silva, 2003). It was originated in the Americas and is currently cultivated on all continents (Gepsts; Debouck, 1991), as it adapts to different soil and climatic variations and to the various production systems. New functional and nutritional characteristics have been demanded by modern society and may be present in the landrace. The characterization and conservation of these will meet the demands and will avoid genetic erosion.

To broaden the genetic basis and maximize the gains from selection of a crop, it is essential to accumulate favorable alleles present in wild populations, cultivated and related species (Gepsts; Debouck, 1991), Land varieties have been used as a source of favorable alleles and at the same time, for direct consumption of both rural populations and, more recently, urban.
The aim of this study was to evaluate the potential productivity of landrace varieties of beans, grouped by seed color.

## MATERIAL AND METHODS

The cultivars were obtained from or through collections made on the spot in bean producing properties throughout the State of Rio Grande do Sul, or through donations from rural extension organs, producers or collaborators.

The experiment consisted of a single 3m row of each of the 153 landraces, 0.5 m apart, with a population of 12 plants.$m^{-1}$. The experimental design was intercropped check cultivars. The checks were BRS Guerreiro and BRS Campeiro for black bean cultivars and Carioca and Iraí for other grain colors. The block was characterized by the presence of ten test lines, with check cultivars at the beginning and at the end of the block. A comparative assessment of cultivars to the checks was made by graphic method. Cultivars that surpassed the line that connects the average mean productivity of the checks in each block was considered superior. The seeds were sown on 2009/10/28 and harvest was variable, according to the cycle of each material, starting on 2010/09/02 and concluded on 2010/02/20 and no disease control was performed. At the base were applied, 250 kg ha-1 of NPK fertilizer, 5:20:20 formula. Weeding with was conducted animal traction. For purposes of assessing the productivity potential, cultivars were grouped by grain color identifying black, green, purple, red, pink, yellow and “mouro”, as in Table 1.

## RESULTS AND DISCUSSION

It is considered suitable to form the next stage of the breeding program of Embrapa Temperate Climate, cultivars that exceed the average production of checks, located at the beginning and end of each block.

It can be seen that, except for the group of cultivars of green seeds in all groups there were cultivars that exceeded the checks, demonstrating the high potential of the groups black, purple and "mouro", of the cultivars that exceed the checks where 48.3%, 52.7% and 40.0% respectively. Equally important is the potential productivity of the red group, whose cultivars that exceeded the average of the checks reached an average of 114.5% of superiority, with a maximum value of 230.3%. In the yellow group noted the potential for productivity is low by offering a very favorable outlook for this class of seeds.

**Table 1.** Productivity performance of materials in Creole evaluated Preliminary Test Procedure I.

| GCG[1] | NCG[2] | PCT(%)[3] | VCT (%)[4] | PSP (%)[5] |
|---|---|---|---|---|
| **Black** | **59** | **48.3** | **47.0** | **109.9** |
| **Green** | **4** | **0.0** | **-** | **-** |
| **Purlpe** | **20** | **40.0** | **54.0** | **81.6** |
| **Red** | **27** | **18.5** | **114.5** | **230.3** |
| **Pink** | **13** | **23.1** | **28.1** | **57.2** |
| **Yellow** | **11** | **27.2** | **2.6** | **4.9** |
| **"Mouro"** | **19** | **52.7** | **51.2** | **104.5** |

**[1] Group of grain color.**
**[2] Number of cultivars in the group.**
**[3] Percentage of cultivars that exceeded the average of the witnesses.**
**[4] Average percentage value of the cultivars that exceeded the average of the witnesses.**
**[5] Pencentage superiority of cultivar maximum productivity within the group over the average of the local cultivars.**

## CONCLUSION

There are different yield potential between groups of color in seed germplasm Creole. Simultaneously, we detected promising cultivars in all groups, except that on cultivars of green seeds.

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# EVALUATION OF HEIRLOOM BEANS FOR PRODUCTION IN NORTHERN COLORADO

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Heirloom dry edible beans (*Phaseolus* spp.) have become popular for their unique seed coat color/patterns and flavor. The term heirloom refers to bean types that have been passed down from generation to generation. In most crop production systems heirloom beans are not widely grown since they have limited market outlets and often are not adapted to field scale production. However, if they are adapted, they can provide a market niche for local and unique markets. The production of heirloom beans also provides diversity to producers. The objectives of this study were to evaluate adaptation, seed yield, maturity, growth habit, and estimated gross income of heirloom beans for potential large scale production in Colorado.

## MATERIAL AND METHODS

Thirty-two heirloom bean types and two checks (pinto beans cultivars UI 114 and Croissant) were evaluated at the Colorado State University Agriculture Research, Development and Education Center in Fort Collins, CO. The heirloom seed was obtained from bean distributers, including Rancho Gordo Napa, CA, Native Seed Search Tucson, AZ, Seed Savers Exchange, Decorah, IA, and the collection in the Colorado State University Dry Bean Breeding Project. Twenty-six entries of common bean (*Phaseolus vulgaris* L.), two entries of lima bean (*Phaseolus lunatis* L.) and four entries of tepary bean (*Phaseilus acutifolius* A. Gary) were evaluated. The seeds were planted on 2 June, 2011 in rows 30cm wide, 7.62m long. The plots received 6.5 cm of rainfall and 12.7 cm of supplemental furrow irrigation to total 19.2cm of water during the growing season. Yield and harvest maturity were evaluated. Growth habit was classified according to Singh et al. (1999). All plots were hand harvested. This research was funded in part by the USDA-NIFA Bean Coordinated Agricultural Project.

## RESULTS AND DISCUSSION

Ten entries had seed yield higher than 1,000 kg/ha (Table). Only Flor de Mayo had higher seed yield than the pinto checks, however seven entries had similar seed yield to the checks. Seed weights range from 21 to 50 g/100 seeds. Six entries were not harvested due to late maturity. Among the highest entries for yield, maturity ranged from 85 to 103 days (Table). Because northern Colorado has a relatively short growing season, beans with more than 100 day maturity are in danger of early season frost damage. Two entries, Yellow Indian Women and Swedish Brown common bean had the shortest time to maturity at 89 and 90 days, respectively. Growth habit among entries ranged from type I to type IV. Entries that exhibited type IV growth habit were trellised.

Estimated gross income among entries was calculated based on one-fourth that of retail price listed by distributors on the internet. When commercial grain price was not available, the seed price was used. Gross incomes for Flor de Mayo and Snowcap Bean were almost 8 times higher than for the commercial pinto beans ‘Croissant’ and ‘UI 114’. Flor de Mayo and Snowcap Bean may provide an opportunity for growers in northern Colorado to produce heirloom beans. All of the top eight entries (Table) had higher gross income than that of the check pinto entries.

Lima bean entry Calico, also known as Christmas Lima Bean due to its red and cream seed coat color, had high yield potential (1606 kg/ha). However because it had 128 days to harvest maturity and type IV growth habit that required trellising and hand harvest, it would not be adapted to commercial field production. It may be of value to small local growers that are willing to invest the labor and time needed. The four tepary beans evaluated developed severe root rot caused by *Fusarium* spp., consequently seed yield was low. Entry Santa Rosa White had the highest yield among tepary (902 kg/ha) and Blue Speckled tepary had the lowest yield (446 kg/ha). Based on these yield levels and the relatively low value of the crop, tepary beans would not be provide an economically viable alternative to pinto bean in northern Colorado.

## CONCLUSIONS

Some heirloom beans have potential for commercial bean production in northern Colorado. The most limiting factor for production of some heirloom beans was harvest maturity. Among long season beans, Flor de Mayo and Snowcap Bean performed well. Among shorter season beans Yellow Indian Women, Kronis Purple, Swedish Brown and African Purple had yield levels similar to the checks. Because some of the entries tested were climbing types, labor to trellis and harvest them would prohibit them from large scale field production in northern Colorado.

| Entry | Yield | | Maturity | | Growth Habit | Estimated Gross Income | |
|---|---|---|---|---|---|---|---|
| | | 100 seed weight | Days to Flower | Days to Maturity | CIAT Scale | Retail Price | Estimate Income (1/4 retail price*Yield) |
| **Common Bean** | **kg/ha** | **g** | | | | **$/kg** | **$/kg** |
| Flor de Mayo | 2339 a | 29 d | 50 b | 103 abc | type III | $9.36 | $5,473 |
| Snowcap Bean | 2182 ab | 70 a | 62 a | 110 a | type III | $9.44 | $5,149 |
| UI 144 Pinto | 1878 bc | 41 c | 44 de | 87 cd | type III | $1.75 | $648 |
| Croissant | 1685 bc | 34 d | 47 bcde | 87 cd | type III | $1.75 | $648 |
| African Yellow | 1668 bc | 35 cd | 48 bcd | 92 cd | type III | $9.44 | $3,936 |
| Swedish Brown | 1477 bc | 33 d | 44 de | 90 cd | type I | $9.44 | $3,485 |
| Amethyst | 1468 bc | 29 d | 50 b | 103 abc | type I | $8.70 | $3,214 |
| Kilimanjaro Speckled | 1330 c | 36 cd | 48 bcd | 110 ab | type III | $12.46 | $4,607 |
| Yellow Indian Women | 1205 c | 21 f | 43 e | 85 d | type III | $10.11 | $3,740 |
| Kronis Purple | 1134 c | 50 b | 47 bcde | 93 bcd | type I | $10.90 | $4,035 |

Means followed by the same letter are not significantly different according to the LSD(0.05

# ARE COMMON BEAN BREEDING LINES AS VARIABLE AS LAND RACES?

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## INTRODUCTION

Common bean breeding programs usually exploit a narrow range of the species available germplasm. The main reason is that efforts are directed towards market types, generally limited in number of classes. As a result, less variability is expected to be found at breeding programs than at land race germplasm. The present article uses the picture found at the Embrapa Temperate Climate (CPACT) breeding program to bring a factual view on this subject.

## MATERIALS AND METHODS

Thirty four representative CPACT common bean program breeding lines (BL) and 107 land races (LR) available at its germplasm bank were characterized for stem anthocianine presence, flower color, growth habit, plant type, vine length, pod string presence and growth cycle according to Silva (2005). Experimental plots comprised an individual 4m row, 0.5m apart, with 12 plants per meter, with no replication. Data were transformed to percent and frequency for each germplasm group, as defined, compared.

## RESULTS AND DISCUSSION

It can be observed from the figure that for the traits stem anthocianine presence, vine length and pod string presence, both germplasms were similar for number of classes and intraclass magnitude. For flower color, growth habit, plant type, and growth cycle, however, LR displayed a greater number of classes. Specifically for stem anthocianine presence, 82% of the land races did not fulfill the characteristic whereas for the breeding lines, 82% did so. All BL presented purple flowers, whereas LR revealed the presence of white and pink flowers besides purple ones. At the same time, LR with determinate habit were found (18%) and all BL had indeterminate one. LR presented plant types I, II, III and IV, whereas 94% of the BL had type II plants. As related to pod string presence, no relevant difference between the two germplasms was detected since the marketable product is the dry bean for which the presence of pod string is a positive fact. For growth cycle only for LR was detected variation, with the presence of cultivars in all three classes defined. BL were all within the long cycle class (above 81 days to maturity). Despite the fact that the larger number of LR studied increased the possibility of detecting more variable genotypes as compared to BL, the results suggest that the germplasm used in family agriculture is really more diverse than that under development at CPACT, a situation similar to that found in other Brazilian common bean breeding programs. This fact support the strategy adopted at CPACT that, through the use of Biodiversity Scores (Partitura de Biodiversidade - PBio, that are LR collections), make available to family farmers this important source of new lines for own use or for marketing.

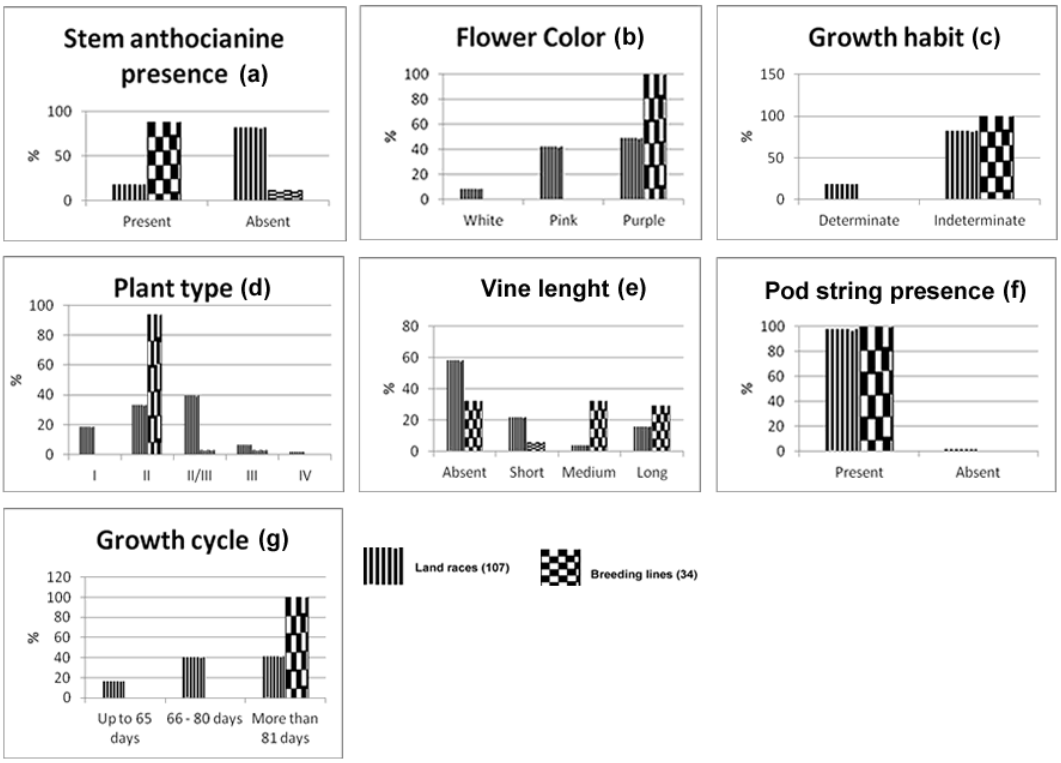


**Figure 1.** Stem anthocianine presence (a), flower color (b), growth habit (c), plant type (d), vine length (e), pod string presence (f) and growth cycle (g) frequency in common

## CONCLUSIONS

Greater variation for the set of characteristics studied was detected in land races as compared to the CPACT common bean breeding lines. The results support the use of Biodiversity Scores under way by the CPACT common bean breeding program to make available more diverse germplasm to family farmers from which new food and market sources can be obtained.

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# SEED YIELD OF 'ROSA DE CASTILLA' LANDRACES FROM THE CENTRAL-WEST AREA OF MEXICO

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The ancestral common bean landrace 'Rosa de Castilla' (RC) is ascribed to the Jalisco race (Singh *et al*., 1991). The seed of this bean type is from medium to large size (32 to 45 g/100-seed), oval shaped of dull or shiny pink-cream colors. The RC bean type is mainly grown under rainfall conditions in small areas by hundreds of farmers in the states of Jalisco, Guanajuato, San Luis Potosi (SLP) and SE Zacatecas. Landraces from Jalisco and the SW of Guanajuato, the area of bean domestication (Kwak *et al.*, 2009; Rossi *et al*., 2009), are of climbing growth habit whereas those from Northern Guanajuato, SLP and Zacatecas are of indeterminate prostrate growth habit. This bean type is locally sold and consumed and can also be found in traditional markets in most large cities in Central-West Mexico, it also is appreciated by Mexican migrants that live in the USA and Canada.

During 2008 and 2009, landraces of the RC bean type were collected from farmers in three of the states above mentioned, mostly from areas located from 1800 to 2200 m asl. Sixty-nine RC landraces plus three improved cultivars of the Flor de Mayo type (M38, Anita and Eugenia) were yield tested under rainfed conditions during 2009 and 2010 at two locations each year. Sowing was carried out after the onset of the rainy season in July. A 9X8 Lattice Design with three replications was used. In 2009 plot were a single 6 m row and two rows in 2010. Data were taken on days to flowering, natural disease incidence, seed yield and 100-seed weight. A Principal Component Analyses (PCA) with the average data from four test sites was performed.

In general, most landraces started flowering at 55 d after planting (data no shown), the checks one week earlier. Landraces were of late maturity and at the two higher sites were slightly damaged by frost. Pod set up in the bred cultivars was accentuated in a short period of time after the onset of flowering as compared to landraces that kept a large vegetative growth and flowering towards physiological maturity, resembling ancestral climbers (Singh et al., 1991). Without exception, all landraces were susceptible to rust and of intermediate reaction to bean common blight, few showed symptoms of halo blight and anthracnose. On the other hand, RC landraces displayed healthy root system in soils infested with Fusarium spp. In spite of some drought spills, particularly at the Villa de Arriaga site in the state of SLP, the seed yield of some landraces was acceptable (Table 1), suggesting a moderate level of drought resistance. In all trials, the average yield of the three improved Flor de Mayo cultivars was higher than the one of landraces. Flor de Mayo Eugenia was the check of higher seed weight and at least six landraces displayed an average 100-seed weight above 40 g (data no shown).

In Figure 1, the vertical dispersion of the RC genotypes indicates large diversity in seed yield, RCG23, RCG25 and RCZ11 resulted outstanding, whereas only RCZ12 is near the checks in regard to earliness. The rust susceptibility of the landraces is also noticeable, while the checks were resistant. Due to the demand by consumers, earliness and disease resistance have to be incorporated into improved versions of best landraces.

**Table 1.** Average seed yield and 100-seed weight of 69 landraces of the RC bean type and three bred Flor de Mayo cultivars grown under rainfall conditions in four location/trials at Central Mexico.

| | Celaya, Gto. 2009 | | J. Iturbide, Gto. 2009 | | Celaya, Gto. 2010 | | V. Arriaga, SLP 2010 | |
|---|---|---|---|---|---|---|---|---|
| Value/genotype | kg ha$^{-1}$ | 100SW g | kg ha$^{-1}$ | 100SW g | kg ha$^{-1}$ | 100SW g | kg ha$^{-1}$ | 100SW g |
| Minimum RC | 229 | 25.3 | 290 | 27.8 | 884 | 25.6 | 506 | 21.9 |
| Maximum RC | 1359 | 40.9 | 1460 | 42.8 | 2949 | 39.3 | 1035 | 42.0 |
| Average RC | 1065 | 31.3 | 903 | 32.1 | 1899 | 29.6 | 764 | 36.0 |
| FM Eugenia | 1840 | 31.6 | 2190 | 36.2 | 2725 | 39.1 | 722 | 35.4 |
| FM Anita | 1374 | 24.9 | 1980 | 25.7 | 1708 | 28.3 | 817 | 24.9 |
| FM M38 | 1131 | 24.7 | 1930 | 24.6 | 1478 | 29.7 | 920 | 27.3 |

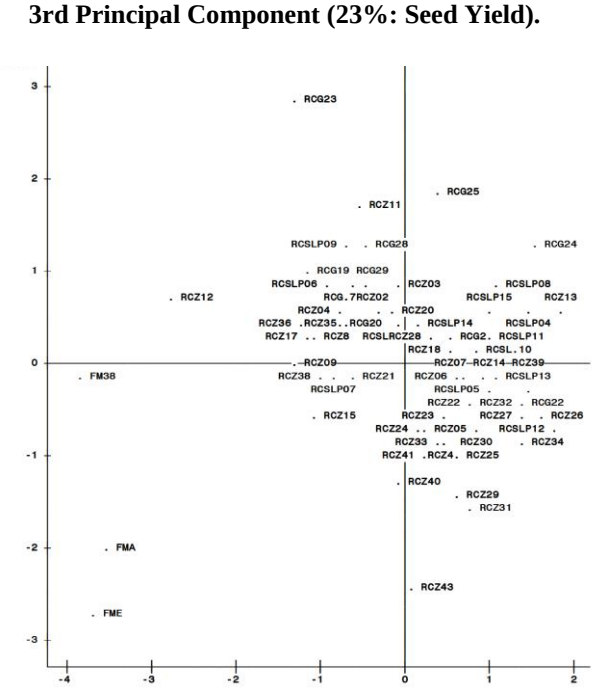


**Figure 1.** Dispersion of 72 bean genotypes on the basis of the First and Third Principal Components of the PCA Analysis.

Data from four trials allowed for the identification of RC landraces of high yield potential, high seed commercial value, and intermediate cycle, but deficient in disease resistance.

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# GENOTYPES OF COMMON BEANS EXPORT TYPE EVALUATED IN BRAZIL

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Traditionally, the common beans most produced in Brazil are Carioca and Black bean types (Del Peloso et al., 2005); however other less consumed types with diverse colors and sizes such as Alubia, Cranberry, Dark Red Kidney, Light Red Kidney, Pinto and Navy are also produced with a great potential for export. At the moment the number of cultivar of those types is still insignificant, and breeding programs are very new if compared with Carioca and Black beans programs. The breeding program of the Embrapa Rice and Beans Research Center started to supply that demand, by identifying genotypes with suitable characteristics for the international market.

Eleven trials were conducted in 2009 and 2010 in the winter cropping season in the states of Goiás (three trials) and Minas Gerais (four trials); and in the wet and dry seasons in the state of Paraná (four trials). The experimental design was a completely randomized block with three replicates. Each trial was composed of 14 genotypes; among them 11 were promising (white, light red kidney, cranberry, dark red kidney and calima), and three controls (BRS Radiante, Hooter and Ouro Branco) (Table 1). These genotypes were selected based on evaluations performed previously by Pereira et al. (2010) and Del Peloso et al. (2010). The following evaluations were performed: lodging; plant architecture; disease resistance (common bacterial blight, anthracnose and oidium) using a scale ranking from 1 (phenotype totally favorable) to 9 (phenotype totally unfavorable); and 100 seed mass. Data collected in each experiment were submitted to individual and joint analysis. Scott Knott test at 10% was used for mean comparison.

The joint analysis detected significant differences ($P<0.01$) among genotypes, environments, as well as for genotype x ambient interaction. There was a good experimental precision with a coefficient of variation (CV=16.6%). The general mean was 1701 kg $ha^{-1}$ and a mean performance variation between 1438 kg $ha^{-1}$ and 2000 kg $ha^{-1}$. The control cultivars BRS Radiante, with stripped seeds and commercially indicated for cropping in the states evaluated, and Ouro Branco, presented the best yield performance, together with genotypes CAL-96, Red Kanner and BRS Embaixador. Therefore, grain yield from these genotypes were similar to those of commercial varieties already being farmed.

The control Ouro Branco yielded the most among white seeded genotypes. Genotype Branco Graúdo was the best among the lines evaluated, but inferior to Ouro Branco in yield, plant architecture, lodging, M100, and reaction to CBC. Del Peloso et al. (2010) reported that these two genotypes showed similar M100. This line was superior to the control regarding anthracnose resistance. The other white seeded lines yielded less than Branco Graudo. However line WAF 75 had the best plant architecture and the best resistance to lodging, besides yielding larger seeds, suggesting a good acceptance by consumers.

Among genotypes with other seed types, CAL-96 of calima type seeds and BRS Embaixador with DRK type seeds excelled, combining high yield, resistance to lodging and good plant architecture. BRS Executivo, seed type cranberry, yielded low. However it is important to point out that the comparison was performed with genotypes of other seed types. This genotype has the largest seeds among the evaluated, highly appreciated by the international market. Therefore, there are promising genotypes that may be recommended as new bean cultivars for the international market.

**Table 1.** Average yield (PROD) (kg $ha^{-1}$); average(1) and maximum(2) grades for lodging (ACA), plant architecture (ARQ), bacterial blight (CBC), anthracnose (AN), and oidium (OI); and 100 seed mass (M100), of 14 genotypes of common beans evaluated in 11 environments in the States of Goiás, Minas Gerais and Paraná (Brazil), in 2009 and 2010.

| Genotype | PROD | | ACA(1)/(2) | ARQ | CBC | AN | OI | M100 |
|---|---|---|---|---|---|---|---|---|
| BRS RADIANTE | 2000 | a | 4.9/6 | 2.9/5 | 4.5/6 | 1.0/1 | 1.4/2 | 41.1 |
| CAL-96 | 1902 | a | 3.9/6 | 2.3/4 | 5.0/6 | 1.0/1 | 6.8/8 | 53.0 |
| RED KANNER | 1874 | a | 5.4/8 | 3.3/5 | 5.0/5 | 1.0/1 | 7.0/8 | 45.2 |
| OURO BRANCO | 1846 | a | 3.7/8 | 2.3/5 | 4.5/5 | 5.5/9 | 6.6/8 | 48.5 |
| BRS EMBAIXADOR | 1841 | a | 3.1/7 | 2.4/5 | 2.5/3 | 1.0/1 | 6.4/8 | 47.2 |
| HOOTER | 1791 | b | 4.6/8 | 2.3/5 | 6.0/7 | 1.0/1 | 6.6/8 | 51.6 |
| CHINOOK | 1757 | b | 4.9/8 | 2.6/5 | 6.5/9 | 1.0/1 | 5.0/8 | 48.5 |
| BRANCO GRAUDO | 1702 | b | 4.7/8 | 2.7/5 | 7.0/8 | 1.0/1 | 6.2/8 | 44.6 |
| LIGHT RED KIDNEY | 1662 | c | 5.9/8 | 6.7/8 | 6.5/9 | 1.0/1 | 5.0/8 | 50.4 |
| BRS EXECUTIVO | 1542 | d | 5.3/8 | 6.4/8 | 2.0/2 | 2.5/4 | 1.6/3 | 58.3 |
| MONTCALM | 1503 | d | 5.4/7 | 2.6/5 | 5.5/6 | 1.0/1 | 6.4/9 | 49.1 |
| WAF 141 | 1484 | d | 2.7/5 | 2.0/4 | 3.0/4 | 1.0/1 | 5.8/8 | 44.3 |
| WAF 75 | 1478 | d | 1.9/3 | 1.7/4 | 5.5/7 | 1.0/1 | 5.6/8 | 56.5 |
| WAF 170 | 1438 | d | 3.6/7 | 2.3/5 | 2.5/3 | 1.0/1 | 7.0/8 | 43.5 |

[1]Means followed by the same letter do not differ among them (Scott-Knott at 10% of probability).

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# GEOGRAPHIC TARGETING OF CLIMBING BEANS IN MID-ELEVATIONS AREAS OF TWO DEPARTMENTS IN COLOMBIA

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**Background:** Climbing beans are a high yielding commodity that is valuable for small-landholders as it is a labor-intensive but profitable crop. Afro-colombian and indigenous communities in South East Colombia can directly benefit from climbing beans as many of these communities are found in areas of adaptation for the crop and farms are generally small. Many different grain types are produced by climbing beans including traditional types such as Cargamanto or Calima beans and gourmet types such as Fabadas. Value addition to the bean production of this region can be through offering a high quality (hand-picked) product or through branding and specialized marketing.

## MATERIALS AND METHODS:

Geographic analysis of climbing bean adaptation was conducted with the following criteria in terms of altitude (<900 masl – marginal due to low altitude, >1500 masl – marginal due to high altitude, between 900 and 1500 ideal altitude) and quarterly precipitation (<400 mm – too dry, >1200 mm – too wet, 400-1200 optimal). Justification for these criteria was that the climbing beans to be used are part of a series of mid-altitude climbing (MAC) beans that are adapted to lower elevation sites and that given their rapid growth cycle of 90 to 100 days, quarterly rainfall of 400 mm or more in 3 months will be sufficient for crop production. These criteria will need to be confirmed by agronomic trials. Data sources for altitude and precipitation were from a Digital Elevation Model for the Southeast Colombia and from the WorldClim (2009) database. Municipality boundaries were overlaid on these models along with information on percent population of Afro-Colombian descent from DANE (2005) as part of the targeting exercise.

## RESULTS AND DISCUSSION:

Municipalities with high Afro-Colombian populations were identified (Table 1) and a geographic analysis of altitude, temperature and quarterly rainfall was performed to find suitable agronomic sites for mid-altitude climbing (MAC) beans across these municipalities (Figure 1a). More detailed analysis of the intersection of majority Afro-Colombian municipalities with climbing bean adaptation were also determined (Figure 1b). Ten of the targeted municipalities were found to have ideal regions for production of MAC lines. The addition of heat tolerance and disease resistance through plant breeding will expand these regions significantly. Irrigation in drier regions would also allow production in other regions of Valle (for example Cerritos, Guacari and Palmira where Afro-Colombian populations also live). Irrigation is also needed for Mulaló and the municipality of Yumbo. Gaupí and coastal regions are not within the zone of adaptation; however, the geographic analysis identified parts of the Patia valley in Cauca as ideal for bean production and a region that is also majority Afro-Colombian. Currently, successful targeting of bean production based on agronomic conditions can be predicted for Jamundi, Suarez, Buenos Aires, Santander de Quilichao and Caloto, all municipalities with “piedemonte” regions and sufficient rainfall that would be ideally suited for CIAT-bred climbing beans.

**Table 1.** Municipalities with high Afro-Colombian populations within Cauca and Valle departments. Source: DANE, 2005.

| Municipio | Total Population | Afro-Colombian population |
|---|---|---|
| 1. Yumbo (Mulalo)* | 92,192 | 13,026 |
| 2. Cali | 2,119,908 | 542,039 |
| 3. Jamundi | 96,993 | 55,608 |
| 4. Suárez | 19,244 | 10,999 |
| 5. Buenos Aires | 26,961 | 15,558 |
| 6. S. Quilichao | 80,282 | 26,717 |
| 7. Villa Rica | 14,326 | 13,796 |
| 8. Puerto Tejada | 44,324 | 43,010 |
| 9. Padilla | 8,336 | 7,741 |
| 10. Caloto (Guachene) | 36,921 | 22,641 |
| 11. Guapi | 28,663 | 24,097 |

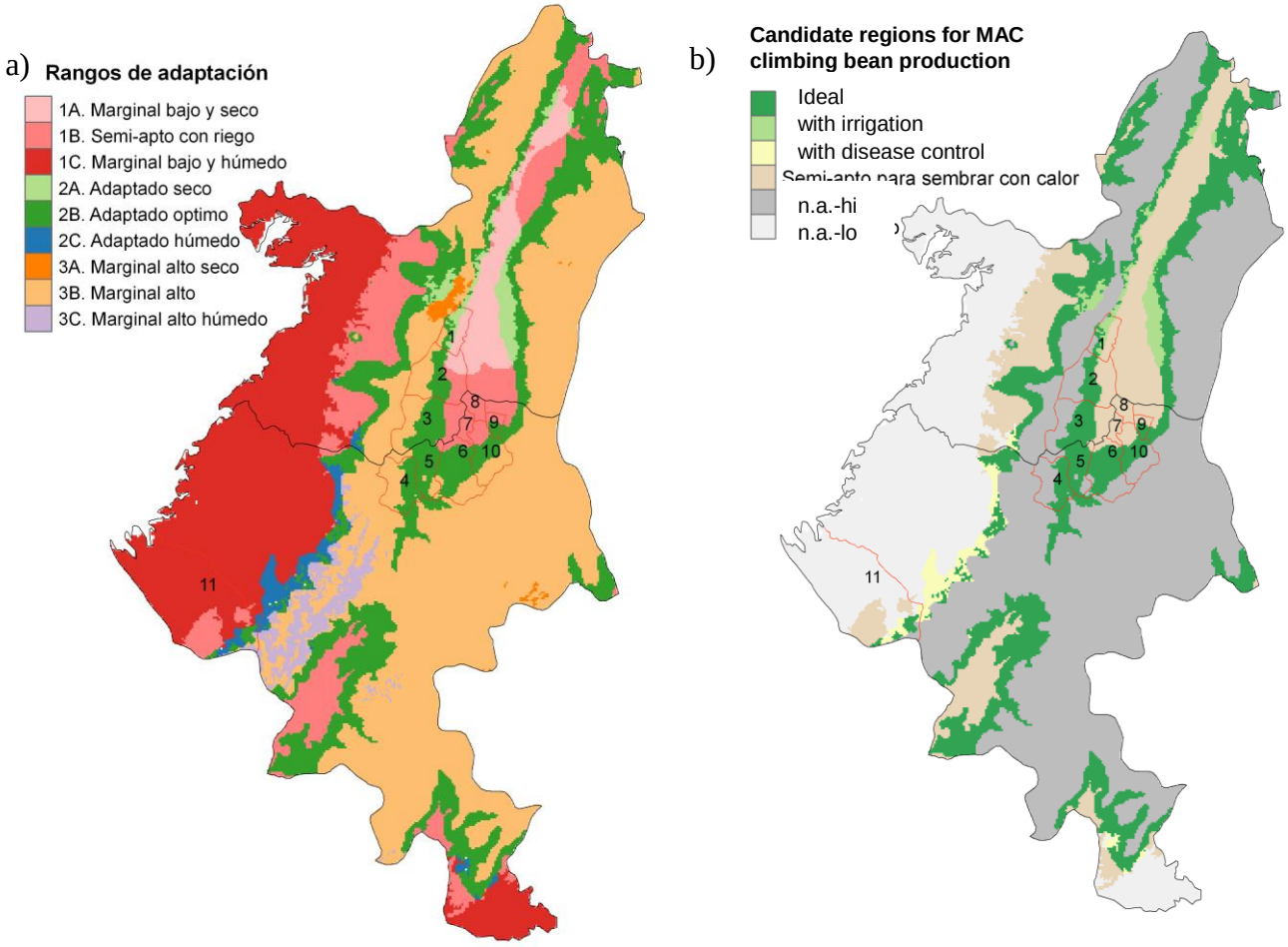


**Figure 1.** Adaptation (a) and candidate (b) regions for mid-altitude climbing beans for Afro-Colombian populations in Valle and Cauca.

# CLIMBING SNAP BEANS YIELD IN RELATION TO SOIL TYPE AND ENVIRONMENTAL INDICES

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## INTRODUCTION

The snap beans (*Phaseolus vulgaris* L.) are important for the human nutrition for its content in vitamins (especially B complex), protein, phosphorus and calcium. The per capita consumption in Mexico is estimated at 0.9 to 1.1 kg per year and snap bean production is estimated at 3 t $ha^{-1}$ which is insufficient to satisfy the demand of the population (Salinas-Ramírez *et al*., 2008). González (1987), says that cultivars of indeterminate climbing growth habit with longer reproductive period and largest number of cuts can be an alternative for increasing the yield per area. Olafujo *et al.* (1981), indicate that the fine and superfine quality bean is linked with the daily court because delaying leaf senescence and gives more opportunity for the formation of a greater number of pods. However, yield may be a function of soil characteristics. The aim of this study was to determine the phenology and production of climbing snap bean and its relationship with the soil type and environmental indices.

## MATERIALS AND METHODS

The study was conducted under rainfed conditions in Montecillo, Mex. of semi-dry climate. The snap bean HAV-14 of indeterminate climbing growth habit (type IV) was sown on May 21, 2004 in a clay soil with pH 8, and May 17, 2005 in sandy loam soil with pH 7.0. The density was 6.5 plants $m^{-2}$ in rows 0.80 m apart. Fertilization of NPK was 100-100-00. The sunflower was used as a tutor. We assessed the phenology and yield (fresh weight and pod number $m^{-2}$). Also, environmental indices as evapotranspiration (mm) and heat units (° C days), and seasonal precipitation was recorded (mm).

## RESULTS AND DISCUSSION

The phenology showed changes in the years of study (Figure 1). The highest snap bean production was achieved in the sandy loam soil with 1000 g $m^{-2}$ and 189 pods $m^{-2}$(2005), and the lowest yield and pod number (190 g 36 $m^{-2}$) in the clay and alkaline soil (2004) and was attributed to the possible flooding and low micronutrient availability (Table 1). This difference between years is attributed to differences in the growth cycle, frequency and number of cuts and crop evapotranspiration, heat units and precipitation (207 and 264 mm, 1048 and 1275 º C and 317 and 578 mm for 2004 and 2005, respectively). The mean pod length was 10.8 cm (2004) and 11.5 cm (2005). These results indicate that soil characteristics, environmental indices and the frequency and number of cuts are determinants to the production snap beans climbing.

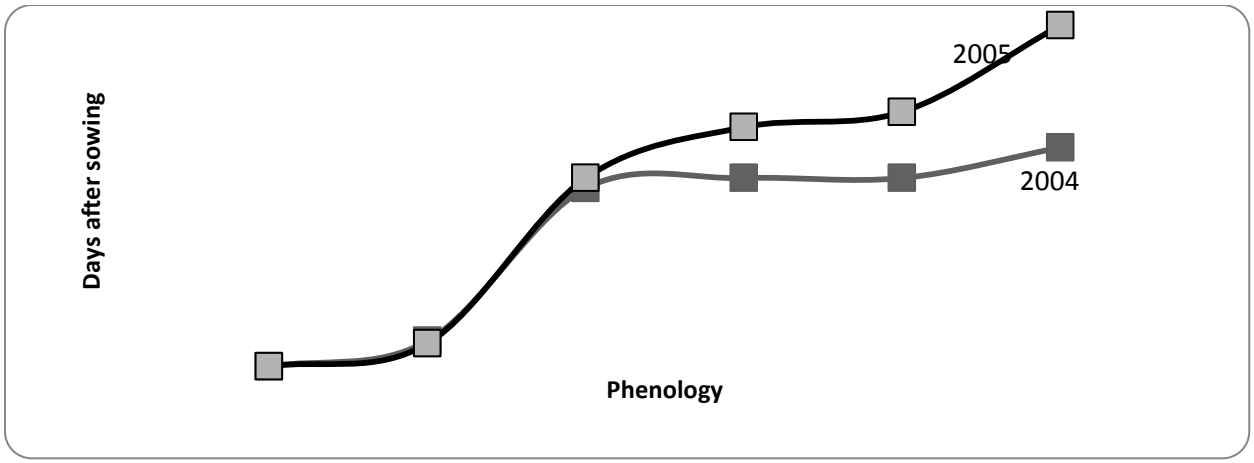


**Figure 1.** Phenology of snap beans and cut stage. 1. Sowing; 2. Seedling emergence; 3. Beginning flowering; 4. Pod formation; 5. First cut; 6. Final cut.

**Table 1.** Yield (fresh weight, g), pod number, cuts number and cutting length of period of snap beans HAV-14 in two soil types. Montecillo, Méx. 2004-2005.

| Year | Soil | Yield ($g\ m^{-2}$) | pod number $m^{-2}$ | Cuts number | Cutting length of period (days) |
|---|---|---|---|---|---|
| **2004** | Clay | 190 c | 36 c | 7 | 34 |
| **2005** | Sandy loam | 1000 a | 189 a | 8 | 17 |

Values in columns with different letters are statistically different (Tukey 0.05).

## CONCLUSIONS

Soil characteristics and environmental indices determine the frequency and number of cuts, length of crop cycle and the snap beans yield. Clay alkaline soil limits the production of climbing snap beans.

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# STRATEGIES FOR SELECTING INDIVIDUALS IN COMMON BEAN BREEDING PROGRAMS

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## INTRODUCTION:

In conducing of segregating populations using Bulk or Bulk within $F_2$ progenies methods, at the time of obtaining the progeny, the choice of individuals is usually performed visually. To improve efficient, the character data could be obtained, and the selection would be made using this information. For this, there are some alternatives, as mass selection, between and within selection, and also the use of mixed models, especially BLUP (Bernardo, 2002; Resende, 2007; Nunes et al., 2008). The aim of this study was to compare selection strategies in identifying the individuals who will lead to the best progenies in order to continue the selection process.

## MATERIALS AND METHODS:

The experiment was conducted at experimental area of Universidade Federal de Lavras, Minas Gerais, Brazil. It was used the data of 51 $F_{2:4}$ progenies derived from the cross between CVIII8511 x RP-26 common bean lines. The experimental design was a random complete block with 20 replications and plots of one plant. The plant architecture and grain yield data were obtained per plant, and, furthermore, the sum of the standardized of those two variables ($\sum Z$) was estimated for simultaneous selection of both characteristics. The analysis of variance was performed by the mixed models method (BLUP) and by the least squares method (LS), and the results were used for comparing different selection strategies.

The phenotypic value per plant data were submitted to different selection strategies for later comparison with the data supplied by BLUP. In the mass selection, the 100 best and 100 worse individuals were selected in the $F_{2:4}$ generation in function of the $\sum Z$ regardless of the progeny or replication to which they belonged. In the stratified mass selection, the plants were divided in strata and each stratum was a replication. Thus, each stratum contained one plant from each progeny and there were a total of 51 plants per stratum. The five best and five worst individuals were selected from each stratum considering the $\sum Z$. As there were 20 replications, there were a total of 100 progenies in each group. To perform the between and within progeny selection, analysis of variance was carried out first using the least squares method to obtain the $\sum Z$ means of the 51 progenies. The six best progenies were selected from these means (11.7% between progenies selection intensity) and within these, the 16 best of the 20 existing plants. The same was done with the group of worst progenies, totaling 96 plants in each group. The selection strategies were compared with BLUP procedure by observing the coincidence of the selected individuals, and also through the selection differential in each strategy.

## RESULTS AND DISCUSSION:

The coincidence of individuals selected by BLUP procedure and LS method was 100%. Bernardo (2002) commented that when the design is completely balanced, BLUP and LS supply the same information. Because each plot consisted of one plant, and 8.6% of the total were lost, it was inferred that with this loss level there was no advantage in BLUP compared to LS.

It was also estimated the coincidence of individuals selected by the different alternatives of selections with BLUP. Because individual BLUP provides an estimate that involves all the model variables, for example, the merit of the progeny, the individual in the progeny and even the replication where it is located (Resende, 2007), should present great coincidence in the individuals selected by between and within selection. Taking as reference the $\sum Z$, the coincidence was over 80%. Mass selection and stratified mass selection had lower concordance compared to BLUP (Table 1).

**Table 1** - Coincidence (in %) of the best and worst plants selected by different selection strategies compared to BLUP, considering the $\sum Z$

| Strategies | 100 Best | 100 Worst |
|---|---|---|
| Mass selection | 44 | 36 |
| Stratified mass selection | 42 | 39 |
| Between and within selection | 83 | 84 |

The results obtained, in the first moment, allowed inference that mass selection was not efficient compared to BLUP. However, the selection by different strategies does not necessarily identify the same progeny/individual, but rather individuals similar in terms of performance. In this condition, the efficiency of the mass selection strategy would be underestimated. To demonstrate this fact, the selection differential of the different strategies was estimated. It was observed that the selection differential was greatest in the mass selection strategy, of 3.63, while for BLUP and between and within selection the selection differential was of 2.6 and 3.14, respectively. It should be emphasized, however, that in the selection gain expression (SG), the selection differential should be multiplied by the heritability ($h^2$). In the case of BLUP, $h^2 = 1.0$. In the between and within progeny selection, the $h^2$ between and $h^2$ within progeny selection should be used as weights. In the case of mass selection, it would be ***SG*** **=** selection differential $_x$ $h_I^2$, where $h_I^2$ is the heritability for selection at individual level, a value that is not normally high (Moreto et al, 2007). It can be inferred that the expected gain from mass selection and BLUP would be similar if $h_I^2$ was 74.3%, that is, 2.6/3.5 = 0.7428. Although this $h_I^2$ estimate was not obtained, it would be difficult to be of this magnitude. However, the efficiency of mass selection compared to BLUP should not be considered of only 40%.

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# PLASTICITY AND STABILITY OF THE PRODUCTIVITY OF NEW COMMON BEAN CULTIVARS (*PHASEOLUS VULGARIS* L.)

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New cultivars should possess high and stable productivity, as well as a number of valuable economic traits which ensure this productivity under a wide range of environments. Productivity is the phenotypic expression of the cultivar's genotype interacting with the environment.

## MATERIALS AND METHODS

A competition varietal trial was performed at DAI – General Toshevo to determine the plasticity ($b_i$) and stability ($\sigma_i^2$) of the yield from 11 common dry bean cultivars (*Phaseolus vulgaris* L.) which belonged to three habit types. The following cultivars of habit type I were included in the investigation: Dunav 1, Tarnovo 13, Trakia, Mizia and GTB Helis; the cultivars of habit type IIa were Abritus and Beslet; and of habit type IIIb – Dobrudzhansky 7, Dobrudzhansky ran, Plovdiv 10 and Elixir. The cultivars used as checks were Dunav 1, Tarnovo 13, Abritus, Dobruzhansky 7, Dobrudzhansky ran and Plovdiv 10. The other five dry bean cultivars were recently developed at DAI – General Toshevo: Trakia, Mizia, GTB Helia, Beslet and Elixir.

The coefficient of regression $b_i$ was calculated using the software product GEST of Ukai (1996), and parameters $YS_i$ (Kang 1993) and $\sigma_i^2$ Shukla (1972) were calculated with the help of STABLE Kang, M. and R. Magari (1995).

## RESULTS AND DISCATION

The cultivars of habit type IIIb Dobrudzhansky 7, Dobrudzhansky ran, Elixir and Plovdiv 10 demonstrated during the period of testing (2005 - 2010) under the conditions of DAI – General Toshevo the highest mean productivity of 1695 kg/ha, within the range 1482 kg/ha – 1906 kg/ha. Cultivar Plovdiv 10 showed the highest productivity: 1906 kg/ha. Cultivar Elixir had the highest stability ($\sigma_i^2$ = 5337) and coefficient of regression almost equal to 1 ($b_i$ = 0,935).

The cultivars of habit type IIa Abritus and Beslet demonstrated mean productivity of 1478 kg/ha, intermediate between that of IIIb (1695 kg/ha) and Ia (1067 kg/ha). The advantage of these varieties to the cultivars of habit IIIb type is related to white mold occurrence and risks of long-lasting rainfalls during harvesting because of the lodging habit type. These problems were not manifested during the test trials we performed and therefore this advantage of habit type IIa to habit type IIIb was not expressed. Therefore both mean yield (1332 kg/ha of cultivar Beslet and 1623 kg/ha of cultivar Abritus) and stability of productivity ($\sigma_i^2$ = 92922 for cultivar Abritus and $\sigma_i^2$ = 120935 for cultivar Beslet) had intermediate values.

The mean yield from the cultivars of I habit type (1067 kg/ha) was the lowest of the three groups of cultivars. Cultivar Trakia had the highest mean yield (1405 kg/ha) followed by cultivar GTB Helis (1306 kg/ha). Although the cultivars of habit type I usually have the lowest adaptability, cultivar Trakia demonstrated one of the highest yield stabilities ($\sigma_i^2$ = 9764) combined with high productivity. Cultivar Trakia showed somewhat higher susceptibility to the variation of the environmental factors ($b_i$ = 1.104). Cultivars Dunav 1 and Mizia showed lowest susceptibility to the environment, with $b_i$ = 0.632 and $b_i$ = 0.447, respectively.

**Table 1.** Growth type, mean yields, coefficient of regression ($b_i$), variance of stability ($\sigma_i^2$) according to Shukla (1972), breeding criterion by yield and stability of yield according to Kang (1993).

| Cultivar | Growth type | Mean yield, kg/ha | Coefficient of regression, $b_i$ | Shukla (1972) $\sigma_i^2$ | Kang (1993) $YS_i$ |
|---|---|---|---|---|---|
| Dobrudzhansky 7 | IIIb | 1727 | 1,312 | 184084*** | 5+ |
| Plovdiv 10 | | 1906 | 1,392 | 114460*** | 6+ |
| Dobrudzhansky ran | | 1482 | 1,125 | 16506** | 8+ |
| Elixir | | 1666 | 0,935 | 5337 | 12+ |
| Abritus | IIa | 1623 | 1,377 | 92922*** | 3+ |
| Beslet | | 1332 | 1,062 | 120935*** | -4 |
| Dunav 1 | Ia | 878 | 0,632 | 83068*** | -9 |
| Tarnovo 13 | | 941 | 0,789 | 47044*** | -8 |
| Trakia | | 1405 | 1,104 | 9764 | 7+ |
| GTB Helis | | 1306 | 0,823 | 13058* | 3+ |
| Mizia | | 806 | 0,447 | 182493*** | -10 |

*, **, *** Denote significance at P = 0.1, 0.05, and 0,01

The concept of phenotypic plasticity was first defined by Bradshaw (1965); it characterizes the quantitative changes in the expression of a given trait under changeable environments. The higher the value of the coefficient, the more susceptible the cultivar is to the changes of the environment. According to Finlay and Wilkinson (1963), a cultivar is most stable at coefficient of regression $b_i = 0$, i.e. yield does not vary by year and location. According to these authors, the cultivar with highest plasticity under certain environment within the group of cultivars involved in the investigation was the cultivar with coefficient of regression $b_i = 1$. In our study (Table 1) cultivar Plovdiv 10 had the highest coefficient of regression ($b_i = 1.392$), but the cultivar gave the highest productivity (1906 kg/ha). Cultivar Mizia had the lowest coefficient of regression and lowest yield (806 kg/ha). Highest plasticity was demonstrated by the cultivars Elixir and Beslet, which had regression coefficients $b_i = 0,935$ and $b_i = 1.062$, respectively.

According to Kang,s selection criterion (1993), most interesting from a breeding point of view is cultivar Elixir ($YS_i = 12+$**).**

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# YIELD STABILITY OF $C_1$ FAMILIES FROM RECURRENT SELECTION OF CARIOCA COMMON BEANS

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Recurrent selection is a breeding method used to obtain a great number of genotypes carrying several alleles of interest, and to allow continuous improvement of the base population. Therefore, one strategy to improve yield is to simultaneously select genotypes for tolerance to other limiting factors affecting crop yield, such as disease susceptibility, low soil fertility and water deficit stress, in addition to develop a new plant ideotype, with erect stature, short and straight branches and high first pod insertion. Yield stability and adaptability of common bean families from the first selection cycle ($C_1$) of recurrent selection were evaluated at Embrapa Rice and Beans Research Center, using carioca bean genotypes, with the objective to select superior families, to provide lines and intercrossing, and to obtain new selection population.

Seven recurrent selection trials were conducted using three controls (BRS Estilo, BRS Cometa e BRS Pontal), and 78 $C_1$ families. Three trials were carried out in 2008 using families from $C_1S_{0:3}$ families (one in the winter season in Santo Antônio de Goiás-GO and two in the wet season in Ponta Grossa-PR and Sete Lagoas-MG). The other four trials were conducted in 2009 using $C_1S_{0:4}$ families: one in the winter season (Santo Antônio de Goiás-GO); two in the dry season (Ponta Grossa- PR and Lavras- MG); and one in the wet season (Frei Paulo- SE). The experimental design was a triple square lattice 9x9 and data were submitted to individual and joint analysis of variance using the Genes program (CRUZ, 2001), a genetics and statistics computer applicative. Analyses of yield stability and adaptability were carried out using the methodology proposed by Annichiarico (1992). To measure family behavior within a particular environment, ambient conditions were decomposed into favorable (yield above overall mean) and unfavorable (below overall mean). This method is based upon the so called genotypic confidence index ($\omega i$), using a coefficient of 75% or $\alpha = 0.25$.

Significant differences were detected among families (at .01 level of probability) in all trials and joint analyses, also presenting significant interactions between families and environments. According to results obtained for stability and adaptability analyses performed for the 20 most productive families (Table 1), family SRC-207103318 presented the highest Wi general value (112.5), indicating 75% probability to produce 12.5% more than the average value obtained for all environments evaluated. Besides, it also presented confidence indexes above favorable (111.9) as well as unfavorable environments (113.7), which demonstrate the stability of this genotype over a varied crop conditions. Family SRC- 207103079 surpassed 29.7% the overall mean obtained from the unfavorable environments (Wi=129.7), revealing its adequacy for family agriculture. Under favorable ambient conditions, family SRC-207103004 (Wi=118.3) presented the highest production stability indicating to be responsive to technology inputs.

The above results lead to the conclusion that it is possible to select high yield families, either with ample or specific stability to both favorable and unfavorable conditions that may be improved

with the objective of obtaining superior cultivars and/or to be recombined for further recurrent selection procedures.

**Table 1.** Overall response and responses to favorable and unfavorable environments; and average yield of selected families obtained in the Carioca Beans Recurrent Selection Program in Santo Antônio de Goiás-GO, Ponta Grossa-PR, Sete Lagoas-MG, Lavras-MG and Frei Paulo-SE during wet, dry and winter seasons in 2008 and 2009.

| Families | Wi Overall | Wi Unfavorable | Wi Favorable | Yield (Kg/ha) |
|---|---|---|---|---|
| SRC-207103318 | 112.5 | 113.7 | 111.9 | 1963 |
| SRC-207103079 | 107 | 129.7 | 97.8 | 1848 |
| SRC-207102999 | 106.8 | 105 | 109.1 | 1942 |
| SRC-207103299 | 106.6 | 109.8 | 104.4 | 1873 |
| SRC-207103296 | 106.5 | 118.3 | 100.4 | 1870 |
| SRC-207103004 | 106.1 | 87.6 | 118.3 | 2077 |
| SRC-207103049 | 105.7 | 106.9 | 106.3 | 1855 |
| SRC-207103169 | 105.6 | 121.9 | 99.1 | 1826 |
| SRC-207103167 | 105.0 | 106.8 | 103.6 | 1867 |
| SRC-207103498 | 104.7 | 104.6 | 105 | 1851 |
| SRC-207103304 | 104.5 | 108.4 | 102.5 | 1834 |
| SRC-207102863 | 104.1 | 96.7 | 109.8 | 1892 |
| SRC-207103102 | 103.8 | 97.5 | 108.1 | 1840 |
| SRC-207102959 | 103.6 | 114.9 | 100.2 | 1814 |
| SRC-207103781 | 100.7 | 104.8 | 98.4 | 1741 |
| SRC-207103757 | 95.6 | 74.7 | 111.7 | 1872 |
| SRC-207103587 | 92.6 | 103.7 | 86.7 | 1704 |
| SRC-207103578 | 90.9 | 89.2 | 91.5 | 1701 |
| SRC-207103459 | 89.4 | 96.4 | 85.3 | 1638 |
| SRC-207103154 | 79.4 | 60.1 | 93 | 1609 |

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# INBREED DEVELOPMENT OF BEANS TYPE "CARIOCA" ORIGINATING FROM TWO CYCLES OF RECURRENT SELECTION

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**INTRODUCTION:** In these last year, the beans plant genetic breeding programs have been giving emphasis to the breeding of cultivars from the commercial group "carioca", especially due to the preference of farmers and consumers for this type of grain. Thus, the development of new inbreed with elevated productive capacity and good grain quality has been the main goal for most of the breeding programs. Taking into consideration the fact that in order to obtain inbreed that associate grains within the commercial pattern of the "carioca" with high productivity, a great number of genes is involved. Therefore, an interesting breeding strategy is the use of recurrent selection cycles (VIEIRA et al., 2005). The aim of the present work was to evaluate the performance of beans inbreed of the type "carioca" extracted from twenty segregating populations, originating from the second recurrent selection cycle ($C_1$) of the beans plant breeding program at the Federal University of Viçosa - UFV, Viçosa, state of Minas Gerais, Brazil.

**MATERIALS AND METHODS:** The experiments were conducted in the experimental field of the Plant Science Department at UFV, in the city of Coimbra, state of Minas Gerais, Brazil, during the winter crop of 2007 and "drought" and winter of 2008. Forty inbreed originating from 20 populations, together with nine controls, among them *Pérola*, *Talismã* and *Majestoso* were evaluated. The experimental design in latice 7 x 7 was used, with plots constituted of two lines of 2 meters, spaced in 50 cm, with density of 12 seeds per meter. Besides the productivity (kg/ha), the commercial aspect of the grains as well as the plant architecture were evaluated. For the grain aspect a scale of scores going from 1 (typical "carioca" grain, light yellow color with light brown marks, light yellow halo and not flat) to 5 (grain with light yellow color with dark brown marks, with a halo that was not light yellow and flat). The plant architecture was also evaluated through scores that varied from 1 (erect plants) to 5 (non-erect plants). The data related to the evaluated characters were, initially, submitted to variance analysis per crop and later on, the group variance analysis involving the three crops.

**RESULTS AND DISCUSSION:** According to the summary of the group variance analysis good experimental precision was observed, with CV of 15.99% for grains productivity, 1.65% for grains aspect and 9.36% for architecture, thus adequate for experiments of this nature. For the productivity and grains aspect, a significant effect was observed ($P<0,01$) for the sources of crop variation as well as treatments, whereas for the plant architecture only crops showed significant effect ($P<0,01$). For the productivity characters and grains aspect, the interaction treatments x crops was significant ($P<0,01$), indicating inconsistency in the inbreed behaviors in the different environments. The inbreed average performance in the three crops is presented in Table 1. the inbreed 304-17, 309-19, 323-19, 306-6 and 323-4 were the ones which stood out in terms of productivity, commercial grains aspect as well as plant architecture. These inbreed have also presented grains of good commercial aspect and plant architecture similar to the ones presented by the controls.

**Table 1.** Productivity averages (kg/ha) and scores of grains aspect and plant architecture, of 40 inbreed of beans of the "carioca" type originating from two recurrent selection cycles. Coimbra-MG, winter of 2007 and "drought" and winter of 2008.

| Inbreeds | Productivity (kg/ha) | Aspect of the grains | Architecture |
|---|---|---|---|
| 304-17 | 4406 | 2.17 abc | 2.95 abc |
| 309-19 | 4382 | 2.37 abc | 3.10 abc |
| 323-19 | 4103 | 2.23 abc | 2.95 abc |
| 306-6 | 4039 | 2.33 abc | 3.10 abc |
| 323-15 | 4019 | 2.13 abc | 2.90 abc |
| 323-4 | 3971 | 2.30 abc | 2.55 abc |
| VC-3 | 3874 | 1.50 | 3.75 abc |
| 308-19 | 3853 a | 1.97 abc | 3.30 abc |
| VC-6 | 3816 a | 2.37 abc | 3.25 abc |
| 323-6 | 3814 a | 2.27 abc | 3.50 abc |
| 323-9 | 3802 a | 3.00 | 2.85 abc |
| 321-18 | 3764 a | 2.30 abc | 3.40 abc |
| 315-3 | 3757 a | 2.40 abc | 3.25 abc |
| 322-7 | 3717 a | 2.03 abc | 3.40 abc |
| 312-7 | 3678 a | 2.30 abc | 2.70 abc |
| 312-9 | 3636 a | 2.37 abc | 2.75 abc |
| 321-13 | 3625 a | 2.20 abc | 3.15 abc |
| 321-3 | 3613 a | 2.27 abc | 3.25 abc |
| 312-14 | 3610 a | 2.30 abc | 2.85 abc |
| 307-16 | 3582 a | 2.23 abc | 2.60 abc |
| 322-2 | 3553 a | 2.43 abc | 3.15 abc |
| 312-11 | 3533 a | 2.33 abc | 2.75 abc |
| 308-8 | 3512 a | 2.43 abc | 2.85 abc |
| 321-1 | 3487 a | 2.27 abc | 3.30 abc |
| 321-19 | 3377 a | 2.43 abc | 3.30 abc |
| 323-7 | 3364 a | 2.20 abc | 2.95 abc |
| 315-6 | 3340 a | 2.13 abc | 3.00 abc |
| 305-9 | 3312 ab | 2.10 abc | 2.35 abc |
| 312-18 | 3296 ab | 2.27 abc | 3.25 abc |
| 304-12 | 3276 ab | 2.20 abc | 3.20 abc |
| 310-8 | 3250 ab | 1.97 abc | 2.80 abc |
| 310-7 | 3223 abc | 1.97 abc | 3.35 abc |
| 319-2 | 3200 abc | 2.33 abc | 2.80 abc |
| 315-1 | 3196 abc | 1.97 abc | 2.90 abc |
| 304-16 | 3170 abc | 2.20 abc | 3.40 abc |
| 315-11 | 3169 abc | 1.97 abc | 3.05 abc |
| 315-2 | 3160 abc | 2.13 abc | 2.85 abc |
| 305-6 | 3157 abc | 2.33 abc | 3.10 abc |
| 315-7 | 3117 abc | 1.93 abc | 2.75 abc |
| Pioneiro | 3017 abc | 3.03 | 3.30 abc |
| 310-12 | 2991 abc | 2.17 abc | 2.50 abc |
| Requinte | 2990 abc | 2.50 abc | 3.30 abc |
| 310-9 | 2975 abc | 2.20 abc | 3.15 abc |
| Horizonte | 2953 abc | 2.17 abc | 2.85 abc |
| 306-13 | 2936 abc | 2.33 abc | 3.15 abc |
| Pérola | 3243 a | 2.17 a | 2.75 a |
| Majestoso | 2693 b | 2.37 c | 2.85 b |
| Talismã | 2595 c | 2.30 b | 3.35 c |

*Averages followed by the letters a, b and c in the same column, do not differ from the controls Talismã, Pérola and Majestoso, respectively (Dunnett, P<0,05).

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# YIELD STABILITY OF $C_1$ FAMILIES FROM RECURRENT SELECTION OF COMMON BLACK BEANS

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Selection of an adequate breeding method to improve yield of common beans is very important, since this is a quantitative trait of low heritability. Recurrent selection, successfully used in allogamous plant species to provide accumulation of desirable alleles of quantitative traits, such as yield, consists of a series of evaluation cycles followed by recombination of the selected families, leading to new genotypic combinations. Advanced selection cycles increases frequency of favorable alleles within the population with consequent increased chances to produce one or more pure lines with improved number of favorable alleles. It also provides the breeder increased opportunities to explore genetic variability by the new lines obtained after each selection cycle. Yield stability and adaptability of common bean families from the first selection cycle ($C_1$) of recurrent selection were evaluated at Embrapa Rice and Beans Research Center, using black bean genotypes, with the objective to select superior families, to obtain lines and intercrossing, and provide the new selection population.

Eight recurrent selection trials were conducted using three controls (BRS Campeiro, BRS Supremo and BRS Esplendor) plus 46 families from the cycle $C_1S_{0:3}$ carried out in 2008 and five from the $C_1S_{0:4}$ cycle conducted in 2009. In 2008, one trial was carried out in Santo Antônio de Goiás - GO, during the winter season, and two during the wet season in Ponta Grossa - PR, and Sete Lagoas - MG. In 2009, one trial was conducted during the winter in Santo Antônio de Goiás - GO, three during the dry season (Ponta Grossa- PR, Lavras- MG, Santo Antônio de Goiás- GO), and one in the wet season in Frei Paulo- SE. Experimental design was a triple square lattice 7x7 and data were submitted to individual and joint analysis of variance using the Genes program (CRUZ, 2001), a genetics and statistics computer applicative. Analysis of yield stability and adaptability was carried out using the methodology proposed by Lin & Binns (1988), estimating general family stability ($P_i$) and also decomposing $P_i$ into groups related to favorable and unfavorable environment conditions classified according to ambient indexes defined by the difference between mean value obtained in each location and the overall mean.

Significant differences were detected among families (at 0.01 level of probability) in all trials and joint analysis, also presenting significant interactions between families and environments. According to results obtained for stability and adaptability analyses performed for the 20 most productive families (Table 1), family SRP-207103873 presented the smallest $P_i$ over the average of all ambient conditions as well as in favorable environments indicating ample adaptability to different cropping conditions but specially to ambients provided with high level of technology and low risk of weather stress conditions. The best adapted family to unfavorable environment conditions was SRP-207104534 that may be recommended for regions subjected to biotic and abiotic stresses.

Recurrent selection programs used in common black type beans presents promising potential to develop high yield cultivars with ample adaptation to all regions as well as to those with or without risk of occurrence of biotic and abiotic stresses.

**Table 1.** Overall response to favorable and unfavorable environments, and average yield of selected families obtained in the Black Beans Recurrent Selection Program in different regions and crop seasons*.

| Families | $P_i$ Overall | $P_i$ Favorable | $P_i$ Unfavorable | Yield (Kg/ha) |
|---|---|---|---|---|
| SRP-207103873 | 158921 | 84553 | 282868 | 2323 |
| SRP-207104593 | 178850 | 199675 | 144141 | 2251 |
| SRP-207104055 | 311206 | 368700 | 61141 | 2072 |
| SRP-207103883 | 326742 | 107591 | 691992 | 2237 |
| SRP-207104586 | 337118 | 378634 | 100856 | 2054 |
| SRP-207104534 | 349932 | 501849 | 96736 | 2018 |
| SRP-207103881 | 365105 | 222504 | 602773 | 2029 |
| SRP-207103913 | 367325 | 187319 | 667334 | 2115 |
| SRP-207104099 | 382660 | 450184 | 270121 | 2105 |
| SRP-207104522 | 403740 | 365441 | 467572 | 2011 |
| SRP-207104488 | 404272 | 244390 | 670742 | 2067 |
| SRP-207103879 | 419964 | 230362 | 735967 | 2067 |
| SRP-207103876 | 421113 | 201484 | 787161 | 2136 |
| SRP-207104594 | 456627 | 554870 | 292887 | 1929 |
| SRP-207104342 | 458300 | 565372 | 279847 | 1987 |
| SRP-207104206 | 495425 | 436272 | 594012 | 1861 |
| SRP-207104098 | 497747 | 550165 | 410383 | 2097 |
| SRP-207103898 | 531535 | 473122 | 628889 | 1883 |
| SRP-207104458 | 542947 | 593163 | 459254 | 1774 |
| SRP-207104521 | 588873 | 394974 | 912038 | 1915 |

* Santo Antônio de Goiás-GO, winter season (2008) and dry season (2009); Ponta Grossa-PR and Sete Lagoas-MG, wet season (2008); Lavras-MG and Ponta Grossa-PR, wet season (2009); Santo Antônio de Goiás-GO, winter season (2009)

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# *IN VITRO* CULTURE TECHNIQUE TO RESCUE GLOBULAR EMBRYOS OF *PHASEOLUS* BY MICROCUTTING

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## INTRODUCTION

Crosses between *Phaseolus coccineus* L. (♀) and *P. vulgaris* L. usually lead to the abortion of hybrid embryos 2 to 8 days after pollination (DAP) at globular stage (Lecomte, 1997). *In vitro* culture is the only way to rescue these embryos. Two major problems occur with the culture of very immature embryos in *Phaseolus*: *(i)* the complexity of the required medium with very small embryos and *(ii)* the difficulty to extract such tiny embryos without damaging the suspensor. To reduce suspensor damage, we opted for a protected embryo culture, *in ovulo* or pod culture, during a limited time (5 to 10 days) before extracting the embryo at a stage when the role of the suspensor is much less important, usually the late heart-shaped or cotyledonary stage. Applying this technique, some embryos at globular developmental stage evolve to the cotyledonary stage. A step by step procedure of *in vitro* culture was developed by Geerts (2001) to rescue immature embryos; in particular, the application of high and variable osmolality conditions similar to those observed *in vivo*, during pod culture (1 week) and before extracting the embryos gave the best results in terms of ovule and embryo development. Following this technique, with the successive transfer of pods and embryos in various media, Geerts (2001) was the first to carry out the regeneration of *P. vulgaris* plantlets from 2 days old embryos. However the plantlets expressed physiological disorders after 14 days of culture with a very low regeneration rate (2.8%). Therefore, the specific objective of our study was to find out an efficient method for regeneration of *Phaseolus* sp. from globular embryos by using microcutting of cotyledonary node of *in vitro* regenerants. .

## MATERIALS AND METHODS

Pods of *P. vulgaris* (NI 637, 2 DAP) and *P. coccineus* (NI 16, 5 DAP) were used as plant material and two protocols designated as P1 and P2 were compared: the control (Geerts scheme) or P1= pod culture technique followed by embryo culture and P2 = P1+ microcutting technique. The media used for the different steps of pod and embryo culture (P1) are explained in details in Geerts (2001): the pods of the 2 genotypes were cultured in Petri dishes; after one week, embryos were extracted and they were successively cultured on maturation, dehydration, root induction and rooting media. Microcuttings containing cotyledonary node were excised from 14 days-old *in vitro* regenerants obtained thanks to the method of Geerts (2011) and were subcultured during 4 weeks on modified Murashige et Skoog (1962) medium, with the addition of $GA_3$(0,18µM) and BAP (0,1µM). This protocol (P2) was applied not only for the two parents (NI 637 and NI 16) but also for interspecific crosses between the two parental species, the embryos being extracted from pods aborting between 10 et 14 DAP. With the protocol P1, for each of the two parental species, 90 embryos were extracted from precultivated pods and distributed in 5 replicates, while with the protocol P2, 108 and 225 embryos were extracted respectively for NI 637 and NI 16 (with 3 replicates for both species) and 11 embryos were extracted from the cross NI 16 (♀) X NI 637.

## RESULTS AND DISCUSSION

In our results, a regenerated plantlet showed stem, trifoliated leaves and roots**.** The regeneration rates vary according to the protocols. With P1, no plantlet was obtained for the two genotypes studied, all *in vitro* regenerants stopped development after 7 days on rooting medium and shoots showed no roots or trifoliated leaves. A similar result was reported with *P. vulgaris* by Geerts (2001). With P2, plantlets were obtained from the 2 genotypes after 4 weeks on microcutting medium; however the rate of *in vitro* plantlets was significantly higher with *P. coccineus* compared to the value obtained with *P. vulgaris* (73, 33% versus 44, 45%) The better success obtained with *P.coccineus* using P2 could be explained by the good development of plantlets regenerated by microcutting. In a study aiming to achieve micropropagation in *Phaseolus* sp., higher rate of plant regeneration from 7 cultivars of *P. coccineus* compared to 10 of *P. vulgaris*, was obtained with microcuts excised from plantlets derived from mature seed germinated *in vitro* (Santalla *et al*., 1998). For the hybrid embryos, only 1 plantlet out of 11 hybrid embryos cultivated was regenerated after 8 weeks using MS medium enriched by activated charcoal (0,5%). The hybrid state of the plantlet was confirmed by PCR, using SSR BM141. The remaining hybrid microcuttings developed shoots showing morphologically abnormal stems and roots, and marked browning of explants. A similar low rate of regeneration was also associated with significant tissue browning in the study conducted by Arellano *et al.* (2009) using indirect organogenesis from cotyledonary node isolated from mature seeds of 10 *P.vulgaris* varieties. However, in the same study, use of an antioxidant, polyvinylpyrrolidone (PVP-360), in culture media prior to the rooting phase prevented callus browning and promoted a good rooting of the regenerated plantlets.

## CONCLUSION AND PROSPECTS

Our study confirmed that plantlet regeneration from *in vitro* culture of globular embryos is difficult. Although regeneration rate is higher in *P.coccineus* than *in P. vulgaris*, microcutting of cotyledonary node has improved the rate of regenerated plantlets in both species. For hybrids, our work currently in progress aim to optimize the pods culture technique followed by microcutting of cotyledonary node: investigations are precisely oriented to the microcutting culture medium in order to reduce significantly explant browning and consequently promote the regeneration of hybrids that abort approximately 4 at 8 DAP.

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# DNA METHYLATION PROFILES DIFFER BETWEEN PARENTS AND *PHASEOLUS* INTERSPECIFIC HYBRIDS

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## INTRODUCTION

*Phaseolus* beans are cultivated worldwide in the tropics, subtropics, and temperate zones. The common bean (*Phaseolus vulgaris* L.) is one of the most important food legume in the world. This species is however susceptible to several viral, bacterial, and fungal diseases. Genetic resistance to some of these major biotic constraints has been identified in related species such as *Phaseolus coccineus* Lam. and *P.polyanthus* Greenm.. Transfer of desirable traits from related *Phaseolus* species to *P. vulgaris* could greatly improve agronomic characteristics of the common bean. Therefore, interspecific hybridization has been used to facilitate genetic exchange between *Phaseolus* species. Although fertilization occurs and embryos are formed, interspecific hybrid embryos are generally limited in their developmental potential. The major reproductive barrier to interspecific hybridization amongst the genus *Phaseolus* occurs post-fertilisation, especially during early embryo development. Several mechanisms have been proposed to explain postzygotic barriers such as genetic and epigenetic mechanisms [1]. Methylation is shown to play an important role in regulation of gene expression during plant embryogenesis and further cell differentiation [2]. Indeed, changes in DNA methylation have been shown to be involved in the transcriptional regulation of gene expression [3]. DNA methylation is recognized as critical for embryogenesis in *Arabidopsis* and is involved in regulating gene expression affecting both auxin responsiveness and embryo cell identity [4]. The purpose of our investigation was to examine and compare changes in cytosine methylation in *Phaseolus* interspecific hybrids in comparison with the parents.

## MATERIEL AND METHODS

Two parental genotypes *P. vulgaris* (cv, NI637) and *P. coccineus* (cv, NI16) and their reciprocal interspecific hybrids were used in this study. Genomic DNA was isolated from the young seeds 3-6 DAP (days after pollination) of the parental lines and interspecific hybrids and were analyzed using methylation-sensitive amplification polymorphism (MSAP). Genomic DNA (1µg) was digested with 10U *Eco*RI (Fermentas) in a final volume of 20 µl of the appropriate buffer. For the second digestion, 10U *Msp*I or *Hpa*II (Fermentas) were used. The digested fragments were ligated to the adapters. Preamplification was performed using 4 µl dilute restriction and ligation product. The PCR was runned with the following protocol: 72°C for 2 min, followed by 12 cycles with a 0.8°C decrease in annealing temperature per cycle, and 23 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 2 min. The amplification products were separated by electrophoresis in 6.5% denaturing polyacrylamid gel using an automated ALF express sequencer (Pharmacia Biotech). MSAP images were analyzed with Allelinks™ analysis software.

## RESULTS AND DISCUSSION

To test if epigenetic modifications occur in the *Phaseolus* interspecific hybrids, we analyzed the methylation status of the hybrids in comparison with the parental plants. The methylation pattern at the 5'-CCGG sites was analysed using the isochizomer methylation-sensitive enzymes *Hpa*II and *Msp*I. *Hpa*II is sensitive to full methylation (both strands methylated) of any cytosine; *Msp*I is sensitive only to methylation of the external cytosine [5]. For MSAP analysis, 8 pairs of primers were used and a total of 547 fragments were amplified in the two parents. Of these fragments, 179 (32.72%) were differentially amplified from the two digests between the parents and the hybrids (Table 1). The following types of change were observed: (i) full methylation of both cytosine residues at the recognition site results in neither *Msp*I nor *Hpa*II cleavage; (ii) full methylation of the internal cytosine results in *Msp*I cleavage, but not *Hpa*II cleavage; (iii) hemimethylation of the external cytosine results in cleavage by *Hpa*II, but not *Msp*I; (iv) full demethylation of both cytosine residues at the recognition site, resulted in both *Hpa*II and *Mspa*I cleavage. Based on these MSAP profiles 13 bands (7.26% of all methylated sites), and 9 bands (5.02% of all methylated sites) displayed methylation alteration in the *P. coccineus* (♀) X *P. vulgaris* (H1) and *P. vulgaris* (♀) X *P. coccineus* (H2) interspecific hybrids respectively. MSAP results revealed an alteration in the methylation pattern in *Phaseolus* interspecific hybrids compared to parents. It is possible that these newly generated bands might be derived from altered parental bands arising from epigenetic modifications such as cytosine methylation induced by interspecific hybridization.

**Table 1.** Number of bands amplified using eight MSAP selective primer combinations in the parental *Phaseolus* genotypes and their interspecific hybrids

| Oligonucleotides | Total number of bands in the two parents[a] | Total number of methylated sites[b] | Methylation alteration in the *P. coccineus* (NI16) X *P. vulgaris* (NI637) hybrid | Methylation alteration in the *P. vulgaris* (NI637) X *P. coccineus* (NI16) hybrid |
|---|---|---|---|---|
| *EcoR*I + ACC-*Hpa*II/*Msp*I + TCAA | 94 | 26 | 2 | 1 |
| *EcoR*I + ACC-*Hpa*II/*Msp*I + TCAC | 72 | 17 | 1 | 0 |
| *EcoR*I + ACT-*Hpa*II/*Msp*I + TCAA | 69 | 24 | 2 | 1 |
| *EcoR*I + ACT-*Hpa*II/*Msp*I + TCAC | 34 | 13 | 0 | 3 |
| *EcoR*I + ACG-*Hpa*II/*Msp*I + TCAA | 78 | 29 | 4 | 0 |
| *EcoR*I + ACG-*Hpa*II/*Msp*I + TCAC | 91 | 35 | 1 | 3 |
| *EcoR*I + AAC-*Hpa*II/*Msp*I + TCAA | 68 | 24 | 2 | 1 |
| *EcoR*I + AAC-*Hpa*II/*Msp*I + TCAC | 41 | 11 | 1 | 0 |
| Total | 547 | 179 | 13 | 9 |

[a]Bands from four lanes were analyzed in each selective primer combination, two lanes for each parent (digested with either *Hpa*II or *Msp*I). Bands that were monomorphic (identical in both parents) were counted only once.
[b]Bands were considered methylated if they showed polymorphism between the two isoschizomers. Bands with the same methylation pattern in both patterns were scored only once.

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# DEVELOPMENT OF TEMPERATURE SWITCH-PCR (TS-PCR) MARKERS FOR SNP GENOTYPING IN COMMON BEAN

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## INTRODUCTION

It was estimated that common bean (*Phaseolus vulgaris* L.) has an average of one single nucleotide polymorphism (SNP) in every 88 base pairs (Gaitan-Solis et al. 2008). Based on an estimated common bean genome size of approximately 588 Mb, there would be 6-7 million SNPs available in the common bean genome (Liu et al.2010). However, effective application of these SNPs is a challenge for the basic molecular breeding programs, because of the cost of special equipments required for efficient SNP genotyping.

Temperature switch Polymerase Chain Reaction (TS-PCR), a robust assay for reliable amplification and genotyping of SNPs, is a biphasic three or four-primer PCR system with a universal primer design that permits amplification of the target locus in the first phase of thermal cycling before switching to the detection of the alleles (Hayden et al. 2009). This type of SNP marker assay system can be used efficiently by the average bean breeding programs for marker-assisted selection.

## MATERIALS AND METHODS

The plant materials used in this study are the BAT93 and Jalo EEP558, the parental lines used to develop the BAT93 and Jalo EEP558 recombinant inbred line population (Nodari et al. 1993). The SNPs tested in this study were identified and described by McConnell et al (2010). The PCR primers design and PCR reactions were performed as described by Hayden,et al. 2009 with some modifications and optimizations (Table 1).

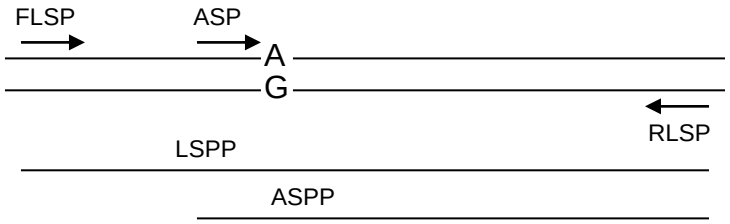


**Fig 1**, a diagram illustrating the positions and orientations of the three PCR primers for the allele-specific TSP marker relative to its matching template DNA. FLSP is the forward locus specific primer; ASP is the allele specific primer; RLSP is the reverse locus specific primer; LSPP is the locus specific PCR product; and ASPP is the allele specific PCR product.

**Table 1.** Primer sequences of 4 TS-PCR markers. Sequences underlined are the added tail at the 5' end and the SNP at the 3'end, respectively. "b" after the name indicates that the SNP is from Bat 93.

| NP name | Forward | Reverse |
|---|---|---|
| **g934b** | **FLSP** CATACTCGAGGATTGTGGAGAAGC<br>**ASP** AACCGTGTACACGGCG | **RLSP** AAACCAAACGTGACATACCAAAA |
| **g1795b** | **FLSP** AGAAAAACGTAGCGAGAGTGGTG<br>**ASP** GAG GCAGTTCCATGGG | **RLSP** CCAGTGACAAACATATCCACATTCA |
| **g774b** | **FLSP** AACGAAAGAGAGAGAAAGGGGAAT<br>**ASP** ATG GATGCGAAAAGCG | **RLSP** TTACTACTGCGGCTTCGAAAATTG |
| **g893b** | **FLSP** CCACCTTCCAGCCTTACAAAACTA<br>**ASP** GACGGTAAGCAGAATGAATTC | **RLSP** GCATTGTCCTTGTTGCTGTTGTAG |

## RESULTS AND DISCUSSION

A number of TS-PCR markers were developed. Modification and optimization of the primer design and PCR conditions are necessary for reproducible amplification of the TS-PCR markers. Although amplification of the locus-specific allele is sometimes visible, the SNP allele is usually amplifiable and distinguishable from the locus-specific allele (Fig. 2). With the TS-PCR amplification approach, detection of SNPs in bean can be carried out using simple lab procedure, available to average breeding programs.

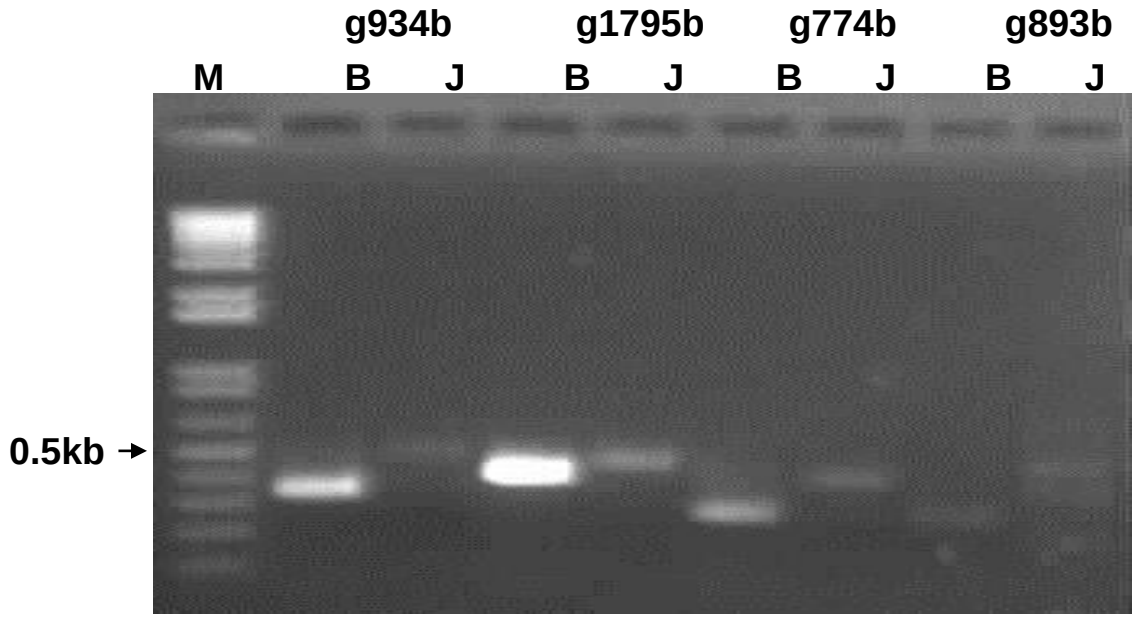


**Fig. 2.** Examples of 4 TS-PCR markers for four SNPs. B is Bat 93, J is Jalo EEP558, M is 1 kb plus molecular weight standard. The names of the SNPs are on the top of the figure.

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# ASSOCIATION MAPPING OF COMMON BACTERIAL BLIGHT RESISTANCE QTL IN ONTARIO BEAN BREEDING POPULATIONS

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## INTRODUCTION

Common bacterial blight (CBB), incited by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*), is a major yield-limiting factor of common bean (*Phaseolus valgaris* L.) production around the world. Host resistance is practically the most effective and environmentally-sound approach to control CBB. Application of association mapping in QTL discovery in plant breeding programs is a promising approach to overcome some of the limitations with conventional QTL mapping strategies. The objectives of this research were to 1) apply association mapping to identify CBB resistance QTL in Ontario bean breeding materials and 2) evaluate whether association mapping can be used effectively to discover CBB resistance QTLs using SNP genotyping of plant materials, routinely developed in a bean breeding program.

## MATERIALS & METHODS

A population of 469 dry bean lines of different market classes representing plant materials routinely developed in a bean breeding program were used. Of them, 395 lines were evaluated for CBB resistance at 14 and 21 DAI (Days After Inoculation) in the summer of 2009 in an artificially inoculated CBB nursery in south-western Ontario. Genotyping was performed using the Sequenom iPLEX Gold Assay (Sequenom, Cambridge, MA) in Genome Quebec (Montreal, Quebec). Association mapping analyses were carried out with TASSEL 2.1 software, available at http://www.maizegenetics.net/index.php?option=com_content&task=view&id=89&Itemid=119. The mapping information of SNP markers was extracted from McClean (NDSU) 2007 genetic map at Legume Information System (www.comparative-legumes.org/index.php/Home) (McConnell et al., 2010).

## RESULTS

All lines were genotyped using 132 SNPs (Single Nucleotide Polymorphisms) evenly distributed across the genome. Of the 132 SNPs, 26 SNPs had more than 20% missing data, 12 SNPs were monomorphic, and 17 SNPs had a MAF (Minor Allelic Frequency) of less than 0.20, therefore only 75 SNPs were used for association studies, based on one SNP per locus. Marker data collected from all lines were used to construct population structure and kinship relationship matrices. The best possible population structure was to assign 36% and 64% of the lines into Andean and Mesoamerican subgroups, respectively. Kinship analysis also revealed complex familial relationships among all lines, which corresponds with the known pedigree history. MLM (Mixed Linear Model) analysis, including population structure and kinship, was used to discover marker-trait associations. Eighteen and 22 markers were significantly associated with CBB rating at 14 and 21 DAI, respectively (Table 1). Fourteen markers were significant for both dates and the markers UBC420, SU91, g321, g471, and g796 were highly significant ($p \leq 0.001$). Meanwhile, g471 on LG (Linkage group) 6 and g796 on LG 8 corroborate that both chromosome 8 and the distal region of the chromosome 6 are carrying major CBB resistance QTLs. Furthermore, 15 significant SNP

markers were co-localized with or close to the CBB-QTLs identified previously by conventional QTL mapping.

## CONCLUSIONS

This study demonstrated that association mapping using a reasonable number of markers, distributed across the genome and with application of plant materials that are routinely developed in a plant breeding program can detect significant QTLs for traits of interest. Unlike conventional QTL discovery strategies, in which limited number of bi-parental populations ($F_2$, RIL, or DH) are used, association mapping-based strategies can use diverse plant breeding populations derived from several bi-parental and/or complex crosses. This may address some of the concerns with conventional QTL mapping that the bi-parental mapping populations rarely give rise to new cultivars, the identified QTLs may not be effective in multiple genetic backgrounds and that the QTL-linked markers are not immediately available for marker-assisted selection.

**Table 1.** Testing of association between marker loci and common bacterial blight severity using unified MLM (Mixed Linear Model) method

| Chr. | CM | Marker[a] | 14 DAI | | 21 DAI | |
|---|---|---|---|---|---|---|
| | | | *p* | $R^2$_marker[b] | *p* | $R^2$_marker |
| 1 | 135 | g934 | n.s. | | * | 0.0061 |
| 2 | 39 | g680 | *** | 0.0098 | * | 0.0094 |
| 2 | 121 | g321 | *** | 0.0151 | *** | 0.0141 |
| 2 | 123 | g2581 | * | 0.0065 | n.s. | |
| 3 | 14 | g1296 | ** | 0.0104 | * | 0.0072 |
| 3 | 93 | g1656 | * | 0.0076 | *** | 0.0208 |
| 5 | 59 | g1689 | * | 0.0090 | ** | 0.0142 |
| 6 | 13 | g1757 | n.s. | | ** | 0.0079 |
| 6 | | UBC420 | *** | 0.0136 | *** | 0.0215 |
| 6 | 105 | g471 | *** | 0.0227 | *** | 0.0471 |
| 6 | 111 | g1436 | n.s. | | * | 0.0049 |
| 6 | 130 | g2538 | * | 0.0075 | * | 0.0088 |
| 7 | 63 | g2531 | n.s. | | *** | 0.0126 |
| 7 | 125 | g290 | *** | 0.0129 | ** | 0.0085 |
| 8 | | SU91 | *** | 0.0495 | *** | 0.0320 |
| 8 | 46 | g1119 | ** | 0.0102 | ** | 0.0136 |
| 8 | 64 | g696 | n.s. | | * | 0.0071 |
| 8 | 134 | g580 | n.s. | | * | 0.0048 |
| 8 | 166 | g1713 | ** | 0.0125 | n.s. | |
| 8 | 182 | g796 | *** | 0.0128 | *** | 0.0148 |
| 9 | 112 | g544 | ** | 0.0101 | * | 0.0068 |
| 9 | 121 | g1286 | n.s. | | * | 0.0045 |
| 10 | 13 | g2521 | n.s. | | * | 0.0109 |
| 10 | 59 | g2600 | ** | 0.0068 | n.s. | |
| 11 | 61 | g1215 | * | 0.0049 | n.s. | |
| 11 | 63 | g1415 | ** | 0.0073 | *** | 0.0171 |

[a] n.s., not statistically significant; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

[b] $R^2$_marker was calculated as the proportion of sum square due to marker after accounting for all other effects in model.

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# CHARACTERIZATION OF COMMON BACTERIAL BLIGHT AND HALO BLIGHT STRAINS ISOLATED IN DURANGO, MÉXICO

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**INTRODUCTION:** In Durango, México, several diseases have been reported as important factors reducing grain yield and seed quality in common beans (*Phaseolus vulgaris* L.) (Ibarra *et al*., 2009). Genetic control has been achieved for antrachnose (*Colletotrichum lindemuthianum*) and rust (*Uromyces appendiculatus* var. *appendiculatus*), but some difficulties have been observed for common bacterial blight (CBB) (*Xanthomonas campestris=axonopodis* pv. *phaseoli*) and halo blight (HB) (*Pseudomonas syringae* pv. *phaseolicola*), due to a lack of reliable gene sources for inducing resistance in commercial cultivars. CBB and HB diversity need to be analyzed and used in selecting parents and implementing efficient breeding programs to develop improved cultivars with enhanced resistance. The objective was to characterize CBB and HB strains isolated from seed samples collected in the state of Durango, México.

**MATERIAL AND METHODS:** During October 2010 seed samples were taken from six cultivars planted on July 16$^{th}$ at INIFAP-Durango experiment station. Plants were harvested separately and seeds were extracted manually from pods and placed in sterile plastic bags. 10g of seed samples were hilum sealed using cyanoacrylate based glue. Seeds were disinfected using sodium hypochlorite 2%, shaking constantly during 3 min, rinsed, and then dried and milled in a mortar with phosphate buffer. Serial dilutions were obtained and sown was then performed by duplicate, on Petri dishes with nutritive agar-cyclohexamide for HB isolation and YCA for CBB. Plaques were incubated in a chamber at a 27°C temperature during 24h and thereafter viable cell count was performed. Plates were incubated again during 24-120 h to allow the development of macroscopic colonies. Selected colonies were replanted to obtain axenic cultures and then characterized using morphological traits. Strains seeming like CBB and HB were used in microscopic and metabolic tests and grown in enriched medium. In each cultivar four replications were used to corroborate disease presence and strain isolation. CBB isolates were re-sown in B-King medium to eliminate non-fluorescent strains and those showing growth were used in oxidase test to separate *Pseudomonas syiringae* from other fluorescent *Pseudomonas*.

**RESULTS AND DISCUSSION:** Cultivars showing the highest bacterial load were Pinto Bravo for *Pseudomonas* spp. (840 CFU $g^{-1}$) and Pinto Libertad for both *Pseudomonas* spp. (561 CFU $g^{-1}$) and *Xanthomonas* spp. (34,500 CFU $g^{-1}$) (Table 1). Some resistance to *Pseudomonas syringae* or low seed contamination was observed in Pinto Centenario. 38 isolates seeming like *Pseudomonas* spp. and only one similar to *Xanthomonas* spp. (strain HC622) were obtained from mixed crops extracted in nutritive agar. From colonies obtained from YCA medium, only one *Xanthomonas* spp. strain was isolated from Pinto Libertad (YCA1) and a *Pseudomonas* strain from Pinto Coloso (YCA31). Metabolic tests revealed 11 *Pseudomonas* flourescent strains; however oxidase test revealed that only the strains HC353, HC427, HC643, and YCA31 were confirmed as *Pseudomonas syringae* (Table 2). The strains HC622 and YCA1 identified as *Xanthomonas* spp. showed growth in nutritive agar supplemented with sodium chloride 5%, dextrose 10% and YCA medium. The isolated strains will be used in experiments to determine genetic diversity using common bean differential cultivars.

**Table 1**. Bacterial load and isolates obtained in six pinto bean cultivars planted in Durango, 2010.

| Cultivar | *Pseudomonas* spp. CFU $g^{-1}$ | Isolates | *Xanthomonas* spp. CFU $g^{-1}$ | Isolates |
|---|---|---|---|---|
| Pinto Libertad | 561 | 9 | 34,500 | 1 |
| Pinto Centauro | 314 | 3 | 0 | 0 |
| Pinto Coloso | 212 | 8 | 0 | 0 |
| Pinto Centenario | 8 | 9 | 0 | 0 |
| Pinto Bravo | 840 | 1 | 0 | 0 |
| Pinto Saltillo | 130 | 8 | 0 | 1 |
| Total | | 38 | | 2 |

CFU= Colony Forming Units

**Table 2**. Morphological, physiological traits and metabolism test performed on *Xanthomonas* spp. and *Pseudomonas* spp. isolations obtained in Durango. 2010.

| TEST | HC353 | HC427 | HC622 | HC643 | YCA1 | YCA31 |
|---|---|---|---|---|---|---|
| Morphology | +P | +P | +X | +P | +X | +P |
| Gram response | - | - | - | - | - | - |
| Shape | B | B | B | B | B | B |
| Glucose use | - | + | + | - | + | - |
| Mixed acid production | - | - | - | - | - | - |
| Acetoine production | - | - | - | - | - | - |
| Sulfhidric acid production | - | - | + | - | + | - |
| Citrate use | + | + | + | + | + | + |
| Mobility | + | + | + | + | + | + |
| Indol production | - | - | - | - | - | - |
| Urease | - | - | - | - | - | - |
| Oxidase | - | - | - | - | - | - |
| Catalase | + | + | + | + | + | + |
| Gelatine Hydrolysis | - | - | + | - | + | - |

B= Bacillus; +X= *Xanthomonas* positive; +P= *Pseudomonas* positive; HC353 isolated from Pinto Coloso; HC427 isolated from Pinto Centenario; HC622 and HC643 isolated from Pinto Saltillo; YCA1 isolated from Pinto Libertad and YCA31 isolated from Pinto Coloso.

**CONCLUSIONS:** Cleaning treatment resulted insufficient to ensure seed disinfection and predominance of HB (*Pseudomonas* spp.), compared to CBB (*Xanthomonas* spp.) was observed in most of the pinto common bean cultivars evaluated in Durango. Diversity observed for *Pseudomonas* spp. based on morphological traits and laboratory tests need to be corroborated using differential cultivars.

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# COMPARISON OF CHOICE VERSUS NO-CHOICE TESTS OF A DRY BEAN IBL POPULATION FOR RESISTANCE TO POTATO LEAFHOPPER

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**INTRODUCTION:** Choice and no-choice tests are commonly used in insect resistance breeding programs in order to control for a number of factors. Choice tests allow pest populations to naturally infest planted material according to preferences, while using forced inoculation of caged samples with the pest of interest in no-choice tests can control for years with reduced pest populations as well as limit the possibility of escapes (Kornegay et al., 1989). In addition, comparisons of choice and no-choice can be very useful in identifying the nature of insect resistance. Resistance to a pest can be the result of antibiosis, antixenosis or tolerance mechanisms. In order to differentiate these mechanisms, plant responses under different conditions must be evaluated.

The temperate potato leafhopper (PLH), *Empoasca fabae*, is currently a major insect pest of dry beans in Michigan. While commercial growers have access to effective chemical control of this pest, organic growers do not. Identification of resistance to potato leafhopper in dry bean germplasm could prove useful in providing growers with resistant commercial varieties.

In this study, resistance to PLH was evaluated in Michigan using a dry bean IBL population generated from a single backcross to Matterhorn, a susceptible Michigan commercial variety, from a cross with EMP 507, a line developed by CIAT for resistance to the tropical leafhopper *E. kraemeri*. Parallel choice and no-choice tests were assessed for PLH feeding damage in the field in 2009 and 2010.

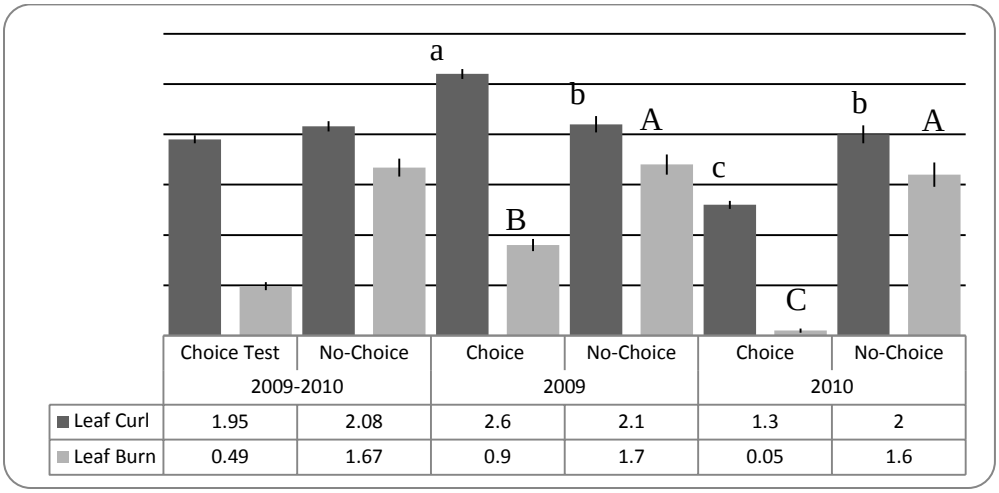
**MATERIALS AND METHODS:**

**Plant Material:** An inbred backcross line (IBL) population developed from a single backcross to Matterhorn from EMP 507 was examined in this study. From the population, 75 individual $F_{4:8}$-derived IBLs, the two parents (EMP 507, Matterhorn) and three check varieties (EMP 509, Sierra, and Santa Fe in 2009; EMP 509, Santa Fe and Swedish Brown in 2010) were planted in the field.

**Field Screening:** Choice and no-choice tests were conducted at Michigan State University, East Lansing, MI in 2009 and 2010. Three replications of the choice test were planted on 15 June 2009 and 18 June 2010 in a randomized complete block design (RCBD). Parallel to the choice test, a no-choice test was conducted with one replication per year. In the no-choice tests, cages were placed over field planted IBLs following germination and thinned to 5 plants per cage. Cages were inoculated with adult PLHs at current industry economic thresholds (1 adult/trifoliate) at 3$^{rd}$ trifoliate stage. Both tests were evaluated for leaf curl and leaf burn at pod fill stage using the damage scale from 0-5 as described in Murray et al. (2004a), where 0= no visible damage and 5= severe damage. The Proc Mixed and Proc ttest procedures of the SAS statistical package 9.1 (SAS Institute, Cary, USA) were used to analyze the data.

**RESULTS:** All damage-related indices were found to be significantly affected by genotypic effects ($p<0.05$). Leaf curl (LC) was normally distributed in both choice and no-choice tests; however, leaf burn (LB) was found to be left-skewed in the choice test with the majority of lines having low scores (0-1). Figure 1 shows overall mean scores for LB and LC across both years as well mean scores for 2009 and 2010 growing seasons. No-choice tests successfully controlled for annual environmental

variation in PLH predation as there are no significant differences in the no-choice tests between 2009 and 2010 for LB or LC while choice test scores vary dramatically for all indices.




| | Choice Test | No-Choice | Choice | No-Choice | Choice | No-Choice |
|---|---|---|---|---|---|---|
| | 2009-2010 | | 2009 | | 2010 | |
| Leaf Curl | 1.95 | 2.08 | 2.6 | 2.1 | 1.3 | 2 |
| Leaf Burn | 0.49 | 1.67 | 0.9 | 1.7 | 0.05 | 1.6 |

**Figure 1.** Mean Damage Scores. Bars with the same letter are not significantly different ($p>0.05$). Error bars = SEM.

In order to identify individual IBLs that responded differently under choice or no-choice conditions, t-tests were conducted to compare the means of each genotype under each condition. A total of 26 individual IBLs were identified. In all 26 cases, the IBLs had higher damage scores in the no-choice test than the choice test. Three IBLs had significantly higher LC damage scores using the Satterthwaite approximation for unequal variances ($p<0.05$), while 23 IBLs had significantly higher LB damage scores under no-choice conditions ($p<0.05$). However, no IBLs were significantly different for both LC and LB scores. These results suggest that antixenosis resistance mechanisms may be involved in these IBLs. Antixenosis occurs when, due to specific traits of the plant, an insect either preferentially feeds on the plant or avoids the plant in favor of other more desirable options (Murray et al., 2004a). In addition, the fact that LB and LC scores showed significantly different levels of variation between the tests and that no IBLs differed significantly for both LB and LC across tests support previous findings that suggest LB and LC plant responses to PLH feeding damage are controlled by separate genetic mechanisms (Murray et al, 2004b). This study will be repeated in 2011 in Michigan to confirm these findings. This data is also being used in a currently ongoing QTL mapping study.

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# BIOLOGICAL CONTROL OF *THRIPS TABACI* LIND. WITH SOME PHYTOPESTICIDES IN BEANS (*PHASEOLUS VULGARIS* L.)

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**INTRODUCTION:** *Thrips tabaci* Lind. is a worldwide pest of vegetable crops. It ranges from tropical and subtropical areas into the temperate regions. This species is highly polyphagous and attacks a wide range of weeds and crops: onion, paper, tomato, beans, peas, cucumber, etc. The use of insecticides results in poor control of thrips because of its often-induced resistibility. Biological control is an adequate and sustainable solution to escape phytotoxicity, and obtaining pesticide –free vegetables [2; 8]. Numerous plant extracts containing alkaloids, esters, glycosides, etc., are used in biological agriculture as bioinsecticides [6]. Azadirachtin is an active ingredient of many of organic pesticides on the market. It is extracted from the seeds and plants of Neem tree (*Azadirachta indica*) and alters the organs of the insects which can make them loose their appetite, cause sterilization and even death [4]. Besides, some plants have a systemic effect, which means that the plants, when absorbing said substances through their roots, will no longer be affected by sucking insects. Such phytopesticides are broad-spectrum and highly efficient against pests from orders *Lepidoptera, Homoptera и Thysanoptera* [3, 5, 7].

The **objects** of this study are 1) to trace the dynamic of population size of *Thrips tabaci* Lind. of bean (*Phaseolus vulgaris* L.) crops, and 2) to establish biological activity of some phytopesticides under field conditions.

**MATERIALS AND METHODS:** Plots in three replications and non - treated control plants were conducted in experimental fields of Maritsa Vegetable Crops Research Institute with *P. vulgaris* cultivar 'Starozagorski tzer' under natural background of infestation from *Thrips tabaci* Lind. in 2009 and 2010. Snap bean 'Starozagorski tzer' is with determinate upright growth habit, flat green pods and black seeds. Observations of bean crops have bean conducted in every 10 days to count number of larva, nymphs and adults on 10 leaves / plot. Number of live ones was recorded before and 1, 5, 10 and 14 days after application with pesticides upon previously chosen plants. Pesticides effectiveness was calculated by formula Henderson-Tilton. The following insecticides were applied: Vaztak 100 EC 0.03% (a. i. alfa cypermethrin)–standard; Piros 0.08% (a. i. pyrethrin, extract from *Chrysanthemum cinerariaefolium*); Pirethrum 0.05% (a. i. pyrethrin); NeemAzal T/S 0.3% (a. i. azadirachtin, extract from *Azadirachta indica*).

**RESULTS AND DISCUSSIONS:**

Dynamics of population: density of *T. tabaci* in bean crops was established. Pests' attack was recorded before blooming, right after plant germination. In 3rd decade of May were red single specimens, but in June their number was increasing rapidly. Highest density of the population was red in 2nd and 3rd decade of July - 9,8-9,6 in 2009 and 8,5-9,0 in 2010. Number of larva, nymphs and adults reduced gradually in August, and single specimens were detected before harvest (fig.1).

Biological activity of the phytopesticides: NeemAzal T/S 0.3% was the most efficient biopesticide for both experimental years - 81,08% on the 5th day after application similar to the standard pesticide Vaztak 100 EC 0.03% (83,36%). Piros 0.08% and Pirethrum 0.05% possess lower effectiveness on the 5th day after application compare to the standard - 78,12% and 78,99%, respectively. All of the tested bioinsecticides decreased significantly their efficacy in 10 to 14 days after application (table 1).

**CONCLUSIONS:** Obtained results showed sufficient biological activity of phytopesticide NeemAzal T/S 0.3% against *Thrips tabaci* Lind. in bean crops. It was registered in Bulgaria as biopesticide against

spider-mites, white flies and aphids in the greenhouses. Even though, our study showed that it is suitable for biological control against *T. tabaci* in the open field as well.

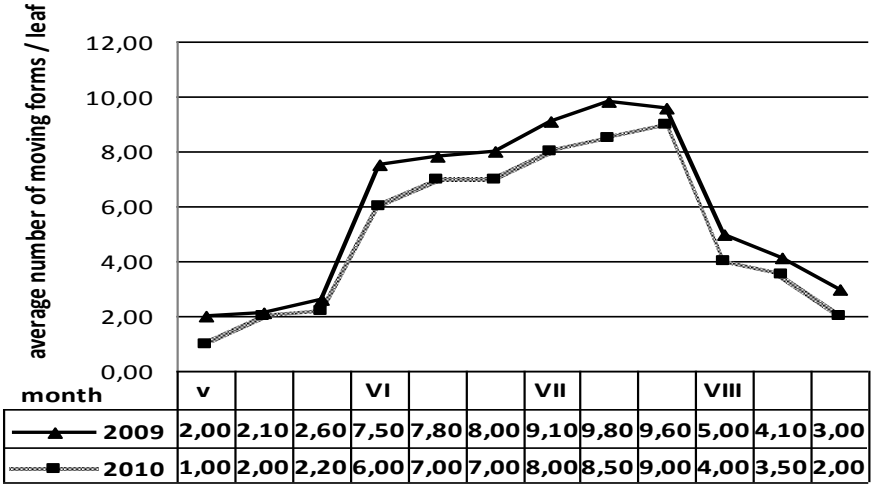


**Figure 1.** Population dynamics of *Thrips tabaci* Lind. in beans

**Table 1.** Efficacy of some phytopesticides against *Thrips tabaci* Lind. in bean crops

| Variant | Efficacy, %/Days after treatment | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | | 5 | | | 10 | | | 14 | | |
| | 2009 | 2010 | mean | 2009 | 2010 | mean | 2009 | 2010 | mean | 2009 | 2010 | mean |
| Vaztak 10 EC 0.03% | 55,06 | 70,43 | 62,75 | 85,75 | 80,96 | 83,36 | 86,31 | 77,16 | 81,74 | 79,42 | 72,31 | 75,87 |
| Piros 0.08% | 51,02 | 55,58 | 53,30 | 82,11 | 74,13 | 78,12 | 79,42 | 73,35 | 76,39 | 69,13 | 73,10 | 71,12 |
| Pirethrum 0.05% | 57,14 | 55,65 | 56,40 | 81,25 | 76,73 | 78,99 | 81,99 | 73,39 | 77,69 | 70,02 | 70,94 | 70,48 |
| NeemAzal T/S 0.3% | 57,14 | 61,93 | 59,54 | 84,34 | 77,82 | 81,08 | 84,96 | 77,16 | 81,06 | 81,99 | 76,95 | 79,47 |

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# BIOLOGICAL ACTIVITY OF SOME PRODUCTS FOR PLANT PROTECTION AGAINST COMMON BEAN WEEVIL (*ACANTHOSCELIDES OBTECTUA* SAY) ON BULGARIAN SNAP BEAN (*PHASEOLUS VULGARIS* L.) CULTIVARS

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**INTRODUCTION:** By using both crop protection potentiality and plant breeding achievements, we could have successful pest control over common bean production. Pest management against common bean weevil (*Acanthoscelides obtectus* Say) initiates during the vegetation, by directing specific efforts towards adults. That includes regular observation of the crops, as first mature pods appeared [6; 7]. Beetles' egg-laying is restricted in very short period, just before harvest time, which is precisely the most appropriate phase for efficient pest management [5]. Utilization of efficient pesticides against bean weevil in the open field is an alternative to obtain healthy seeds for storage. Previous tests have bean reviled various pesticides' efficacy against adults. Two of them- Decis and Hostation were reported to have high level of toxicity after 48 hours [2]. Synthetic pyrethroid insecticides Decis 2.5 EC and new formulation Decis 25 WG (active ingredient deltamethrin) have been used against numerous pests, including bean weevil. Here, the results of a series of studies we conducted that examined the effects of alternative application of the pesticides against bean weevil on some Bulgarian snap bean cultivars will be discussed.

**MATERIALS AND METHODS:** Plots were established at 'Maritsa' Vegetable Crops Research Institute (VCRI) under field conditions and natural background of infestation from bean weevil in 2009 and 2010 vegetation.

Treated plant material: Snap bean cultivars (*P. vulgaris* L.) from 'Maritsa' VCRI collection, including two market classes- flat pod cultivars (Starozagorski tzer, Tangra, Pagane) and oval pod cultivars (Oreol and Zaria).

Tested insecticides: Decis 2.5 EC 0.04% (a. i .deltamethrin 25 g/l) as standard and Decis 25 WG 0.004% (a. i. deltamethrin 250 g/l). One - time application was made with working solutions at 100 l/da consumption at early maturity stage of pod development and population density of 2 beetles / 100 blows with entomology bag. Non-treated plants from each plot were used as a control.

Statistical Analysis: 1) seed index of infestation (%)[3] based on 50 days after harvest were taken samples of 100 pods from every treatment and cultivar; 2) insecticides efficacy was estimated [1].

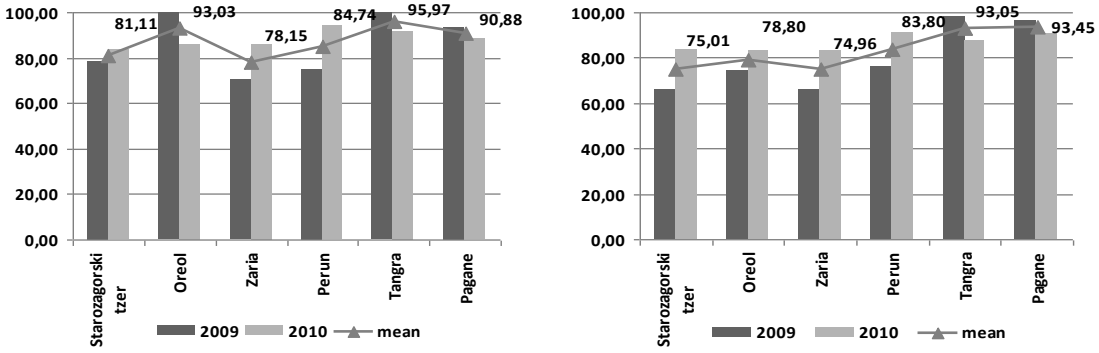
**RESULTS AND DISCUSSION:** Bean weevil damages were estimated by index of infestation (table 1). Obtained results from non-treated control plants showed that comparatively low index of infestation had Tangra and Pagane with mean index of infestation from both experimental years of 3,54% and 4,06%, respectively. Highest index of infestation for both experimental years was recorded for Starozagorski tzer- 7,17%. Our conclusion that bean weevil less preferred Tangra and Pagane in the field is corroborated with our previous studies that prove their enhanced resistance [4].

From two years - treated experimental plots with both insecticides, Decis 2.5 EC 0.04% (standard) was the most efficient one against bean weevil for Tangra (95,97%). Decis 25 WG 0.004% was most efficient for Tangra (93,05%) and Pagane (93,45%) too, with mean values very closely to that recorded for the standard (fig. 1).

**CONCLUSIONS:** Decis 2.5 EC 0.04% and Decis 25 WG 0.004% are efficient pesticides against bean weevil in the bean field crops. Application of these insecticides on schedule, in combination with cultivars having enhanced tolerance as Tangra and Pagane, can secure bean seed production for storage.

**Table 1**. Index of infestation (%) from bean weevil on snap bean cultivars

| Treatment | Cultivar | Index of infestation, % | | |
|---|---|---|---|---|
| | | 2009 | 2010 | mean |
| Control (non-treated) | Starozagorski tzer | 3,87 | 10,47 | 7,17 |
| | Oreol | 2,37 | 8,96 | 5,67 |
| | Zaria | 2,03 | 8,56 | 5,30 |
| | Perun | 0,88 | 9,24 | 5,06 |
| | Tangra | 2,98 | 4,09 | 3,54 |
| | Pagane | 4,27 | 3,85 | 4,06 |
| Decis 2.5 EC 0.04% | Starozagorski tzer | 0,83 | 1,71 | 1,27 |
| | Oreol | 0,00 | 1,25 | 0,63 |
| | Zaria | 0,60 | 1,21 | 0,91 |
| | Perun | 0,22 | 0,51 | 0,37 |
| | Tangra | 0,00 | 0,33 | 0,17 |
| | Pagane | 0,28 | 0,45 | 0,37 |
| Decis 25 WG 0.004% | Starozagorski tzer | 1,31 | 1,69 | 1,50 |
| | Oreol | 0,60 | 1,53 | 1,07 |
| | Zaria | 0,68 | 1,42 | 1,05 |
| | Perun | 0,21 | 0,79 | 0,50 |
| | Tangra | 0,05 | 0,50 | 0,28 |
| | Pagane | 0,16 | 0,36 | 0,26 |




**Decis 2.5 EC 0.04%** **Decis 25 WG 0.004%**

**Figure 1**. Efficacy (%) of Decis 2.5 EC 0.04% and Decis 25 WG 0.004% against bean weevil

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# EVALUATION OF INTA'S IMPROVED BEAN LINES BEHAVIOR TO BGMV AND CPMMV NATURAL FIELD INFECTION, TRANSMITTED BY *BEMICIA TABACI*

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## INTRODUCTION

There are many factors affecting production stability on bean crops in North West Argentina, among them, viral diseases. Viruses are one of the principal health limitations in beans extensive production.

Begomovirus and CpMMV infections were confirmed by serology test and its relative concentrations were quantified. *Bemisia tabaci* biotype was identified using RAPD´s technique.

This research´s objective was to evaluate improved bean (*Phaseolus vulgaris* L.) lines behavior to Bean Golden Mosaic Virus (BGMV) and Cowpea Mild Mottle Virus (CpMMV) natural field infection, and identify *Bemisia tabaci* biotype as transmitter.

## MATERIALS AND METHODS

These trails were carried out during 2007 and 2008, in sites with different climate conditions, in North of Salta Province (Argentina); in locations: Ballivián (S 22º 55 0.29) and Las Varas (S 23º 21 0.54 W 64º 03 0.71).

Field experiments including 6 genotypes: large white´s Alubia and CEB 003/4; red kidney´s LR 135 and CER 99/17; and small black´s CEGRO 99/15-12 and TUC 500; were conducted under two conditions/ locations, humid and sub humid respectivelly. In both sites a Blocks completely leathery design (4x4) was used, with three replications.

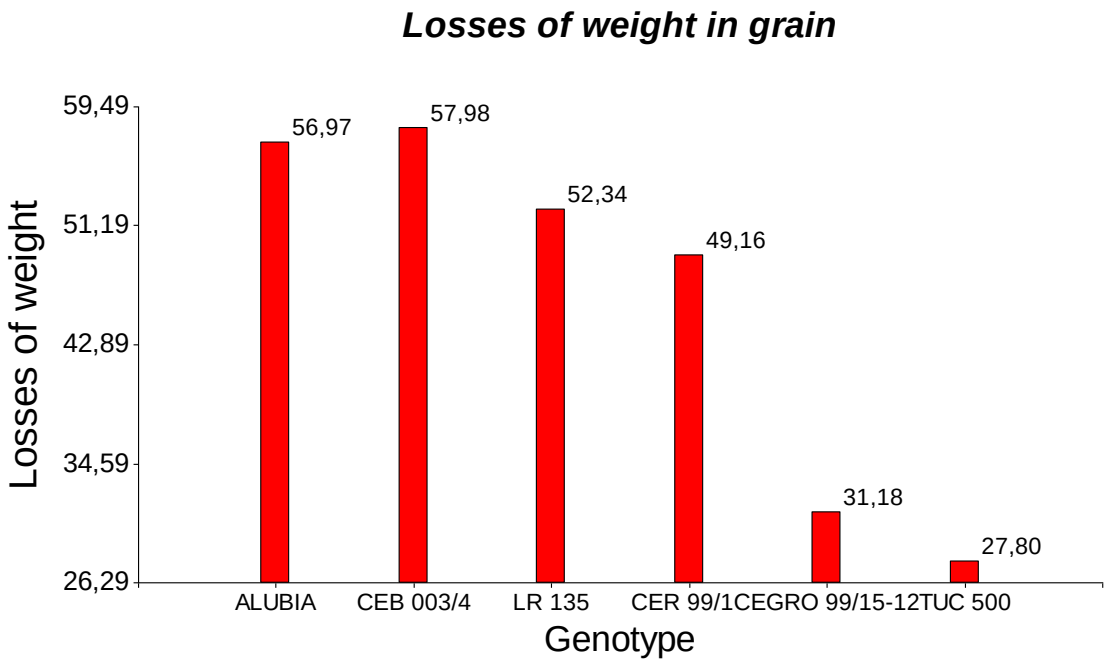
Incidence, number of plants with symptoms, and severity, degree symptom´s range, were evaluated in each unit. BGMV and CpMMV relative concentrations were determinated by serology tests.

Plants´yield or grain´s weight by plant, and yield components: number of pods by plant, number of grains by pod, and weight of 10 seeds, after harvest, were measured and analized in 5 paired plants´ comparison per genotype, with and without virus symptoms.

White fly (*Bemicia tabaci*), transmitter of these viruses, were collected near the trails, female fly were selected, and its DNA was extracted with Barro y Driver protocoll (1997) and Truol et al., (2003); the molecular identification was realized with RAPD-PCR, Lima *et al*. (2002).

**RESULTS**

The black genotypes presented the lower losses in grains´ yield and in their components, red kidneys presented intermediate losses and white genotypes had the higher losses.




**CONCLUSIONS**

All genotypes showed fewer pods by plant, less number of grains by pod, loss in grain weight and yield per plant, in pair comparison between plants with syntoms caused by virus infection and without them.

Black genotypes showed the more tolerant; red kidneys had intermediate tolerance, while large white genotypes showed the more susceptible. The determination of relative concentration was important, as more exact method compared with visual estimation.

On the first year of experiments, a positive correlation was found between CpMMV concentration and yield losses, however, this correlation was not found in BGMV. On second year, neither CpMMV nor BGMV concentrations were association with yield losses, suggesting the possible existence of others viruses transmitted by *Bemisia tabaci* reducing crop yields.

*Bemisia tabaci w*hite flies´ biotype determined was “A” similar to “Br”, found in Brazil.

# MERREMIA MILD MOSAIC AND BEAN YELLOW MOSAIC MEXICO VIRUSES: TWO, NEW DISTINCT BEAN-INFECTING BEGOMOVIRUS SPECIES FROM PUERTO RICO

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## INTRODUCTION

Geminiviruses are a large family of plant viruses with circular, single-stranded DNA (ssDNA) genomes packaged within twinned, or 'geminate' particles. The family *Geminiviridae* is divided into four genera (*Mastrevirus, Curtovirus, Topocuvirus, and Begomovirus*) according to viral genome organization and biological properties. Geminiviruses are among the most widespread and damaging of plant viruses world, worldwide and many have emerged only recently to cause untold losses in yield and quality (Brown, 2000). Members of the genus, *Begomovirus* have a genome of circular, ssDNA (~2.8-5.2 kb). Many begomovirus species are bipartite, having two components (chromosomes), which are referred to as DNA-A and DNA-B, both which are required for systemic infection and disease development, and share a common Rep binding site and other crucial functional elements in the intergenic common region. In contrast, some begomoviruses have only one component (monopartite), which is functionally equivalent to the DNA-A and DNA-B components of their bipartite counterparts. Bipartite begomoviruses occur in both the Eastern and Western Hemisphere, but monopartite viruses are found only in the Eastern Hemisphere (Lazarowitz, 1992).

Previously, three begomoviruses of the common bean (*Phaseolus vulgaris*) were identified in common bean and common weeds in Puerto Rico (PR) and were fully characterized. *Bean golden yellow mosaic virus* (BGYMV) (Bird and Sanchez, 1971; Bird and Maramorosch, 1978) is widespread in common bean and induces bright yellow mosaic symptoms. *Macroptilium mosaic Puerto Rico virus* (MaMPRV) was identified in an indigenous common weed that showing mild mosaic and stunting symptoms (Idris et al., 2003), while *Rhynchosia mild mosaic virus* (RhMMV) was cloned from another perennial weed, also from PR. Experimental and natural plant hosts of RhMMV included common bean and *Rhyncosai minima*. Variant of BGYMV are widely distributed in the Caribbean islands, Florida and Mesoamerica (Blair et al., 1995).

## MATERIALS AND METHODS

*Merremia quinquefolia* plants exhibiting caused yellow mosaic and vein yellowing symptoms reminiscent of begomovirus infection were observed in Maricao, PR during the summer, 1998. Common bean plants showing yellow mosaic symptoms were collected from Mexico in 2006. Total nucleic acids were extracted and circular ssDNA was subjected to rolling circle amplification (RCA). RCA products obtained using *M. quinquefolia* were linearize with *Sac*I (~2.6 kb) and cloned into pGEM7Zf+. Four clones carrying inserts of about 2.6 kb were subjected to capillary DNA sequencing. The RCA products of the common bean from Mexico were linearized with *Pst*I and cloned into pGEM5Zf+. Five clones carrying apparently full-length genomic component (~2.6 kb) were sequenced.

## RESULTS AND DISCUSSION

LASTn analysis using the GenBank database with the nucleotide sequences obtained from *M. quinquefolia* indicated the presence of two begomoviral DNA-A and two DNA-B components each. One was represented by two clones each of a DNA-A (99.9%) and DNA-B (99.9%) components that shared high nucleotide identity indicating that the genome organization of both components is typical 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 component, respectively. Inspection of the common region (CR, length 205 nucleotides) revealed that DNA-A and DNA-B molecules shared 97.6% nucleotide identity indicating that they are cognate components of the same viral species.

Pairwise sequence alignment showed that DNA-A shared its' the highest nucleotide identity, at 84.7% and 77.5%, with homologous RhMMV and *Rhynchosia golden mosaic virus* (RhGMV), respectively, also from PR and hence the virus was tentatively named Merremia mild mosaic virus (MeMMV). The distance analyses for the DNA-B indicated that these isolates shared 68.4% and 61.8% nucleotide identity with their closest relatives, RhMMV and RhGMV, respectively.

Comparative analysis of the nucleotide sequence of DNA-A obtained from the common bean indicated that these five molecules shared 99-100% nucleotide identity with each other and shared the highest nucleotide identity with *Chino del tomate virus* (CdTV) and Okra yellow mosaic Mexico virus at 81.3%, also from Mexico. Therefore, according to the ICTV guidelines (Fauquet et al., 2003) these are considered isolates of a single begomovirus and tentatively termed Bean yellow mosaic Mexico virus (BYMMXV). The clones of DNA-A and DNA-B of MeMMV were released with *Sac*I and biolistically inoculated into common bean and *Nicotiana benthamiana*. Inoculated *N. benthamiana* seedlings developed mild mosaic symptoms. Inoculated common bean seedlings developed yellow mosaic symptoms. Efforts are underway to clone the DNA-B of BYMMXV to prove causality.

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# AN INVESTIGATION OF THE SOURCES OF RESISTANCE TO LIMA BEAN DOWNY MILDEW (*PHYTOPHTHORA PHASEOLI)* RACES E AND F, AND IDENTIFICATION OF FORDHOOK LIMA BEAN CULTIVARS WITH DOWNY MILDEW RESISTANCE

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**INTRODUCTION:** Lima beans (*Phaseolus lunatus*), grown for consumption as a succulent shelled bean, are an economically important vegetable crop in Delaware. One of the major constraints of lima production in Delaware is downy mildew caused by *Phytophthora phaseoli.* This disease was first reported in 1889 in New Haven, Connecticut and subsequently spread to lima producing areas in New Jersey and later Delaware.[1] Downy mildew of lima beans has still not been reported outside of the Mid-Atlantic region of the United states.

Downy mildew began to cause serious losses for lima growers and vegetable processors in the 1940s, which prompted the initiation of a downy mildew resistance breeding program at the US Department of Agriculture in 1948.[1] The USDA breeding program identified several sources of resistance and continued to release cultivars with resistance as new races of the pathogen emerged from the 1950s through the 1970s.[2] Race D of downy mildew was first detected in 1975[3] and the USDA breeding program released 'B2C' which had resistance to all of the know races of downy mildew at the time (A, B, C & D) in 1976.[2]

After the discovery of race D in 1975, no new races of downy mildew were detected until 1995, when race E was first found in Delaware.[4] In the 20 years between the emergence of races D and E, growers relied almost exclusively on resistant varieties to control the disease. Another new race of downy mildew (race F) was detected in Delaware in 2000.[4] Various green baby lima bean varieties were screened for resistance to the new races of downy mildew and several were found to be resistant to race E (Bridgeton, Dover Tucker, C-elite Select, Cypress, 184-85) and several were found to be resistant to race F (B2C, M-15, Eastland, 8-78).[5] No varieties were identified with resistance to both races E and F. The downy mildew resistance in these varieties is conferred by single dominant genes.[6]

Some of the green baby limas with resistance to races E and F are USDA downy mildew resistant varieties or germplasm releases, others are known to be derived from these USDA lines. Given that it seemed likely that the USDA lines were the source of the genes conferring resistance to downy mildew races E and F, field and growth chamber inoculations were conducted with two objectives: (1) determine which of the PIs that the USDA used as sources of downy mildew resistance carry genes for resistance to races E and/or F; and (2) determine if any of USDA's downy mildew resistant Fordhook lima germplasm releases have resistance to downy mildew race E or F.

**MATERIALS AND METHODS:** The lines listed in Table 1 were planted in the field at University of Delaware's research farm in Newark, DE on July 6, 2009 and were planted at the UD research farm in Georgetown, DE on July 7, 2009. The green baby lima varieties B2C and Bridgeton were included as checks. Susceptible spreader rows were planted to border one side of each plot. The plots were inoculated with sporangial suspensions of race E (at Newark) or race F (at Georgetown) in early September. After inoculation, plants were misted at night to increase humidity and leaf wetness. Plants were rated for their disease reactions on Sept. 16 & 25, 2009.

The disease reactions of PI 189403 and PI 195342 in the field were suspect because of delayed flowering due to photoperiod response. They were subsequently screened at the hypocotyl stage in growth chambers.

**RESULTS AND DISCUSSION:** PI 195432 did not flower in the field and PI 189403 began flowering in mid-September. In the field, racemes on PI 189403 became infected with race F but did not become infected with race E. Young, tender stems of PI 195432 became infected with race E in the field, but did not become infected with race F. Because downy mildew affects primarily pods and racemes and not leaf tissue, the field screening results for these two PIs were suspect; however subsequent growth chamber screens confirmed the field results. PI 189403 is the likely source of resistance to race E and PI 195342 is the likely source of resistance to race F.

Of the ten Fordhook varieties tested, one was resistant to race E (F-169) and one was resistant to race F (MRF-79). Both resistant Fordhooks are products of the USDA breeding program. F-169 has PI 189403 in its pedigree and MRF-79 has both PI 189403 and PI 195342.[2] MRF-79 and F-169 are being used in the University of Delaware lima breeding program to develop Fordhook lines with resistance to both races.

**Table 1. Disease Reactions for Lima Lines Screened for Resistance to Downy Mildew Races E and F**

| Variety | Source[a] | Description[2] | Rxn to Race E | Rxn to Race F |
|---|---|---|---|---|
| B2C | PI 549515 | 1976 USDA release; ABCD resistant | S | R |
| Bridgeton | PI 549508 | 1972 USDA release; ABD resistant | R | S |
| PI 189403 | | ABD resistance source; Guatemala | R | S |
| PI 195342 | | ABC resistance source; Guatemala | S | R |
| Concentrated Fordhook | Charter Seed | | S | S |
| Concentrated Fordhook | PI 549479 | | S | S |
| Fordhook Concentrated | PI 549471 | | S | S |
| Fordhook 242 | PI 549464 | | S | S |
| Fordhook Bush | PI 549465 | | S | S |
| Sussex | ADM Seedwest | | S | S |
| Fordhook 1072 | PI 549419 | 1978 USDA release; ABC resistant | S | S |
| Fordhook 90-1 | Ben Fish | 1991 USDA release; ABCD resistant | S | S |
| MRF-79 | PI 549524 | 1981 USDA release; ABCD resistant | S | R[b] |
| F-169 | PI 549514 | 1975 USDA release; ABD resistant | R | S |

[a] all plant introductions obtained from the USDA Germplasm Collection, others from listed lima seed supplier

[b] One plant out of 18 was infected. Seed was collected from the resistant plants.

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# INHERITANCE OF THE RESISTANCE TO POWDERY MILDEW IN A RIL POPULATION DERIVED FROM THE CROSS XANA/CORNELL 49242

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## INTRODUCTION

Powdery mildew is a devastating disease of common bean in northern Spain, particularly in the market class fabada. Its incidence is increasing in recent years. Control with fungicides is possible but uneconomic, what reinforce the need of powdery mildew resistant cultivars. Some sources of resistance to this pathogen have been identified (Schwartz et al, 1981) but there is little knowledge on inheritance of such resistance. Previous reports suggest a qualitative inheritance (Dundas, 1936; Bett and Michaels, 1995; Rezende et al., 1999). In this preliminary work, we investigate the inheritance of the resistance to powdery mildew in a RIL population derived from the cross Xana/Cornell 49242.

## MATERIALS AND METHODS

The inheritance of the resistance to powdery mildew was analyzed in a population of 104 $F_7$ recombinant inbred lines (RILs) derived from the cross Xana/Cornell 49242, obtained by the single-seed descent method (Pérez-Vega et al. 2009). Xana is a fabada market class cultivar, susceptible to powdery mildew. Cornell 49242 is a small black-seeded line, resistant to powdery mildew.

The resistance evaluations were carried out in greenhouse using a local isolate obtained from naturally infected bean plants collected in Villaviciosa, Asturias, Spain (43º29´01´´N; 05º26´11´´W; elevation 6,5 m). The fungus was maintained on living plants of cv. Xana. Three consecutive replications were performed in the greenhouse during summer 2009. Each replication consisted of two pots per RIL, four plants per pot. Seedlings were inoculated when the primary leaves were fully expanded by blowing conidia from infected leaves with the help of a hairdryer. Plants were assessed 10 days after inoculation when the symptoms were clearly distinguished on the parent Xana, using a IT0-4 scale where IT0 = no visible sign of disease, and IT4 = well developed, freely sporulating colonies.

A linkage map previously developed in this RIL population (Pérez-Vega et al. 2009) was used to map the resistance loci to powdery mildew. The linkage map consist of 175 AFLPs, 27 microsatellites, 30 SCARs, 33 ISSRs, 12 RAPDs, 13 loci codifying for seed proteins, and four morphological loci. The map has a total length of 1,042 cM distributed across 11 linkage groups, aligned to these of the core linkage map of bean, using common molecular markers as anchor points. Chi-square was used to test goodness-of-fit of observed to expected ratios. Linkage analysis of the markers and the resistance genes was performed using JoinMap V 3.0 (van Ooijen and Voorrips 2001).

## RESULTS AND DISCUSSION

Symptoms of the disease were clearly distinguished on the inoculated plants by the development of white and powdery spots on the leaves. Reaction of lines was consistent across the different replications. A total of 77 lines showed IT0 (like Cornell 49242), whereas 26 lines showed IT4 (like Xana). Segregation for resistance to powdery mildew showed a good fit to the 3:1 expected ratio for two independent genes ($\chi^2 = 0.003$; $p>0.05$). Other works have also shown a qualitative inheritance of the resistance to powdery mildew, and the implication of at least a dominant gene has been suggested (Dundas, 1936; Rezende et al., 1999).

The location of the two resistance genes (designated as PM-1 and PM-2) in the genetic map was investigated first by means of contingence chi-square tests of the joint segregations of the powdery mildew resistance and other marker loci included in the genetic map. The resistance was significantly associated ($p<0.05$) with two groups of loci: one of them (corresponding to PM-1) formed by five linked loci at linkage group B4 (markers SW12, SI19, SBA8, $(AC)_8YT^{424}$, and $(AC)_8YC^{860}$) and the other (corresponding to PM-2) formed by five linked loci at linkage group B11 (markers SH13, SCAreoli, Pv-ag001, $MCAGEAC^{73}$, and $MCTGEAT^{187}$).

The relative positions of these resistance loci were investigated by means of a linkage analysis in two sub-populations: sub-population A, formed by 44 lines without the allele of Cornell 49242 for the locus SW12 (probably lacking the resistance gene PM-1, linked to SW12), and sub-population B, formed by 54 lines without the allele of Cornell 49242 for the locus SH13 (probably lacking the resistance gene PM-2, linked to SH13). In the sub-population A, a segregation of 19 resistant: 22 susceptible was observed and the resistance (probably due to the PM-2 gene) was linked to the SCAR markers SH13 (RF= 0.02; LOD= 8.28) and SCArioli (RF= 0.04; LOD= 7.43). In the sub-population B, a segregation of 28 resistant: 23 susceptible was observed and the resistance (probably due to the PM-1 gene) was linked to the SCAR markers SW12 (RF= 0.04; LOD= 9.13) and SI19 (RF= 0.07; LOD= 7.24).

The chromosome regions carrying the resistance genes PM-1 and PM-2 at linkage groups B4 and B11, respectively, are also involved in the genetic control of the resistance to anthracnose (Kelly and Vallejo 2004) and rust (Miklas et al. 2002).

Crosses between recombinant inbred lines will be carried out in order to identify the dominant or recessive inheritance of these two loci.

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# MAPPING OF THE ALLELE FOR RESISTANCE TO ANTHRACNOSE IN THE COMMON BEAN MSU 7-1 LINE

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## INTRODUCTION

The anthracnose caused by the fungal agent *Colletotrichum lindemuthianum* (Sacc. & Magn.) Scrib., is one of the most important fungal diseases in common bean (*Phaseolus vulgaris* L.). Nowadays, a total of 15 genes and four allelic series in common bean have been characterized as resistant sources to anthracnose (BIC, 2010). Microsatellites molecular markers are abundant in the genome, co-dominants and they also show high heterozygosis, which is useful for mapping economical traits, such as resistance to diseases in plant species. The MSU 7-1 ((Black Magic x SEL 111) line confers resistance to races 7, 9, 23, 55, 64, 65, 73, 89, 448 and 453 of *C. lindemuthianum* (Gonçalves-Vidigal et al. 2008, 2009; Vallejo and Kelly, 2009). Thus, the objective of this work was to investigate the utility of the microsatellites markers linked to resistance *Co-5* locus, for genetic mapping of the allele present in MSU 7-1.

## MATERIALS AND METHODS

The molecular-genetic analyses were conducted on $F_2$ population derived from cross between MSU 7-1 (resistant parent) × México 222 (susceptible parent), inoculated with race 64 of C. *lindemuthianum*. Young trifoliate of each $F_2$ plant from both parents were collected, and DNA extraction was carried out based on the methodology proposed by Afanador et al. (1993) with modifications.

Among the screened markers (g1175, g1233, g1378, g2416, g2459 and g2531) located on linkage group Pv7 (McClean et al. 2010) only the STS g1233 marker was polymorphic in parental plants and in resistant and susceptible bulks. This was also polymorphic in the BAT 93/Jalo EEP 558 (BJ) mapping population. The segregation of marker g1233 was determined among the $F_2$ plants from MSU 7-1 × Mexico 222. The primer sequences used for the g1233 marker was 'TGAAGGTGGATGTACAGGAAGACA' (forward) TACCTTCATTGGCTTGGTCAGCTA' (reverse) (McClean et al. 2010, available from PhaseolusGenes: http://phaseolusgenes.bioinformatics.ucdavis.edu/markers/766. A minimum likelihood of the odds ratio score (LOD) ≥ 3.0 and a maximum distance of 30 centiMorgans (cM) were used to test linkages among these markers. The linkage group containing the *Co-5* locus and the g1233 marker were labeled and oriented according to the map nomenclature (Pedrosa-Harand et al. 2008).

## RESULTS AND DISCUSSION

The $F_2$ population, inoculated with race 64 of C. *lindemuthianum*, was evaluated in relation to the presence of gene *Co-5*$^2$ (line MSU 7-1). Phenotypic segregation was observed in 69 resistant plants and 21 susceptible plants, exhibiting a segregation ratio of 3R:1S ($p$ = 0.71). This fact elucidates that resistance showed by MSU-7 line is dominant monogenic and conditioned by gene *Co-5*$^2$. Genetic

mapping of individuals from the $F_2$ population of the cross MSU 7-1 × Mexico 222, inoculated with race 64 of *C. lindemuthianum*, revealed the presence of g1233 marker in the resistant plants.

The genetic linkage analysis resulted in a segregation of 68(+):22(-), indicating a good fit to the expected ratio of 3:1 ($P$ = 0.90). Utilizing the g1233 marker, a fragment of 3250 bp was amplified in all $F_2$ resistant individuals from cross MSU 7-1 × Mexico 222. This dominant marker segregated in a ratio of 3:1 and was linked in coupling phase to the resistance *Co-5*$^2$ allele (*Co-5* locus) at a distance of 1.2 cM on LG Pv7. A segregation analysis, using this marker in mapping population BAT93 x Jalo EEP558, revealed a ratio of 1:1 ($p$ = 0.64). Additionally, tests carried out in our laboratory demonstrated that $g1233_{3250}$ marker is present in both G2333 and MSU7-1 cultivars. However, TU cultivar did not present $g1233_{3250}$ marker. Furthermore, Sousa et al. (2009) observed the allelism in $F_2$ population from the cross MSU 7-1 × G 2333, suggesting that MSU 7-1 and G 2333 have alleles in the same locus. Therefore, it can be concluded that the allele present in MSU 7-1 is the same *Co-5*$^2$ allele of G 2333.

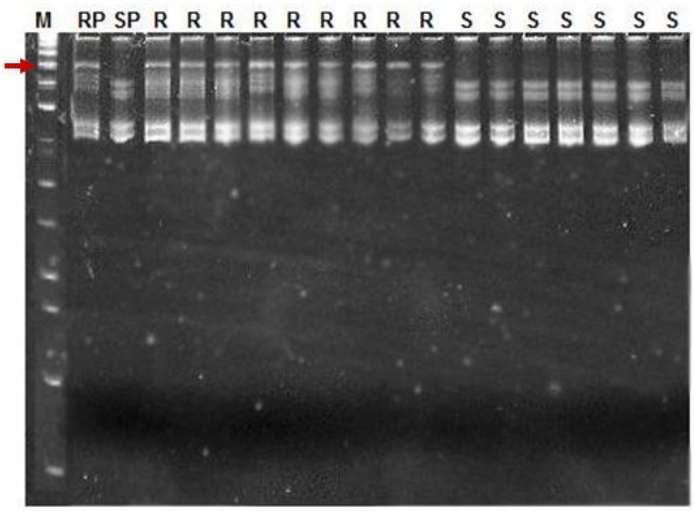


**Figure 1** - Electrophoretic analysis of amplification products of the g1233 marker. Lanes: M, 1 Kb Plus DNA Ladder; RP, MSU 7-1; SP, Mexico 222; R, individuals of the resistant bulk; S, individuals of the susceptible bulk to *C .lindemuthianum.* The arrow indicates a DNA band of 3250 bp linked to the resistance allele *Co-5*$^2$.

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# ALTERNATIVE METHOD TO ASSESS THE REACTION OF COMMON BEAN LINES TO *PSEUDOCERCOSPORA GRISEOLA*

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## INTRODUCTION

*Pseudocercospora griseola* (Sacc) Crus & U. Brown is the causal agent of angular leaf spot in common bean, one of the most severe diseases attacking this crop. This pathogen has great pathogenic variability and constant monitoring it is necessary. However, the methodologies used to identify races of pathogen and resistance sources have some difficulties, such as the inoculation is carried out in the V3 stage of the plant. Therefore, the objective of this study was to develop a new methodology that evaluated the disease symptoms in the initial stages of development of the common bean plant.

## MATERIAL AND METHODS

Were used eight common bean lines, Carioca MG, ESAL-686, Pérola, BRS Horizonte, Jalo, MAII-16, MAIII-3.6 e M-20, and a strain of *P. griseola* (race 63-63).The isolate was cultivated in tomato medium (Sanglard et al 2009) and incubated at 24°C for 14 days with 12 hours of photoperiod. Lines reaction to *P.griseola* was assessed using traditional and a alternative methodology. The traditional method was proposed by CIAT (1987). For the alternative methodology were used 16 seeds from each line. Spores were suspended at two concentrations (2. $10^4$ and 4. $10^4$ conidia $mL^{-1}$). After eight days in the greenhouse, plants were inoculated at the V2 physiological stage and incubated in a moist chamber (RH 95%) for 48 hours with a photoperiod of 16 hours. Later plants were transferred to greenhouse. Lines reaction to *P. griseola* was evaluated 13 to 14 days after inoculation according to the descriptive scale 1-9 developed by CIAT (1987).

## RESULTS AND DISCUSSION

The results from two physiological stages of inoculation evaluated (V2 and V3) were similar to the concentration of 2. $10^4$ conidia $mL^{-1}$. Only the M-20 line showed resistance, and three lines (MA-16, MA Jalo and 3-36) were moderately resistant. Symptoms were observed in plants stems inoculated at the V2 stage (Figure 1). A descriptive scale presented by CIAT (1987), does not consider the presence of symptoms on stems, branches and pestioles. It was possible to discriminate susceptible and resistant lines 13 days after inoculation. The early assessment to angular leaf spot was made by Cardona-Alvarez (1956) who observed plants with a minimum age of ten days showed symptoms. However data from CIAT (Anonymous 1985) showed that most plants do not develop symptoms before 30 days after sowing under field conditions. Plants have been inoculated with 15-20 days (Schwartz et al, 1982) and 21 days after sowing (Inglis et al., 1988) in greenhouse. It is important to emphasize that one advantage of the evaluation of the disease in V2 stage is that the plant remains little time in the greenhouse before inoculation, decreasing chances of contamination with spores of this and other pathogens. Another advantage is that using the same amount of inoculum, the proposed methodology allows to evaluate a larger number of plants due to reduced cotyledon leaf area. Furthermore, the inoculum reaches the stem and branches, enabling the emergence of

symptoms in these areas. The results showed that the concentration 2 x $10^4$ conidia $mL^{-1}$ did not discriminate susceptible from resistant lines at V2 stage. The plants inoculated with the suspension at a concentration of 4. $10^4$ conidia $mL^{-1}$had a high infection rate in these leaves and consequently it was possible to separate resistant and susceptible lines.

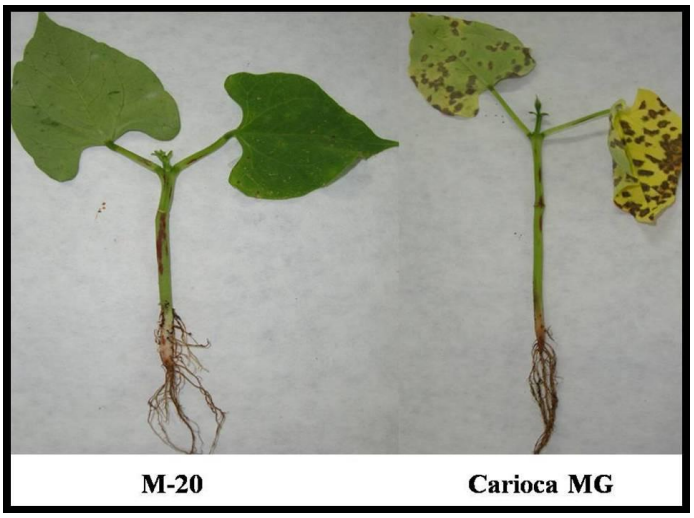


**Figure 1.** Symptoms of angular leaf spot in stem and leaves of common bean seedlings.

**ACKNOWLEDGEMENTS**

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# INHERITANCE OF RESISTANCE TO THREE ANTHRACNOSE RACES IN THE COMMON BEAN DIFFERENTIAL CULTIVAR TO

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## INTRODUCTION:

Anthracnose, caused by the fungus *Colletotrichum lindemuthianum* (Sacc. and Magn.) Scrib., is one of the most important diseases in common bean (*Phaseolus vulgaris* L.). Most of the anthracnose resistance loci in common bean (designated as *Co-* followed by a number or a letter) are organized as clusters of individual genes conferring race-specific resistance (Campa *et al.* 2009; David *et al.* 2008; Rodríguez-Suárez *et al.* 2007; 2008). This pathogen shows a broad pathogenic variability, being identified more than 100 different races based on the disease reaction of the 12 differential common bean cultivars (Pastor-Corrales 1991). Cultivar TO is among the differential cultivars showing the highest resistance spectrum. The *Co-4* gene was first described in this cultivar (Fouilloux, 1976) and, up to date, is the only anthracnose resistance locus identified in TO. The aim of this work was to investigate the inheritance of resistance to three anthracnose races in the common bean differential cultivar TO.

## MATERIALS AND METHODS:

Resistance to races 7, 19 and 39 was independently evaluated using 86 $F_{2:3}$ families (16 plants per $F_{2:3}$ family) derived from the cross TO x MDRK. Plants were inoculated and evaluated according to standard methods (Pastor-Corrales *et al.* 1994). Cultivar TO is resistant to the three races while cultivar MDRK is susceptible to all of them.

Molecular markers linked to *Co-* loci located in the common bean genetic map were analyzed in order to identify the loci involved in the resistance to specific races: markers TGA 1.1 and CV542014 linked to *Co-1* locus in linkage group (LG) 1 (Gonçalves-Vidigal *et al*. 2011); PVag001 linked to *Co-2* locus in LG 11 (Pérez-Vega *et al.* 2010); $OAH18^{600}$ and 254-G15F linked to *Co-3/9* locus in LG 4 (Rodríguez-Suárez *et al.* 2008; David *et al*. 2008); SAS13 linked to *Co-4* locus in LG 8 (Young *et al*. 1998); and SZ04 linked to *Co-5* locus in LG 7 (Campa *et al*. 2007). PCR amplifications were performed according to the instructions of the respective authors. PCR products were resolved on 8% polyacrylamide or 2% agarose gels, stained with SYBR Safe DNA© and visualized under UV light. Linkage analyses were carried out using MAPMAKER 2.0 software and a LOD score minimum of 2.5 (Lander *et al*., 1987).

## RESULTS AND DISCUSSION:

Segregations for the seven molecular markers showed a good fit to the expected ratio for a single locus (3:1 or 1:2:1). Table 1 shows the segregations for the resistance to races 7, 19 and 39 in the $F_{2:3}$ population derived from the cross TO x MDRK. Segregation for resistance to race 7 showed a good fit to the expected ratio for a single dominant gene (1R: 2R/S: 1S). This resistance locus is significantly linked to SAS13 (RF= 0.29; LOD> 2.5), a molecular marker previously linked to the *Co-4* locus (Young *et al*., 1998). This linkage relationship indicates that the gene conferring resistance to race 7 in TO is included at the *Co-4* cluster.

Segregation for resistance to race 39 showed a good fit to the expected ratio for two independent dominant genes (7R: 8R/S: 1S). In order to determine the identity of these loci, contingency chi-square tests were carried out between the segregation for resistance to race 39, and the loci showing monogenic segregation (race 7 and molecular markers). Contingency test showed a

significant association ($p< 0.01$) with the reaction to race 7 and the molecular marker SAS13. This result suggests that one of the loci conferring resistance to race 39 in TO is included in the *Co-4* cluster. Contingency tests also revealed that the resistance to race 39 segregates independently from the molecular markers linked to *Co-1* locus (TGA1.1 and CV542014), to *Co-2* locus (PVag001), to *Co-3/9* locus (OAH18$^{600}$, and 254-G15F), and to *Co-5* locus (SZ04). The position of this second resistance locus present in TO remains unknown.

Segregation for resistance to race 19 showed a good fit to the expected ratio for two independent, dominant and complementary genes (1R: 8 R/S: 7 S). Contingency chi-square tests were carried out between the segregation for the resistance to race 19 and the loci showing monogenic segregation (race 7 and molecular markers). The contingency chi-square values deviate significantly ($p< 0.01$) from the expectation of random segregation when compared the resistance to race 7 and marker SAS13. This result suggests that one complementary dominant gene conferring resistance to race 19 is included at the *Co-4* cluster. Resistance to race 19 segregates independently from the molecular markers linked to *Co-1* locus (TGA 1.1 and CV542014), to *Co-2* locus (PVag001), to *Co-3/9* locus (OAH18$^{600}$ and 254-G15F), and to *Co-5* locus (SZ04). The relative position of this second dominant complementary gene present in TO remains unknown.

**Table 1.** Segregation analysis for the resistance to anthracnose races 7, 19, and 39 in the $F_{2:3}$ population derived from the cross TO x MDRK. R = $F_{2:3}$ families having all individuals resistant; R/S = $F_{2:3}$ families having individuals resistant and susceptible; S = $F_{2:3}$ families having all individuals susceptible.

| | $F_{2:3}$ TO x MDRK families | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Parental phenotypes | | Observed | | | Expected ratio | | |
| Race | TO | MDRK | R | R/S | S | R:R/S:S | $\chi^2$ | p |
| 7 | R | S | 16 | 41 | 23 | 1:2:1 | 1.28 | 0.53 |
| 19 | R | S | 5 | 43 | 26 | 1:8:7 | 2.26 | 0.32 |
| 39 | R | S | 31 | 24 | 6 | 7:8:1 | 3.34 | 0.19 |

In sum, these results indicate that the *Co-4* anthracnose resistance cluster present in the differential cultivar TO includes two dominant genes conferring specific resistance against races 7 and 39, respectively, and one complementary dominant gene against the race 19. Apart from the genes included in the *Co-4* cluster, cultivar TO carries other resistance genes: a second complementary dominant gene conferring resistance against race 19, and a second dominant genes conferring specific resistance against race 39. These preliminary results suggest that the genetic control of the anthracnose resistance present in the differential cultivar TO is more complex than previously considered.

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# INTRASPECIFIC VARIABILITY OF *COLLETOTRICHUM LINDEMUTHIANUM*

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Anthracnosis is one of the most expressive disease that affect productivity and grain quality of common bean. The pathogenic variability and amount of pathotypes of *Colletotrichum lindemuthianum* within species becomes a limiting factor in the selection of resistant bean genotypes to the major pathotypes found in the field. Among the control measures, genetic resistance is the most effective, both because of lower production costs as the reduction of damage to the environment.

This study aimed to evaluate the change in the resistance reaction of 41 bean genotypes inoculated with two isolates representing the same pathotype, and a mixture of eight different pathotypes in the same inoculum suspension.

Two representatives from eight pathotypes isolated from different regions of bean were inoculated into 41 genotypes and twelve differential cultivars planted in trays (Figure 1). The representatives of the two pathotypes: isolates CNPAFCl 1304 and 1355 (representing pathotype 65), CNPAFCl 1143 and 1224 (pathotype 73), CNPAFCl 1333 and 1334 (pathotype 77), CNPAFCl 1164 and 1251 (pathotype 81), CNPAFCl 1247 and 1312 (pathotype 91), CNPAFCl 1315 and 1322 (pathotype 475), CNPAFCl 1324 and 1328 (pathotype 479) and Cl CNPAF 1294 and 1311 (pathotype 1609) were inoculated individually and mixed in the same suspension ($1.2 \times 10^6$ spores/mL). Inoculation was performed seven days after planting and the plants were maintained at a temperature of 24 ° C in a greenhouse. The evaluation was performed seven days after inoculation using a scale ranging from 1 to 9. Plants with scores 1, 2, 3 were considered resistant and plants with a score greater than or equal to 4 were considered susceptible.

Intraspecific variability was observed for the pathotypes 77, 81, 479 and 1609 of *Colletotrichum lindemuthianum*, through the presence of contrasting reaction in the genotypes BRSMG União, Aporé, Vereda, BRS Marfim, CNFC 10408 and CNFC 10470, respectively (Table 1). These genotypes showed susceptibility to an isolate and resistance to another isolate representative of the same pathotype. Other genotypes showed no reversal in the reaction of resistance and susceptibility among isolates of the same race (pathotype).

Pathotype 65 showed no symptoms in the differential series, so the mixture was obtained only with a combination of pathotypes 73, 77 and 81 and mixing with the combination of the two pathotypes 91, 475, 479 and 1609 in the same inoculum suspension, which resulted in the identification of races 93 and 2015 respectively, completely distinct from those that were inoculated. This fact can be explained by the combination of joint reaction symptoms in the differential cultivars in comparison with the reaction isolated from each pathotype. It was found that the set of differential cultivars is not efficient to identify the pathogen variability and to determine the pathotypes of *C. lindemuthianum* with the precision necessary for common bean breeding program. Therefore, the genotypes genotypes BRSMG União, Aporé, Vereda, BRS Marfim, CNFC 10408 and CNFC 10470 are candidates for the formation of a new set of differential cultivars and the inoculation of a mixture of races is feasible for the selection of genotypes resistant to diseases.

**Table 1:** Evaluation of some genotypes that showed variability in pathogenicity

| Isolates | CNPAF Cl 1333 | | CNPAF Cl 1334 | | CNPAF Cl 1164 | | CNPAF Cl 1251 | | CNPAF Cl 1324 | | CNPAF Cl 1328 | | CNPAF Cl 1294 | | CNPAF Cl 1311 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cultivars/ Pathotypes | 77 | | | | 81 | | | | 479 | | | | 1609 | | | |
| BRS Horizonte | 9* | S | 9 | S | 4 | MR | 4 | MR | 9 | S | 9 | S | 9 | S | 6 | S |
| BRS Pontal | 1 | R | 1 | R | 2 | R | 3 | MR | 9 | S | 9 | S | 4 | MR | 1 | R |
| BRS Requinte | 1 | R | 1 | R | 2 | R | 2 | R | 9 | S | 9 | S | 3 | MR | 2 | R |
| BRS Talismã | 1 | R | 1 | R | 2 | R | 3 | MR | 9 | S | 9 | S | 2 | R | 2 | R |
| Pérola | 8 | S | 6 | S | 9 | S | 9 | S | 9 | S | 9 | S | 5 | S | 7 | S |
| BRS MG Magestoso | 9 | S | 9 | S | 2 | R | 2 | R | 9 | S | 9 | S | 1 | R | 4 | MR |
| BRS 9435 Cometa | 9 | S | 9 | S | 2 | R | 2 | R | 9 | S | 9 | S | 4 | MR | 4 | MR |
| BRS Estilo | 9 | S | 9 | S | 1 | R | 1 | R | 9 | S | 9 | S | 2 | R | 3 | MR |
| BRS Campeiro | 9 | S | 9 | S | 9 | S | 8 | S | 9 | S | 9 | S | 8 | S | 8 | S |
| BRS Grafite | 1 | R | 1 | R | 1 | R | 1 | R | 9 | S | 9 | S | 1 | R | 1 | R |
| BRS Supremo | 9 | S | 9 | S | 3 | MR | 3 | MR | 9 | S | 9 | S | 2 | R | 4 | MR |
| BRS Valente | 1 | R | 1 | R | 1 | R | 1 | R | 9 | S | 9 | S | 1 | R | 1 | R |
| Diamante Negro | 9 | S | 9 | S | 9 | S | 9 | S | 9 | S | 9 | S | 9 | S | 9 | S |
| BRS Esplendor | 2 | R | 3 | MR | 1 | R | 1 | R | 1 | R | 1 | R | 9 | S | 9 | S |
| Emgopa 201-Ouro | 1 | R | 1 | R | 1 | R | 1 | R | 9 | S | 9 | S | 1 | R | 2 | R |
| BRS Vereda | 9 | S | 9 | S | 1 | R | 2 | R | 9 | S | 9 | S | 2 | R | 4 | MR |
| BRS Pitanga | 1 | R | 1 | R | 4 | MR | 4 | MR | 9 | S | 9 | S | 4 | MR | 4 | MR |
| BRS Timbó | 1 | R | 1 | R | 2 | R | 4 | MR | 9 | S | 9 | S | 1 | R | 4 | MR |
| **BRS Marfim** | 1 | R | 1 | R | 1 | R | 4 | MR | **9** | **S** | **1** | **R** | 1 | R | 4 | MR |
| BRS Executivo | 9 | S | 9 | S | 9 | S | 9 | S | 9 | S | 9 | S | 9 | S | 9 | S |
| BRS Embaixador | 6 | S | 6 | S | 1 | R | 3 | MR | 9 | S | 9 | S | 2 | R | 3 | MR |
| BRS Radiante | 4 | MR | 8 | S | 8 | S | 7 | S | 4 | MR | 8 | S | 9 | S | 9 | S |
| BRS MG Realce | 1 | R | 1 | R | 1 | R | 2 | R | 1 | R | 1 | R | 3 | MR | 3 | MR |
| BRS MG Tesouro | 1 | R | 1 | R | 1 | R | 1 | R | 9 | S | 9 | S | 1 | R | 2 | R |
| **BRS MG União** | **2** | **R** | **8** | **S** | 9 | S | 9 | S | 4 | MR | 8 | S | 9 | S | 9 | S |
| **Aporé** | 9 | S | 8 | S | **1** | **R** | **9** | **S** | **9** | **S** | **1** | **R** | 8 | S | 7 | S |
| **Vereda** | 9 | S | 9 | S | 3 | MR | 1 | R | 9 | S | 9 | S | **1** | **R** | **5** | **S** |
| Pioneiro | 9 | S | 9 | S | 2 | R | 1 | R | 9 | S | 7 | S | 4 | MR | 4 | MR |
| Ouro Branco | 9 | S | 9 | S | 2 | R | 3 | MR | 9 | S | 9 | S | 4 | MR | 4 | MR |
| Corrente | 1 | R | 1 | R | 3 | MR | 3 | MR | 9 | S | 9 | S | 4 | MR | 4 | MR |
| Ouro Vermelho | 1 | R | 1 | R | 1 | R | 2 | R | 6 | S | 8 | S | 3 | MR | 1 | R |
| Expedito | 9 | S | 9 | S | 1 | R | 1 | R | 9 | S | 9 | S | 4 | MR | 1 | R |
| CNFC 10429 | 9 | S | 9 | S | 2 | R | 2 | R | 9 | S | 9 | S | 2 | R | 3 | MR |
| CNFC 10729 | 9 | S | 9 | S | 1 | R | 2 | R | 9 | S | 9 | S | 2 | R | 3 | MR |
| CNFC 10733 | 9 | S | 9 | S | 1 | R | 2 | R | 9 | S | 9 | S | 1 | R | 3 | MR |
| CNFC 10120 | 1 | R | 1 | R | 1 | R | 1 | R | 1 | R | 1 | R | 9 | S | 9 | S |
| CNFC 10762 | 1 | R | 1 | R | 1 | R | 1 | R | 8 | S | 9 | S | 1 | R | 1 | R |
| **CNFC 10408** | 1 | R | 1 | R | 1 | R | 1 | R | **7** | **S** | **1** | **R** | 1 | R | 2 | R |
| **CNFC 10470** | 1 | R | 1 | R | 1 | R | 1 | R | **9** | **S** | **1** | **R** | 1 | R | 1 | R |
| CNFP 10132 | 5 | S | 8 | S | 1 | R | 1 | R | 2 | R | 1 | R | 9 | S | 9 | S |
| Rosinha G2 | 6 | S | 6 | S | 9 | S | 9 | S | 7 | S | 7 | S | 9 | S | 9 | S |

*Score 1: A total absence of symptoms; Score 2: A minimum of two little dark streaks in some veins of the lower leaf surface (very little perceived); Score 3: A greater number of already blackened grooves in the ribs of the underside of the leaves (easily perceived) Score 4: Score 3 identical symptoms, but already showing a minimum of grooves in the face Superor some veins of the leaves (very little perceived); Score 5: A large number of dark streaks in both the lower face but also on the upper surface of leaves (easily perceived); Score 6: Score 5 identical symptoms, but with some lesions on stems and stems of most plants; Score 7: The vast majority of the black veins with wilted leaves; Score 8: Symptoms similar to Score 7, but already showing some dead plants; Score 9: Most plant killed;

# MORPHOLOGICAL AND PHYLOGENETIC ANALYSIS OF *GLOMERELLA* AND *C. LINDEMUTHIANUM* STRAINS ISOLATED FROM COMMON BEAN ANTHRACNOSE LESIONS

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## INTRODUCTION

*Colletotrichum lindemuthianum* causes anthracnose disease on common bean (*Phaseolus vulgaris*), resulting in severe economic losses in Brazil and worldwide. Genetic resistance is the best way to control the disease, but the high degree of genetic variability in the pathogen makes breeding cultivars with durable resistance difficult. Sexual recombination is thought to increase the probability that a pathogen will overcome host resistance. We isolated a large number of teleomorphic *Glomerella* strains from anthracnose lesions on common bean plants in fields in Brazil. We isolated anamorphic *C. lindemuthianum* strains from the same lesions. Differences in symptoms, colony morphology and germination rates between the *Glomerella* and *C. lindemuthianum* strains were observed previously (Ishikawa at al 2010). Our aim is to elucidate the genetic relationship between these *Glomerella* and *C. lindemuthianum* strains.

## MATERIAL AND METHODS

We included four *C. lindemuthianum* (conidia) and 10 *Glomerella* (conidia and ascospores) strains in our studies. Spore suspensions were made and the concentration was adjusted to 1 x $10^4$ spores/ml. Septum formation in germinating spores was analyzed after adding 10 µl Calcofluor (concentration) to spores that had been germinated in drops of deionized water on plastic cover slips in a moist chamber overnight. To elucidate the genetic relationship of the teleomorphic and anamorphic strains, we performed a phylogenetic analysis based on the internal transcribed spacer (ITS variable regions) of the ribosomal DNA and on the high mobility group (HMG)-encoding sequence of the MAT1-2-1 mating type gene. DNA sequences of other *Colletotrichum* species from Genebank were included on our phylogenetic analysis. We used degenerate primers NcHMG1 and NcHMG2, described by Arie et al (1997) to amplify HMG regions of the MAT1–2 mating type genes of *Glomerella* strains. PCR products were cloned and sequenced with primers complementary to the cloning vector. Multiple alignments were prepared, and nondegenerate primer pairs were developed for *Glomerella* strains. These nondegenerate primer pairs were used to amplify and sequence HMG fragments from *Glomerella* strains. The specific primers HMGCLF and HMGCLR created by García-Serrano et al. (1998) were used to amplify and sequence HMG regions of *C. lindemuthianum* strains. Phylogenetic analyses were performed by using the Phylogeny.fr platform (Dereeper et al, 2008), and included the following steps: sequences alignment with MUSCLE (v3.7); removal of ambiguous regions with Gblocks (v0.91b); construction of a phylogenetic tree using the maximum likelihood method with PhyML (v3.0 aLRT); and evaluation of internal branch reliability using the aLRT test (SH-Like). Phylogenetic trees were drawn and edited using TreeDyn (v198.3).

## RESULTS AND DISCUSSION

*C. lindemuthianum* and its close relatives *C. orbiculare*, *C. trifolii* and *C. malvarum* do not form a septum in germinated conidia (Liu at al 2007). We observed that our *C.lindemuthianum* strains did not form a septum during conidial germination, as expected, whereas the *Glomerella* strains did form a septum during germination of both ascospores and conidia, suggesting that these are not members of the *C. lindemuthianum* clade (Figure 1). The phylogenetic analysis also

suggested that the teleomorphic strains are not closely related to *Colletotrichum lindemuthianum*. The teleomorphic strains could not be positively identified as another known species of *Colletotrichum* or *Glomerella*, and may be a new species (Figure 2 and 3). New studies including phylogeny, infection and pathogenicity have been done to elucidate the relationship of these strains to *Colletotrichum lindemuthianum* and bean anthracnose disease on common bean.

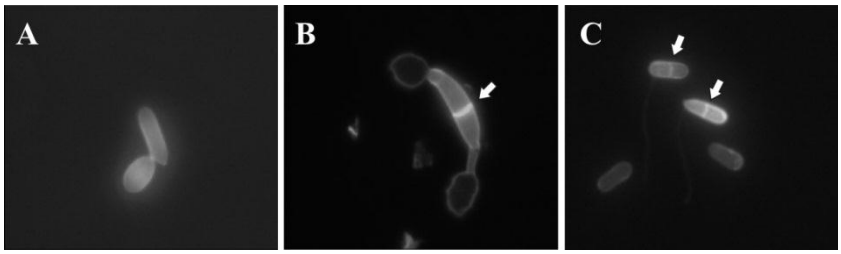


**Figure 1:** (A) Germinated conidia of *C.lindemuthianum* strain LV117, no septum formation. (B) Ascospore of *Glomerella* strain UFLAG08, white arrow indicates the septum after germination. (C) Conidia of *Glomerella* strain UFLAG0815.5, white arrows showing septum formation after germination.

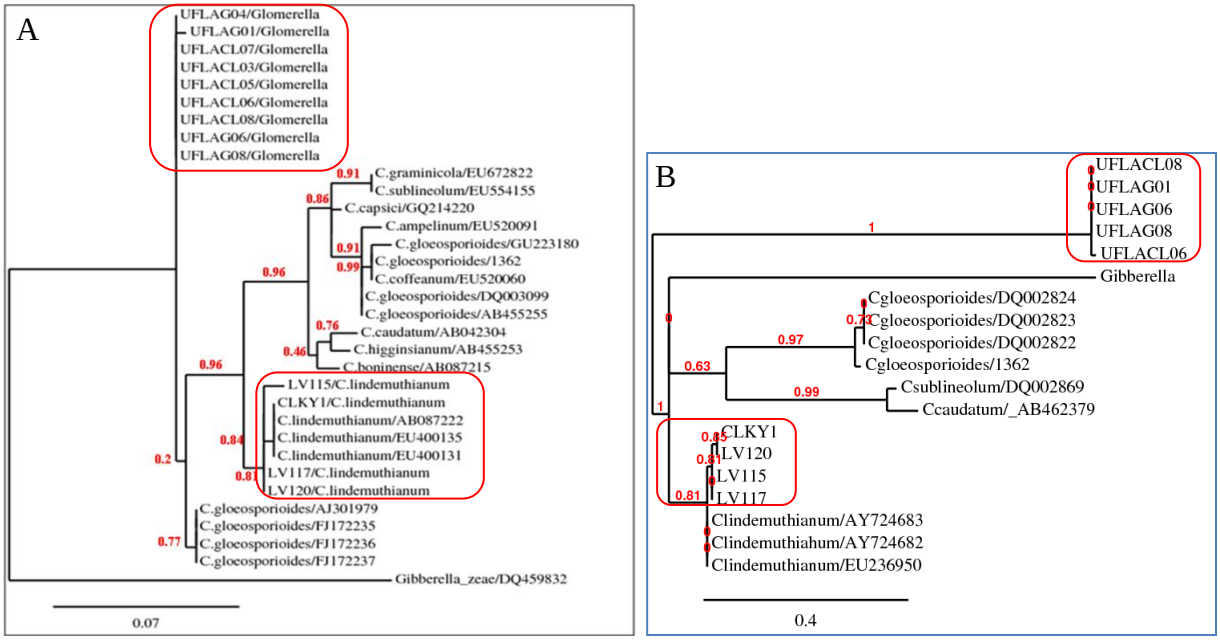


**Figure 2:** (A) Phylogenetic tree based on ITS sequences from *Glomerella* and *C.lindemuthianum* strains and sequences of *Colletrotrichum* from Genebank. (B) Phylogenetic tree based on HMG sequences from *Glomerella* and *C.lindemuthianum* strains and sequences from different species of *Colletrotrichum* from Genebank. Red squares are showing our strains of *Glomerella* and *C. lindemuthianum.*


## ACKNOWLEDGEMENTS

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# MODIFICATIONS IN GAS EXCHANGE OF COMMON BEAN PLANTS INFECTED BY PATHOGEN *COLLETOTRICHUM LINDEMUTIANUM*

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## INTRODUCTION

Anthracnose is a disease promoted by the pathogen *Colletotrichum lindemuthianum* that attack *Phaseolus vulgaris* plants. This fungus under favorable conditions as high temperature and humidity can infect common bean crop in all growth stages (Broughton et al., 2003). In addition, this pathogen causes morphological, physiological and biochemical alterations that has been previously described in *Phaseolus vulgaris*, such as the significant decrease in photosynthetic pigments (Lobato et al., 2009a), photosynthesis rate, and consequent yield reduction (Gonçalves-Vidigal et al., 2009).

Aim of this study was to evaluate infection effects promoted by pathogen *Colletotrichum lindemuthianum* (race 2047) in gas exchange of *Phaseolus vulgaris* (cv. Mexico 222) plants.

## MATERIALS AND METHODS

The study was conducted in greenhouse and growth chamber conditions located in Núcleo de Pesquisa Aplicada à Agricultura (Nupagri). Photosynthesis rate and photosynthetic water use efficiency were evaluated in well expanded trifoliolate leaves 3$^{rd}$, located at the middle of the main branch at the stage $V_4$. So, the plants presented three trifoliolate leaves during $V_4$ stage and photosynthetic water use efficiency was estimated according to Osmond et al. (1980). Radiation level and temperature in the chamber were previously evaluated to this crop, and adjusted to 1100 µmol $m^{-2}$ $s^{-1}$ and 32ºC, respectively. Gas exchange parameters were evaluated between 09:00 and 12:00 h. Data were analyzed employing a variance analysis, and using Tukey test at 5% level of probability. Standard errors were also calculated in all treatments evaluated.

## RESULTS AND DISCUSSION

Stomatal conductance was influenced by the pathogen infection, because inoculated plants showed lower values in all evaluated dates, compared with uninfected plants. A significant reduction of 78.9% was showed for the 12$^{th}$ day after pathogen inoculation (Figure 1 A).

Transpiration rate in infected plants was lower in all the evaluated periods when control and infected plants were compared. Inoculated plants showed reductions of 12.7, 23.2, and 67.7% at the 4, 8, and 12$^{th}$ day after the pathogen inoculation, respectively (Figure 1 B), but just transpiration rate had presented significant differences at the 8$^{th}$ and 12$^{th}$ day.

Photosynthetic rate was reduced by the infection of C*olletotrichum lindemuthianum* pathogen showing significant reductions of 22, 49.9, and 77.3% at the 4, 8 and 12$^{th}$ after inoculation, respectively (Figure 1 C). Inoculated plants were reduced in all the evaluated periods indicating that *Colletotrichum lindemuthianum* pathogen caused strong negative effects over influence under photosynthesis rate and photosynthetic pigment. Pigment reduction and consequent lower capacity to absorb light promotes decrease in photosynthesis rate. Low photosynthesis on leaves is the reason of the lower yield in infected plants by this fungus (Jesus Junior et al., 2001). Lobato et al. (2009b)

reported that *Phaseolus vulgaris* plants presented lower amounts of carbohydrates and sucrose as a consequence of low the reduction in photosynthesis, because the transport and partitioning of carbon compounds from leaf to others organs is dependent of photosynthesis. In addition, lower photosynthesis rates, means low carbohydrate source for apical and root meristems, flower and seed.

Photosynthetic water use efficiency presented similar behavior as photosynthesis rate in control and inoculated plants. But just it is showed significant differences between control and infected plants at the 8th and 12th day, when compared to 4th day after inoculation (Figure 1 D).

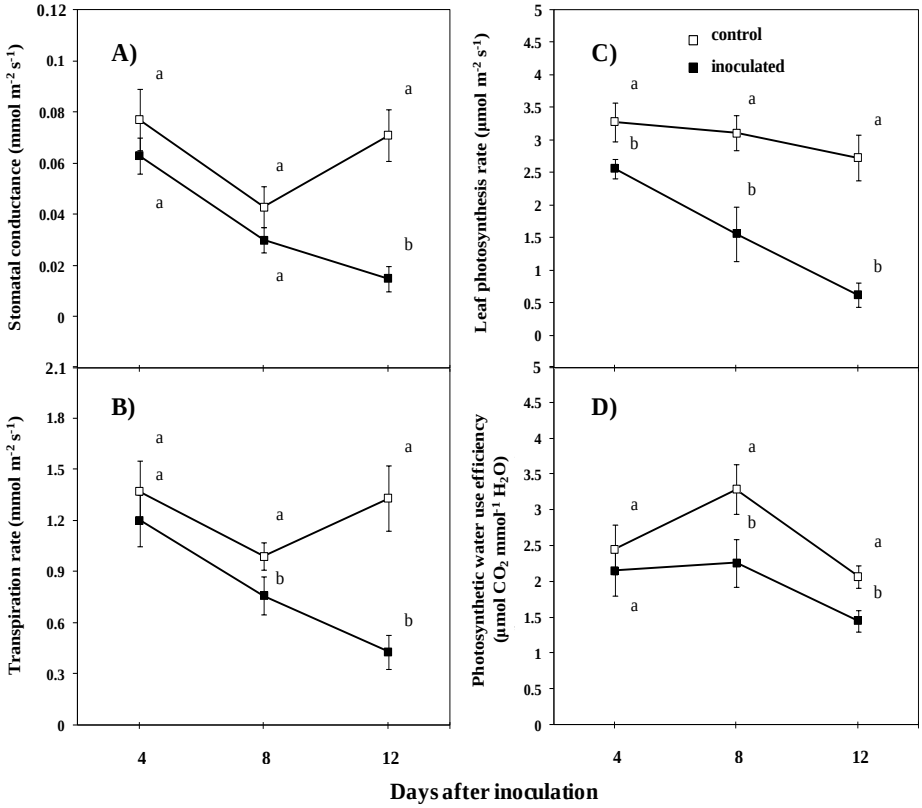


**Fig. 1:** (A) Stomatal conductance, (B) transpiration rate, (C) Photosynthesis, and (D) photosynthetic water use efficiency in *Phaseolus vulgaris* (cv. Mexico 222) inoculated with fungus *C. lindemuthianum* race 2047. Same letters do not show significant differences at Tukey test ($P < 0.05$). Bars represent the mean standard error.

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# CHANGES IN CHLOROPHYLL CONTENTS PROMOTED BY *C. LINDEMUTHIANUM* (RACE 2047) IN COMMON BEAN PLANTS

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## INTRODUCTION

Anthracnose is a disease promoted by the pathogen *Colletotrichum lindemuthianum* that attack *Phaseolus vulgaris* plants. This fungus under favorable conditions as high temperature and humidity can infect common bean crop in all growth stages (Broughton et al., 2003). In addition, this pathogen causes morphological, physiological and biochemical alterations that has been previously described in *Phaseolus vulgaris*, such as the significant decrease in photosynthetic pigments (Lobato et al., 2009a), and yield reduction (Gonçalves-Vidigal et al., 2009a). Other disorder in carbon and nitrogen metabolism has also been reported by Lobato et al. (2009b) and Gonçalves-Vidigal et al. (2009b).

The objective of this study was to evaluate infection effects promoted by *Colletotrichum lindemutianum* pathogen (race 2047) on leaf pigments in *Phaseolus vulgaris* plants (cv. Mexico 222).

## MATERIALS AND METHODS

The study was conducted in greenhouse and growth chamber conditions located in Núcleo de Pesquisa Aplicada à Agricultura (Nupagri) that is situated in Research Station of the Universidade Estadual de Maringá (UEM). Seeds of *Phaseolus vulgaris* Mexico 222 cultivar, which presents a determined growth, it was obtained from the Nupagri seed bank. Pathogen employed to infect the plants was the fungus *Colletotrichum lindemuthianum* race 2047, and cultivar Mexico 222 was to this fungus.

The experimental design employed was completely randomized in factorial, with 2 factors (condition and evaluation period). The two conditions (control and inoculated) were combined with three evaluation periods (4, 8 and 12$^{th}$ day after inoculation). The experiment was composed of six replicates, and thirty six experimental units (1 plant in each unit).

Chlorophyll a and b was determined using 25 mg of leaf tissue taken from first trifoliate leaf that presented progressive infection symptoms during 4, 8 and 12$^{th}$ day after the pathogen inoculation. Tissue samples were homogenized in dark with 2 mL of acetone 80% (Nuclear) and centrifuged at 5.000 *g*, for 10 minutes at 5ºC. Chlorophyll a and b, carotenoids and total chlorophyll were quantified using spectrophotometer in the supernatant phase, according to the methodology described by Lichthenthaler (1987). Data were analyzed employing a variance analysis, and using Tukey test at 5% level of probability. Standard errors were also calculated in all treatments evaluated. All statistical procedures were carried out using software SAS.

## RESULTS AND DISCUSSION

The chlorophyll a/b ratio presented significant differences only at the 12$^{th}$ day after pathogen inoculation showing a significant reduction in the chlorophyll a/b ratio, if compared with the 8$^{th}$ day (Figure 1 A). Similarly, carotenoids contents was altered by the pathogen presence, which showed significant reductions at the in 8 and 12$^{th}$ day after the pathogen inoculation (Figure 1 B). Total chlorophyll level also was modified (significant differences) at the 8 and 12$^{th}$ day after infection.

Inoculated plants a decreases of 6.4, 20.6 and 21.3% at the 4, 8 and 12$^{th}$ respectively, when they are compared with the control plants (Figure 1 C).

A reduction on the chlorophyll a/b ratio on infected plants by the fungical pathogen occurred mainly by minor chlorophyll a content. Carotenoids level presented significant reductions in 8 and 12$^{th}$ day as consequence of the infection expansion and consequent cell death produced by the pathogen in leaf tissue (Tománkova et al., 2006). Total chlorophyll amount in inoculated plants presented significant decrease by two factors, means as less leaf photosynthetic area, less light absorption, and chloroplast disorders during the pathogen infection (Radwan et al., 2008).

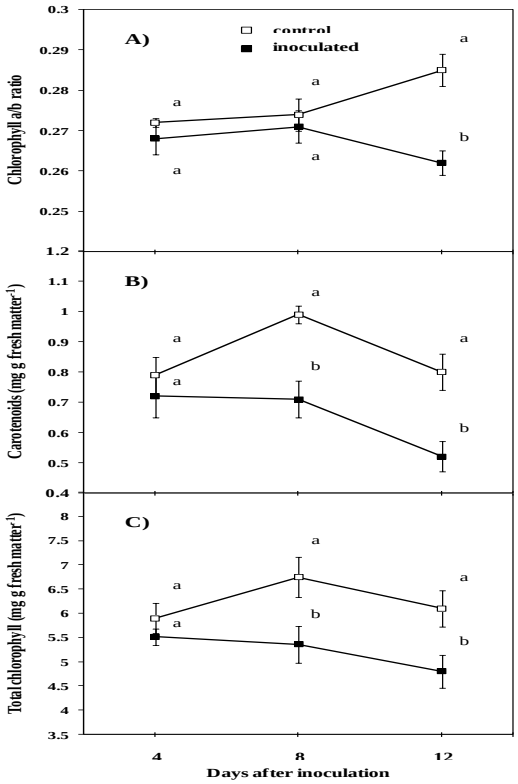


**Fig. 1:** (A) Chlorophyll a/b ratio, (B) carotenoids and (C) total chlorophyll in *Phaseolus vulgaris* (cv. Mexico 222) inoculated with fungus *C. lindemuthianum* race 2047. Same letters do not show significant differences at Tukey test ($P < 0.05$). Bars represent the mean standard error.

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# PHOTOSYNTHETIC PIGMENTS OF COMMON BEAN EXPOSED TO TWO *COLLETOTRICHUM LINDEMUTHIANUM* RACES

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## INTRODUCTION

Photosynthetic pigments in plants are comprised by chlorophylls *a* and *b*, and these pigments are responsible mainly by capture light in the antenna complex via photosystem II, with consequent electron transport (Candan and Tarhan 2003). Berova et al. (2007) investigating *Phaseolus vulgaris* plants exposed to pathogen infection described significant pigment loss.

Aim of this study was to investigate impact on photosynthetic pigments in *Phaseolus vulgaris* leaf induced by two *Colletotrichum lindemuthianum* races, which promote resistance and susceptibility in Mexico 222 cultivar.

## MATERIALS AND METHODS

The study was conducted in greenhouse and growth chamber conditions located in Núcleo de Pesquisa Aplicada à Agricultura (Nupagri), Universidade Estadual de Maringá (UEM).

Seeds of *Phaseolus vulgaris* Mexico 222 cultivar, which presents a determined growth, it was obtained from the Nupagri seed bank. Pathogen employed to infect the plants was the fungus *Colletotrichum lindemuthianum* races 7 and 2047, where Mexico 222 is resistant and susceptible to races 7 and 2047, respectively. Plants were grown in greenhouse conditions controlled by the local weather minimum/maximum temperature was 34.8/13.5ºC and relative humidity was 55/81%, respectively, during the experimental period. Inoculum obtained from Nupagri was transferred culture tubes that contained growing medium and immature pod beans.

Experimental design employed was completely randomized with 3 treatments (control, resistant and susceptible). The experiment was composed of six replicates, and thirty six experimental units (1 plant in each unit).

In this study were evaluated chlorophylls *a*, *b*, carotenoids, and total chlorophyll in 8$^{th}$ after inoculation, being measured in well expanded trifoliolate leaves 3$^{rd}$, located at the middle of the main branch at the stage $V_4$. Pigments were quantified according to Lichthenthaler (1987). Data were analyzed employing a variance analysis, and using Scott-Knott test at 5% level of probability. Standard errors were also calculated in all treatments evaluated.

## RESULTS AND DISCUSSION

Chlorophyll *a* levels showed in control, resistant, and susceptible treatments were 3.64, 3.37, and 2.90 mg g $FM^{-1}$, respectively, and statistically control and resistant treatments were similar (Figure 1A), despite reduction promoted by pathogen contact with leaf superficies.

In relation to chlorophyll *b* were obtained 3.31, 3.08, and 2.83 mg g $FM^{-1}$ in control, resistant, and susceptible treatments, respectively (Figure 1B). In addition, significant reduction was showed only in susceptible treatment. Reductions in chlorophylls *a* and *b* showed in susceptible treatment probably will have consequence in carbon metabolism, because damage in chloroplast structures promoted by *C. lindemuthianum* pathogen reduces capacity of light absorption carried by these pigments.

Carotenoids levels present 0.88, 0.71, and 0.69 mg g $FM^{-1}$ in control, resistant and susceptible treatments (Figure 1C), respectively, and these data reveal reduction not significant among treatments. Lobato et al. (2009) reported similar lower carotenoid levels when plants of *Phaseolus vulgaris* were infected by *Colletotrichum lindemuthianum* race 23.

Total chlorophyll was showed significant interference promoted by *Colletotrichum lindemuthianum* races when infected Mexico 222 (Figure 1D). In addition, decreases produced by 7 and 2047 races were of 19.3 and 21.5%, respectively, with respect to control. Fall significant obtained in resistant treatment can be associated to lower decreases in clorophylls a and b. However, reduction revealed in susceptible treatment is promoted by pathogen infection and consequent disorders in leaf pigments.

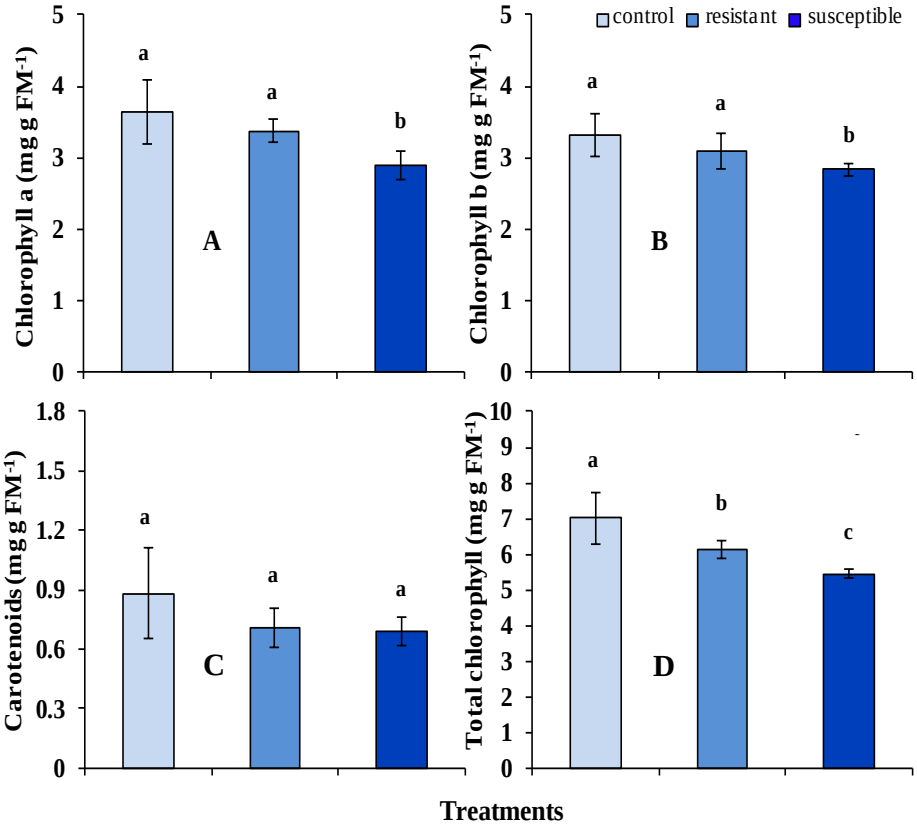


**Fig. 1:** (A) Chlorophyll *a*, (B) chlorophyll *b*, (C) carotenoids, and (D) total chlorophyll in *Phaseolus vulgaris* (cv. Mexico 222) inoculated with two *C. lindemuthianum* races 7 (resistant) and 2047 (susceptible). Same letters do not show significant differences at Scott-Knott test ($P < 0.05$). Bars represent the mean standard error.

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# RESISTANCE TO AND DIVERSITY OF ASCOCHYTA BLIGHT IN COMMON BEANS

**Matthew W. Blair[1], Luz Nayibe Garzón G.[2], Carlos Jara[1], Guillermo Castellanos[1], Gloria Mosquera[1], Gustavo A. Ligarreto[2], Oscar A. Oliveros G.[2], Carlos M. Rivera[2] and Paola Villarrraga[3]**

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**INTRODUCTION:** Disease losses in common bean (*Phaseolus vulgaris* L.) to Ascochyta blight caused by the fungal pathogen *Phoma exigua* var. *diversispora* can amount to 84% in highland environments of the Andes and is especially prevalent in the Departments of Antioquia, Boyacá, Cauca, Cundinamarca and Nariño in Colombia and in many parts of Ecuador and Peru as well (CIAT, 1984). Few studies have analyzed any suspected resistance sources or local varieties for their level of disease infection nor the inheritance of resistance to Ascochyta blight in beans. In some initial screening in Peru, F. Camarena identified a set of genebank accessions with some level of resistance mostly from a relative species, *P. coccinues*. Two other studies in common bean, however, found that resistance is never very high and only a few genotypes present intermediate levels of resistance (De la Cruz 1990; Erazo and Pastor-Corrales 1990). Meanwhile, the existance of pathogenic races for the disease has not been confirmed and no genotypes defined as differentials for the analysis of differential infection exist. Despite all this we have recently made a collection of isolates for the pathogen in an attempt to differentiate strains from different regions of Colombia based on differential reactions with previously described resistance sources. The objective of this study has been to describe the diversity of these strains and to differentiate the reaction of local cultivars and possible resistance source to Ascochyta blight, through pathogenicity tests with four new isolates of the disease.

**MATERIALS AND METHODS:**

*Plant material:* A set of ten common bean (*P. vulgaris*) landraces were used in this study along with four control genotypes from CIAT mapping populations. Among the landraces were six from the CIAT-managed genebank for common beans (G10747, G3367, G3991, G4032, G6436, G9603) which were recommended as resistant to Ascochyta blight based on De la Cruz (1990) and four important climbing bean accessions/varieties collected by the National Univ. of Colombia (Agrario, Cabrera, Cargamanto Rojo, D. Moreno) which are important in production systems that are often heavily affected by Ascochyta blight. Apart from these 14 genotypes four bi-parental mapping population parents were used as checks since some have shown resistance to anthracnose and possibly to Ascochyta blight (G2333, G19839, G19833 and DOR364).

*Infection and pathogenicity tests:* Four isolates were used in pathogenicity tests and to determine the level of resistance or susceptibility of the genotypes described above. These were from four different production regions in four separate departments of Colombia: Popayan, Cauca (isolate ASC1), Rionegro, Antioquia (isolate ASC3), Silvania, Cundinamarca (isolate ASC 35) and Socorro, Santander (isolate ASC236).Plant were inoculated at 14 days after planting in an isolated humidity chamber within a CIAT greenhouse in Palmira, Valle de Cauca. Plants were rated at eight days post-inoculation based on a severity scale of 1 to 9 as recommended in the CIAT evaluation handbook.

**RESULTS AND DISCUSSION:** A differential response was found for the genotypes and strains of the pathogen tested in the disease screening tests (Table 1), indicating that races of Ascochyta blight

exist with different pathogenicity profiles. Among the four strains of the disease the most aggressive was the one from Popayán. The strain from Santander was the weakest, causing severe disease in only four genotypes while the rest were intermediate in susceptibility. A *P. coccineus* (scarlet runner bean) accession (G35182) was also tested and showed higher levels of resistance as expected since this species unlike *P. vulgaris* (common bean) is rarely attacked by Ascochyta blight. Among the common bean genotypes, the four Colombian landraces were variable in susceptibility with D. Moreno more susceptible than the three other accessions which were only attacked severely by the strongest strain as mentioned above. Among the sources of resistance the most resistant against all strains were G4032 and G6436 while differential responses were found in the other genotypes except G9603 which was susceptible to all strains of the disease. It was notable that the variability in resistance sources was found within the Mesoamerican genepool while the Colombian landraces were all of the Andean genepool; making it important to screen for differential response before making crosses among the two genepools. Establishment of a differential set including the genotypes tested here would be valuable for further work in Ascochyta blight resistance breeding. Finally a set of genetic mapping parents were found to vary for resistance to the weakest strain mentioned above with the resistant allele coming from the Andean genotypes (G19833 and G19839) and the susceptible allele coming from the Mesoamerican genotypes (DOR364 and G2333). This is important since G2333 has been used for crosses to improve anthracnose resistance and it is important to monitor that susceptibility to Ascochyta blight is not co-inherited with that resistance.

**Table 1.** Sources of resistance evaluated against four Colombian isolates of the disease Ascochyta blight casued by *Phoma exigua* var. *diversisporum*.

| Genotype | Gene Pool (*P. vulgaris*) | Infection level with corresponding strains | | | |
|---|---|---|---|---|---|
| | | Popayán ASC1 | Antioquia ASC3 | Cundinamarca ASC35 | Santander ASC 236 |
| Agrario | A | S | I | I | I |
| Cabrera | A | S | I | I | I |
| Cargamanto | A | I | I | I | I |
| D. Moreno | A | S | S | S | I |
| G3367 | MA | S | I | S | S |
| G3991 | MA | S | S | S | I |
| G9603 | - | S | S | S | S |
| G4032 | MA | I | I | I | I |
| G6436 | MA | I | I | I | I |
| G10747 | MA | I | I | S | I |
| G19833 | A | S | S | S | I |
| DOR364 | MA | S | S | S | S |
| G2333 | MA | S | S | S | S |
| G19839 | A | S | S | S | I |
| G35182 | *P. cocc.* | R | R | R | R |

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# DISEASE RESPONSE OF ANDEAN BREEDING LINES TESTED FOR ASCOCHYTA BLIGHT RESISTANCE

**Luz Nayibe Garzón G.[1], Matthew W. Blair[2], Carlos Jara[2], Guillermo Castellanos[2], Agobardo Hoyos[2], Gloria Mosquera[2], Gustavo A. Ligarreto[1], Oscar A. Oliveros G.[1] and Paola Villarrraga[3]**

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## INTRODUCTION:

Ascochyta blight in common bean is caused by the pathogen *Phoma exigua* var. *diversispora* (Bubak) Boerema (synonymous with: *Phoma diversispora* Bubak) and is among the most important fungal diseases of highland production regions of the tropics. Levels of infestation can be very severe causing 100% yield loss in bush beans In Ecuador, Colombia and Peru among countries of the Andean region of South America and is also important in high elevation areas of Guatemala in Central America. For this reason, climbing beans which outgrow or outlast the disease, which is more prevalent in the rainy season are often grown, especially in mixtures with maize or other crops which tend to limit the spread of the pathogen which lasts for several cycles in the soil.

Disease resistance sources have tended to be lacking in the Andean genepool native to the Andean region, while some resistance has been observed in Mesoamerican climbing beans. In addition ICTA (Guatemala) has been successful in breeding a resistant bush bean named Hunapu. Apart from this the International Center for Tropical Agriculture produced a series of Ascochyta blight resistant small-seeded bush beans (ASC series). In this work our goal was to evaluate a new set of large-seeded bush beans (ASR series) bred with ICTA Hunapu and ASC lines as a resistance sources. The lines were especially innovative as they were derived from multiple four parent crosses and gamete selection. Our hope therefore was that the new lines would contain multiple genes for resistance and that this would be a useful method for integrating Mesoamerican resistance sources into an Andean background.

## MATERIALS AND METHODS:

*Pathogen strains:* Evaluations were carried out in an enclosed glass greenhouse using four isolates of Ascochyta blight pathogen collected from individual farmers´ fields across four departments of Colombia: ASC1-Cauca, ASC3-Antioquia, ASC35-Cundinamarca and ASC236-Santander. Each isolate was grown and infected separately onto plants of fourteen-day old seedlings

*Plant materials:* Ten replicate plants were used per genotypes and a total of 49 breeding lines (39 of the new ASR series and 10 of the older ASC series) were tested against each of the isolates described above. The ASR lines were produced from three different four-parent crosses and gamete selection as will be described in results.

*Disease scoring:* Standard inoculation conditions and a humidity chamber were used for infection of the seedlings and then the plants were moved to glasshouse tables where disease ratings were taken 28 days after planting (two weeks after inoculation) using a 1 to 9 scale as recommended by van Schoonhoven, A.; Pastor, M. (eds) ¨Sistema estándar para evaluación de germoplasma de frijol¨, CIAT (1987).

**RESULTS:**

The ASR lines were developed from two different multiple cross populations (Table 1) involving three different ASC lines and ICTA Hunapu a resistance source from Guatemala. Although the disease resistant parents were small seeded and mostly climbing beans except for ICTA Hunapu which is a type IIb bush bean, the three-way cross was topcrosses to an Andean parent with intermediate resistance, G20523. From these crosses the ASR lines were derived from single plant selections followed by bulking to the F3:5 generation and were selected to have Andean seed types (mostly red mottled and cream mottled). Meanwhile, as mentioned above, the ASC lines were all of Mesoamerican origin and varied in their pedigrees. Table 2 shows the disease reaction of the ASR (Andean) and ASC (Meosamerican) lines and their differential response to the four isolates of the disease pathogen. Only three lines were highly resistant for any isolate and of these two were ASC lines challenged with ASC1 and one was an ASR line challenged with ASC236. Meanwhile, a larger number of both ASC and ASR lines (37 in total) showed intermediate reactions against one to three isolates. It was more common to find intermediate reactions to one (12 lines) or two (14 lines) isolates than to three (only 8 lines) isolates. Susceptibility was found in the reactions of 12 lines to all isolates, 15 lines to three isolates and 20 lines to only two isolates.

**CONCLUSIONS:**

Variability for the disease isolates in their capacity to infect and cause disease in different advanced lines of common beans is evident showing that breeding programs must take into account the differential reaction to various isolates. Greater emphasis is needed in the Andean genepool where resistance is lacking and for which seed type requirements are less easily achieved than in the Mesoamerican genepool.

**Table 1.** Characteristics of two sets of advanced lines bred for Ascochyta resistance.

| Series | Seed Type | No. Lines Evaluated | Pedigrees |
|---|---|---|---|
| **ASR** | And | 26 | G 20523 x ( ICTA HUNAPU x ( ASC 72 x ASC 77 )) |
| | And | 13 | G 20523 x ( ICTA HUNAPU x ( ASC 73 x ASC 77 )) |
| **ASC** | Meso | 10 | Various as found in IPHIS |

**Table 2.** Summary of disease reaction of the advanced lines upon inoculation with four isolates of the pathogen *Phoma exigua*, causal agent of Ascochyta blight.

| Isolate | Lines tested | Defense Response | | |
|---|---|---|---|---|
| | | Resistant | Intermediate | Susceptible |
| **ASC1 - Cauca** | ASR | 0 | 17 | 22 |
| **ASC 3 - Antioquia** | ASR | 0 | 9 | 30 |
| **ASC 35- Cundinamarca** | ASR | 0 | 7 | 32 |
| **ASC 236 - Santander** | ASR | 1 | 13 | 25 |
| **ASC1 - Cauca** | ASC | 2 | 8 | 0 |
| **ASC 3 - Antioquia** | ASC | 0 | 10 | 0 |
| **ASC 35- Cundinamarca** | ASC | 0 | 1 | 9 |
| **ASC 236 - Santander** | **ASC1 - Cauca** | 0 | 4 | 6 |

# DISEASE RESPONSE OF INTER-SPECIFIC COMMON BEAN (*PHASEOLUS VULGARIS*) X SCARLET RUNNER OR YEAR-LONG BEAN (*P. COCCINEUS* AND *P. DUMOSUS*) BREEDING LINES FOR ASCOCHYTA BLIGHT RESISTANCE

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## INTRODUCTION

Common beans (*Phaseolus vulgaris* L.) of both the Andean and Mesoamerican genepools are mostly susceptible to Ascochyta blight with some exceptions among Guatemalan black beans, while high levels of resistance are found in the secondary genepool species scarlet runner bean (*P. coccineus*) and year-long bean (*P. dumosus*). Inter-specific crosses are possible between these species although most segregants are more similar to one or the other species and repeated backcrossing is needed to return to the self-pollinating common bean type. Negative attributes of scarlet runner bean are its outcrossing nature, its poor seed set and its thick seed coat. Year long bean similary has a thick seed coat but is less of an outcrossing species. Large seededness, high mineral concentration in terms of the micronutrients iron and zinc as well as adaptation to cool rainy conditions are desirable traits of scarlet runner bean, however almost all scarlet runner beans have climbing bean architecture or are photoperiod sensitive and are difficult to work with. Although some scarlet runner beans are type III bush beans with slightly less photoperiod sensitivity these generally do not carry the desirable traits. Finally, scarlet runner beans are known for producing high vegetative biomass and thick roots which provides it with some measure of tolerance to root rots and aluminum toxicity as well. The goal of this report is to describe the level of Ascochyta blight resistance in some populations derived from interspecific *P. vulgaris* x *P. coccineus P. vulgaris* x *P. dumosus* crosses that were made to incorporate high iron and aluminum tolerance into the former species. Most of the lines are derived from at least one backcross and have Mesoamerican genepool background. The populations have nominally been named through their cross names (FEIN and ASIN) although they are still segregating for some traits.

## MATERIALS AND METHODS

*Plant materials:* Ten replicate plants were used per population and a total of 30 FEIN and five ASIN populations were tested against each of the isolates described below. Of the ASIN lines four were from the cross 13439 and one was from the cross 13438. Of the FEIN lines almost all were from the cross 15528 with the pedigree CAL96 x G35575 while one population was derived from a backcross 15550 with pedigree CAL96 x (CAL96 x G35999). A *P. dumosus* accession G35182 used as a check was resistant to all isolates.

*Pathogen strains:* Evaluations were carried out in an enclosed glass greenhouse using four isolates of Ascochyta blight pathogen collected from individual farmers´ fields across four departments of Colombia: ASC1-Cauca, ASC3-Antioquia, ASC35-Cundinamarca y ASC236-Santander. Each isolate was grown and infected separately onto plants of fourteen-day old seedlings (Table 1).

*Disease scoring:* Standard inoculation conditions and a humidity chamber were used for infection of the seedlings and then the plants were moved to glasshouse tables where disease ratings were taken 14 days after inoculation using a 1 to 9 scale as recommended by CIAT (1987).

## RESULTS

The highest Ascochyta blight resistance was observed in the CAL96 x G35575 lines where nine populations were highly resistant to three isolates: ASC3, ASC35 and ASC236. Meanwhile, the ASC1 isolate was the most virulent and none of the populations were resistant to this strain of the pathogen (Table 1). The other three less virulent strains infected only 40% of the lines overall. These results confirm the variability in the pathogenic capacity of different isolates of the causal agent of Ascochyta blight and the need for differential varieties for a more accurate description of the isolates and categorization into races as has been done for other common bean disease causing fungi. Geographic differentiation of the isolates between the common bean and coffee growing regions of Antioquia, Cauca and Santander versus the highland regions of Cundinamarca also indicate that different resistant genes may function for breeding programs targeting each region. Overall the results suggest that *P. coccineus* and *P. dumosus* may be providing resistance genes against Ascochyta blight since the Andean genotype CAL96 common to all the crosses is not resistant to this disease. Further pathogenicity tests are planned to confirm resistance or susceptibility of both sources from the secondary gene pool and Andean common bean parents against new isolates of the pathogen.

**Table 1.** Summary of Ascochyta blight disease response for four populations of interspecific beans against four isolates of the disease pathogen *Phoma exigua*.

| Population | No. of lines | Resistance Response | | |
|---|---|---|---|---|
| | | R | I | S |
| ASIN13438 | 1 | 0 | 4 | 0 |
| | 1 | 0 | 2 | 2 |
| | 2 | 0 | 1 | 3 |
| ASIN13439 | 1 | 0 | 0 | 4 |
| | 3 | 0 | 1 | 3 |
| | 7 | 0 | 2 | 2 |
| | 6 | 0 | 3 | 1 |
| FEIN15528 | 4 | 0 | 4 | 0 |
| | 2 | 1 | 1 | 2 |
| | 5 | 1 | 2 | 1 |
| | 2 | 1 | 3 | 0 |
| FEIN15550 | 1 | 0 | 0 | 4 |
| **Total** | **35** | **3** | **23** | **21** |

## REFERENCE

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# THE DOMESTICATED TEPARY BEAN ACCESSION G40022 HAS BROADER RESISTANCE TO THE HIGHLY VARIABLE BEAN RUST PATHOGEN THAN THE KNOWN RUST RESISTANCE GENES IN COMMON BEAN

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**INTRODUCTION:** Resistance in common bean (*Phaseolus vulgaris*) to the rust pathogen (*Uromyces appendiculatus*) could be fleeting. This highly variable pathogen is known for continuously producing new virulent strains, called races (Stavely and Pastor-Corrales, 1989). Two new races appeared in 2007 in Michigan and in 2008 in North Dakota (Wright et al. 2008; Markell et al., 2009). Bean cultivars combining two or more rust resistance genes have been more effective for rust management than cultivars with single genes. However, the multiple resistance gene strategy requires of an assortment of effective genes. There are nine named and mapped single and dominant rust resistance genes in common bean (*Ur-3*, *Ur-4*, *Ur-5*, *Ur-6*, *Ur-7*, *Ur-9*, *Ur-11*, *Ur-12*, and *Ur-13*). None of these genes is resistant to all races. The genes *Ur-3*, *Ur-4*, *Ur-5*, *Ur-6*, and *Ur-11* are resistant to 44, 30, 70, 22, and 89 of 90 races respectively, maintained at Beltsville. Recently while evaluating the rust reaction of the parents of a RIL population obtained from crossing the domesticated tepary bean (*P. acutifolius*) accession G40022 with the wild accession G40186 of *P. parvifolius*, we discovered that G40022 has broader resistance than all of the known rust resistance genes present in common bean. Here we compare the rust resistance of G40022 with that of several important rust resistance genes present in common bean.

**MATERIALS AND METHODS:** Using published methodologies we inoculated approximately 20 plants each of the tepary bean accession G40022, several common bean cultivars with single rust resistance genes, and common bean cultivars with broad rust resistance but with unnamed rust resistance genes. All were inoculated with 10 Mesoamerican and six Andean races of the rust pathogen (Tables 1 and 2). Several of these races are used as phenotypic markers to identify rust resistance genes alone or in combinations. Race 47 is used to identify *Ur-3*, race 49 for *Ur-4*, race 53 for *Ur-3*, race 67 for *Ur-11*. The other races are used to confirm the presence of these genes. Race 67 overcomes the resistance of the *Ur-3*, *Ur-4*, *Ur-5*, *Ur-6*, and *Ur-12* genes and the resistance of CNC.

**RESULTS AND DISCUSSION:** Only the tepary bean G40022 was resistant to all 10 Mesoamerican and 6 Andean races of the rust pathogen (Tables 1 and 2). All common beans carrying single rust resistance and the other sources of rust resistance were susceptible to one or more races. PI 181996 (source of the highly effective *Ur-11* gene) and PI 310762 (with an unnamed gene) were each susceptible only to one Middle American race; PI 181996 to race 108 and PI 310762 to race 85 (Table 1). On the other hand, the Andean bean PI 260418 was susceptible only to the Andean race 84 (Table 2). The PI bean Introductions PI 181996, PI 310762 and PI 260418 were the most resistant common beans. The Middle American bean CNC was also resistant to most races but it was susceptible to three Middle American races (Tables 1 and 2).

**Table 1.** Comparing the reaction tepary bean G40022, common beans with single rust resistance genes, and sources of rust resistance to 10 Mesoamerican races of the rust pathogen

| Tepary and common beans | Rust Resist Genes | Reaction to Mesoamerican Races* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 41 | 44 | 47 | 49 | 53 | 63 | 67 | 73 | 85 | 108 |
| G40022 | | 1 | f2cl | f2cl | f2cl | f2cl | 1 | f2cl | 1 | f2cl | f2cl |
| Aurora | *Ur-3* | HR | S | S | S | HR | S | S | S | S | HR |
| Early Gallatin | *Ur-4* | S | HR | S | HR | S | HR | S | HR | HR | HR |
| Mexico309 | *Ur-5* | R | R | R | S | R | R | S | S | S | S |
| Golden Gate Wax | *Ur-6* | HR | HR | HR | S | S | HR | S | HR | HR | S |
| Great Nort.1140 | *Ur-7* | S | R | S | R | S | R | R | S | S | R |
| PI181996 | *Ur-11* | R | R | R | R | R | R | R | R | R | S |
| Redlands Pioneer | *Ur-13* | S | HR | S | S | S | S | S | S | S | S |
| CNC | | R | R | R | R | R | R | S | R | S | S |
| PI 260418 | | R | R | R | R | R | R | R | R | R | R |
| PI 310762 | | R | R | R | R | R | R | R | R | S | R |

*The rust resistance in G40022 was expressed as 1 (No visible rust symptoms) and as f2cl (faint chlorotic spots); in common bean rust resistance was expressed as HR (Necrotic spots without sporulation) or as R (tiny sporualting pustules accompanied by faint chlorotic spots). All susceptible reactions were expressed as S (Large and very large sporulating pustules

**Table 2**. Comparing the reactions of tepary bean G40022, common beans with single rust resistance genes, and sources of rust resistance to six Andean races of the rust pathogen

| Tepary and common beans | Rust Resist Genes | Reactions to Andean Races | | | | | |
|---|---|---|---|---|---|---|---|
| | | 72 | 84 | 89 | 98 | 102 | 105 |
| G 40022 | | f2cl | f2cl | f2cl | f2cl | 1 | f2cl |
| Aurora | *Ur-3* | HR | HR | HR | HR | HR | HR |
| Early Gallatin | *Ur-4* | S | S | S | S | S | S |
| Mexico309 | *Ur-5* | R | R | R | R | R | R |
| Golden Gate Wax | *Ur-6* | S | HR | S | HR | S | S |
| GN1140 | *Ur-7* | R | S | R | R | R | R |
| PI181996 | *Ur-11* | R | R | R | R | R | R |
| Redlands Pioneer | *Ur-13* | HR | HR | HR | HR | HR | HR |
| Compuesto Negro Chimaltenango - CNC | | R | R | R | R | R | R |
| PI 260418 | | R | S | R | R | R | R |
| PI 310762 | | R | R | R | R | R | R |

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# IDENTIFYING PLANTS OF STAMPEDE PINTO BEAN WITH RESISTANCE TO NEW RACES OF RUST PATHOGEN

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## INTRODUCTION

A new race of the bean rust pathogen (*Uromyces appendiculatus*) appeared in 2008 in North Dakota infecting dry bean cultivars carrying the *Ur-3* rust resistance gene that previously had been resistant to the rust disease (Markell et al., 2009). The recently released pinto bean Stampede was susceptible to this race and to another race that appeared in Michigan in 2007 (Wright et al., 2008). Stampede is a selection from a cross between 94-029-01-01 x BelDakMi-RMR-14 (Osorno et al., 2010). The BelDakMi-RMR-14 parent has the *Ur-3*, *Ur-6*, and *Ur-11* rust resistance genes and it is resistant to the new races from North Dakota and Michigan. While evaluating the reaction of U.S. dry bean cultivars to the Michigan and North Dakota races, Stampede plants with resistance to both races, about 50% of plants evaluated, were discovered. The objective of this study was to identify the resistance genes present in the newly discovered rust-resistant Stampede plants.

## MATERIALS AND METHODS

At the ARS-USDA Bean Project at Beltsville, MD, we use eight races of the rust pathogen as phenotypic markers to identify bean plants with the *Ur-3*, *Ur-4*, *Ur-6*, and *Ur-11* genes alone on in combination (Pastor-Corrales and Stavely, 2002). The identification of these plants is based on the specific reaction of the resistance genes to the eight races (Table 1). Because the inoculation of many bean plants with eight races is very laborious, we used four races that accomplish the same results (Table 2). The tip of one primary leaf of each bean plant was cut and inoculated with races 47 and 67. The other uncut primary leaf was inoculated with races 73 and 108. Check cultivars included Aurora (*Ur-3*), Golden Gate Wax (*Ur-6*), BMD-RR-7 (*Ur-11*), Coyne (*Ur-3*, *Ur-6*), BDM-RR-8 (*Ur-6*, *Ur-11*), and BDM-RMR-14 (*Ur-3*, *Ur-6*, *Ur-11*).

## RESULTS AND DISCUSSION

The resistant reactions of the *Ur-3* and *Ur-6* genes are different from that of the *Ur-11* gene. The reactions of *Ur-3* and *Ur-6* are grade "2" necrotic spots without sporulation while the reaction of the *Ur-11* gene is a grade "3" tiny sporulating pustules accompanied by faint chlorotic spots referred as "f2" (Table 1). The resistant reactions of *Ur-3* and *Ur-6* mask the resistant reaction of *Ur-11* when either *Ur-3* or *Ur-6* is combined with *Ur-11*. Thus, bean plants combining the *Ur-3* and *Ur-11* genes, are resistant to races 41, 53, and 108 but only their reaction to races 41 and 53 is controlled by both genes (Table 1). Since the resistant reaction of *Ur-3* masks the resistant reaction of *Ur-11*, plants with *Ur-3* and *Ur-11* display the grade "2" typical of *Ur-3* gene to races 41 and 53. Similarly, plants combining the *Ur-6* and *Ur-11* are resistant to seven of the eight races but are susceptible only to race 108. Only the reaction to races 41, 44, 47 and 73 are controlled by both genes. Because the resistant reaction of *Ur-6* masks the resistant reaction of *Ur-11*, plants with *Ur-6* and *Ur-11* display the grade "2" reaction typical of *Ur-6* to races 41, 44, 47, and 73 (Table 1). In this study we found

many Stampede plants with two (*Ur-3*, *Ur-11*) and some with three (*Ur-3*, *Ur-6*, *Ur-11*) rust resistance genes. Both groups of plants were also resistant to all eight races used as phenotypic markers and to the two new races from North Dakota and Michigan. The Stampede plants with *Ur-3* and *Ur-11* had the same reactions as the check navy bean germplasm line BelDakMi-RR-8, while the Stampede plants with *Ur-3*, *Ur-6* and *Ur-11* had the same reactions as the check pinto bean germplasm line BelDaKMi-RMR14.

**Table 1.** Reactions of bean cultivars with single rust resistance genes and with combinations of these genes to the eight races of the rust pathogen used as phenotypic markers

| Bean Cultivar | Resistance Gene | *Reaction to rust races used as phenotypic markers | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **41** | **44** | **47** | **49** | **53** | **67** | **73** | **108** |
| Aurora | *Ur-3* | 2,2$^+$ | 4,5 | 4,5,6 | 5,4 | 2,2$^+$ | 4,5 | 5,6 | 2,2$^+$ |
| G. Gate Wax | *Ur-6* | 2,2$^+$ | 2,2$^+$ | 2,2$^+$ | 5,4 | 5,4 | 5,4 | 2 | 5,6,4 |
| BMD-RR-7 | *Ur-11* | f2,3 | f2,3 | f2,3 | f2,3 | f2,3 | f2,3 | f2,3 | 5,6,4 |
| | | | | | | | | | |
| Stampede-2R | *Ur-3, Ur-11* | 2,2$^+$ | f2,3 | f2,3 | f2,3 | 2,2$^+$ | f2,3 | f2,3 | 2,2$^+$ |
| Coyne | *Ur-3, Ur-6* | 2,2$^+$ | 2,2$^+$ | 2,2$^+$ | 5,4 | 2,2$^+$ | 5,4 | 2 | 2,2$^+$ |
| BMD-RR-8 | *Ur-6, Ur-11* | 2,2$^+$ | 2,2$^+$ | 2,2$^+$ | f2,3 | f2,3 | f2,3 | 2 | 5,6,4 |
| BDM-RMR-14 | *Ur-3, Ur-6, Ur-11* | 2,2$^+$ | 2$^+$,2 | 2,2$^+$ | f2,3 | 2,2$^+$ | f2,3 | 2 | 2,2$^+$ |

* Resistant rust reactions of the *Ur-3* and *Ur-6* genes are necrotic spots (2) and of *Ur-11* are tiny pustules (3) with faint chlorotic spots (f2). All susceptible reactions are large pustules (4, 5, 6). The resistant reactions of *Ur-3* and *Ur-6* mask the resistant reaction of *Ur-11*.

**Table 2.** Reaction of checks and Stampede plants with two and three rust resistance genes to selected races of the rust pathogen and new races from Michigan and North Dakota

| Checks and Stampede plants with various rust resistance genes | Races to ID Stampede-R | | | | | New Races | |
|---|---|---|---|---|---|---|---|
| | **47** | **67** | **73** | **108** | | **MI** | **ND** |
| Aurora (*Ur-3*) | 4,56 | 4,5 | 5,6 | 2,2$^+$ | | 5,6 | 5,6 |
| Golden Gate Wax (*Ur-6*) | 2,2$^+$ | 5,6 | 2 | 5,6 | | 5,6 | 5,6 |
| PI 181996 (*Ur-11*) | f2,3 | f2,3 | f2,3 | 5,6,4 | | f2,3 | f2,3 |
| | | | | | | | |
| Stampede-2R (Ur-3, Ur-11) | f2,3 | f2,3 | f2,3 | 2,2$^+$ | | f2,3 | f2,3 |
| Stampede-3R (*Ur-3, Ur-6, Ur-11*) | 2,2$^+$ | f2,3 | 2 | 2,2$^+$ | | f2,3 | f2,3 |

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# MORPHOLOGICAL VARIABILITY OF *SCLEROTINIA SCLEROTIORUM* SAMPLED FROM BEAN FIELDS IN MINAS GERAIS STATE, BRAZIL

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## INTRODUCTION

White mold caused by *Sclerotinia sclerotiorum* is a serious disease of common beans. Variability among *S. sclerotiorum* populations has been reported (Barari et al., 2010; Gomes et al., 2011). This research was undertaken to study the morphological variability within populations of *S. sclerotiorum* on bean fields in the state of Minas Gerais, Brazil.

## MATERIALS AND METHODS

Thirty isolates of *S. sclerotiorum* were collected in areas cultivated with common beans. Each isolate (a single sclerotium) was purified by transferring a single hyphal tip onto potato dextrose agar (PDA) medium. Mycelial plugs (5.0 mm) of each isolate were taken from the growing margins of colonies grown on PDA for three days and transferred to Petri plates containing 25 mL of PDA medium (pH 5.6). One Petri plate with one mycelium plug was considered an experimental unit. Treatments (isolates) were replicated three times in a completely randomized design. The cultures were incubated at 23°C in the darkness. Colony diameter was measured after 24 hours of incubation. Twenty-five days later color of colonies and sclerotia characteristics (number, weight, dimensions and distribution on the plates) were evaluated. The length, width, and thickness of 10 sclerotia per plate were assessed.

## RESULTS AND DISCUSSION

The main colony colors observed were white, beige, and brown (Table 1). The colony color varied among experimental units of the same isolate. On the other hand, distribution of sclerotia on Petri plate was consistent within each isolate. Sclerotia weight and width differentiated the isolates better than number, length, and thickness (Table 1). Our results indicated a high variability among the isolates. The morphological characterization of colonies and sclerotia of *S. sclerotiorum* could be useful as a complementary descriptor in studies aimed at assessing pathogen variability. Further studies using molecular markers (SSR) are now being conducted to better characterize the population of *S. sclerotiorum* from Minas Gerais State.

**Table 1** – Characterization of colony and sclerotia of *Sclerotinia sclerotiorum* isolates of Minas Gerais, Brazil

| Isolate | Colony | | Sclerotia | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Diameter (cm) | Color (number of plates) | Number | Weight (g) | Length (mm) | Width (mm) | Thickness (mm) | Distribution on PDA |
| 1 | 4.68 a | Br (2), W (1) | 41 a | 0.34 c | 3.26 b | 2.30 c | 1.56 | I |
| 2 | 4.33 a | W (2), Br (1) | 32 a | 0.33 c | 4.05 b | 2.62 c | 1.71 | I |
| 3 | 4.58 a | W (3) | 20 b | 0.29 c | 3.52 b | 2.52 c | 1.63 | II |
| 4 | 4.92 a | W (2), Be (1) | 40 a | 0.41 c | 4.54 a | 2.39 c | 1.26 | I |
| 5 | 4.47 a | Br (2), Be (1) | 59 a | 0.50 b | 3.25 b | 2.33 c | 1.63 | I |
| 6 | 4.58 a | W (3) | 9 b | 0.13 d | 4.62 a | 2.61 c | 1.32 | I |
| 7 | 4.50 a | W (2), Br (1) | 27 b | 0.43 c | 3.88 b | 2.70 b | 1.63 | I |
| 8 | 4.50 a | Br (3) | 52 a | 0.41 c | 3.27 b | 2.29 c | 1.76 | I |
| 9 | 3.95 b | Be (1), Br (1), W (1) | 14 b | 0.11 d | 3.88 b | 2.09 c | 1.41 | I |
| 10 | 4.45 a | Br (2), W (1) | 34 a | 0.29 c | 3.45 b | 2.31 c | 1.51 | I |
| 11 | 4.52 a | W (2), Br (1) | 47 a | 0.41 c | 3.21 b | 2.29 c | 1.56 | I |
| 12 | 4.23 a | Br (2), W (1) | 42 a | 0.35 c | 3.87 b | 2.56 c | 1.68 | I |
| 13 | 3.02 c | Br (3) | 20 b | 0.40 c | 5.67 a | 2.83 b | 1.57 | I |
| 14 | 4.20 a | W (3) | 18 b | 0.53 b | 6.46 a | 3.57 a | 1.54 | I |
| 15 | 4.05 a | Be (3) | 38 a | 0.80 a | 4.58 a | 3.01 b | 1.60 | I |
| 16 | 4.58 a | Br (2), W (1) | 47 a | 0.49 b | 3.92 b | 2.54 c | 1.57 | II |
| 17 | 4.20 a | Be (2), W (1) | 49 a | 0.52 b | 3.95 b | 2.52 c | 1.46 | I |
| 18 | 4.35 a | Br (3) | 34 a | 0.58 b | 5.12 a | 2.73 b | 1.61 | I |
| 19 | 4.25 a | W (3) | 30 b | 0.34 c | 3.57 b | 2.39 c | 1.45 | I |
| 20 | 3.70 b | W (3) | 15 b | 0.32 c | 5.35 a | 2.76 b | 1.89 | I |
| 21 | 3.83 b | W (3) | 27 b | 0.43 c | 3.50 b | 2.47 c | 1.57 | II |
| 22 | 4.12 a | Be (3) | 39 a | 0.77 a | 5.31 a | 2.74 b | 1.57 | I |
| 23 | 4.12 a | W (3) | 37 a | 0.39 c | 4.15 b | 2.76 b | 1.69 | II |
| 24 | 3.80 b | W (3) | 16 b | 0.50 b | 5.24 a | 3.36 a | 1.60 | I |
| 25 | 4.37 a | W (2), Br (1) | 30 b | 0.38 c | 3.66 b | 2.41 c | 1.78 | I |
| 26 | 4.30 a | W (3) | 22 b | 0.36 c | 3.89 b | 2.39 c | 1.32 | II |
| 27 | 4.13 a | W (3) | 25 b | 0.35 c | 3.90 b | 2.79 b | 1.53 | II |
| 28 | 4.17 a | Br (2), W (1) | 43 a | 0.47 b | 3.69 b | 2.44 c | 1.49 | II |
| 29 | 4.48 a | W (3) | 32 a | 0.35 c | 4.19 b | 2.34 c | 1.46 | I |
| 30 | 4.05 a | Be (3) | 33 a | 0.47 b | 4.00 b | 2.81 b | 1.64 | II |

Br = brown; W = white; Be = beige; I = in the edge of Petri plate; II = irregularly. A Scott-Knott test was used to separate treatment means into discrete, non-overlapping groups or clusters ($P < 0.05$).

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# USE OF MUTI SITE SCREENING TO IDENTIFY PARTIAL RESISTANCE TO WHITE MOLD IN COMMON BEAN IN 2010

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The development of common bean cultivars with partial resistance and/ or avoidance to white mold (*Sclerotinia sclerotiorum*) would benefit producers by reducing yield loss due to this disease and reducing input costs for fungicides. Our main objective in this study is to identify bean germplasm with broad partial resistance to white mold.

Bean breeders sent 20 bean lines with putative sources of resistance to our central location where the seeds were divided in equal amounts for field and/or greenhouse tests and then sent to nine locations to be evaluated by standardized greenhouse and field screening methods.

The field tests consisted of two rows of each of the 12 entries and one row of 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 six field tests conducted in six locations. 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 1). Wisconsin, Nebraska and Oregon were not included in the results table due to lack of plants, no infection and gray mold infection that out competed white mold, respectively. These problems that resulted in no data were disappointing, but demonstrate the importance of testing in multiple locations. The field results indicate that A195 was a more resistant bean line than G122 (resistant check); however, it was tested in only one location. The other lines tested in the field all showed intermediate resistance. Testing in multiple locations provides some security that data will be collected for that year so breeders can continue to move forward with developing future bean cultivars.

The greenhouse tests were conducted on 23 entries using a straw test method of inoculating 21-28 day old plants. The plants were infected one inch above the fourth node with a plug of PDA media and young *S. sclerotiorum* mycelia inserted in a one inch piece of clear drinking straw sealed at one end and slid over the cut internode. The infected plants were evaluated eight days later using a modified Petzoldt and Dickson scale for straw tests (Teran et al, 2006) (Table 2). The greenhouse tests results indicate that bean lines VCW54 and A195 were more resistant that G122. The actual straw test mean for these lines indicates they have only intermediate resistance; however, greenhouse conditions are more stable and allow the fungus to grow in optimal conditions which is less likely to be encountered in the field. Lines that showed intermediate resistance in the field also demonstrated intermediate resistance in the greenhouse test. Thus, no lines exhibited escape.

The 2010 screening season, overall, was not very favorable for field screening, but data from greenhouse screening was very good and still provides information for bean breeders and pathologists to make more informed decisions on their lines.

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

| ENTRY | SEED CLASS | COLLABORATOR | ND | MI | WA | Mean | t Grouping |
|---|---|---|---|---|---|---|---|
| **A195** | BAYO | S. SINGH-ID | 1.7 | - | - | 1.7 | A |
| **G122** | CRAN | Resistant Check | 2.4 | 3.0 | 2.7 | 2.7 | A B |
| **EX RICO (BUNSI)** | NAVY | Intermediate Check | 2.6 | 2.3 | 4.5 | 3.1 | A B |
| **VCW54** | BLACK | S. SINGH-ID | 3.3 | - | - | 3.3 | A B |
| **P07863** | PINTO | J. KELLY-MI | 3 | 3.0 | 4.8 | 3.6 | A B |
| **37-2** | PINTO | P. MIKLAS-WA | 3.2 | 3.3 | 4.7 | 3.7 | A B C |
| **C08709** | CRANBERRY | J. KELLY-MI | 4.8 | 2.3 | 4.2 | 3.8 | A B C |
| **ND080547** | SMALL RED | J. OSORNO-ND | 2.4 | 3.7 | 5.3 | 3.8 | A B C |
| **P07751** | PINTO | J. KELLY-MI | 3.5 | 4.7 | 4.3 | 4.2 | B C D |
| **ND060514** | NAVY | J. OSORNO-ND | 2.8 | 5.0 | 4.7 | 4.2 | B C D |
| **70-1** | PINTO | P. MIKLAS-WA | 4.1 | 5.0 | 5.2 | 4.8 | B C D |
| **50-2** | SMALL RED | P. MIKLAS-WA | 4.4 | 5.3 | 4.7 | 4.8 | B C D |
| **NE2-06-8** | PINTO | C. URREA-NE | 5.8 | - | - | 5.8 | C D |
| **BERYL** | G. NORTHERN | Susceptible Check | 7.6 | 3.3 | 7.5 | 6.1 | D |

*1 = no disease, 9 = plants dead **Alpha = 0.05, LSD = 2.14

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

| ENTRY | SEED CLASS | COLLABORATOR | CO | ID | MI | NE | NY | OR | WA | WI | Mean | t Grouping |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **VCW54** | BLACK | S. SINGH-ID | 3.8 | 5.7 | 2.0 | 3.5 | 3.1 | 4.6 | 3.1 | 4.4 | 3.8 | A |
| **A195** | BAYO | S. SINGH-ID | 4.0 | 4.4 | 3.8 | 4.0 | 4.5 | 4.8 | 3.6 | 3.5 | 4.1 | A B |
| **G122** | CRANBERRY | Resistant Check | 5.2 | 4.8 | 1.8 | 4.4 | 3.9 | 4.2 | 3.8 | 4.7 | 4.1 | A B |
| **37-2** | PINTO | P. MIKLAS-WA | 4.2 | 5.1 | 3.3 | 5.1 | 8.2 | 5.3 | 4.0 | 4.4 | 4.9 | A B C |
| **ND060514** | NAVY | J. OSORNO - ND | 6.8 | 4.1 | 2.8 | 5.0 | 8.4 | 5.1 | 3.9 | 4.2 | 5.0 | A B C |
| **ND080547** | CRANBERRY | J. OSORNO - ND | 6.4 | 5.0 | 4.0 | 6.1 | 5.6 | 6.2 | 3.7 | 5.6 | 5.3 | B C D |
| **NE2-09-16** | PINTO | C. URREA-NE | 6.1 | 5.2 | 5.8 | 5.3 | 5.8 | 6.2 | 5.3 | 3.8 | 5.4 | C D |
| **C08709** | CRANBERRY | J. KELLY-MI | 7.1 | 5.2 | 4.0 | 6.5 | 4.0 | 6.8 | 5.7 | 6.1 | 5.7 | C D E |
| **NE2-06-8** | PINTO | C. URREA-NE | 4.9 | 5.8 | 5.0 | 6.7 | 6.4 | 7.9 | 5.9 | 5.7 | 6.0 | C D E F |
| **NE2-09-19** | PINTO | C. URREA-NE | 7.2 | 5.6 | 3.3 | 7.0 | 7.3 | 6.8 | 6.0 | 5.2 | 6.0 | C D E F |
| **NE2-09-4** | PINTO | C. URREA-NE | 5.8 | 6.5 | 2.8 | 6.7 | 7.2 | 6.9 | 5.9 | 7.2 | 6.1 | C D E F |
| **P07751** | PINTO | J. KELLY-MI | 6.6 | 5.7 | 4.3 | 7.0 | 6.2 | 6.8 | 6.2 | 6.4 | 6.1 | C D E F G |
| **NE2-09-14** | PINTO | C. URREA-NE | 5.2 | 5.9 | 7.3 | 6.7 | 8.9 | 7.2 | 4.8 | 6.1 | 6.5 | D E F G H |
| **P07863** | PINTO | J. KELLY-MI | 6.4 | 6.0 | 4.5 | 8.0 | 7.3 | 7.9 | 6.4 | 8.0 | 6.8 | E F G H I |
| **NE1-09-20** | G. NORTHERN | C. URREA-NE | 7.7 | 6.1 | 3.8 | 8.3 | 8.5 | 7.0 | 6.3 | 8.3 | 7.0 | F G H I |
| **70-1** | PINTO | P. MIKLAS-WA | 6.9 | 6.6 | 3.8 | 8.7 | 9.0 | 8.2 | 6.3 | 7.4 | 7.1 | F G H I |
| **NE2-09-10** | PINTO | C. URREA-NE | 7.2 | 6.2 | 4.3 | 7.6 | 8.9 | 7.8 | 7.7 | 7.4 | 7.1 | F G H I |
| **50-2** | SMALL RED | P. MIKLAS-WA | 7.7 | 6.3 | 5.5 | 6.8 | 8.8 | 8.4 | 6.5 | 7.1 | 7.1 | F G H I |
| **NE2-09-1** | PINTO | C. URREA-NE | 7.1 | 6.7 | 3.3 | 8.5 | 8.3 | 8.7 | 5.8 | 8.7 | 7.1 | F G H I |
| **NE1-09-12** | G. NORTHERN | C. URREA-NE | 7.4 | 6.0 | 3.8 | 9.0 | 8.9 | 7.1 | 8.2 | 9.0 | 7.4 | G H I |
| **NE2-09-6** | PINTO | C. URREA-NE | 8.6 | 6.4 | 5.0 | 7.8 | 8.5 | 8.7 | 7.0 | 8.1 | 7.5 | H I |
| **BERYL** | G. NORTHERN | Susceptible Check | 8.3 | 6.4 | 7.3 | 8.2 | 7.9 | 8.2 | 5.7 | 8.3 | 7.5 | H I |
| **EX RICO (BUNSI)** | NAVY | Intermediate Check | 8.2 | 6.6 | 8.3 | 6.8 | 9.0 | 7.9 | 8.0 | 8.5 | 7.9 | I |

*Straw test rating scale based on modified Petzoldt and Dickson scale (Teran et al, 2006)
(1-3 = resistant, 4-6 = intermediate, 7-9 = susceptible) **Alpha = 0.05, LSD = 1.28

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# ASSESSMENT OF THE AGGRESSIVENESS OF ISOLATES OF *SCLEROTINIA SCLEROTIORUM* AND OF THE RESISTANCE OF COMMON BEANS

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## INTRODUCTION

Among the measures of controlling the white mold in common bean, genetic resistance is the most useful by reducing production costs and environmental damage. However, obtaining resistant cultivars has been hampered by the low resistance level presented in the germoplasm, and some resistance sources are poorly adapted to cultivation conditions in Brazil. Besides, the methods used for identifying the resistant genotypes are not very efficient. Among the factors that affect the success of evaluation of resistant genotypes, is the variability of the isolates of the pathogen. So, the variation assessment of the aggressiveness of *Sclerotinia sclerotiorum* isolates may help to identify some more useful for selecting resistant genotypes. Besides the use of some promising parents it can give an idea of the disease resistance (Melo and Santos 1999). Thus, the objectives of this study were to investigate the aggressiveness of different isolates of *S. sclerotiorum* and assess the level of resistance of lines/cultivars.

## MATERIALS AND METHODS

The experiments were conducted at the Federal University of Lavras, Minas Gerais, Brazil, in the 2009/2010 rainy season and in the 2010 dry season. Thirteen cultivars/lines were inoculated with six isolates of *S. sclerotiorum* from different regions in the rainy season, and other four isolates plus two used in the previous evaluation in the dry season. Each isolate was used per experiment, therefore, 12 experiments were conducted in randomized block design with three replications. The experimental plot had fifteen plants in one meter, and the ten more vigorous plants were inoculated through the straw test method. The evaluation was done per plant in accordance with Terán and Singh (2009). The mean score per plot was used in the analyses of variance performed with SAS ® software (SAS Institute, 2005), PROC GLM.

## RESULTS AND DISCUSSION

Significant difference among cultivars/lines in both seasons ($P \leq 0.01$) indicates different resistance alleles. Also, significant differences among isolates ($P \leq 0.01$), indicate different alleles for aggressiveness of the *S. sclerotiorum* isolates. In the rainy season, the interaction isolates x lines was significant, indicating the reaction of the lines was not coincident when inoculated with different isolates. However, in the dry season the interaction was not significant, indicating the reaction of the lines was coincident when inoculated with different isolates, e.i the lines reacted similarly to all isolates. Therefore, probably the resistance is of horizontal type. It is worthy observe the correlation among the cultivar/line means in the two seasons that was of average magnitude (64%), indicating the reaction of the cultivars/lines was relatively consistent regardless of the isolates tested. Also,

there was consistency of the aggressiveness of the common isolates in the two seasons, and it can be inferred that the genetic control of the pathogenicity is polygenic. Considering the joint analysis of variance the interaction isolates x lines was significant, however, cultivars/lines that were classified in the most resistant and susceptible group were the same in both seasons, confirming the horizontal resistance and the identification of those more promising. It also confirms that the genetic control of pathogen aggressiveness is polygenic, as expected due to the lower specificity of the interaction of *S. sclerotiorum* with common bean.

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# HERITABILITY ESTIMATES AND PHENOTYPIC CORRELATIONS FOR WHITE MOLD RESISTANCE AND AGRONOMIC TRAITS IN PINTO BEAN

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## INTRODUCTION

White mold caused by *Sclerotinia sclerotiorum* is a serious disease that reduces yield and seed quality in common bean (*Phaseolus vulgaris*). The use of resistant varieties is the most economical way to manage the disease but white mold resistance is a complex quantitative trait that is affected by plant architectural traits and the environment. Pinto beans which belong to the medium-seeded Middle American Durango race are the most common market class grown in North America; however traditional cultivars lack physiological resistance and possess indeterminate, prostrate, type III growth habit that favors disease development. In recent years, new type II indeterminate upright cultivars have been bred to avoid white mold. The objective of this study was to analyze the heritability of and correlations between white mold resistance and agronomic traits in a type II pinto bean RIL population.

## MATERIALS AND METHODS

The study was conducted on a RIL population of 94 F4:7 lines from two parents: AN-37 (Miklas et al. 2006) which has Type IIb growth habit, open canopy, stay-green trait, with an unacceptable dark pinto seed; and P02630, a MSU breeding line with high yield, superior seed quality, upright plant habit, but is susceptible to white mold. The RILs were grown in a naturally infested field in Michigan. Data on height, lodging, days to maturity and white mold incidence was collected at physiological maturity for four years (2007-2010). Greenhouse straw tests were conducted from 2008-2010 to detect physiological resistance as described by Petzoldt and Dickson (1996). Data generated from all greenhouse and field experiments were analyzed as randomized complete block designs using PROC MIXED both individually and across field and greenhouse tests (SAS 1995). Narrow sense heritability $h^2$ was estimated on a progeny mean basis for the traits (Hallauer and Miranda 1981). Exact 90% confidence intervals were calculated for $h^2$ estimates were calculated as described by Knapp et al. (1985).

## RESULTS AND DISCUSSION

Significant variation was observed as shown by the data ranges among all the traits in the RIL population which suggests that initial selection in the segregating population was unbiased. Disease incidence was consistent across four years except for 2008 when it was particularly low due to hot and dry weather (Table 1). These data support the major role that weather plays in the incidence and development of white mold. Yield was high in 2008 (due to low disease incidence) and the RILs took longer to reach physiological maturity in 2009 possibly due to high rainfall and excessive vegetative growth. All the other traits were fairly consistent across the years.

Heritability estimates for most of the traits were low to moderate except for the straw test, yield, seed weight and days to flowering (Table 1). Field disease incidence was noticeably low in comparison to greenhouse tests indicating that only 23% of the observed variation in the field was

due to genotypic differences, suggesting the role of architectural avoidance. Plant height and lodging also exhibited low heritability estimates. Seed weight and yield exhibited relatively higher heritability. Similar estimates of heritability have been previously reported (Kolkman and Kelly 2002, Miklas et al. 2003).

The Pearson correlation coefficient (r) was calculated for field disease incidence and all the other traits (Table 1). All of the traits were negatively correlated with disease incidence in the field except for lodging. Excessive lodging is associated with high disease incidence as it creates favorable conditions for disease development and spread. There was small but significant negative correlation with the straw test which indicates that the straw test detects different resistance mechanism for white mold in common bean than is observed in the field.

**Table 1.** Means and heritability estimates for white mold resistance and agronomic traits.

| Trait | 2007 mean (range) | 2008 mean (range) | 2009 mean (range) | 2010 mean (range) | Heritability h2 (90% CI) | Corr. with DI (r)† |
|---|---|---|---|---|---|---|
| Disease Incidence (%) | 53.2 (11.1-88.9) | 20.2 (11.9-36.3) | 43.5 (16.4-79.4) | 52.2 (18.87-92.13) | 0.23 (0.03-0.41) | 1.00 |
| Straw Test (1-9) | - | 5.23 (1.0-6.0) | 4.96 (1.0-8.0) | 3.37 (3.0-8.0) | 0.46 (0.29-0.58) | -0.14* |
| Seed Yield (cwt/acre) | 37.3 (28.6-46.0) | 40.3 (29.6-52.0) | 27.4 (17.7-33.3) | 25.7 (16.2-32.1) | 0.53 (0.37-0.64) | -0.30** |
| 100-Seed weight (g) | 35.9 (29.4-44.9) | 36.8 (30.8-42.8) | 37.6 (26.5-44.7) | 33.6 (26.4-40.0) | 0.74 (0.65-0.80) | -0.06 |
| Flowering (days) | 43.0 (41.1-47.5) | 40.0 (34.5-45.5) | 47.6 (41.5-53) | 38.6 (36.7-41.3) | 0.62 (0.49-0.70) | -0.17* |
| Maturity (days) | 97.7 (92.0-110.0) | 94.3 (92.0-99.5) | 101.5 (94.0-108.0) | 94.3 (90.0-102.7) | 0.34 (0.11-0.49) | -0.47** |
| Lodging (1-5) | 2.00 (1.0-3.0) | 1.50 (1.0-2.5) | 2.00 (1.0-3.0) | 2.90 (1.0-4.7) | 0.23 (0.03-0.41) | 0.44** |
| Canopy Height (cm) | 47.5 (42.5-52.5) | 50.4 (45.5-53.5) | 55.7 (51.5-66.0) | 51.3 (44.7-57.0) | 0.25 (0.01-0.43) | -0.45** |

Significant at *p= 0.05, **p=0.01; † Pearson correlation coefficient (r) with DI=disease incidence in field

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# REACTION OF COMMON BEAN GENOTYPES AFTER INOCULATION OF PLANTS WITH *SCLEROTINIA SCLEROTIORUM*

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## INTRODUCTION

The irrigated dry bean (*Phaseolus vulgaris*) areas of the state Minas Gerais, Brazil, have moderate temperatures (15-22°C) during the fall-winter season. These conditions favor the development and spreading of the white mold caused by *Sclerotinia sclerotiorum*. Commercial cultivars are generally susceptible to the disease. Fungicides are efficient to control the disease, but they increase the production costs and are ecologically harmful. The strategy of combining upright cultivars with physiological resistance has a great potential. The purpose of this work was to identify physiologically resistant genotypes to *S. sclerotiorum*. They could be used in breeding programs to incorporate resistance to white mold in upright cultivars.

## MATERIALS AND METHODS

Thirty common bean genotypes were tested in greenhouse. Plants were grown in 5.0 L-pots. Each plot was a pot with three plants. Treatments were replicated three times in a randomized block design. Inoculation of plants with an isolate of *S. sclerotiorum* from Oratórios, Minas Gerais, Brazil, was done according to Petzoldt & Dickson (1996). Plants were rated for disease severity at 7 and 14 days after inoculation (DAI) using a scale adapted from Terán et al. (2006): 1 – no symptoms; 2 – stem/branch infected, but invasion of the first internode < 1 inch; 3 – stem/branch invasion of the first internode > 1 inch, but not reached the first node; 4 – stem/branch invasion reached the first node, but not further; 5 – stem/branch invasion passed the first node, but invasion of the second internode < 1 inch; 6 – stem/branch invasion of the second internode > 1 inch, but not reached the second node; 7 – stem/branch invasion reached the second node, but not further; 8 – stem/branch invasion passed the second node, but invasion of the third internode < 1 inch; 9 – stem/branch invasion of the third internode > 1 inch, leading to plant death.

## RESULTS AND DISCUSSION

The genotype 11A-39 had the highest level of resistance to *S. sclerotiorum* (Table 1). Lines with scores less than 5 are considered potential sources of resistance to white mold. Thus, the Brazilian cultivars Carnaval MG, Jalo MG 65 (Andean), and Ouro Negro (Mesoamerican) showed some physiological resistance to the fungus. Jalo MG 65 and Ouro Negro are type III cultivars, whereas Carnaval MG is type I. The most susceptible genotypes were Eclipse, NE2-06-08, BRS Valente, Orion, and VC-6.

**Table 1** – Mean severity scores ± SD for invasion of *Sclerotinia sclerotiorum* on common bean plants at 7 and 14 days after inoculation (DAI)

| Genotype | 7 DAI | 14 DAI |
|---|---|---|
| 11A-39 | 2.9 ± 0.51 | 3.3 ± 0.33 |
| A195 | 4.2 ± 0.38 | 4.7 ± 0.33 |
| Cornell 603 | 4.4 ± 0.69 | 4.6 ± 0.77 |
| Ouro Negro | 4.4 ± 0.51 | 4.7 ± 0.58 |
| Carnaval MG | 4.6 ± 0.10 | 4.8 ± 0.29 |
| 37-2 | 4.9 ± 1.54 | 5.9 ± 0.96 |
| G-122 | 4.9 ± 0.19 | 6.1 ± 0.84 |
| Manteigão Fosco11 | 4.9 ± 0.51 | 6.4 ± 0.51 |
| Jalo MG 65 | 5.0 ± 0.00 | 5.0 ± 0.00 |
| NE2-07-10 | 5.0 ± 0.58 | 5.4 ± 1.02 |
| Tapia | 5.0 ± 0.33 | 5.8 ± 0.51 |
| Beryl | 5.1 ± 1.02 | 5.9 ± 1.83 |
| Roma II | 5.2 ± 0.69 | 6.0 ± 0.67 |
| Ex-Rico (Bunsi) | 5.7 ± 1.00 | 5.8 ± 0.83 |
| Avalanche | 5.9 ± 0.84 | 6.0 ± 1.45 |
| 38-4 | 5.9 ± 1.84 | 6.5 ± 1.59 |
| 29 C-6 | 5.9 ± 0.82 | 6.9 ± 1.35 |
| WM31 | 6.0 ± 0.00 | 6.8 ± 0.50 |
| NE1-06-12 | 6.1 ± 0.38 | 7.9 ± 1.39 |
| Stampede | 6.3 ± 0.33 | 7.7 ± 0.75 |
| P 07863 | 6.4 ± 1.40 | 6.8 ± 1.55 |
| BRS Supremo | 6.4 ± 0.77 | 6.8 ± 1.04 |
| NE1-07-12 | 6.4 ± 1.33 | 7.4 ± 1.50 |
| Pérola | 6.5 ± 1.32 | 6.9 ± 1.42 |
| B 07104 | 6.5 ± 1.50 | 7.0 ± 1.00 |
| VC-6 | 7.1 ± 0.77 | 7.3 ± 0.88 |
| Orion | 7.2 ± 0.69 | 8.1 ± 0.63 |
| BRS Valente | 7.5 ± 0.50 | 7.5 ± 1.00 |
| NE2-06-8 | 7.7 ± 0.30 | 8.1 ± 0.51 |
| Eclipse | 7.9 ± 0.19 | 8.1 ± 0.19 |

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# INHERITANCE OF WHITE MOLD RESISTANCE IN THE COMMON BEAN LINE A195

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## INTRODUCTION

White mold, caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, is a frequent and destructive disease in common bean. The resistance against this pathogen has quantitative nature implicating both, avoidance and physiological mechanisms. Quantitative trait loci (QTL) associated to the response to white mold were coalesced into 21 distinct regions across nine linkage groups (Soule *et al*. 2011). Using greenhouse tests, the common bean line A195 showed moderate levels of resistance to white mold (Singh *et al.* 2007; Pascual *et al.* 2010). In this work, the association between the responses to white mold, and specific molecular markers mapped in the relative position of 18 QTLs controlling the reaction to this pathogen is investigated in a $F_{2:3}$ population derived from the cross A195 x Cornell 49242.

## MATERIALS AND METHODS

A population of 99 $F_{2:3}$ families derived from the cross between the line A195 and the cultivar Cornell 49242 was used to investigate the inheritance of the response to white mold. Cornell 49242 is a very small black-seeded line included in the black turtle market class; it has indeterminate postrate growth habit, and is susceptible to white mold.

The local isolate 1, obtained from tissues of naturally infected bean plants and derived from one sclerotia (Pascual *et al.* 2010), was used in this study. Fresh inoculum was obtained from sclerotia germinated on potato dextrose agar medium in Petri plates. Resistance tests were developed using the greenhouse straw method (Petzoldt and Dickson, 1996). Plants were evaluated 8-10 days after inoculation considering the invasion in the main stem, and the disease reactions were assessed using a 1 - 9 severity scale, where 1= no symptoms and 9 = total plant collapse (Miklas, 2007). Twenty $F_3$ plants derived from each $F_2$ plants were evaluated in two tests. In each test there were two pots with 4-5 plants.

A total of 33 markers (molecular or morphological), selected according to the comparative QTL map reported by Soule et al. (2011) or inferred by comparison of genetic maps, were analyzed in the $F_{2:3}$ population. These markers were previously associated to 18 QTLs conferring resistance to white mold: **WM1.1**$^{\mathbf{AG}}$ QTL (*Fin* gene, BMd45); **WM1.2**$^{\mathbf{GC}}$ (PVBR250, PVBR233, PVBR107); **WM2.1**$^{\mathbf{PX}}$ (BM156); **WM2.2**$^{\mathbf{BN,\ HN,}}$ (BMd17); **WM2.3**$^{\mathbf{BR,\ GC}}$ (PVM115, BM143); **WM3.1**$^{\mathbf{AN}}$ (PVM148, SBD5, SD8); **WM4.2**$^{\mathbf{R31}}$ (Pv-ag-004, BMd16); **WM4.1**$^{\mathbf{PX}}$ (PV-gaat001); **WM5.1**$^{\mathbf{PX}}$ (BMd20);**WM5.3**$^{\mathbf{R31}}$**/ WM5.4**$^{\mathbf{R31}}$ (BM175, BMd53); **WM6.1**$^{\mathbf{B60,\ R31}}$ (PV-at004, BM170, PVBR5); **WM7.1**$^{\mathbf{AG,\ PX}}$ (Phs); **WM7.2**$^{\mathbf{BN,\ BR}}$ (PVBR35, PVBR167); **WM7.3**$^{\mathbf{R31}}$ (BM210, BM185); **WM8.3**$^{\mathbf{B60,\ GC,\ BV}}$ BM189; **WM8.4**$^{\mathbf{BR,GC,R31}}$ (BM151, BM167, PVBR45); **WM9.1**$^{\mathbf{GC}}$ (BMd46, PVBR101, PVBR168). QTLs are named according to Miklas and Porch (2010). The name reflects the trait abbreviation (WM = white mold), linkage group (1–11), order of discovery, and originating population in superscript. Molecular markers were amplified using the programs described by the respective authors. PCR products were resolved on 8% polyacrylamide or 2% agarose gels, stained with SYBR Safe DNA© and visualized under UV light.

Linkage analyses were carried out using MAPMAKER 2.0 software (Lander *et al*., 1987). Significant association (-log p(F)> 3) between the markers and the response to white mold was identified by single-marker regression using the software Qgene 4.3.8 (Joehanes and Nelson, 2008).

Significant associations identified in at least one test and in the mean obtained from both tests, were considered.

## RESULTS AND DISCUSSION

Observed segregation of the markers did not deviate significantly from the ratio expected for a single gene (3:1 or 1:2:1), except for the microsatellite BM143. Recombination was not observed between the loci BM210 and BM185. The loci previously mapped in the same linkage group (LG), showed a linkage relationship between them in this analysis.

Figure 1 shows the distributions of the response to white mold (isolate 1) in the $F_{2:3}$ population. No significant deviation from the corresponding normal distribution was observed using the Kolmogorov–Smirnov test. Single-marker regression analysis revealed a significant association between the response to white mold and four loci mapped in LG 1 and LG 7 (Table 1). The genetic distances between the loci *Fin/fin* - BMd45 (LG1) and Phs - BM210/BM185 (LG7) were 3.0 and 21.3 cM, respectively. The QTLs WM1.1$^{AG}$, WM7.1$^{AG, PX}$ and WM7.3$^{R31}$ were previously identified in the the relative positions where these markers were mapped (Soule et al., 2011). These results did not reveal evidences about the qualitative inheritance to white mold described by Genchev and Kiryakov (2002) in A195, and they suggest that at least three QTLs are involved in the response to white mold (isolate 1) in this genotype.

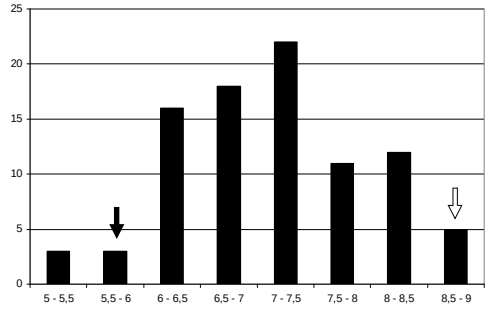


**Figure 1.** Histogram showing the distributions (mean obtained from the two evaluations) for the reaction to the local isolate 1of white mold Black and white arrows indicate the phenotypic values of parents A195 and Cornell 49242, respectively.

**Table 1.** Test statistics for single-marker regression in the loci showing a significant association with the response to white mold.

| LG | Locus name | -log p(F) | Additive effect | $R^2$ (%) |
|---|---|---|---|---|
| 1 | BMd45 | 3.211 | -0.45 | 15.0 |
| 1 | *Fin* gene | 3.294 | -0.49 | 15.4 |
| 7 | Phs | 7.557 | -1.09 | 31.8 |
| 7 | BM210/BM185 | 6.077 | -0.59 | 26.5 |

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# OXALIC ACID REACTION AND *PHS* SCAR MARKER OF COMMON BEAN PROGENIES DERIVED FROM RECURRENT SELECTION BASED ON GRAIN YIELD

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## INTRODUCTION

Some recurrent selection programs in common bean usually consider only grain yield for selecting the best progenies (Ramalho et al. 2005). Considering that white mold causes drastic reduction in grain yield if preventive measures are not properly taken, it is interesting to check if higher yield as a selection criteria helps to select more resistant genotypes. Genetic resistance to white mold is useful for helping to control the disease. An indirect way of assessing the resistance is through the evaluation of genotypes in the solution of oxalic acid, as described by Kolkman & Kelly (2000). Thus, the objectives were to evaluate the reaction to oxalic acid of the selected progenies of higher grain yield from a recurrent selection program, and also to verify if the *Phs* SCAR marker (Miklas, 2005) is associated to a QTL that confer resistance to white mold, originally presented in three of the parents.

## MATERIALS AND METHODS

From a recurrent selection program aiming to increase the grain yield, there were evaluated fifteen parental lines and the six best progenies of the cycles I, II, III, IV, V, VII, VIII and IX (Ramalho et al. 2005), for resistance to white mold through the *Phs* SCAR developed specifically for this type of resistance (Miklas, 2005). Those progenies and parents were also evaluated for the reaction to oxalic acid using the methodology of Kolkman & Kelly (2000). It was used the randomized complete block design with three replications and each plot consisted of five plants. The evaluation was performed according to the key of scores proposed by Kolkman & Kelly (2000). Analyses of variance were set up considering the average score of the plot, and the mean scores of the progenies were grouped by the Scott-Knott procedure using the GENES software (Cruz, 2006).

## RESULTS AND DISCUSSION

Only three parental lines and one progeny from the first cycle of recurrent selection presented the *Phs* SCAR, which confers resistance to the pathogen in temperate conditions (Miklas, 2005). Those genotypes confirm their resistance to oxalic acid, but other parents and progenies exhibited the same level of resistance and did not have the marker. Considering the reaction of the parents and all progenies to oxalic acid it was found that there was significant difference among the cycles of recurrent selection, among the parental lines and among the progenies within each cycle, except the fourth cycle. Within each cycle, four progenies from the first cycle, two from the cycle II, two from the cycle III, six from the cycle IV, five from the cycle V, three from the cycle VII, four from the cycle VIII and five from the cycle IX, belong to the resistant group, i.e. 64.58% of the progeny have a higher resistance level. It is noteworthy that only in cycle II and III most of the progenies is present in the susceptible group, otherwise in cycle IV all of them belong to the resistant group. It is inferred, therefore, that the QTL identified by the SCAR did not expressed in the southern region of Minas Gerais, as noted, upon inoculation, by Carneiro et al. (2010). Indeed it is more likely that

others QTLs have expressed their effect of partial resistance instead of the QTL identified by the *Phs* SCAR, as long as it was eliminated during the last cycles of recurrent selection. Considering the twenty-four progenies in the first four cycles of recurrent selection, 58% are among the most resistant to oxalic acid. Among the twenty-four selected in the last four cycles, which were superior in yield, 71% were more resistant to the acid, indicating a tendency of association of the two characters. However, no correlation was found between the performances of the progenies. This lack of association probably is related to the absence of the disease during the conduction of the experiments in the recurrent selection program.

**ACKNOWLEDGEMENTS**

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# FIELD AND GREENHOUSE EVALUATION OF BEAN GERMPLASM FOR ROOT ROT AND OTHER DISEASES IN NEW YORK, 2010

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Root rot is a significant production constraint in beans worldwide in both temperate and tropical soils (Abawi and Pastor-Corrales, 1990) leading to complete crop loss under severe conditions. Two trials were conducted on common bean lines under root rot (RR) conditions in the field at the Cornell's Vegetable Research Farm near Geneva, NY and in a follow-up greenhouse test. A total of 21 bean lines: 10 RR lines provided by USDA-ARS (Mayaguez, PR); nine breeding lines provided by Michigan State University (East Lansing, MI); and 2 controls, Pink panther and CLRK, were evaluated in both trials.

The greenhouse evaluation was conducted using soil from the same RR field. Each bean line was planted in four, 10-cm clay pots (7 seeds/pot) and maintained for 6 weeks. Both Fusarium root rot (Fsp; caused by *Fusarium solani* f. sp. *phaseoli*) and black root rot (Tb; caused by *Thielaviopsis basicola*) were observed on all plants. Symptoms of *Pythium ultimum* (Pu) root rot were also frequently observed, but *Rhizoctonia solani* (Rs) infection symptoms only occasionally. Emergence (# or % emerged); above ground biomass (g); root biomass (g); and root rot severity, 1 (normal roots/healthy) to 9 (>75% of root and stem tissues affected) were assessed (Table 1). The results indicate significant differences in root rot severity ratings, above ground biomass, and root biomass among the materials tested. RR08 and B09135 showed the lowest root rot severity scores; Red Hawk, P7863, and, RR09 had the highest above ground biomass; and RR09, G08256, and P07863 had the highest root biomass. Pasteurized soil controls (data not shown) had higher values for biomass than those grown in RR soil and all root were healthy (RRS = 1).

The field trial was conducted in the root rot evaluation nursery, established about 15 years ago, that is heavily infested with the bean root pathogens: Fsp, Tb, Pu, and Rs. Two trials in a RCBD with 4 replications were conducted, and used single 10 ft. rows (Trial 1, MSU materials) or two 20 ft. rows (Trial 2, ARS materials) spaced 2.5 ft. apart. All the seeds were treated with the fungicides Apron and Maxim as well as the insecticide Cruiser at recommended rates, while the MSU materials were also treated with Streptomycin. All additional maintenance practices were performed according to recommended commercial guidelines. As a result of dry and warm weather conditions early in the growing season, root rot development was low to moderate. Symptoms observed on roots were those diagnostic of Fsp, Pu, Rs, and Tb, in descending order. Common Bacterial Blight (CBB) was also evaluated, as severe natural incidence was observed. Limited symptoms of Bean Yellow Mosaic Virus (BYMV) were present. Emergence; root rot severity; root weight; vigor (1 = most vigorous, high yield potential and 9 = least growth, poor yield potential); CBB (1 = no symptoms observed to 9 = most severe), data were recorded (Table 1). RRS ratings ranged from 3.1 (Zorro) to 5.3 (Red Hawk). RR05, RR07, RR09, RR30, and RR31 showed high rates of emergence and RR01, RR05, RR06, RR07 showed high vigor and yield potential (scores <=3) in Trial 2. In addition, several lines exhibited a resistant reaction (<=3) to CBB including B09135, RR16, RR07, RR31, B05055, B09197, and P07863. RR08 and RR031 exhibited the least and most severe BYMV symptoms, respectively.

**Table 1.** Results of greenhouse and field evaluations of bean germplasm under root rot conditions at the Vegetable Research Farm, NYSAES, Geneva, NY, 2010.

| | 2010 Greenhouse Study | | | | 2010 Field Study | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Genotype | Emergence | Above ground biomass | Root biomass | Root rot severity | Emergence | Root rot severity | Common bacterial blight | Vigor | BYMV Virus |
| **Trial 1** | No./pot | g | g | 1-9 scale | % in 20' | 1-9 scale | 1-9 scale | 1-9 scale | 1-9 scale |
| Red Hawk | 7 | 40.2 | 7.8 | 7.8 | 37.3 | 5.3 | 4.5 | 5 | 3.5 |
| B09135 | 6.8 | 20.8 | 4.2 | 3.8 | 37.8 | 3.8 | 1 | 5.3 | 4 |
| B09197 | 6.5 | 20.6 | 4.4 | 4.8 | 40.5 | 4.8 | 1.5 | 4.8 | 3 |
| G08256 | 7 | 29.7 | 8.2 | 7 | 46 | 4.5 | 7.3 | 5 | 3.8 |
| S08419 | 7 | 32.1 | 5.1 | 7.5 | 28.5 | 4 | 7.3 | 4.5 | 2.5 |
| Zorro | 7 | 19.1 | 4.3 | 4.5 | 40 | 3.1 | 6.3 | 4.8 | 3.5 |
| P07863 | 6.5 | 37.9 | 8.1 | 6.8 | 34.5 | 3.5 | 2.5 | 5.3 | 1.8 |
| Pink panther | 6.3 | 27 | 5.8 | 7.8 | | | | | |
| CLRK | 7 | 28.1 | 6.4 | 7.8 | | | | | |
| Mean | 6.8 | 28.4 | 6.0 | 6.4 | 37.8 | 4.1 | 4.3 | 5.0 | 3.2 |
| LSD | 0.6 | 5.8 | 1.9 | 0.9 | 7.5 | 1.3 | 1.9 | 1.6 | 1.1 |
| **Trial 2** | | | | | | | | | |
| RR001 | 7 | 23.5 | 4.6 | 4.8 | 88.5 | 4.02 | 5 | 2.8 | 2.5 |
| RR005 | 7 | 29.5 | 4.2 | 5.8 | 100 | 3.68 | 4.5 | 2.5 | 2.8 |
| RR006 | 7 | 32.3 | 6.5 | 4.8 | 96.3 | 3.69 | 5 | 3 | 2.3 |
| RR007 | 7 | 30.6 | 7.3 | 4 | 100.3 | 3.74 | 1.3 | 2.5 | 2.5 |
| RR008 | 6.5 | 16.6 | 5 | 2.8 | 92.5 | 3.48 | 4 | 5.8 | 1.3 |
| RR009 | 7 | 36.4 | 9.4 | 5.3 | 100.5 | 4.08 | 6.8 | 4 | 2.3 |
| RR016 | 6.8 | 26.6 | 5.4 | 5.3 | 95 | 3.26 | 1 | 3.8 | 1.5 |
| RR019 | 5.8 | 21 | 4.4 | 7.8 | 82.3 | 4.07 | 4.3 | 4.5 | 1.8 |
| RR030 | 7 | 24 | 5.3 | 5.5 | 102.5 | 3.8 | 6.5 | 4.5 | 2.8 |
| RR031 | 7 | 24.7 | 5.1 | 4 | 101.5 | 4.61 | 1.3 | 4 | 4.5 |
| B05055 | 7 | 21.6 | 4 | 4.5 | 79.3 | 4.48 | 1.5 | 4.3 | 2.8 |
| N05311 | 6.5 | 19.4 | 3.2 | 7.3 | 76.8 | 3.59 | 7 | 5 | 3.5 |
| Pink panther | 7 | 34 | 7.6 | 7.5 | 82.5 | 4.24 | 8.3 | 4.8 | 3.3 |
| CLRK | 7 | 29.1 | 5.8 | 8 | 94.7 | 5.21 | 8.3 | 5.3 | 4 |
| Mean | 6.8 | 26.4 | 5.6 | 5.5 | 92.3 | 4.0 | 4.6 | 4.1 | 2.7 |
| LSD | 0.6 | 5.8 | 1.9 | 0.9 | 9.8 | 0.8 | 1.5 | 1.6 | 1.2 |

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# RESISTANCE OF COMMON BEAN TO FUSARIUM ROOT ROT

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## INTRODUCTION

Fusarium root rot caused by *Fusarium solani* f. sp. *phaseoli* (*Fsp*) is one of the most important common bean diseases caused by soilborne pathogens. There is no source of complete resistance to this disease. Although partial resistance is available in the common bean germplasm and probably among the common bean lines and cultivars originated from Brazilian breeding programs, evaluation of reaction of these lines and cultivars to the disease has not been done. This study aimed to test bean lines and cultivars for reaction to Fusarium root rot.

## MATERIALS AND METHODS

Ninety six common bean lines and cultivars (Table 1) were tested in greenhouse and in field for reaction to Fusarium root rot. These genotypes are from the bean breeding program of the Agricultural Research Institute of the State of Minas Gerais (Epamig), Viçosa Federal University (UFV), Lavras Federal University (UFLA) and Brazilian Agricultural Research Corporation (Embrapa). In greenhouse, treatments were replicated five times in a randomized block design. Each replication consisted of one 1.0 L-pot with three plants per pot. Soil was infested with 4,000 chlamydospores/g of soil before sowing. Two field experiments were carried out at an area naturally infested with *Fsp*. Treatments were replicated three times in a randomized block design. Each plot was two rows 0.5 m apart and 2 m long with 15 plants per meter. Plants were rated for disease severity according to the scale (1 to 9) proposed by Abawi & Pastor-Corrales (1990) at 15, 30 and 45 days after emergence. The area under the disease progress curve (AUDPC) was calculated using severity data from each treatment. Later, the variance analysis was carried out and the averages were compared by the Scott-Knott test ($P < 0.05$).

## RESULTS AND DISCUSSION

Disease severity scores varied with the genotype. The most susceptible and resistant genotypes are showed in Table 2. Andean genotypes (jalo type) were related to the group of the most susceptible genotypes and some of the black group (Mesoamerican genotypes) to the more resistant. Results of greenhouse and field experiments were significantly correlated, indicating that the use of 4,000 chlamydospores $g^{-1}$ of substrate is reliable to evaluate large-scale screenings of bean germplasm. These results will be considered before release of new cultivars.

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**Table 1-** Genotypes tested for reaction to Fusarium root rot.

| Genotype | Market class | Genotype | Market class | Genotype | Market class |
|---|---|---|---|---|---|
| CNFP 9328 | Black | MN-34-46 | Black | VI 16-3-4 | Red |
| CNFP 7994 | Black | MN-34-44 | Black | Ouro Vermelho | Red |
| CNFP 10798 | Black | CNFC 10720 | Carioca | DOR 371 | Red |
| CNFP 7966 | Black | CNFC 9504 | Carioca | RAB 94 | Red |
| CNFP 8096 | Black | CNFC 9506 | Carioca | CNFRX 8144 | Violet |
| CNFP 10773 | Black | CNFC 10764 | Carioca | CNFRX 10535 | Violet |
| CNFP 10117 | Black | CNFC 10722 | Carioca | CNFRX 10531 | Violet |
| CNFP 10180 | Black | CNFC 9500 | Carioca | CNFR 8149 | Violet |
| CNFP 8108 | Black | BRS 9461 | Carioca | CNFR 7847 | Violet |
| CNFP 7726 | Black | Cometa | Carioca | VR-3 | Violet |
| CNFP 7677 | Black | Pérola | Carioca | VR-12 | Violet |
| CNFP 10047 | Black | RP-2 | Carioca | BRS Pitanga | Violet |
| BRS 8000 | Black | BRSMG Madrepérola | Carioca | Roxo 90 | Violet |
| BRS Supremo | Black | VC-13 | Carioca | Timbó (FEB 163) | Violet |
| BRS Valente | Black | VC-14 | Carioca | Bambuí | Beige |
| BRS Campeiro | Black | VC-15 | Carioca | A-300 | Beige |
| VP-14 | Black | VCIII-39-24 | Carioca | BAT 332 | Beige |
| VP-15 | Black | BP-31 | Carioca | IAC Bico de Ouro | Beige |
| VP-16 | Black | Talismã | Carioca | Costa Rica | Beige |
| VP-17 | Black | BRSMG Majestoso | Carioca | CNFRJ 10571* | Jalo |
| VP-18 | Black | VC-16 | Carioca | CNFRJ 10564* | Jalo |
| VP-19 | Black | Pioneiro | Carioca | CNFRJ 10556* | Jalo |
| VP-20 | Black | MAII-2 | Carioca | Jalo EEP558* | Jalo |
| VP-21 | Black | MAII-16 | Carioca | BJ-1* | Jalo |
| VP-22 | Black | MAII-22 | Carioca | BJ-2* | Jalo |
| VP-23 | Black | RP-1 | Carioca | BJ-3* | Jalo |
| Ouro Negro | Black | CVIII-85-11 | Carioca | BRSMG União* | Jalo |
| CNFP 10802 | Black | VCIII-119-4 | Carioca | BJ-5* | Jalo |
| MN-37-2 | Black | Pitoco | Carioca | BJ-6* | Jalo |
| MN-34-20 | Black | AB-136 | Red | BJ-7* | Jalo |
| MN-34-66 | Black | BP-9116396 | Red | BJ-8* | Jalo |
| MN-34-53 | Black | AFR 188 | Red | Jalo MG 65* | Jalo |

*large seeds

**Table 2** - Most resistant and susceptible genotypes to Fusarium root rot.

| | Greenhouse experiment | | First field experiment | | Second field experiment | |
|---|---|---|---|---|---|---|
| | Severity (%)[1] | AUDPC[2] | Severity (%) | AUDPC | Severity (%) | AUDPC |
| Susceptible | | | | | | |
| BJ-8 | 45.0 | 1256.3 a | 45.7 | 1022.8 a | 49.9 | 1338.0 a |
| BRSMG Majestoso | 35.0 | 1050.0 a | 34.5 | 1084.0 a | 40.5 | 1194.3 a |
| BJ-2 | 35.0 | 1016.3 a | 40.2 | 1069.3 a | 43.0 | 1111.8 a |
| CNFRJ 10564 | 32.5 | 1001.3 a | 37.1 | 1131.3 a | 40.0 | 1065.3 a |
| CNFRJ 10556 | 35.0 | 1038.8 a | 29.3 | 1010.8 a | 42.4 | 1094.5 a |
| BJ-5 | 35.0 | 1027.5 a | 37.8 | 1006.3 a | 42.0 | 1089.8 a |
| BJ-1 | 32.5 | 1008.8 a | 37.6 | 1011.5 a | 38.5 | 1059.3 a |
| Resistant | | | | | | |
| VP-17 | 22.0 | 615.0 d | 21.6 | 712.3 b | 24.6 | 788.5 b |
| VP-18 | 17.5 | 566.3 d | 22.6 | 740.8 b | 25.1 | 804.3 b |
| MN-34-44 | 17,5 | 528.8 d | 28.9 | 804.5 b | 26.8 | 769.3 b |
| BP-9116396 | 19.0 | 536.3 d | 24.3 | 605.3 b | 22.3 | 771.8 b |
| MN-37-2 | 19.0 | 517.5 d | 16.8 | 618.3 b | 23.2 | 722.0 b |
| MN-34-20 | 17.5 | 506.3 d | 14.7 | 580.5 b | 22.5 | 758.0 b |
| A-300 | 17,5 | 506.3 d | 20.8 | 758.8 b | 18.1 | 696.3 b |
| MN-34-66 | 13.0 | 416.3 d | 22.0 | 698.3 b | 12.2 | 588.0 b |
| RP-2 | 19.0 | 577.5 d | 14.9 | 547.5 b | 21.8 | 757.0 b |
| RP-1 | 13.0 | 408.8 d | 21.8 | 541.8 b | 18.0 | 662.3 b |

[1] Percentage of lesions on hypocotyls and root tissues at 30 days after emergence; [2] AUDPC = area under disease progress curve. Averages followed by the same letter in column do not differ by Scott-Knott test ($P < 0.05$).

# ASSESSMENT OF *PYTHIUM ULTIMUM* TROW PATHOGENICITY IN DIFFERENT BACKCROSS PROGENIES OF COMMON BEAN

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## INTRODUCTION

Bean root rot disease caused by *Pythium* spp. is an important constraint of the cultivation of *Phaseolus vulgaris* L. in East and Central Africa (Otsyula et al., 2003). Different control methods (chemical, biological, cultural and genetic) can be used to limit the damages due to this disease (Spence, 2003). RWR 719, a small seeded variety from the Mesoamerican gene pool, and AND1062, a large seeded variety from the Andean gene pool, are known as resistant to all species of *Pythium* isolated in the Great Lakes Region (Mukalazi et al., 2001). Resistance to bean root rot is controlled by a single dominant gene (Mahuku et al., 2007). A SCAR marker named PYAA $19_{800}$ was found to be associated with *Pythium* root rot resistance gene in RWR 719 and AND 1062 (Mahuku, 2007).To introduce this genetic resistance to common bean, a backcrossing program was undertaken between the donor resistant varieties and different recurrent susceptible varieties grown in Rwanda. Our objective was to carry out pathogenicity tests to evaluate the efficiency of this breeding strategy.

## MATERIALS AND METHODS

Prior to the pathogenicity tests, molecular analyses were performed from backcross progenies, generation 1 (BC1) to generation 4 (BC4) to identify individuals having the molecular marker linked to the resistance gene. At each BC generation, only individuals with this molecular marker were included in backcrosses with the recurrent varieties. The BC4 progenies having the molecular marker linked to the resistance gene were submitted to the ultimate pathogenicity tests carried out in screen house. Seeds of three susceptible cultivars (R617-97A, RWR 1668 and Urugezi) were provided by the Rwanda National Bean Program, while seeds of two resistant varieties (RWR 719 and AND 1062) were provided by the CIAT Regional Office in Uganda. Seeds obtained from the crosses between each of the 3 susceptible varieties and each of the 2 resistant varieties were planted in inoculated soil in wooden trays.The experimental investigations were carried out in a screen house at Kawanda (Uganda), a CIAT regional centre. This site is located at 0°25′05″ N and 32°31′54″ E and at 1190 meters above sea level (masl) with an average rainfall of 1224 mm per annum and average daily temperatures of 15.3°C (minimum) and 27.3°C (maximum). In the pathogenicity study, two genotypes: CAL 96, a susceptible line from CIAT and the resistant RWR 719 were used as reference for the inoculation tests (Otsyula et al., 2003). Inoculum of P. ultimum strain was multiplied by plating mycelia on autoclaved millet grains (100 g) mixed with 200 ml of water in 500 ml bottles. After two weeks of incubation under darkness at 25°C, pre sterilized soil was mixed with the infested millet at a ratio of 1:10 v/v in wooden trays of 42 cm x 72 cm. Each tray contained 10 plants of each bean class of materials, i.e. 2 controls and 6 BC 4 progenies (table 1). The trays were set up in a completely randomized block design with three replications. After germination, the seedlings were watered two times per day to provide a favorable environment for the pathogen establishment and development. Three weeks after emergence of the seedlings, the surviving plants were uprooted and washed with water to remove soil. Severity of root rot symptoms was then

assessed using the CIAT visual 1-9 scale (Abawi and Pastor- Corrales, 1990). Genotypes that had an average disease score of 1-2 were considered as resistant while those with an average score of 3-5 were considered as tolerant and those with an average score of 6-9 were considered as susceptible (Abawi and Pastor-Corrales, 1990).

## RESULTS AND DISCUSSION

Disease severity scoring from the pathogenicity tests on BC4 progenies are presented in table 1. According to the scores, CAL 96 and RWR 719 were confirmed as the susceptible and resistant references, respectively. The 6 BC4 progenies showed an average level of root rot severity intermediate between values observed in the 2 references. Two BC4 progenies were classified as tolerant and the other four were classified as resistant.

**Table 1.** Bean root rot severity in 6 BC4 progenies and 2 references (30 plants per class of materials)

| Classes of material | Mean | t Grouping | Reaction to disease |
|---|---|---|---|
| CAL 96 | 8.47 | A | Susceptible |
| URUGEZI X RWR 719 | 3.73 | B | Tolerant |
| R 617-97A X RWR 719 | 3.20 | BC | Tolerant |
| R 617-97A X AND 1062 | 2.90 | DC | Resistant |
| URUGEZI X AND 1062 | 2.73 | DC | Resistant |
| RWR 1668 X AND 1062 | 2.67 | DC | Resistant |
| RWR 1668 X RWR 719 | 2.23 | D | Resistant |
| RWR 719 | 1.33 | E | Resistant |

Means with the same letter are not significantly different, $\alpha$= 0.05, LSD= 0.76, SE=0.27, Pr> |t|<.0001.

The backcrossing program allows us to select recurrent common bean varieties keeping the resistant gene. On the basis of our results and those from Buruchara and Kimani (1999) and Buruchara et al. (2001), segregation of disease reaction in each combination is similar, whatever the origin of parental genotypes. The implication of resistant varieties from the two common bean gene pool does not modify the disease reaction of the progenies.

## ACKNOWLEDGEMENT

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# EFFICACY OF TRICHODERMA-BASED COMMERCIAL PRODUCTS IN CONTROLLING FUSARIUM ROOT ROT ON COMMON BEANS

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## INTRODUCTION

Fusarium root rot caused by *Fusarium solani* f. sp. *phaseoli* (*Fsp*) is a widely distributed disease of common bean in Brazil, especially in irrigated areas. Seed treatment with fungicide, crop rotation, and reduction of soil compaction are among the main strategies to control the disease (Abawi and Pastor-Corrales, 1990). One of the most effective fungicides for chemical seed treatment against *Fsp* is fludioxonil. Interest in biological control has increased because of concerns about the economic, environmental, and health costs of chemical crop protection practices. *Trichoderma* species are among the fungi with the highest potential for soilborne disease control. The aim of this study was to test the efficacy of six Trichoderma-based commercial products (TCP) available in Brazil for Fusarium root rot control.

## MATERIALS AND METHODS

An experiment was carried out during the winter at an area naturally infested with *Fsp* in Oratórios, State of Minas Gerais, Brazil. One TCP was used for seed treatment, three for distribution in the furrow, and two for both methods of application (Table 1). A seed treatment with the fungicide fludioxonil and an untreated control were used for comparisons. The cultivar Ouro Vermelho (type II, red seeds) was sown in plots with four rows 0.5 m apart and 2 m long, with 10 plants per meter. The statistical design was a randomized complete block with six replicates. At planting, a commercial fertilizer (4N:14P:8K) was applied in bands along with the seeds at 600 kg $ha^{-1}$. Urea was applied as a side dressing at 15 DAE. At this time, plants were also sprayed with sodium molybdate solution at 200 g $ha^{-1}$. The area was sprinkler irrigated weekly with 40 mm of water. Plants were preventively sprayed with the fungicide azoxystrobin (50 g $ha^{-1}$) at 44 DAE in order to control angular leaf spot. Incidence was calculated as the percentage of plants with Fusarium root rot symptoms. Plants were rated for disease severity index (DSI) by means of a scale (Van Schoonhoven & Pastor-Corrales, 1987). DSI was calculated on a percentage basis:

$$DSI(\%) = \frac{\sum(scores\ of\ all\ plants)}{9\ x\ (total\ number\ of\ plants)} \times 100$$

Yield was estimated based on mass of seeds with 12% moisture (w/w) harvested in the two central lines. Analysis of variance was performed.

## RESULTS AND DISCUSSION

In general, fungicide and TCP improved seedling emergence compared with untreated control (Table 2). Three TCP, embracing distribution in the furrow and seed treatment, were as effective as the

fungicide in increasing the percentage of emerged seedlings. Treatments did not affect disease incidence, but all TCP and the fungicide decreased disease severity relative to untreated control. Yield was not affect by the treatments. We conclude that, regardless of the method of application, all TCP are as effective as fungicide for the Fusarium root rot management.

**Table 1** - Trichoderma-based commercial products and fungicide tested for Fusarium root rot control on common beans. Oratórios, MG, Brazil, 2008.

| Treatment | Formulation | Application mode | Rate |
|---|---|---|---|
| Trichodermil SC® | SC | In the furrow | 1000 mL $ha^{-1}$ |
| Trichodermax Plus® | DP | Seed treatment | 200 g 100 $kg^{-1}$ seeds |
| Trichodermax CE® | EC | In the furrow | 1000 mL $ha^{-1}$ |
| Quality WG® | WG | Seed treatment | 75 g 100 $kg^{-1}$ seeds |
| Quality WG® | WG | In the furrow | 100 g $ha^{-1}$ |
| Trichoderma JCO® | WSP | In the furrow | 2 kg $ha^{-1}$ |
| Trichodel Solo® | SC | Seed treatment | 500 mL 100 $kg^{-1}$ seeds |
| Trichodel Solo® | SC | In the furrow | 1500 mL $ha^{-1}$ |
| Fludioxonil (Maxim®) | SC | Seed treatment | 200 mL 100 $kg^{-1}$ seeds |
| Untreated control[a] | --- | --- | --- |

[a] Water was applied in the furrow (0.5 L $m^{-1}$).

**Table 2 -** Effects of Trichoderma-based commercial products on seedlings emergence, disease intensity and yield. Oratórios, MG, Brazil, 2008.

| Treatment | Seedlings emerged (%) | *Fsp* population density at 25 DAE ($10^4$ cfu $g^{-1}$ of soil) | Incidence (%) | | DSI[1] (%) | | Yield (kg $ha^{-1}$) |
|---|---|---|---|---|---|---|---|
| | | | 15 DAE[2] | 25 DAE | 15 DAE | 25 DAE | |
| Trichodermil SC®[3] | 68.7 † | 3.60 | 92 | 98 | 18.3 † | 15.2 † | 2690 |
| Trichodermax Plus®[4] | 62.4 * | 3.85 | 88 | 98 | 12.2 † | 19.1 † | 2110 |
| Trichodermax CE®[3] | 67.2 † | 4.00 | 90 | 98 | 17.4 † | 15.8 † | 2533 |
| Quality WG®[4] | 62.0 * | 4.70 | 88 | 98 | 17.1 † | 14.8 † | 2145 |
| Quality WG®[3] | 66.1 † * | 4.70 | 86 | 100 | 19.6 † | 23.8 † | 2279 |
| Trichoderma JCO®[3] | 61.4 * | 4.80 | 92 | 96 | 19.9 † | 18.5 † | 2442 |
| Trichodel Solo®[4] | 69.9 † | 5.55 | 86 | 98 | 16.4 † | 17.8 † | 2327 |
| Trichodel Solo®[3] | 66.4 † * | 4.00 | 92 | 100 | 17.1 † | 18.9 † | 2094 |
| Fludioxonil [4] | 72.3 † | 2.65 | 86 | 100 | 11.7 † | 17.2 † | 2266 |
| Untreated control | 56.5 * | 6.25 | 100 | 100 | 32.8 * | 37.1 * | 2637 |
| CV (%) | 4.7 | 28.1 | 16.1 | 8.2 | 18.5 | 12.6 | 16.4 |

*Fsp* = *Fusarium solani* f. sp. *phaseoli*; in columns, means followed by † differ significantly from untreated control and means followed by * differ significantly from fludioxonil according to Dunnett's test ($P < 0.05$); [1]DSI (%) = disease severity index; [2]DAE = days after seedling emergence; [3]application in the furrow; [4]seed treatment. Data of seedlings emerged and cfu of *Fsp* $g^{-1}$ of soil were transformed using square root before analysis, but untransformed means are presented. Data of incidence and DSI were transformed using arcsine square root before analysis, but untransformed means are presented.

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# SEED MASS AFFECTS ROOT CHARACTERISTICS OF COMMON BEAN

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## INTRODUCTION

Studies related to root characteristics of common beans have increased in the past 15 years. In many of these studies, attention is given to the uniformity of seed mass used in the trials whereas other studies give no importance to this issue. The objective of this study was to evaluate the effect of seed mass on root characteristics of common bean.

## MATERIALS AND METHODS

Seeds of the cultivar 'Ouro Negro' (black seeds, type III plants) were harvested and threshed by hand. Next, each seed was weighed and classified in five classes (see Table). Seeds were then germinated according to methodology described by Vieira et al. (2007). Five-day seedlings were use to evaluate root characteristics. Data were analyzed as a completely randomized design, with six replicates. Each replicate consisted of four seedlings.

## RESULTS AND DISCUSSION

Seedlings originated from the heaviest seed class (0.36 to 0.39 g) had more basal roots than seedlings from seed classes below 0.27 g (Table 1). Root length and mass of basal roots were affected more clearly by seed mass than their numbers. Primary root dry mass was affected markedly by the treatments: seedlings from the heaviest seeds presented more than twice the primary root mass compared with those from the lightest seeds. Seed mass between 0.28 and 0.35 g had no effect on the root characteristics. These results suggest that seeds used for studies related to root characteristics should be strictly selected according to mass.

**Table 1** - Root characteristics of common bean 'Ouro Negro' in response to five classes of seed mass. Values shown are means ± SD. Mean comparisons in the columns were performed using Duncan test ($\alpha = 0.05$)

| Seed mass (g) | Number of whorl | Number of basal root | Basal root lenght (cm) | Basal root dry mass (mg) | Primary root dry mass (mg) |
|---|---|---|---|---|---|
| 0.36 to 0.39 | 2.60 ± 0.43 a | 9.43 ± 1.47 a | 68.4 ± 10.2 a | 12.3 ± 0.3 a | 14.7 ± 2.8 a |
| 0.32 to 0.35 | 2.33 ± 0.48 ab | 8.68 ± 0.75 abc | 58.9 ± 6.7 b | 11.1 ± 0.6 b | 12.2 ± 2.3 b |
| 0.28 to 0.31 | 2.48 ± 0.35 a | 9.10 ± 1.02 ab | 57.3 ± 7.5 b | 10.5 ± 1.1 bc | 10.6 ± 1.3 bc |
| 0.24 to 0.27 | 2.05 ± 0.16 b | 8.00 ± 0.33 c | 52.4 ± 11.4 bc | 9.8 ± 1.2 c | 9.1 ± 2.2 c |
| 0.20 to 0.23 | 2.28 ± 0.36 ab | 8.29 ± 0.69 bc | 47.9 ± 6.3 c | 7.9 ± 1.3 d | 6.9 ± 1.6 d |

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# MIXTURE OF CULTIVAR OF CONTRASTING ROOT ARCHITECTURE INCREASES COMMON BEAN SEED YIELD

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## INTRODUCTION

Diseases, drought, and low soil fertility are among the most widespread and endemic production problems of common bean in Brazil. The capacity of plants to absorb both water and mineral nutrients from the soil is related to the development of an extensive and well-positioned root system. Among the nutrients needed by plants, phosphorus (P) is the main constraint to plant growth. Because the topsoil is generally the soil stratum with the greatest P availability, the extent of topsoil foraging is an important aspect of P acquisition in most soils. Basal root shallowness is related to differences in genotypic tolerance to low P, but this characteristic is inversely related to tolerance to drought. The use of multilines (cultivar mixtures) in which each line has contrasting root architecture could optimize soil resources utilization and decrease belowground competition. We evaluated the benefit of using these multilines on common bean seed yield under no water stress.

## MATERIALS AND METHODS

One experiment was conducted in a clay soil in Coimbra, MG, Brazil, in an area under no-tillage system for at least 10 years. Soil pH (in water) was 5.2 and P (Mehlich 1), in mg $dm^3$, decreased from 9.9 to 5.9 from 0-5 to 15-20 cm soil layer. Treatments allocation to experimental units followed a completely randomized block design with four replications and a split-plot arrangement. Fertilization (with or without) were allocated to plots and cultivars (Diamante Negro, Vi-1-2-1 and D. Negro + Vi-10-2-1) to sub-plots. Fertilized plots received 350 kg $ha^{-1}$ of the formulation 8-28-16 ($N - P_2O_5 - K_2O$). Both cultivars have black seeds and type II indeterminate plants. D. Negro was identified as shallow rooted and Vi-10-2-1 as deep rooted (Vieira et al., 2008). Both have weak root hairs in both basal and primary roots (Vieira et al., 2007). Sowing of each genotype was done by hand in plot with four rows 4 m in length, 50 cm apart. For the treatment D. Negro + Vi-10-2-1, plants of different genotypes were intercalated (10 cm between plants) in the row and were harvested separately. Sprinkler irrigation was used and application of herbicide, insecticide and fungicide were made as needed.

## RESULTS AND DISCUSSION

In the fertilized plots, cultivar mixture provided higher yield than that achieved by one of the components (see Figure 1). In the unfertilized plots, cultivar mixture yield exceeded yield of the other treatments. Diversity can play an important role in the control of pest and pathogens. However, in this experiment, disease, pest, and weeds were controlled. Furthermore, water was not a limiting factor. The data of nutrient acquisition by the cultivars in monoculture and in mixture and of root by soil layers (not available yet) will be helpful in understanding these results.

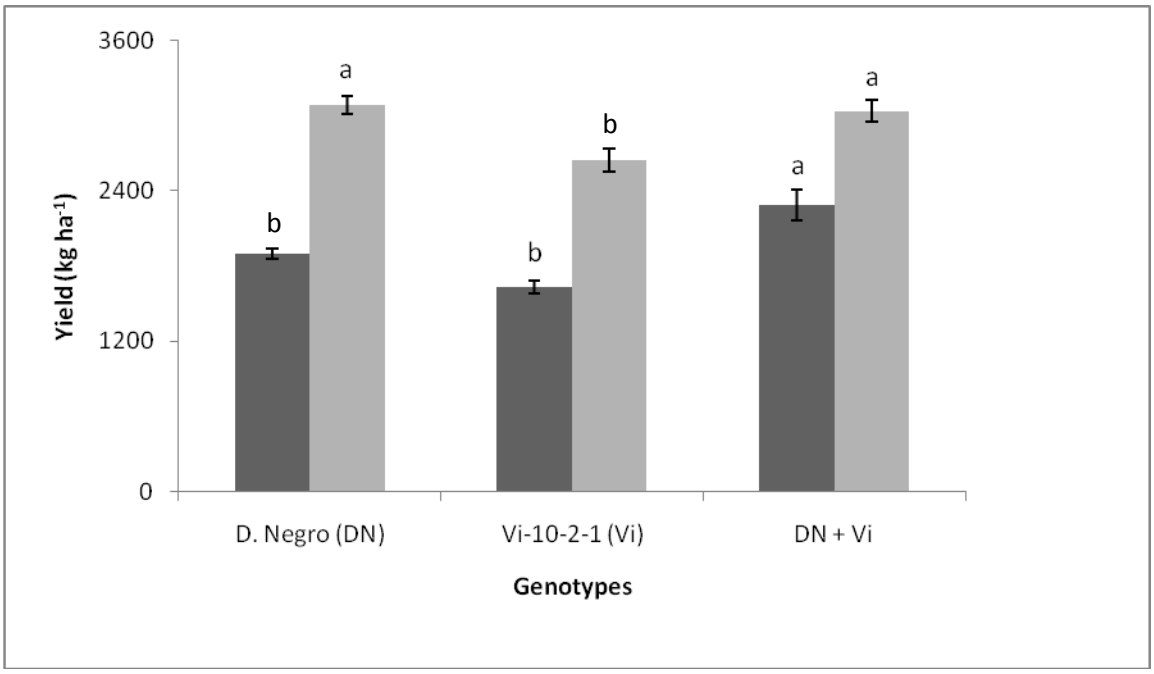


**Figure 1 -** Mean yield (± SD) of dry bean cultivars with contrasting root characteristics in monoculture and in mixture (DN + Vi) in unfertilized (dark gray) and fertilized (light gray) plots. Letters over each bar within each fertilizer treatment compare genotypes by Duncan test ($P < 0.05$).

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# IDENTIFICATION OF SOURCES OF RHIZOCTONIA ROOT ROT RESISTANCE IN DROUGHT TOLERANT DRY BEANS

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## INTRODUCTION

*Rhizoctonia solani* causes root rot, a potentially serious disease that may cause severe economic losses if it has the right environmental conditions. Since root health is a vital factor in plant development, root diseases would influence water and nutrient uptake. Urrea (2010) previously evaluated two sets of bean entries for drought tolerance at Mitchell, NE and Fortuna, PR. The first set coded as NE-08 contained USA dry bean breeding lines, commercial cultivars, tepary beans (*Phaseolus acutifolius* A. Gray) and germplasm accessions from the National Plant Germplasm System. The second set (NE-14) was composed of 111 entries from a shuttle breeding program between Nebraska and Puerto Rico initiated in 2007. The goal of this research was to identify drought tolerant bean germplasm that also exhibits rhizoctonia root rot resistance.

## METHODOLOGY

An efficient rhizoctonia root rot screening method was developed and used to identify bean lines resistant to this disease (Peña et al, 2010). Two sets of dry bean germplasm and breeding lines (NE-08 and NE-14) were analyzed separately to identify sources of root rot resistance in an Alpha Lattice design. The NE-08 and NE-14 sets were composed of 163 and 111 entries respectively. The isolate WN-11 (subspecies group AG-2-2 IV) of *R. solani*, was used for inoculation. Plants were grown in the greenhouse at 24 ± 2°C and evaluated 15 days after soil inoculation. Each plant was scored based on above ground plant symptoms and root lesions using the CIAT scale of 1 (resistant) to 9 (susceptible) (Schoonhoven and Pastor-Corrales, 1987). The lines with the best performance were identified and tested again to confirm root rot resistance. This root rot data was also correlated with yield data under drought stress and non stress conditions from Urrea and Porch (2010) using Pearson's correlation.

## RESULTS AND DISCUSION

The entries from NE-08 with the lowest *R. solani* root rot scores exhibited mostly intermediate resistance with an overall mean of 7. Most of these are from Middle America with a growth habit type III or II, and black was the predominant seed class (Table 1). The 15 entries with the lowest scores were screened again to confirm their intermediate resistance reaction and the mean score for these ranged from 1.7 to 3.9. The overall mean of the root rot scores for the NE-14 set was 6.4 (Table 2), indicating overall high susceptibility of these entries. Some entries from this nursery were selected and tested again for partial or intermediate resistance and the resistance was confirmed, with mean scores from 2.6 to 5.7. There was no significant correlation between drought tolerance and rhizoctonia root rot resistance in either NE-08 or NE-14 lines. However, there were drought tolerant lines that also had root rot resistance. Entries with both traits can be used as parents in breeding programs where improvement of both drought tolerance and rhizoctonia root rot resistance is needed.

**Table 1.** Entries from the NE-08 set selected for partial resistance to rhizoctonia root rot and drought tolerance (Yield Kg ha$^{-1}$)

| Line | Source | Origin | Growth habit | Seed color | Root rot mean† | NSC* | DSC** | GMC*** |
|---|---|---|---|---|---|---|---|---|
| A774 | Cultivar CIAT | Brazil | III | Cream | 4.6 | 2194 | 1429 | 1771 |
| SEA 15 | Exp. lines | CIAT | III | Red | 5.3 | 2146 | 1517 | 1804 |
| LEF 2RB | Exp. lines | CO, US | III | Cream stripped | 5.3 | 2430 | 1725 | 2047 |
| PI 310739 | NPGS | Guatemala | III/IV | Black | 5.4 | 1714 | 1416 | 1558 |
| VAX 1 | Exp. lines | ID, US | III | Carioca | 5.6 | 2327 | 1404 | 1807 |
| NE25-07-17 | Exp. lines | NE, US | II | Pinto | 5.7 | 2476 | 1574 | 1974 |
| NE25-07-18 | Exp. Lines | NE, US | II | Pinto | 6.5 | 2179 | 1715 | 1933 |

†Mean of root score based on a scale 1 to 9. 1 to 3= resistant; 4 to 6= intermediate; 7 to 9= susceptible (Schoonhoven and Pastor-Corrales, 1987); * NSC: Non stressed combined. **DSC: Drought stress combined. *** GMC: Geometric mean combined. Growth habit: I= determinate bush; II= indeterminate upright; III= indeterminate semipostrate vine; IV= indeterminate climbing vine. Overall mean: 7.02; LSD (0.05*): 1.74; CV%: 17.8. Confirmation of resistance: Overall mean: 6.43; LSD (0.05*): 1.79; CV%: 19.9; $^{**}P < 0.01$

**Table 2.** Entries from the NE-14 set selected for partial resistance to rhizoctonia root rot and drought tolerance (Yield kg ha$^{-1}$).

| Line code | Seed type | Root rot mean† | NSC* | DSC** | GMC*** |
|---|---|---|---|---|---|
| NE14-08-176 | Black | 4.3 | 1867 | 2573 | 2192 |
| NE14-08-225 | Black | 4.7 | 1502 | 2576 | 1967 |
| NE14-08-307 | Black | 6.3 | 1488 | 3084 | 2142 |
| NE14-08-314 | Black | 6.3 | 1609 | 2807 | 2125 |
| NE14-08-253 | Great northern | 7.5 | 1966 | 2270 | 2113 |
| NE14-08-76 | Navy | 7.7 | 1808 | 2321 | 2048 |
| NE14-08-79 | Navy | 7.9 | 2010 | 2660 | 2312 |

†Mean of root score based on a scale 1 to 9. 1 to 3= resistant; 4 to 6= intermediate; 7 to 9= susceptible (Schoonhoven and Pastor-Corrales, 1987); * NSC: Non stressed combined. **DSC: Drought stress combined. *** GMC: Geometric mean combined. Overall mean: 6.43; LSD (0.05*): 1.79; CV%: 19.9; $^{**}P < 0.01$

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# IMPROVING DRY BEAN PRODUCTION SYSTEMS UNDER LIMITED IRRIGATION BY INTEGRATING VARIETY DROUGHT TOLERANCE, SOIL WATER BASED IRRIGATION SCHEDULING, AND ALLEVIATION OF SOIL COMPACTION

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## INTRODUCTION

Previous research has examined individual factors that may affect dry bean yield under limited irrigation. Urrea and Yonts are currently evaluating dry bean germplasm under different limited irrigation regimes. They are finding that varieties differ in their response to the timing and amount of irrigation. Yonts (personal communication) evaluated the impact of withholding irrigation during different portions of the growing season using a single dry bean variety. Delaying the initiation of irrigation by one and two weeks delayed maturity by 3 and 6 days, and reduced yield by 5 and 15%, respectively. Water stress induced at the end of the growing season also delayed maturity and suppressed yield, but the impact was less severe because the plants had more extensive root systems later in the season. Harveson et al. (2005) found that soil compaction (one level) substantially reduced yield of one bean variety under full irrigation. The next step to improve dry bean yield and quality under limited irrigation is to evaluate these factors in combination to develop the optimal combination of variety, irrigation scheduling, and alleviation of compaction.

## METHODOLOGY

In June 24, 2009, plots were established at the University of Nebraska Panhandle Research and Extension Center-Scottsbluff (41°53.6′ N, 103°40.7′ W, 1200 m elevation). Treatments included combinations of variety, irrigation scheduling, and soil compaction. A strip-split plot design was used to test the treatments. The strip corresponded to levels of compaction (non-compacted, moderately compacted, and heavily compacted). Compaction was achieved by driving over the appropriate strips with a tandem axle truck weighing 10,500 kg and 25,500 kg for the moderately and heavily compacted treatments, respectively. Four irrigation treatments were assigned to subplots, including full irrigation (100%), two limited irrigation schemes (75%, 50%), and no supplemental irrigation (0%). Nine varieties, Marquis, Matterhorn, 99-131, Emerson, Orion, Tara, Beryl-R, Roza, and UI-537 were assigned to the sub-plots. Each variety was planted in two 6.7-m rows spaced 55.9 cm apart. Measurements included, soil water content, crop emergence, days to flowering and maturity, soil cone penetrometer resistance, and yield and its components.

## RESULTS AND DSICUSSION

Yield declined with increasing compaction and decreasing irrigation. Yield was reduced 62 and 65% when soil was moderately and heavily compacted, respectively (Table 1). Average yield was reduced 46% when irrigation was reduced from 100 to 0% (Table 2). Yield also varied with genotype (range: 1394 to 1808 kg/ha) (Table 3). UI-537 had the highest overall yield and performed significantly better than the other genotypes (Table 3).

One hundred-seed weight was greater in non-compacted plots than in compacted plots (Table 1) and varied within genotype (range: 23.9 to 34.6 g) (Table 3). Seed size was reduced an average of 5.5 % when soils were heavily or moderately compacted (Table 1). Emerson had the largest seed size (Table 3).

Days to flowering and maturity varied within genotype (range: 40.2-45.0 and 76.2-90.1 days, respectively). Gemini and UI-537 flowered and matured earliest (Table 3).

**Table 1.** Effect of soil compaction on yield (lbs/a), days to flowering and maturity, and 100-seed weight (g) at Scottsbluff during 2009.

| Soil Compaction | Yield | Days to Flowering | Days to Maturity | 100-seed Weight |
|---|---|---|---|---|
| | ---kg/ha--- | --------days-------- | -------days-------- | ----------g-------- |
| **Non-compacted** | 2740a† | 43a | 82a | 28.9a |
| **Moderately** | 1036b | 43a | 82a | 27.4b |
| **Heavily** | 969b | 43a | 82a | 27.2b |

† Within columns, data followed by the same letter are not significantly different in the Duncan test at $p$=0.05

**Table 2.** Effect of irrigation scheduling on yield (lbs/a) at Scottsbluff during 2009.

| Irrigation Scheduling | Yield | 100-seed weight |
|---|---|---|
| | ----------ka/ha---------- | ----g---- |
| **100%** | 1767a† | 28.1a |
| **75%** | 1849a | 28.6a |
| **50%** | 1708a | 28.6a |
| **0%** | 1001b | 26.1b |

† Within columns, data followed by the same letter are not significantly different in the Duncan test at $p$=0.05

**Table 3.** Effect of cultivar on yield (lbs/A), days to flowering and maturity (days), and 100-seed weight (g) at Scottsbluff during 2009.

| Variety | Yield | Days to Flowering | Days to Maturity | 100-seed Weight |
|---|---|---|---|---|
| | ----kg/ha---- | ------days----- | -------days------- | ----------g--------- |
| **UI-537** | 1808a | 40.3d | 76.2d | 29.9b |
| **Beryl-R** | 1635b | 42.2c | 77.5d | 23.9f |
| **Marquis** | 1618b | 43.0b | 80.7c | 26.1e |
| **Gemini** | 1603b | 40.2d | 76.7d | 26.7d |
| **Matterhorn** | 1594b | 44.9a | 85.4b | 28.5c |
| **Tara** | 1555b | 45.0a | 86.3b | 29.9b |
| **Orion** | 1529b | 45.0a | 86.4b | 25.6e |
| **Emerson** | 1525b | 42.2c | 81.2c | 34.6a |
| **Roza** | 1394b | 45.0a | 90.1a | 25.7e |

† Within columns, data followed by the same letter are not significantly different in the Duncan test at $p$=0.05

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# GRAIN YIELD OF BEAN VARIETIES UNDER DROUGHT STRESS

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## INTRODUCTION

The bean (*Phaseolus vulgaris* L.) is one of the world's important crops, originating from Mexico. Since its domestication, bean has represented an important part of the daily diet of Mexicans. Drought is the main factor that limits the production of bean in Mexico. Low yields in drought conditions, are mainly due to erratic and bad distributed rainfall Acosta *et al*. (2000). The aim of this study was to identify more resistant varieties to drought that bean variety Pinto Villa.

## MATERIALS AND METHODS

The study was conducted at the Experimental Station of INIFAP in Bachiniva, Chihuahua: 28 ° 47 '19.32 " North Latitude, 107 ° 16' 11.64" West Longitude, at an altitude of 2012 meters. In a clay loam soil with 43 % sand, 28.72 % silt and 28.28 % clay, salt free, high organic matter content (2.01 %), the slope ranged from 0.16 % to 0.64 %. Applied 30-50-00 fertilizer, in both levels of humidity. The rainfall during the crop was 351 mm, adding from a rain before planting date experiment that took place July 4, 2010. Applied two irrigations of 25 mm on 2 August and 11 September. Evaluated the days to flowering and maturity and yield in kg $ha^{-1}$. Experimental design was a randomized complete block with three replications. Each replication consisted of 2 rows of 5 m length. The grain was standardized to 11 % humidity to calculate the grain yield per hectare. An analysis of variance using Statistical Analysis System. In this research we used 3 Tepary varieties harvested in the Sonoran Desert, Pinto Saltillo better quality control and production, Pinto Villa drought resistant control, Azufrado Higuera variety tropical and Rosa La Bufa, collected in 2009 in the town Bufa, Chihuahua. The intensity index of drought [IIS = 1 - (XS / XR)] was also calculated, XS is the average yield under drought and XR is the average yield under irrigation (Fischer and Maurer, 1978).

## RESULTS AND DISCUSSION

Under drought treatment showed a reduction in yield in all varieties compared with the irrigated treatment, reducing the intensity of drought will represented by the value IIS = 0.27. This tension is comparable with previous experiments performed with beans under rainfed conditions in the highlands of Mexico (IIS = 0.49, Schneider *et al*. 1997; 0.48, Rosales-Serna *et al*. 2004). The varieties tested responded to drought in several ways. In general, the varieties showed a tendency to escape the effects of drought through a more rapid development in response to stress. Similar effects of drought on plant phenology have been observed before (Ramirez Vallejo and Kelly, 1998). Therefore, the adaptation of phenology to environmental conditions, particularly rainfall, has been recognized as an important criterion to improve drought adaptation in common bean (Acosta Gallegos and White, 1995; Ramírez Vallejo and Kelly, 1998; Rosales-Serna *et al*. 2000; Rosales-Serna *et al*. 2004). Significant differences in days to flowering, days to maturity and yield. Under drought treatment caused a reduction in yield in all varieties compared with the irrigated treatment. In general, lower yields due to drought was greater in the varieties Tepary Café, Pinto Saltillo, Azufrado Higuera and Tepary RS. The best variety was Rosa La Bufa, presenting only the 10.5 % increase in yield (kg $ha^{-1}$) with respect to under drought treatment, followed by the variety Tepary

Pinto with 20.80% and Pinto Villa with 21.06% increase, reported as resistant variety under drought by Foster *et al*. (1995), Rosales-Serna *et al*. (2000), Rosales-Serna *et al*. (2004).

Days to flowering, physiological maturity, yield (kg $ha^{-1}$) and percent yield increase irrigated treatments compared to treatments under drought of seven varieties of beans.

| Variety | Days to flowering | | Days to maturity | | Yield (kg $ha^{-1}$) | | % increase (kg $ha^{-1}$) respect to drought |
|---|---|---|---|---|---|---|---|
| | Drought | Irrigation | Drought | Irrigation | Drought | Irrigation | |
| Tepary RS | 51.6 bc | 51.6 de | 73.0 d | 73.0 d | 701 c | 913 d | 30.20 |
| Pinto Saltillo | 52.6 a | 52.6 a | 90.0 a | 90.0 a | 1192 ab | 1691 a | 41.80 |
| Rosa Bufa | 51.0 c | 51.0 e | 80.0 c | 80.0 c | 1484 a | 1641 ab | 10.50 |
| Pinto Villa | 52.0 ab | 52.0 b | 84.0 b | 84.0 b | 1201 ab | 1454 abc | 21.06 |
| Tepary Café | 51.0 c | 51.0 cd | 73.0 d | 73.0 d | 1070 abc | 2237 bcd | 109.06 |
| Azufrado Higuera | 52.0 ab | 52.0 bc | 90.0 a | 90.0 a | 144 d | 195 e | 35.41 |
| Tepary Pinto | 51.3 bc | 51.3 de | 75.0 d | 73.0 d | 963 bc | 1164 cd | 20.80 |
| **Mean** | **51.6** | **51.9** | **80.76** | **80.42** | **965.4** | **1185.5** | **------** |
| **DMS** | **1.37** | **0.98** | **4.36** | **0.00** | **649.0** | **650.2** | **------** |
| **CV** | **0.92** | **0.66** | **1.89** | **0.00** | **23.5** | **19.2** | **------** |

## CONCLUSIONS

The cultivars showed a tendency to escape the effects of drought through a more rapid development, especially reducing the number of days to maturity. The best variety was Rosa La Bufa, presenting only the 10.5% increase in yield (kg $ha^{-1}$) with respect to under drought treatment, followed by the variety Tepary Pinto with 20.80% and Pinto Villa with 21.06% increase, reported as resistant variety under drought.

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# SHOOT DRY WEIGHT AND SEED YIELD OF FAMILIES DERIVED FROM TWO DROUGHT RESISTANT PINTO CULTIVARS

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In the bean breeding process is important to take into consideration the physiological yield components: rate of biomass accumulation, harvest index and the time to physiological maturity as a function of the time available for crop growth (Wallace *et al*., 1993). In this research several plant phenology and biomass accumulation traits were recorded and correlated to the seed yield in a population of $F_4$ families derived from the drought resistant, photoperiod sensitive cultivars, Pinto Villa (PV) and Pinto Saltillo (PS), grown under rainfed conditions in central Mexico. Photoperiod sensitivity may be part of mechanisms related to adaptation in rainfall-drought prone environments (Acosta and White, 1995).

The cross of PV/PS was made in the spring of 2009 and during the rainfall summer-fall season, 400 $F_2$ individual plants were taken at random from a large population. The seed from each individual plant was row planted under irrigation early in 2010 and after harvesting, 289 families with enough seed for replicated trials were taken. The 289 $F_4$ families were tested under rainfed conditions in two different trials, one was established on july $10^{th}$ in a loamy soil and the second on july $17^{th}$ in a sandy soil at 'El Bajio' Experiment Station of INIFAP, Celaya, Guanajuato. Each family was sown in a 4-m row plot 0.76 m apart. The crop was rainfed and the recorded rainfall a month before and during the growth season accumulated 400 mm. Following traits were recorded: days to flowering and physiological maturity and after harvest, seed yield and aboveground biomass, as well as 100-seed weight. Rate of biomass accumulation per day of the reproductive period and for the whole cycle was calculated along with the harvest index (HI). Data averaged from six replications were used to conduct a stepwise multiple regression analysis to identify traits related to seed yield. Data from traits that did not add significance to the model were eliminated from the analysis.

Traits that showed to be significant in the model were, biomass, HI, length of the reproductive stage, days to flowering and 100-seed weight. From these, biomass and HI contributed largely on the observed variation in seed yield (53 and 33%). Nevertheless, all traits contributed towards the seed yield predictive model (92%), which was structured as:

Yield= -888.951 + 0.46596(Biomass) + 36.937(HI) - 9.11889(Reproductive stage) - 9.03018(Days to Flowering) - 3.18969(100-Seed weight). Biomass and HI had previously show high association with seed yield under moisture stress (Muñoz *et al*., 2006). Since this trial was conformed to families, the correlations might have a high genetic component and the heritability and repeatability across environments should be determined in 2011 for some secondary traits to be considered as desirable in the breeding process (Laffite *et al.*, 2002). 100-seed weight, an important trait for consumers had displayed intermediate heritability (0.61), higher than that for biomass and HI (0.49) (Singh and Urrea, 1994), however, biomass can also be useful in the selection process under drought stress. A histogram of the yield seed frequency indicated a normal distribution with the Shapiro-Wilk test (Figure 1). The yield of both parents was similar to the highest yielding families. In the

Figure 2 the relationship between seed and biomass yield shows a coefficient of determination ($R^2$) of 0.58 that reinforces the use of biomass as an indicator of seed yield under rainfed conditions. Although both parents belong to the Durango race, an important source of drought resistance, they were developed from different parental stocks and might have different mechanisms for drought adaptation. Therefore, with the aid of molecular markers we will identify those families with superior alleles for further recombination (Ribout *et al*., 2010).

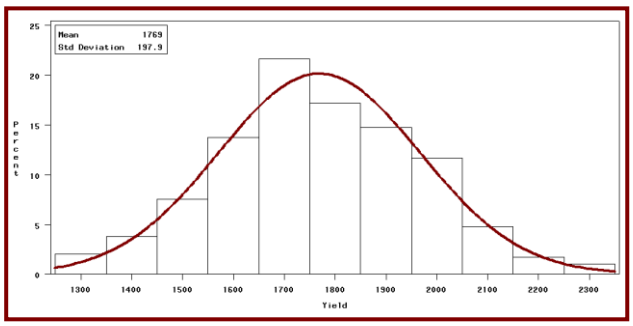


**Figure 1.** Seed yield frequency distribution of 289 $F_4$ families grown under rainfed conditions.

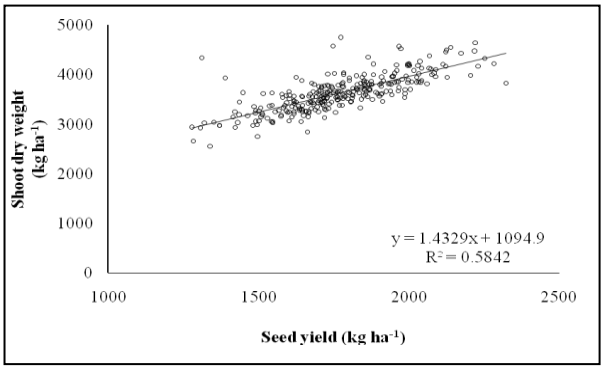


**Figure 2.** Relationship between seed yield and shoot biomass of 289 $F_4$ families grown under rainfed conditions.

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# SOIL WATER AVAILABILITY: ITS EFFECT ON PHOTOSYNTHESIS, TRANSPIRATION AND LEAF AREA IN BEAN (*PHASEOLUS VULGARIS* L.)

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## INTRODUCTION

The deficit of soil water availability induces a series of alterations in plant physiological processes among them photosynthesis, transpiration and growth (Taiz and Zeiger, 2006). Its effect depends upon the intensity, duration, rhythm of establishment, plant preconditioning and developmental stage of the plant. The present work is part of a study which addressed the anatomical and physiological changes in bean under soil water deficit. Here we report the rates of photosynthesis, transpiration and leaf area.

## MATERIALS AND METHODS

Three bean varieties Bayo Madero (BM, type III, drought susceptible), Pinto Villa (PV, type III, drought tolerant,) and G4523 (type I, drought tolerant) were employed. The plants were grown, one per plastic pot filled with 1.4 kg of sandy soil, and brought into an environmental control chamber with 25/20$^{O}$C, 12h light/dark. The photosynthetic photon flux was 670 µmol $m^{-2}$ $s^{-1}$. The experiment was a completely randomized design with six treatments [three bean varieties and two water regimes (suspension of watering SW, and control watering W)]. There were **four** replications for photosynthesis and transpiration rates and the determinations were performed once a day (d) in the morning from d one up to eleven with an infrared gas analyzer (IRGA, model CIRAS-1). For leaf area there were **three** replications and for the determination was employed an electronic area meter at 6, 10, and 16d after SW. The plants were watered with Steiner complete nutrient solution (Steiner, 1984). The experimental unit was represented by a pot with one plant. Thirteen days **after the emergence** when the plants displayed the second compound leaf, the SW treatments started by withholding watering during 6, 10 and 16d. Their respective controls were watered to keep the soil at field capacity. The soil water potential was determined at 6, 10 and 16d after SW with psichrometric chambers (C-52, Wescor Inc.).

## RESULTS AND DISCUSSION

The *soil water potential* (average of soil with SW in the three varieties) were: -0.75 at the 6 d, -2.7 at the 10 d and -3.9 MPa at the 16 d.

The three varieties showed a significant reduction of the *photosynthetic rate* with respect to their controls. In BM these reductions started from the fifth day after the SW and in general continued up to the eleventh. In PV only at the third and restarts from the day seventh onwards. In G4523 from the eighth day onwards (Table 1). At the eleventh day BM and PV exhibited a negative value, which indicates that the rate of respiration was higher than the rate of photosynthesis. In contrast, in G4523 these values remained positive throughout.

*Transpiration rate*. The three varieties showed a significant reduction of the transpiration rate with respect to their control: BM started from the third day after the SW and continued to the 11$^{th}$ d PV and G4523 started from the fifth d and continued also to the eleventh d (Table 2).

*Leaf area***.** It is one of the more sensitive variables to the soil water deficit. Its response (lowering of leaf area) was manifested from the sampling at 6 d after the SW in the tolerant varieties (G4523 and PV). This difference increases as the water suspension period lengthens with the concomitant reduction of the soil water potential from -0.75 to -3.9 MPa. BM (susceptible) showed difference only at the sampling at the 16 d (Table 3).

The intensity of the soil water deficit increases with the duration of the SW with the correlative reduction of photosynthesis, transpiration and leaf area for the three varieties.

The leaf area was reduced with respect to the control, to a higher degree at the 16$^{th}$ day in the tolerant varieties as compared to the susceptible. This might be a mechanism to which the plant resorts to reduce the loss of water by transpiration.

**Table 1.** Photosyntetic rate (µmol $CO_2$ $m^{-2}$ $s^{-1}$) in three bean varieties under soil water deficit

| Treatment | | Days after suspension of watering | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Variety** | **WR** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** |
| **BM** | W | 2.5a[#] | 3.6a | 2.8a | 1.8a | 2.1a | 2.3a | 2.7a | 2.9a | 3.1a | 1.6a | 0.7a |
| | SW | 1.9a | 4.2a | 2.9a | 4.5a | 0.6b | 0.1b | 1.5a | 0.7b | 1.2b | 0.2b | -1.7b |
| **PV** | W | 2.8a | 4.1a | 3.0a | 2.5a | 3.3a | 3.2a | 3.4a | 3.3a | 3.4a | 2.6a | 1.4a |
| | SW | 3.0a | 4.6a | 4.8b | 3.6a | 3.3a | 2.6a | 1.1b | 1.5b | 1.5b | 0.7b | -0.9b |
| **G4523** | W | 2.8a | 3.6a | 2.9a | 3.6a | 3.7a | 3.5a | 3.1a | 3.8a | 3.4a | 3.1a | 3.3a |
| | SW | 3.6a | 3.7a | 3.4a | 4.2a | 2.4b | 2.9a | 3.0a | 1.1b | 2.1b | 1.0b | 1.3b |

BM=Bayo Madero PV=Pinto Villa WR=Water regime W=Watering SW=Suspension of watering [#]Different letters within each variety indicate statistical difference between water regimes (Tukey test P≤0.05)

**Table 2.** Transpiration rate (mmol $H_2O$ $m^{-2}$ $s^{-1}$) in three bean varieties under soil water deficit

| Treatment | | Days after suspension of watering | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Variety** | **WR** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** |
| **BM** | W | 3.2a[#] | 3.1a | 3.7a | 3.0a | 2.8a | 2.8a | 3.3a | 3.2a | 2.9a | 2.9a | 2.5a |
| | SW | 2.4a | 2.8a | 2.6b | 1.9b | 1.5b | 1.8b | 1.4b | 1.4b | 1.2b | 1.2b | 1.3b |
| **PV** | W | 3.6a | 3.0a | 3.3a | 2.7a | 3.9a | 2.7a | 3.1a | 2.8a | 2.8a | 2.2a | 2.1a |
| | SW | 3.0a | 3.1a | 2.7a | 2.5a | 1.7b | 1.8b | 1.6b | 1.5b | 1.4b | 1.5b | 1.5b |
| **G4523** | W | 3.0a | 2.9a | 3.0a | 2.4a | 2.9a | 2.7a | 2.6a | 2.4a | 2.3a | 2.1a | 1.6a |
| | SW | 3.1a | 2.9a | 3.0a | 2.1a | 2.1b | 1.7b | 1.7b | 1.4b | 1.4b | 1.1b | 1.1b |

BM=Bayo Madero PV=Pinto Villa WR=Water regime W=Watering SW=Suspension of watering [#]Different letters within each variety indicate statistical difference between water regimes (Tukey test P≤0.05)

**Table 3.** Leaf area ($cm^2$ $pl^{-1}$) in three bean varieties under soil water deficit

| Treatment | | Days after suspension of watering | | |
|---|---|---|---|---|
| **Variety** | **WR** | **6** | **10** | **16** |
| **BM** | W | 241.82a[#] | 309.67a | 353.45a |
| | SW | 200.82a | 205.19a | 172.72b |
| **PV** | W | 227.14a | 254.37a | 403.80a |
| | SW | 184.23b | 234.93a | 127.01b |
| **G4523** | W | 395.13.a | 449.75a | 573.17a |
| | SW | 257.11b | 244.45b | 205.85b |

pl=plant BM=Bayo Madero PV=Pinto Villa WR=Water regime W=Watering SW=Suspension of watering [#]Different letters within each variety indicate statistical difference between water regimes (Tukey test P≤0.05)

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# TRANSPIRATION AND PHOTOSYNTHETIC WATER USE EFFICIENCY IN COMMON BEAN UNDER WATER DEFICIT

**V.S.R. Martins[1], M.C. Gonçalves-Vidigal[1*], V. Moda-Cirino[2], P.S. Vidigal Filho[1], M.P. Caixeta[1], D.V. Costa[3], E.B. Uchôa[3], G.F. Lacanallo[1] and A.K.S. Lobato[3]**

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## INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is considered one of the most important cultivated leguminous in the world. Its grains present high protein and carbohydrates concentration. Due to adequate climatic conditions and large consumer market, this culture is economically favorable to Brazil, placing this country as main producer and consumer worldwide (Broughton et al., 2003; Vieira, 2005).

Adequate environmental conditions are fundamental for the growth of this crop. Drought is one of the most affecting environmental stresses, causing great yield reductions (Souza et al. 2003). The lack of water promotes decreases in photosynthesis and transpiration rates (Inamullah and Isoda, 2005), as well as modifications in stomatal mechanism which will influence plants water use efficiency. Therefore, the objective of this study was to verify plant response to occasioned water deficit in two common bean cultivars (Pérola and LP 9728), and identify the cultivar which better develops under such condition.

## MATERIALS AND METHODS

Study was conducted at the Laboratory of Plant Physiology, Núcleo de Pesquisa Aplicada a Agricultura (Nupagri), Universidade Estadual de Maringá (UEM), Paraná, Brazil, during the period of October to December 2010. Seedlings of Pérola and LP 9728 cultivars were grown under greenhouse conditions. The minimum and maximum temperature variation of 12.4°C to 36.7°C and relative humidity variation of 42% to 85%, respectively. The photoperiod medium was of 12 h of light and photosynthesis radiation active maximum of 890 μmol $m^{-2}$ $s^{-1}$ (at 12:00 h). The treatments were Pérola and LP 9728 common bean cultivars under stress and Pérola and LP 9728 irrigated (non-stressful) conditions, and the experimental unit consisted of one plant per pot. The experiment was conducted in a complete randomized design with five replications.

The evaluation of water consumption efficiency rates in the processes of transpiration and photosynthesis was estimated in 4$^{th}$ after water restriction according to Osmond et al. (1987). These evaluations were carried out in well expanded trifoliolate leaves 3$^{rd}$, located at the middle of the main branch at the stage $V_4$. Data were analyzed employing a variance analysis, and using Scott-Knott test at 5% level of probability.

## RESULTS AND DISCUSSION

The evaluation of photosynthetic process in common bean Pérola cultivar revealed the values of 5.55 and 12.70 μmol $CO_2$ $mmol^{-1}$ $H_2O$ in control and drought, respectively. On the other hand, LP 9728 cultivar presented 7.95 and 11.65 μmol $CO_2$ $mmol^{-1}$ $H_2O$ when plants were exposed to control and drought stress, respectively (Figure 1B). Therefore, the numbers presented indicate that Pérola has higher photosynthetic efficiency when compared with LP 9728 cultivar under drought conditions.

Meanwhile for transpiration measurements, under control condition, Pérola and LP9728 common bean cultivars showed the highest mean values when compared with results from drought stress condition. The transpiration measurements were reduced significantly at a rate of 81.3 and 73.2%, respectively, demonstrating that both common bean cultivars presented similar reaction (Figure 1A).

These results indicate that decrease more intense was showed in Pérola common bean cultivar (Figure 1A). Transpiration rate is controlled by stomatal mechanism, and under conditions of water deficiency normally these stomatal are partially closed, provoking reduction in transpiration with consequences on water ($H_2O$) exchange among plant and environment. Other function important linked to transpiration is related with plant/leaf thermoregulation, because through adequate transpiration is possible to reduce internal temperature during day periods under heat more intense.

Based in results obtained in transpiration rate and photosynthetic water use efficiency is indicated uses of LP 9728 common bean cultivar to situations under water deficiency, once this cultivar suffer lower changes, when compared with Pérola cultivar.

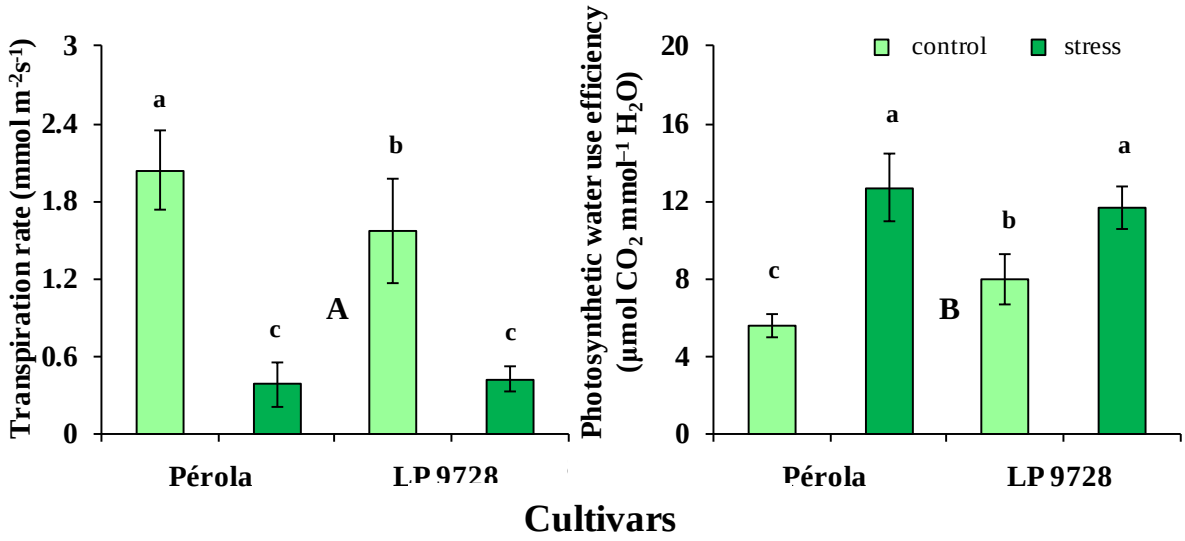


**Fig. 1:** (A) Transpiration rate and (B) photosynthetic water use efficiency in Pérola and LP 9728 cultivars exposed to 4 days of water deficiency. Same letters do not show significant differences at Scott-Knott test ($P < 0.05$). Bars represent the mean standard error.

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Vieira, C. 2005. Memory of half century of study on bean crop. Editora UFV.

# GAS EXCHANGE IN PÉROLA AND LP9728 COMMON BEAN CULTIVARS EXPOSED TO WATER STRESS

**V.S.R. Martins[1], M.C. Gonçalves-Vidigal[1*], V. Moda-Cirino[2], P.S. Vidigal Filho[1], M.P. Caixeta[1], D.V. Costa[3], E.B. Uchôa[3], G.F. Lacanallo[1] and A.K.S. Lobato[3]**

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## INTRODUCTION

Common bean (*Phaseolus vulgaris L.*) is very sensible to environmental conditions, and the drought stress is considered one of the most important factor causing severe yield reduction (Tan et al. 1999; Leport et al., 1999; Souza et al. 2003; Oliveira Neto et al., 2009).

Water deficit is characterized by water losses which exceed absorption rate, and it has direct effect on plant gas exchange (Costa et al., 2008). Under such conditions, plant damages are intrinsically related to intensity of drought and period of exposure. Depending on the scarcity of water, it may cause changes on stomatal mechanism, interfering, consequently, on photosynthesis rates (Ribas-Carbo et al., 2005).

Thus, the objective of this experiment was to investigate the effects of drought on process of gas exchange in common bean cultivars Pérola and LP 9728.

## MATERIALS AND METHODS

The experiments were carried out in a greenhouse and at the Laboratory of Plant Physiology, Núcleo de Pesquisa Aplicada a Agricultura, at Universidade Estadual de Maringá (UEM), Paraná, Brazil. Seedlings of dry bean Pérola and LP 9728 cultivars were grown under greenhouse conditions. The temperature varied from 12.4°C to 36.7°C and the relative humidity ranged from 42% to 85%. The photoperiod was of 12 h of light and photosynthesis maximum active radiation of 890 μmol $m^{-2}$ $s^{-1}$ (at 12:00 h). The treatments used were Pérola and LP 9728 cultivars under stress and Pérola and LP 9728 non-stress (irrigated) conditions. The experimental unit was made up of one plant per pot, and the treatments were evaluated in a complete randomized design with five replications (plants).

The rates of stomatal conductance and photosynthesis were evaluated at 4 days without irrigation, and those measurements were taken in the first trifoliolate leaves (V3) stage. Data were analyzed through a variance analysis, and Scott-Knott test at 5% level of probability. Standard errors were also calculated in all treatments evaluated.

## RESULTS AND DISCUSSION

Photosynthesis rate in Pérola cultivar presented higher value under control treatment (11.24 μmol $m^{-2}$ $s^{-1}$) than under drought stress (4.64 μmol $m^{-2}$ $s^{-1}$ (Figure 1A). Likewise, LP 9728 cultivar presented the magnitude value of 11.95 μmol $m^{-2}$ $s^{-1}$ under control and 4.95 μmol $m^{-2}$ $s^{-1}$ under drought conditions. These results suggested that both common bean cultivars presented similar fall during water shortage. Similar results on reduction of photosynthesis in plants under drought stress were reported by Souza et al. (2003) and Santos et al. (2009).

Stomatal conductance in Pérola cultivar was higher than in LP 9728 when under control treatment (Figure 1B). However, experiment revealed significant decrease in stomatal conductance

in both cultivars under drought. In this condition, the observed values of conductance for LP9628 were higher than for Pérola cultivar.

This study revealed that restriction linked to compounds such as $CO_2$ and water are extremely negative for common bean plants, once it jeopardizes the photosynthetic process. Based on the gas exchange data analysis the Pérola cultivar was considered the most sensible to drought.

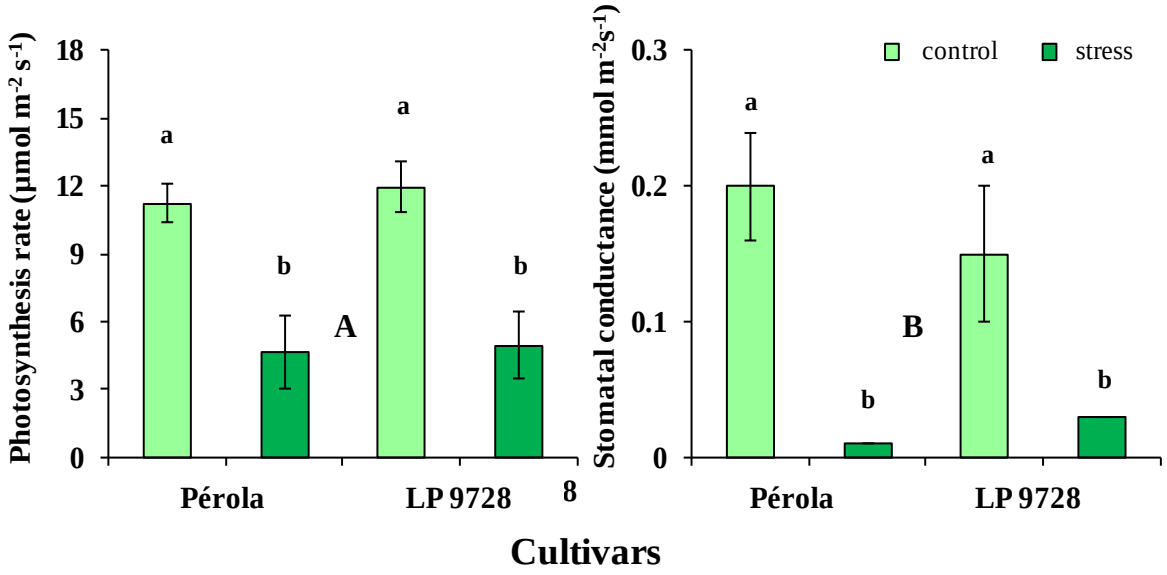


**Fig. 1:** (A) Photosynthesis rate and (B) stomatal conductance in common bean (*Phaseolus vulgaris* L.) Pérola and LP 9728 cultivars exposed to water stress. Same letters do not show significant differences at Scott-Knott test ($P < 0.05$). Thin bars represent the mean standard error.


**ACKNOWLEDGEMENTS**

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# SPEED OF NODULATION OF UMR 1899 AND UMR 1597 IN COMMON BEAN BREEDING LINES

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The speed of nodulation of *Rhizobium tropici* UMR 1899 syn. CIAT 899 and *Rhizobium etli* UMR 1597 syn. CIAT 161 were examined with thirteen different common bean (*Phaseolus vulgaris* L.) genotypes and breeding lines. Rhizobia strains were obtained from the University of Minnesota rhizobia culture collection and they were grown in Yeast-Mannitol-Agar media (Somasegaran and Hoben, 1994). Using a growth pouch assay, strains UMR 1899 and UMR 1597 were inoculated using common bean in the seedling stage (Table 1). Seeds were disinfected with 2.5% sodium hypochlorite and place in 10% water-agar and incubated for 48 hours at 28°C. Growth pouches had a nitrogen free nutrient solution (Broughton and Dilworth, 1970) with seedlings transferred with sterile forceps. The root tip marking technique was used to estimate the position of the uppermost nodule and the number of nodules above the root tip mark (Oliveira and Graham, 1990). Both rhizobia strains were inoculated after 24 h. using a concentration of $10^8$ cells/ml. applied to each seedling. Growth pouches were maintained at room temperature, 22-27°C. The evaluation was carried out seven days after inoculation, and the upper-most nodule (UPM) and the number of nodules above the root tip mark (RTM) were assessed. The first nodules were observed in 'Verano' and PR0737-1 with inoculation of UMR 1899. Both common bean line and line x rhizobia strain interaction were significant in the ANOVA of nodule number, while the strain effect was not. The results also indicated that UMR 1899 inoculated with PR0737-2 had the upper most nodule position and had a lower nodule number when compared to inoculation with UMR 1597, while inoculation with UMR 1597 resulted in the highest nodule number in PR0737-2 (Table 1). The pink bean PR0401-259 produced the highest number of nodules among the lines when inoculated with UMR 1899 and the lowest number of nodules when inoculated with UMR 1597. The red mottled line PR0737-2 produced the greatest number of nodules among the lines when inoculated with UMR 1597 and the lowest number of nodules when inoculated with UMR 1899. Both PR0737-1 and TARS10IS-2421 showed good nodulation in the uppermost root region when inoculated with either strain. The inoculation experiments thus indicated a cultivar preference. Therefore, it is important to select specific strains for specific common bean genotypes.

**Table 1**. Nodule number and nodule position of bean breeding lines after inoculation with UMR 1899 and UMR 1597.

| Line | Breeding line | Origin | Nodule Number above the root tip mark | | Nodule Position uppermost nodule (cm.) | |
|---|---|---|---|---|---|---|
| | | | 1597 | 1899 | 1597 | 1899 |
| **1** | PR9745-232 | Andean x MA | 25.5 | 15.5 | 0.45 | 0.35 |
| **2** | PR0401-259 | Middle American | 9.8 | 43.5 | 0.90 | 0.40 |
| **3** | PR0737-1 | Andean x MA | 41.3 | 35.3 | 0.45 | 0.40 |
| **4** | PR0737-2 | Andean x MA | 52.5 | 11.8 | 0.35 | 0.13 |
| **5** | PR0661-77 | Andean x MA | 32.3 | 15.3 | 0.40 | 0.35 |
| **6** | PR0634-13 | Middle American | 17.8 | 19.0 | 0.05 | 0.60 |
| **7** | Badillo | Andean | 12.0 | 14.3 | 0.55 | 0.53 |
| **8** | Verano | Middle American | 23.3 | 21.8 | 0.48 | 0.35 |
| **9** | DPC 40 | Middle American | 10.5 | 13.3 | 0.15 | 0.38 |
| **10** | Morales | Middle American | 13.3 | 9.0 | 0.23 | 0.50 |
| **11** | TARS10IS-2423 | Middle American | 10.8 | 28.5 | 0.20 | 0.53 |
| **12** | TARS10IS-2435 | Middle American | 21.3 | 21.5 | 0.43 | 0.35 |
| **13** | TARS10IS-2421 | Middle American | 36.0 | 34.0 | 0.60 | 0.50 |

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# INOCULATION OF BEAN PLANTS CULTIVARS WITH RHIZOBIUM STRAINS

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**INTRODUCTION:** Nitrogen (N) is the most widely extracted and imported nutrient by the bean plant, constituting the greatest limitation to the biomass production, development and yield of this species. This requirement is met by the use of nitrogen fertilizers which, however, have proved highly expensive, of low efficient and may cause environmental problems. One alternative to the N supply to the bean plant is the biological nitrogen fixation (BNF), performed by nitrogen fixing bacteria (BFNN or rhizobia) inoculated to the seeds. Works have shown benefits to the bean crop from the FBN in the field (Ferreira et al., 2009), but to maximize their effects, there is still the need to investigate a number of factors which affect the process, including if there is any differential behavior of the cultivars (cvs.) as regarding the inoculation with rhizobium strains.

The objective of the present work was to evaluate the response of cvs. of different commercial groups of common bean to the inoculation of the seeds with two rhizobium strains.

**MATERIAL AND METHODS:** The experiment was conducted in the field in the winter-spring on a typical clayey–textured Oxisol in Lavras, MG, Brazil, under irrigation. The statistical design was that of randomized blocks with three replicates and factorial scheme 6 x 3, involving six cultivars (Talismã, Supremo, Radiante, Bolinha, Ouro Vermelho and Majestoso) and three inoculations treatments: strain *Rhizobium tropici* strain CIAT 899 (recommended officially in Brazil), strain *Rhizobium sp* UFLA 04-173 and one control with no inoculation. The inoculant was prepared with peat sterilized in autoclave at the ration of 3:2 of peat and cultures in liquid medium 79, containing about $10^9$ cells of BFNN $mL^{-1}$, inoculation was performed in the ratio 250g of the inoculant to each 10kg of seeds.

Sowing was manual, adopting 0.5m inter-rows and 17 seeds $m^{-1}$. Each plot had 6 rows of 4.0 m. All the plots were given identical sowing fertilization (NPK 20-70-20) and the topdressing nitrogen fertilization was not done.

At flowering ($R_6$ stage) ten plants ($2^{nd}$ and $3^{rd}$ rows) were collection to determine the number (NN) and dry matter (DMN) of nodules as well as shoot dry matter (SDM), the content (NiL) and accumulation of N in the shoot (NAS). At harvest ($R_9$ stage) ($4^{th}$ and $5^{th}$ rows ) the final stand (FS) and grain yield (GY) with their primary components (number of pods per plant - PP, number of grains per pod - GP and one-hundred grain weight - W) in addition to the content (Ngr) and accumulation of N in the grains (ANGr) were evaluated.

Whenever necessary to meet the requirements for the analysis of variance, the data were previously transformed. The effects of cultivars and of inoculation when significant by the F test, were evaluated by clustering the means by the Scott-Knott test.

**RESULTS AND DISCUSSION:** The analysis of variance revealed that there was effect of cultivars upon the characteristics NN, DMN, PP, GP, W, ANGr and GY, and of inoculation treatments on both FS and PP. The interaction was significant only in relation to PP.

Cultivars Majestoso (group carioca), Supremo (black) and Ouro Vermelho (red) stood out as to the NN, outyielding cvs. Bolinha (yellow) and Radiante (pinto). Out of the first ones, cv. Ouro Vermelho presented smaller nodules, the DMN of which did not differ from those which nodulated

the least. The greatest FS presented by the control was ascribed to the reduction of the size of the seed by the absence of the inoculant, since to make the hand sowing easier, volume of seeds corresponding to the wanted density was utilized for each cultivar. This effect, nevertheless, did not interfere on the grain yield (Table 1).

The splitting of the interaction for the PP within each inoculating treatment revealed that the cultivars differ when inoculated with strain CIAT 899, situation in which cvs. Ouro Vermelho and Majestoso presented PP larger than the others. In the absence of inoculation and in the inoculation with strain UFLA 04-173 the cultivars presented the same behavior. Within each cultivar, the splitting revealed that strain CIAT 899 provided greater PP in cv. Majestoso, whilst both the strains presented superior PP in cv. Ouro Vermelho (Table 2).

**Table 1.** Average values concerning the characteristics of the common bean plant – number (NN) and dry mass of the nodules (DMN), dry matter of the shoot (SDM), content (NiL) and accumulation of nitrogen in the shoot (NAS); final stand of plants (FS), number of pods per plant (PP) and grains per pod (PG), average weight of one hundred grains (W), content (Ngr) and accumulation of nitrogen in gains (ANgr) and grain yield (GY). Lavras, MG, Brazil. 2010

| C[1] | NN | DMN | SDM | NiL | NAS | FS | PP | GP | W | Ngr | ANgr | GY |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (n°/10pls.) | (g/10pls.) | (g/10pls.) | (%) | (g/g) | (mil pl.ha$^{-1}$) | (nº) | (n°) | (g) | (%) | (g/g) | (kg.ha$^{-1)}$ |
| **T** | 159.67 b | 0.17 b | 23.45 | 2.03 | 0.48 | 257 | 4.49 | 4.29 b | 21.65 d | 3.06 | 11.13 a | 954 a |
| **S** | 286.33a | 0.41a | 25.65 | 1.55 | 0.37 | 253 | 5.09 | 5.24 a | 19.85 e | 2.63 | 11.04 a | 979 a |
| **R** | 115.44 b | 0.11 b | 25.99 | 1.34 | 0.39 | 276 | 4.52 | 2.45 d | 33.70 a | 2.18 | 6.13 b | 728 b |
| **B** | 162.78 b | 0.12 b | 27.29 | 2.17 | 0.60 | 246 | 3.42 | 3.38 c | 26.88 b | 2.34 | 5.66 b | 590 b |
| **OV** | 263.44a | 0.20 b | 31.81 | 1.82 | 0.56 | 233 | 6.40 | 5.25 a | 23.17 d | 2.77 | 14.58 a | 1351a |
| **M** | 309.22a | 0.34a | 33.88 | 1.73 | 0.57 | 219 | 5.04 | 4.90a | 24.88 c | 2.79 | 12.36 a | 11071a |
| **FN[2]** | | | | | | | | | | | | |
| **Control** | 26.44 | 0.24 | 27.75 | 1.77 | 0.50 | 274 a | 3.91 | 4.05 | 24.77 | 2.57 | 10.00 | 941 |
| **CIAT 899** | 17.05 | 0.20 | 30.79 | 1.72 | 0.52 | 240 b | 4.99 | 4.36 | 24.74 | 2.61 | 10.00 | 961 |
| **UFLA 04-173** | 26.87 | 0.24 | 25.90 | 1.84 | 0.46 | 227 b | 5.57 | 4.35 | 25.55 | 2.70 | 10.46 | 952 |

Means followed by the same letter belong to a same group according to the Scott-Knott test at the 5% level of probability. [1]Cultivars: T=Talismã, S=Supremo, R=Radiante, B= Bolinha, OV=Ouro Vermelho, M=Majestoso. [2]IN= Inoculation.

**Table 2.** Average number of pods per plant based upon the cultivars and inoculation. Lavras, MG, Brazil. 2010

| Cultivars | Inoculation | | |
|---|---|---|---|
| | Control | CIAT 899 | UFLA 04-173 |
| | (unit.) | | |
| **T** | 3.20Aa | 4.27Ba | 6.00Aa |
| **S** | 5.93Aa | 3.60Ba | 5.73Aa |
| **R** | 4.60Aa | 4.00Ba | 4.97Aa |
| **B** | 2.33Aa | 3.05Ba | 4.87Aa |
| **OV** | 4.20Ab | 8.03Aa | 6.97Aa |
| **M** | 3.20Ab | 7.03Aa | 4.90Ab |

Means followed by the same capital letter in the column and small in the row belong to a same group according to the Scott-Knott test at the 5% level of probability. [1]Cultivars: T=Talismã. S=Supremo. R=Radiante, B= Bolinha, OV=Ouro Vermelho, M=Majestoso. [2]NS= Nitrogen source.

**CONCLUSIONS:** Both cultivars Supremo and Majestoso presented greater nodulation than the others. Supremo, Majestoso, Talismã and Ouro Vermelho presents highest yields than Radiante and Bolinha. The number of pods per plant is influenced by inoculation and this effect dependent upon bean cultivar. In cultivars Ouro Vermelho and Majestoso, increased number of pods is given by strain CIAT 899.

**ACKNOWLEDGEMENTS:** The authors thank the financial support of CNPq, CAPES and FAPEMIG.

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# SCREENING BEAN GENOTYPES FOR BIOLOGICAL NITROGEN RESPONSE TO LOW N UNDER FIELD CONDITIONS

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A biological nitrogen fixation (BNF) experiment was conducted in the field in Paterson, Washington in 2010. The objective was to survey bean genotypes for biological nitrogen response under low soil N conditions. Twenty-eight genotypes were screened from all major U.S. market classes and include checks P-152, a high nitrogen fixer, and a non-nod check R99 (Park and Buttery, 2006). However, data for P-152 is not included in this report due to late maturity.

## MATERIALS AND METHODS

The Paterson trial site is a Quincy Sand. Low residual N (25lbs/A) was confirmed prior to planting. There were three treatments: i) NT=no nitrogen or rhizobium inoculum, ii) BS=Biostacked rhizobium inoculum only, and iii) N=75 lbs of additional N only in the form of urea (46-0-0). BeckerUnderwood BioStacked® inoculant was applied at planting and nitrogen was applied by hand at 22 DAP. The field was planted in a split-block design and yield data was taken from 3-m sections of the two center rows of four row plots. Genotypes were grouped by Andean, Mesoamerican, and Durango races and statistically analyzed using SAS (Proc GLM).

## RESULTS AND DISCUSSION

The non-nodulating genotypic check R99 had 43% more seed yield in the N (3460 $kgha^{-1}$) than the NT (1966 $kgha^{-1}$) which suggests that response to supplemental N in the absence of nodulation was detectable in this field trial. Across the Andean genotypes tested there was a significant treatment effect on yield resulting in a 10% reduction between N and BS treatments, and 4% between N and NT, though insignificant. For Durango genotypes there was no significant difference between NT and N treatments and a 9% reduction for yield between N and BS treatments. Mesoamerican genotypes had no significant differences among all three treatments. Across all genotypes there was a consistent significant effect for the BS inoculant treatment which unexpectedly resulted in 7 and 8% less yield than the NT & N treatments, respectively (data not shown). Perhaps the added Rhizobia were less effective, in part, by inhibiting root colonization by the endemic strains.

## CONCLUSION

Preliminary data show a significant effect between N and BS treatments for Andean and Durango groups (Table 1), as well as for all genotypes analyzed together (data not shown). Nodules for typing Rhizobium strains were not collected from the WA trials this season, but will be collected and characterized across treatments from select genotypes next season. Additional data is required to determine if the insignificant effect between N and NT for many of the tested genotypes is due to environmental conditions or good adaptation to low N conditions. Further studies with these and other genotypes are ongoing to identify contrasting parents with high and low BNF in order to develop RIL populations for QTL analysis of BNF efficiency in dry bean.

**Table 1.** Summary of results for BNF study by origin group, Paterson, WA.

| Andean | | Treatment | | Yield Reduction (%) | |
|---|---|---|---|---|---|
| **Genotype** | **NT†** | **BS** | **N** | **NT vs. N** | **BS vs. N** |
| Blush | 4241 | 3801 | 3924 | 0 | 3 |
| Cardinal | 3917 | 3146 | 4033 | 3 | 22 |
| Eagle | 1480 | 1561 | 1724 | 14 | 9 |
| G-122 | 4156 | 4230 | 4438 | 6 | 5 |
| Montcalm | 3175 | 2769 | 3197 | 1 | 13 |
| THort | 2264 | 2362 | 2631 | 14 | 10 |
| Mean‡ | 3205[ab] | 2978[b] | 3324[a] | 4 | 10 |
| **Mesoamerican** | | | | | |
| A-55 | 3387 | 2890 | 3106 | 0 | 7 |
| Albion | 4057 | 4254 | 3971 | 0 | 0 |
| Black Magic | 3767 | 4237 | 4400 | 14 | 4 |
| I9365-25 | 2824 | 3579 | 2928 | 4 | 0 |
| I9365-31 | 4148 | 3747 | 4812 | 14 | 22 |
| Raven | 3495 | 3184 | 3402 | 0 | 6 |
| Sanilac | 4730 | 4420 | 4380 | 0 | 0 |
| Voyager | 4390 | 4448 | 4684 | 6 | 5 |
| Mean | 3850 | 3845 | 3960 | 3 | 3 |
| **Durango** | | | | | |
| 3138 | 4643 | 3349 | 4695 | 1 | 29 |
| BelNeb-RR-1 | 4084 | 3901 | 4334 | 6 | 10 |
| Buster | 5843 | 5027 | 5563 | 0 | 10 |
| LaPaz | 4732 | 4406 | 4739 | 0 | 7 |
| Montrose | 5087 | 4054 | 4417 | 0 | 8 |
| Othello | 4348 | 4030 | 3792 | 0 | 0 |
| PT8-3 | 5490 | 5095 | 5571 | 1 | 9 |
| Roza | 4225 | 3734 | 5141 | 18 | 27 |
| Shiny Crow | 4269 | 4175 | 3883 | 0 | 0 |
| UI-537 | 4122 | 4055 | 4508 | 9 | 10 |
| USRM-20 | 4265 | 4132 | 4579 | 7 | 10 |
| Viva | 4969 | 4656 | 4639 | 0 | 0 |
| Mean | 4673[a] | 4218[b] | 4655[a] | 0 | 9 |
| R99§ | 1966 | 1756 | 3461 | 43 | 49 |

†NT=no trt, BS=biostacked inoculant, N=nitrogen (75lbs/A).

‡Means followed by a different letter are significant at P=0.05.

§Non-nodulating check.

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# PARTITIONING OF DRY MATTER, CALCIUM, MAGNESIUM AND SULFUR IN BEAN GENOTYPES

**Albuquerque, A.[1], Oliveira, D.P.[1], Vieira, N.M.B.[2], Andrade, C.A.B.[3*] and Andrade, M.J.B.[1*]**

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## INTRODUCTION

The study of the absorption rate and of final accumulation of nutrients allows us to know the amounts of absorbed nutrients and the relative absorption, supplying basic information to the improvement of fertilizer application. In short-cycle species, with little deep roots and highly requiring as the bean plant, that information is still more valuable and must be obtained on the basis of the different genotypes, since a lot of their characteristics such the growth habit, can interfere on the results.

That work was intended to evaluate, in four bean cultivars of different growth habits, the distribution of dry matter, Ca, Mg and S in different organs of the plant.

## MATERIAL AND METHODS

Originally, the study consisted of four experiments (cultivars), conducted in Lavras, state of Minas Gerais, Brazil, on Oxisol. Two neighboring areas were utilized, one at no-tillage planting and another in conventional crop system. In the rainy season of 2006/2007, characterized by excess rainfall (1,143mm), daily average temperatures between 11.8ºC and 32.8ºC (mean of 22.2ºC) and relative air moisture of 80.6% the experiments were conducted. At no-tillage system, sowing was carried out under brachiaria grass straw and, at the conventional; the soil was given a plowing and two harrowings. The bean cultivars were Bolinha (habit II), BRS Radiante (I), Ouro Vermelho (II/III) and Jalo EEP 558 (III). Each experiment had statistical design of randomized blocks, with three replicates and split plot scheme; in the plots were the two systems (either direct or conventional) and, in the subplot, a factorial 5x8, involving five plant densities (75, 145, 215, 285 and 355 thousand plants.ha$^{-1}$) and eight collecting dates (13, 23, 33, 43, 53, 63, 73 and 83 DAE). Each plot was made up of 4 rows of 5 m, with useful plot of 5m$^2$ (the central rows). All the plots were given identical fertilization (400 kg ha$^{-1}$ of 8-28-16, further 30 kg.ha$^{-1}$ of N at 21 days after emergence)

In the present work were compared the accumulations and partitioning of dry matter and Ca, Mg and S obtained on the occasion of the last sampling, taking into account the means of the management systems and plant population.

## RESULTS AND DISCUSSION

As regards dry matter (Table 1), the average values suggest that the growing order of dry matter accumulation on the occasion of the last sampling was leaf<pod<stem<grain. Both cultivars Jalo EEP 558 and BRS Radiante, those of greatest grain size, proved more efficient, accumulating, respectively, 877 kg ha$^{-1}$ and 735 kg ha$^{-1}$ of the dry matter in the grains. Cultivar Ouro Vermelho accumulated only 618 kg.ha$^{-1}$ of dry matter in the grains, but presented greatest accumulation in the stems (873 kg ha$^{-1}$) in relation to the other cultivars.

In general, the accumulations of Ca, Mg and S presented themselves in a manner similar to the dry matter accumulations in the four cultivars. Both in the different organs and in the whole plant, the decreasing order of secondary macronutrients was Ca>Mg>S (Table 1).

**Table 1.** Means of the final accumulation (kg.ha$^{-1}$ e %) of dry matter and secondary macronutrients in cultivars Bolinha (B), Jalo EEP 558 (J), BRS Radiante (R) and Ouro Vermelho (OV) with their partitioning in different parts of the plant, crop of the rainy season of 2006/2007. Lavras, MG, Brazil

| Cv | Part of the plant | Dry matter | | Calcium | | Magnesium | | Sulfur | |
|---|---|---|---|---|---|---|---|---|---|
| | | kg ha$^{-1}$ | % | kg ha$^{-1}$ | % | kg ha$^{-1}$ | % | kg ha$^{-1}$ | % |
| **B** | Stem | 591 | 30% | 5.4 | 33% | 2.1 | 36% | 1.1 | 39% |
| | Leaf | 227 | 11% | 5.0 | 30% | 0.7 | 12% | 0.5 | 18% |
| | Pod | 392 | 32% | 5.1 | 31% | 2.0 | 34% | 0.4 | 15% |
| | Grain | 711 | 27% | 1.0 | 6% | 1.1 | 18% | 0.8 | 29% |
| | **Total** | 1921 | 100% | 16.5 | 100% | 5.8 | 100% | 2.9 | 100% |
| **J** | Stem | 683 | 32% | 6.5 | 44% | 2.2 | 39% | 1.0 | 36% |
| | Leaf | 143 | 7% | 3.3 | 22% | 0.4 | 7% | 0.3 | 12% |
| | Pod | 456 | 21% | 4.0 | 27% | 1.5 | 27% | 0.4 | 13% |
| | Grain | 877 | 41% | 1.2 | 8% | 1.5 | 27% | 1.1 | 38% |
| | **Total** | 2159 | 100% | 15.0 | 100% | 5.6 | 100% | 2.8 | 100% |
| **R** | Stem | 396 | 20% | 3.6 | 27% | 0.9 | 19% | 0.6 | 28% |
| | Leaf | 137 | 7% | 3.3 | 25% | 0.4 | 9% | 0.3 | 12% |
| | Pod | 684 | 35% | 5.4 | 40% | 2.1 | 45% | 0.6 | 26% |
| | Grain | 735 | 38% | 1.1 | 8% | 1.3 | 27% | 0.8 | 35% |
| | **Total** | 1952 | 100% | 13.4 | 100% | 4.6 | 100% | 2.3 | 100% |
| **OV** | Stem | 873 | 36% | 10.2 | 46% | 3.1 | 41% | 3.1 | 59% |
| | Leaf | 152 | 6% | 4.3 | 19% | 0.6 | 8% | 0.4 | 7% |
| | Pod | 812 | 33% | 6.3 | 28% | 2.6 | 34% | 0.7 | 13% |
| | Grain | 618 | 25% | 1.5 | 7% | 1.3 | 17% | 1.1 | 21% |
| | **Total** | 2455 | 100% | 22.2 | 100% | 7.6 | 100% | 5.4 | 100% |
| **M** | Stem | 635.8 | 30% | 6.4 | 38% | 2.1 | 35% | 1.5 | 44% |
| | Leaf | 164.8 | 8% | 4.0 | 24% | 0.5 | 9% | 0.4 | 11% |
| | Pod | 586 | 30% | 5.2 | 31% | 2.0 | 34% | 0.5 | 16% |
| | Grain | 735.3 | 32% | 1.2 | 7% | 1.3 | 22% | 1.0 | 29% |
| | **Total** | 2122 | 100% | 16.8 | 100% | 5.9 | 100% | 3.3 | 100% |

## CONCLUSIONS

Both cultivars, Jalo EEP 558 and Radiante, presented greatest values of final accumulation of dry matter, Ca, Mg and S in their grains. The general order of dry matter accumulation was growing in the leaf<pod<stem<grain direction. The order of accumulation of secondary macronutrients in the bean plant, independently of the organ, was Ca>Mg>S.

## ACKNOWLEDGEMENTS

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# PARTITIONING OF DRY MATTER, NITROGEN, PHOSPHORUS AND POTASSIUM IN BEAN GENOTYPES

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## INTRODUCTION

The study of the absorption rate and of the final accumulation of nutrients enables us to know the amounts of absorbed nutrients and the relative absorption, supplying basic information to the improvement of fertilizer application. In short cycle species, with little deep roots and highly demanding such as the bean plant, that information is further valuable and must be obtained on the basis of different genotypes, since a lot of their characteristics such as growth habit, may impact on the results.

This work was intended to evaluate, in four bean plants of different growth habits, the distribution of dry matter, N, P and K in different organs of the plant.

## MATERIAL AND METHODS

At first, the study consisted of four experiments (cultivars), conducted in Lavras, state of Minas Gerais, Brazil, on Oxisol. Two neighboring areas were utilized, one at no-tillage system and another at conventional crop system. In the rainy season of 2006/2007, characterized by excess rainfall (1,143mm), daily average temperatures between 11.8ºC and 32.8ºC (mean of 22.2ºC) and average air relative humidity of 80.6% the experiments were conducted. At no-tillage system, sowing was conducted under brachiaria grass straw and, at conventional, the soil was given one tillage and two harrowings. The bean cultivars were Bolinha (habit II), BRS Radiante (I), Ouro Vermelho (II/III) and Jalo EEP 558 (III). Each experiment had statistical design of randomized blocks, with three replicates and split plot scheme; in the plots lay the two systems (no-tillage and conventional) and, in the subplot, a factorial 5x8, involving five plant densities (75, 145, 215, 285 and 355 thousand plants.ha$^{-1}$) and eight collecting dates (13, 23, 33, 43, 53, 63, 73 and 83 DAE). Each plot was made up of 4 rows of 5 m, with useful plot of 5m$^2$ (the two central rows). All the plots were given identical fertilization (400 kg ha$^{-1}$ of 8-28-16, more 30 kg ha$^{-1}$ of N at 21 days after emergence).

In the present work the accumulation and partitioning of dry matter and NPK obtained on the occasion of the sampling were compared, taking into account the means of the management systems and plant populations.

## RESULTS AND DISCUSSION

With relation to the dry matter (Table 1), the average values suggest that the growing order of dry matter accumulation on the occasion of the sampling was leaf<pod<stem<grain. Both cultivars, Jalo EEP 558 and BRS Radiante, those with largest grain size, proved more efficient, accumulating, respectively, 877 kg ha$^{-1}$ and 735 kg ha$^{-1}$ of the dry matter in the grains. Cultivar Ouro Vermelho accumulated only 618 kg.ha$^{-1}$ of dry matter in the grains, but it presented greater accumulation in the stems (873 kg ha$^{-1}$) relative to the other cultivars.

Generally, the N, P and K accumulations presented themselves in a similar manner similar to the dry matter accumulations in the four cultivars. The decreasing order of nutrient accumulation,

mean of the four cultivars, was distinct in the leaves and stems (N>K>P), pods (K>N>P>), grains (N>K>P) and total (N>K>P) (Table 1).

**Table 1**. Average values of final accumulation (kg $ha^{-1}$ and %) of dry matter and primary macronutrients in cultivars Bolinha (B), Jalo EEP 558 (J), BRS Radiante (R), Ouro Vermelho (OV), with their partitioning in different parts of the plant, crop Spring-Summer of 2006/2007. UFLA, 2010

| Cv | Part of the plant | Dry matter | | Nitrogen | | Phosphorus | | Potassium | |
|---|---|---|---|---|---|---|---|---|---|
| | | kg $ha^{-1}$ | % | kg $ha^{-1}$ | % | kg $ha^{-1}$ | % | kg $ha^{-1}$ | % |
| B | Stem | 591 | 30% | 8.9 | 20% | 1.2 | 22% | 7.6 | 25% |
| | Folha | 227 | 11% | 5.4 | 12% | 0.6 | 11% | 3.3 | 11% |
| | Pod | 392 | 32% | 10.8 | 25% | 1.2 | 21% | 11.9 | 39% |
| | Grain | 711 | 27% | 18.2 | 42% | 2.6 | 47% | 8.1 | 26% |
| | **Total** | 1921 | 100% | 43.3 | 100% | 5.5 | 100% | 31.0 | 100% |
| J | Stem | 683 | 32% | 8.8 | 17% | 0.8 | 14% | 8.0 | 27% |
| | Leaf | 143 | 7% | 3.6 | 7% | 0.3 | 6% | 1.7 | 6% |
| | Pod | 456 | 21% | 8.2 | 16% | 0.9 | 16% | 8.6 | 29% |
| | Grain | 877 | 41% | 30.2 | 60% | 3.7 | 64% | 11.5 | 39% |
| | **Total** | 2159 | 100% | 50.8 | 100% | 5.8 | 100% | 29.7 | 100% |
| R | Stem | 396 | 20% | 3.4 | 8% | 0.3 | 7% | 4.2 | 17% |
| | Leaf | 137 | 7% | 3.4 | 8% | 0.4 | 8% | 2.1 | 9% |
| | Pod | 684 | 35% | 10.0 | 25% | 1.0 | 21% | 7.5 | 31% |
| | Grain | 735 | 38% | 23.9 | 59% | 3.0 | 63% | 10.7 | 44% |
| | **Total** | 1952 | 100% | 40.7 | 100% | 4.7 | 100% | 24.4 | 100% |
| OV | Stem | 873 | 36% | 11.6 | 25% | 1.3 | 24% | 10.4 | 27% |
| | Leaf | 152 | 6% | 4.0 | 9% | 0.4 | 7% | 2.1 | 5% |
| | Pod | 812 | 33% | 10.4 | 23% | 1.1 | 19% | 16.9 | 44% |
| | Grain | 618 | 25% | 20.0 | 44% | 2.8 | 50% | 9.2 | 24% |
| | **Total** | 2455 | 100% | 46.0 | 100% | 5.6 | 100% | 38.5 | 100% |
| M | Stem | 636 | 30% | 8,1 | 18% | 0.9 | 17% | 7.5 | 24% |
| | Leaf | 165 | 8% | 4.1 | 9% | 0.4 | 8% | 2.3 | 7% |
| | Pod | 586 | 30% | 9.8 | 22% | 1.0 | 19% | 11.2 | 36% |
| | Grain | 735 | 32% | 23.1 | 51% | 3.0 | 56% | 9.9 | 32% |
| | **Total** | 2122 | 100% | 45.2 | 100% | 5.4 | 100% | 30.9 | 100% |

## CONCLUSIONS

Both cultivars, Jalo EEP 558 and Radiante, presented the highest values of final accumulation of dry matter, N, P and K. The general order of dry matter accumulation was growing in the leaf<pod<stem<grain direction. The order the primary macronutrients order of accumulation in the leaves, stems, grains and total was N>K>P, while in the leaves was K>N>P.


## ACKNOWLEDGEMENTS

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ALVES, A.F.; Densidades populacionais para cultivares alternativas de feijoeiro no Norte de Minas Gerais. **Ciência e Agrotecnologia**, Lavras, v.33, n.6, p. 1495-1502, nov./dez

# PHOSPHATE FERTILIZATION OF BEAN CULTIVARS IN THE SOUTH OF MINAS GERAIS

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## INTRODUCTION

The common bean is an important protein source in the Brazilians' diet. In spite of the consumers' preference for the carioca type grain, other commercial types could be used in the country to diversify the offer and, so to add value to the final product. However, to make the use of alternative cultivars by farmers viable, there is the need to adapt the present production systems of the legume, and also the fertilization recommendations.

The objective of this work was to evaluate the response of bean cultivars from different commercial groups to the doses of phosphorus at sowing, aiming to contribute to improve or validate the recommendations of fertilization in the south of Minas Gerais, Brazil.

## MATERIAL AND METHODS

The experiment was conducted in the drought crop of 2008 on a Red Yellow Podzolic soil of the Agriculture Department of the Federal University of Lavras (UFLA), with conventional sprinkling irrigation. The statistical design utilized was randomized blocks with three replicates and factorial scheme 4 x 5, involving four cultivars **(**BRS Radiante, BRS Ouro Vermelho, Bolinha and Jalo EEP 558) and five doses of phosphorus (0, 75, 150, 225 and 300 kg $ha^{-1}$ of $P_2O_5$, source triple superphosphate-37% de $P_2O_5$, applied into the sowing furrow).

Sowing was manual, adopting the spacing of 0.5 m inter-rows, density of 15 seeds $m^{-1}$ at depth of 3 to 4 cm, with 4 rows of 5.0 m per plot. All the plots were given 40 kg $ha^{-1}$ of N + 20 kg $ha^{-1}$ of $K_2O$ at sowing, 30 kg $ha^{-1}$ of N + 20 kg $ha^{-1}$ of $K_2O$ topdressing at $V_3$ stage of the cultural cycle ($1^{st}$ open trifoliate leaf) and 30 Kg $ha^{-1}$ de N at $V_4$ stage ($3^{rd}$ fully open trifoliate open), by utilizing as sources urea (44% of N) and potassium chlorite (58% of KCl).

On the occasion of harvest, final stand (FS) and grain yield (GY) were evaluated with their primary components (number of pods per plant - PP, number of grains per pod -GP and e weight of one hundred grains - W). The data were submitted to the analysis of variance, the effects of cultivars being evaluated by means of the clustering of means by the Scott-Knott test and the effects of the doses of phosphorus were significant by the test, were studied by means of the analysis of regression.

## RESULTS AND DISCUSSION

The analysis of variance revealed that there was a good experimental precision (Oliveira et al., 2009) and that all the characteristics were affected by cultivars (Table 1) by the doses of phosphorus (except FS) and by the interaction among the factors (except GP).

Cultivar Jalo EEP 558, with VP and GV smaller, was the one which reached the highest P100, whilst cultivar Ouro Vermelho presented highest PP and GP and reduced W. Such results reflect negative correlation between gain size and number of grains per pod already reported previously (Souza et al., 2008). The highest GY was obtained by cultivar Bolinha (Table 1), in which the greatest final population obtained may have taken part in the definition of the greatest yield (Souza et al., 2008).

In cultivar Radiante, there was a linear increase of PP (Figure 1) when raising the doses of $P_2O_5$; in the other cultivars, the effect was quadratic, with maximum point between 150 and 225 kg $ha^{-1}$ of $P_2O_5$. GP, independent of the cultivar, increased linearly with the dose of $P_2O_5$ (Figure 2). Only cultivars Jalo and Radiante were influenced significantly by the dose of $P_2O_5$; in the former, the behavior was linear and, in the latter, maximum values occurred with the dose of 270 kg $ha^{-1}$ of $P_2O_5$ (Figure 3). This same dose was responsible by the maximum GY, independent of the cultivar (Figure 4).

**TABLE 1.** Average values of the final stand (FS), number of pods per plant (PP) and grains per pod (GP), weight of one hundred grains (W) and yield (GY) of the bean plan based on the cultivars and doses of $P_2O_5$, UFLA, Lavras, MG, 2008

| Treatments Cultivars | FS (one thousand plants $ha^{-1}$) | PP (number) | GP (number) | W (g) | GY (Kg $ha^{-1}$) |
|---|---|---|---|---|---|
| Bolinha | 305 a[(1)] | 6.27 b | 3.43 b | 35.83 b | 1.783 a |
| Jalo EEP 558 | 286 b | 5.46 c | 2.66 c | 41.46 a | 1.404 b |
| BRS Radiante | 287 b | 8.04 a | 2.53 c | 35.86 b | 1.437 b |
| BRS Ouro Vermelho | 305 a | 8.31 a | 4.55 a | 19.35 c | 1.546 b |
| **Means** | **294** | **7.02** | **3.29** | **33.12** | **1.543** |

In each column, means followed by letters belong to different groups by the Scott-knott test at the 5% level of probability.

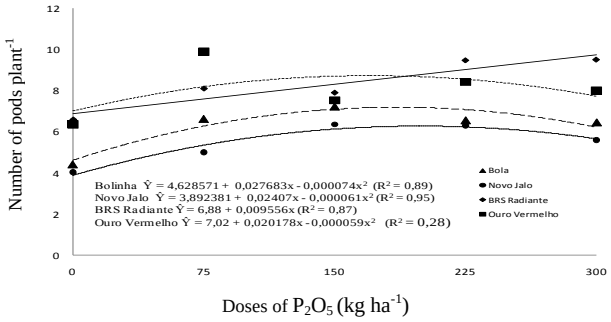


**FIGURE 1** Average number of pods $plant^{-1}$ of bean cultivars on the based on the doses of $P_2O_5$

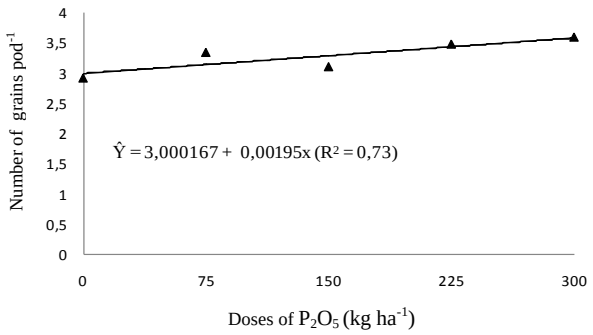


**FIGURE 2** Average number of grains $pod^{-1}$ of bean plant based on the doses of $P_2O_5$ (means of four cultivars)

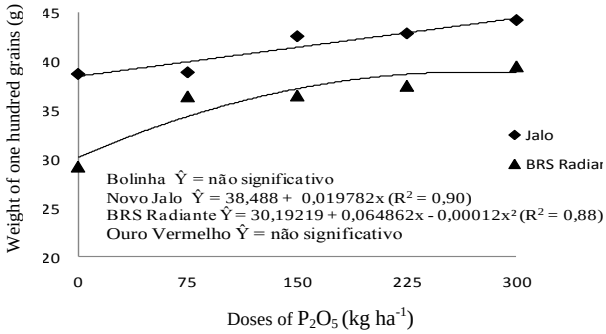


**FIGURE 3** Average weight of one hundred grains of bean cultivars based on the doses of $P_2O_5$

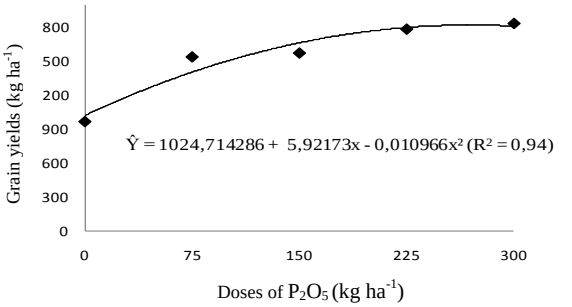


**FIGURE 4** Average grain yields based on the doses of $P_2O_5$ (means of four cultivars)

## CONCLUSIONS

The grain yield of the evaluated cultivars increases with the addition of doses of $P_2O_5$ of up to 270 kg $ha^{-1}$.

The maximum economical efficiency of phosphate fertilization is reached with 141 kg $ha^{-1}$ of $P_2O_5$, dose higher than that recommended at present for the bean plant in the State of Minas Gerais, Brazil.


## ACKNOWLEDGEMENTS

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# DIFFERENT SOIL PHOSPHATE LEVELS AND FOLIAR-APPLIED PHOSPHORUS FORMS ON P UPTAKE EFFICIENCY, P UTILIZATION EFFICIENCY, AND P TRANSLOCATION IN COMMON BEAN PLANTS

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## INTRODUCTION

Aim of this research was to evaluate different soil phosphate levels and foliar-applied phosphorus forms on P uptake efficiency, P utilization efficiency, and P translocation in common bean *Phaseolus vulgaris* cv. Radiante) plants.

## MATERIALS AND METHODS

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

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

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

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

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

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

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

Shoot and root dry mass were ground and analyzed for total P concentration colorimetrically (Murphy and Riley, 1962) after nitric-perchloric digestion of the plant material. Data from shoot and root dry wt and total P concentration were used to calculate P uptake efficiency (P total accumulation in plant / root dry wt), P utilization efficiency according to Siddiqi and Glass (1981) ((plant dry wt)$^2$/ (P total accumulation in plant)), and P translocation from root to shoot (P total accumulation in shoot / P total accumulation in plant). Results were submitted to variance analysis

and applied to Tukey test at 5% level, as well as the standard errors were calculated in all evaluated points.

## RESULTS AND DISCUSSION

Either one or two foliar applications of potassium phosphate and phosphite had not significant effects ($p > 0.05$) on P uptake efficiency such as ability to take up P from soil, and also P translocation such as ability to transporter P from root to shoot (Figure 1). Nevertheless, limiting phosphate availability in soil reduced P uptake efficiency and P translocation by common bean. When the phosphate availability to plants is insufficient, P translocation from root to shoot decreases, increasing the root growth rate in detriment shoot growth rate. Foliar-applied treatments did not affect P utilization efficiency of phosphate-sufficient plants, whereas under limiting phosphate availability in soil, foliar-applied phosphite decreased P utilization efficiency of common bean. This result was due to the inhibitory effect of phosphite on biomass yield of the phosphate-starved plants, since accumulated P of the plants was not affect (data not shown).

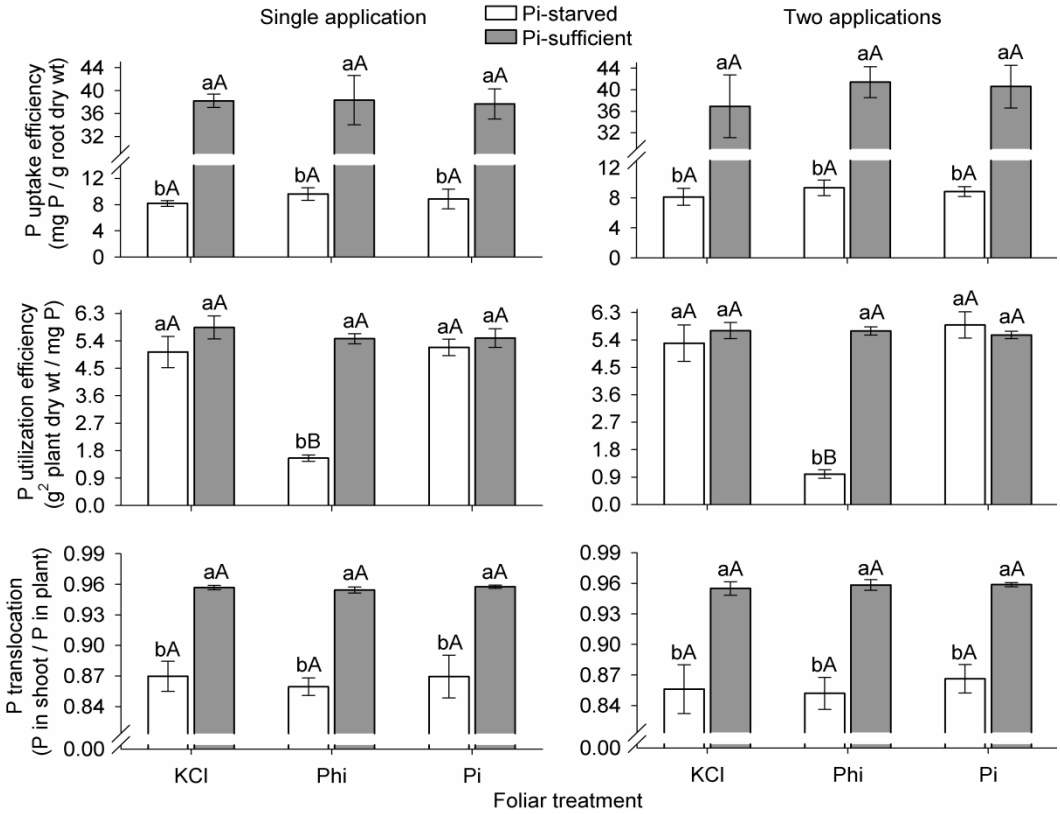


**Fig. 1:** P uptake efficiency, P utilization efficiency, and P translocation in common bean grown in Oxisol under 2 soil phosphate levels (Pi-starved and Pi-sufficient), 3 nutrient sources supplied via foliar application ($KH_2PO_3$, $KH_2PO_4$, and KCl), and 2 foliar application numbers (single and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

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# GROWTH IN COMMON BEAN EXPOSED TO DIFFERENT SOIL PHOSPHATE LEVELS AND FOLIAR-APPLIED PHOSPHORUS FORMS

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## INTRODUCTION

Aim of this study was to investigate interference produced by different soil phosphate levels, as well as action produced by foliar-applied phosphorus forms (phosphite and phosphate), and if number of foliar application can act on growth in common bean (*Phaseolus vulgaris* cv. Radiante) plants.

## MATERIAL AND METHODS

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

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| 5.4 | 0.9 | 22 | 0.5 | 0.7 | 0.4 | 27.4 | 20.5 | 0.1 | 0.1 | 0.1 | 1.7 | 2 | 28 | 13.3 | 396 |

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| $SiO_2$ | $Al_2O_3$ | $Fe_2O_3$ | $TiO_2$ | $P_2O_5$ | $Fe_d$ | $Fe_o$ | Ct | Gb | Ki | Kr |
| g $kg^{-1}$ of clay | | | | | | | | | | |
| 95.1 | 97.4 | 36.2 | 6.2 | 0.0 | 10.8 | 0.1 | 752.0 | 63.0 | 0.98 | 0.71 |

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

Plants were harvested at full flowering stage and separated into shoot and root. Both shoot and root were rinsed in deionized water and dried at 60°C for 72 h prior to dry weight determination. Results were submitted to variance analysis and applied to Tukey test at 5% level, as well as the standard errors were calculated in all evaluated points.

## RESULTS AND DISCUSSION

Variables in this study were not significant affected by foliar application numbers during single application timing and two application timings (Figure 1). Common bean plants grown under limiting phosphate availability (Pi-starved) showed considerable reductions in the root and shoot dry wt and increased root to shoot ratio. The increase root to shoot ratio by phosphate-starved plants is a mechanism for overcoming P deficiency (Devaiah et al., 2007).

Foliar application of potassium phosphite and phosphate had no significant effect on biomass yield in phosphate-sufficient common bean, when compared with the control. However, for plants grown under limiting phosphate availability, shoot and root dry wt were significantly decreased by foliar-applied potassium phosphite. The inhibiting effect of the phosphite anion on growth of phosphate-starved plants also was reported by Thao and Yamakawa (2009), and most plausible hypothesis to date is that plants do not metabolize phosphite anion. Furthermore, phosphite inhibits some mechanisms involved in overcoming of phosphate deprivation, such as increased synthesis of phosphatases, phosphodiesterases, and high-affinity P transporters.

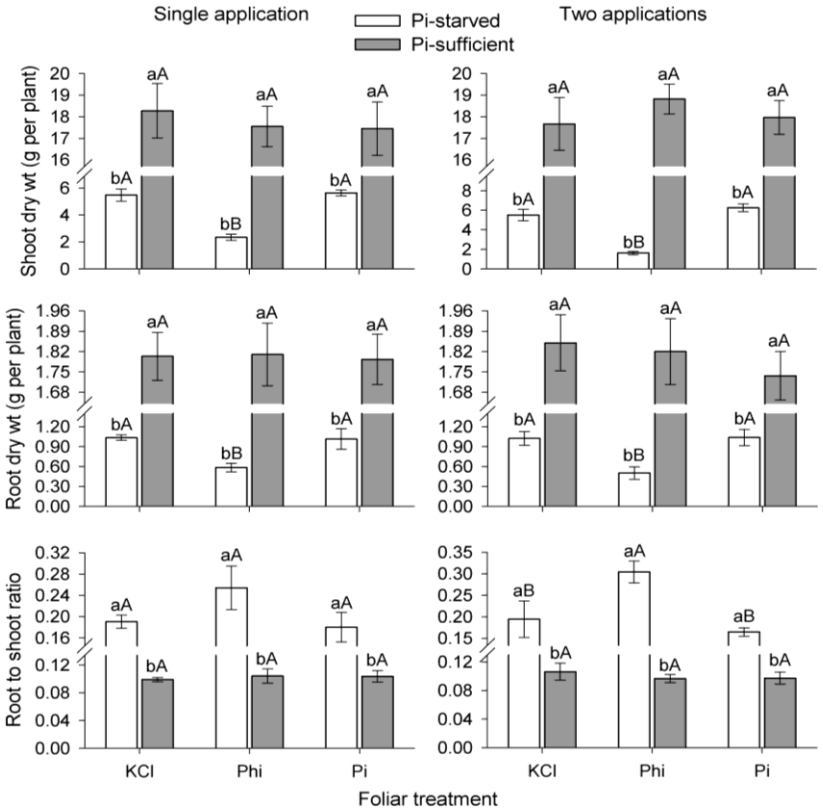


**Fig. 1:** Root dry weight, shoot dry weight, and root to shoot ratio in common bean grown in Oxisol under 2 soil phosphate levels (Pi-starved and Pi-sufficient), 3 nutrient sources supplied via foliar application ($KH_2PO_3$, $KH_2PO_4$, and KCl), and 2 foliar application numbers (single and two applications).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

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# BEAN YIELD IN RELATION TO NITROGEN FOLIAR APPLICATION

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## INTRODUCTION

The dry bean (*Phaseolus vulgaris* L.) for its nutritional and medicinal properties is important for the Mexican diet. Several studies have shown that application of nitrogen (N) to soil, increases the pod number, seed number and yield (Escalante *et al*., 1999). The pods and seeds are strong demand for N, which when the root system ceases its activity, is supplied mainly by the leaves (Sinclair and Horie, 1989). The output of nutrients from leaves, causes a decrease in the size and activity of the photosynthetic apparatus and the senescence, limiting the production of photosynthate for fruit and seed growth (Sinclair and De Wit, 1976), Thus, the supply of N foliar could correct such limitation resulting in a longer leaf duration and activity and consequently higher number of pods and bean yield. The aim of this study was to determine the effect of supply N foliar on biomass, harvest index, grain yield and its components in bean cv. Michoacan 12A3 under rainfed conditions in warm climate.

## MATERIALS AND METHODS

The study was conducted in Iguala, Gro. (18 º 25 'N, 99 º 35 `W and 731 m altitude) of warm climate. The bean cultivar Michoacán 12A3 (Michoacán) of indeterminate bush habit and purple flower color of black grain was sown on 30 June in a loam clay soil with pH 7.5, to density of 13.3 plants $m^{-2}$ in rows 75 cm apart. The fertilizer applied before planting was 40-40-00 of NPK. The treatments consisted of spraying solution with 0, 1, 2, 3 and 4 g of N $L^{-1}$ $m^{-2}$ as urea (46% N), at the beginning and end flowering. Experimental design was randomized blocks with four replications. Was recorded the days after sowing (das) to: emergency, flowering (BF), end of flowering (EF) and physiological maturity (PM) in accordance with criteria presented in Escalante and Kohashi (1993), the temperature (ºC) maximum, minimum, and accumulated rainfall (mm). At PM per $m^2$ : biomass (g dry matter, DM), harvest index (HI), yield (MS grain, g), grains and pods number, the size grain (average grain weight, mg) and grains per pod (Escalante and Kohashi, 1993).

## RESULTS AND DISCUSSION

The occurrence of phenological stages was similar between treatments. The seedling emergence at 6 das, the BF at 40 das, the EF at 70 das and the PM at 90 das. The conditions of maximum and minimum temperatures during bean development were: from sowing to IF 28 to 33ºC; 19 to 20°C, respectively. From IF to FF 30 to 33ºC and 19 to 21ºC, and from FF to MF 31 and 32ºC; 18 and 19ºC, respectively. The accumulated rainfall was 510 mm. We observed a drought period of 10 days at the BF and EF that limited the yield by a possible increase in the fall of reproductive organs and lower pod filling. In the PM, in contrast, the grain size and the grains per pod, biomass (g $m^{-2}$), HI, yield, grain number and pods $m^{-2}$ showed significant changes with application leaf N (Table 1). These last had a quadratic trend as increased the dose of N, the maximum values were found with 2

g of N $L^{-1}$. So, achieved the highest biomass and yield with 154 and 86 $gm^{-2}$, respectively and higher number of seeds and pods (Table 1). Also, the increased demand for photosynthate represented by a greater number of pods and seeds modulated dry matter allocation to seed and was reflected in a higher HI (Escalante and Rodriguez, 2010). The decrease in biomass and yield by 2 g N $L^{-1}$ is possibly due to damage to plants.

**Table 1.** Biomass, harvest index, yield and components of the bean (*Phaseolus vulgaris* L) cv. Michoacan 12A3 in relation to foliar application of nitrogen. Data average of four replications.

| N (g $L^{-1}$) | Biomass g $m^{-2}$ | Harvest Index | Yield g $m^{-2}$ | Grain $m^{-2}$ | Grain size (g) | Pods $m^{-2}$ | Grain /pod |
|---|---|---|---|---|---|---|---|
| 0 | 113 c | 0.38 c | 43 c | 286 c | 0.167 | 89 | 3.1 |
| 1 | 123 bc | 0.49 ab | 61 b | 337 bc | 0.185 | 108 | 3.1 |
| 2 | 154 a | 0..56 a | 86 a | 494 a | 0.174 | 145 | 3.4 |
| 3 | 130 b | 0.47 b | 61 b | 386 b | 0.163 | 113 | 3.4 |
| 4 | 116 bc | 0.44 b | 45 bc | 350 bc | 0.157 | 106 | 3.3 |
| Tukey 0.05 | 14 | 0.08 | 13 | 57 | 0.05 | 16 | 0.7 |

Values in columns with similar letter are statistically the same.

## CONCLUSIONS

Under rainfed conditions and warm climate, biomass, harvest index and yield of bean cv. Michoacan 12A3 increases with the application until 2 g $L^{-1}$ of nitrogen foliar. The increase in the yield is associated with an increased number of grains and pods. Higher doses do not cause increases in these variables

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# GRAIN YIELD WITH TWELVE TECHNOLOGIES IN RAINFED DRY BEANS IN CHIHUAHUA STATE

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## INTRODUCTION

The bean (*Phaseolus vulgaris* L.) is one of the most important crops in Chihuahua, based on crop area (134,872 ha) and production obtained (117,328 ton) (SIAP, 2010). In this state, the increase in chemical fertilizer prices has reduced its use and thus has diminished productivity. It is considered that the use of chemical fertilizer is 24% of the total cost of bean production and use is a problem of contamination of groundwater producing areas. There inoculants market low cost, containing microorganisms such as *Trichoderma* spp. and Micorriza INIFAP$^{MR}$, which are recommended for enhancing the growth of plants. Is no conclusive evidence to demonstrate the usefulness of inoculants and organic fertilizers on bean production in the Chihuahua state. The objective was to evaluate the effect on grain yield of beans Pinto Saltillo with twelve different technologies in the Chihuahua state.

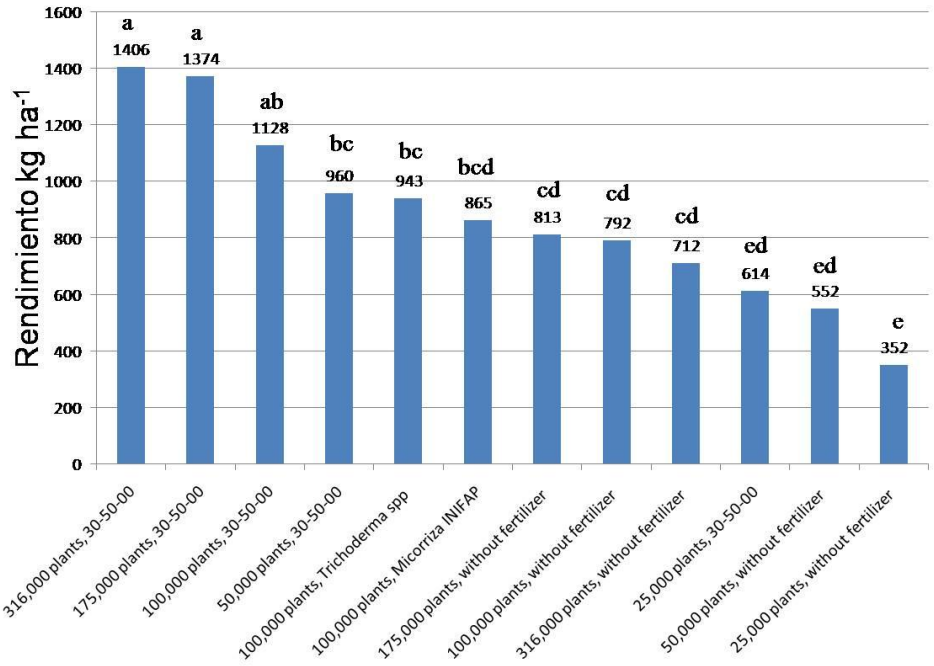
## MATERIALS AND METHODS

Twelve treatments were used with the bean cultivar Pinto Saltillo 1) without fertilizer and 2 pl $m^{-1}$ linear, 2) without fertilizer and 4 pl $m^{-1}$, 3) without fertilizer and 8 pl $m^{-1}$, 4) without fertilizer and 14 pl $m^{-1}$, 5) without fertilizer and 25 pl $m^{-1}$, 6) 30-50-00 and 2 pl $m^{-1}$ linear, 7) 30-50-00 and 4 pl $m^{-1}$, 8) 30-50-00 and 8 pl $m^{-1}$, 9) 30-50-00 and 14 pl $m^{-1}$, 10) 30-50-00 and 25 pl $m^{-1}$, 11) 8 pl $m^{-1}$and Micorriza INIFAP$^{MR}$ y 12) 8 pl $m^{-1}$and *Trichoderma* spp. The research was conducted during the spring-summer 2010 on the land of the Experimental Station of INIFAP in Bachiniva, Chihuahua, Mexico: 28 ° 47 '19.32 "North Latitude, 107 ° 16' 11.64" West Longitude, altitude of 2012 meters. In a clay loam soil with 43 % sand, silt 28.72% and 28.28% clay, salt free, high organic matter content (2.01%). The rainfall during the crop was 351 mm. The experiment was planted on July 6, 2010. Experimental design was a randomized complete block with four replications. Each replication consisted of two rows of 5 m. The grain was standardized to 13% humidity to calculate the yield per hectare. Was performed an analysis of variance using Statistical Analysis System.

## RESULTS AND DISCUSSION

No significant differences were found between treatments with the 30-50-00 and 14 pl $m^{-1}$ and 25 pl $m^{-1}$, these results are consistent with Fernandez *et al*. (2007), indicating that the plant population is a range of 60,000 to 100,000 pl $ha^{-1}$; Ibarra *et al*. (2003) and Ibarra *et al*. (2000) indicates that the density of plants per hectare should be 130,000 pl $ha^{-1}$ to obtain higher yields and Acosta (2010) (personal communication) indicating that should be planted between 12 and 16 seeds per meter (corresponding to 150,000 and 200,000 seeds per hectare respectively, to obtain a good grain yield). Were found highly significant differences between treatment with the 30-50-00 formula and 14 pl $m^{-1}$ and treatment with 30-50-00 and 4 pl $m^{-1}$ and without fertilizer and 4 pl $m^{-1}$ which are the two

common scenarios with the farmers. There were no significant differences between treatments 30-50-00 and 4 pl $m^{-1}$, 8 pl $m^{-1}$ and *Trichoderma* and 8 pl $m^{-1}$ and Micorriza INIFAP. Highly significant differences were found between treatments with *Trichoderma* and Micorriza INIFAP inoculants regarding treatment with 4 pl $m^{-1}$ without fertilizer.




Bean yield Pinto Saltillo with twelve different technologies in the Chihuahua state .$R^2$ = 0.78, C. V. = 22.1, mean= 876.3

## CONCLUSIONS

The best technology for bean production in the Chihuahua state with the bean cultivar Pinto Saltillo is 14 pl $m^{-1}$ (175,000 pl $ha^{-1}$) and fertilizer 30-50-00. The use inoculants (Micorriza INIFAP$^{MR}$ and *Trichoderma* spp.) represents an alternative to increase grain yield and economic benefit of beans in Chihuahua state, although it is necessary to validate the economic feasibility of their extensive application.

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# ALTERNATIVE CULTIVARS OF BEAN PLANT IN NORTHERN MINAS GERAIS BASED UPON POPULATION DENSITIES

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## INTRODUCTION

In Brazilian bean market, the Carioca type is the most accepted. Nevertheless, there has been interest also for other grain types the growing of which will be able to stand for income options for the farmers, to enable the commercialization of a distinct product and exploração of new market niches with increased pay. However, to make the use of alternative cultivars viable, there is the need to adapt the present systems of the production of the alternative legumes, including the population density recommendations.

The objective of the work was investigating the performance of alternative bean cultivars in the North of the state of Minas Gerais, Brazil, by means of ajuste of the best plant populations.

## MATERIAL AND METHODS

Two field experiments were conducted in the Experimental Estações Experimentais of Empresa de Pesquisa Agropecuária de Minas Gerais (Minas Gerais Agricultural Research Corporation), in the localities of Jaíba and Mocambinho, in the Spring-Winter crop of 2007. the utilized experimental design was in randomized blocks with three replicates and factorial scheme 4 x 5, encompassing four cultivars (Radiante, Ouro Vermelho, Bolinha and Novo Jalo) and five population densities (100, 200, 300, 400 and 500 thousand plants $ha^{-1}$).

Sowing was by hand, adopting the 0.5 m inter-row spacing and densities enough for, after thinning, obtaining the wished populations. At sowing, 400 kg $ha^{-1}$ of the formulation NPK 8-28-16 and, at topdressing, 30 kg $ha^{-1}$ of N, source urea, were applied.

On the occasion of harvest, final stand (EF) and grain yield (REND) with their primary components: number of pods per plant - VP, number of grains pr plot - GV and one-hundred grain weigh - P100, were evaluated. The data were submitted to the singe factor and joint analysis of variance, the effect of cultivars being evaluated through the clustering of means by the Scott-Knott test and the effects of population densities when significant by the F test, studied by means of regression analysis.

## RESULTS AND DISCUSSION

The joint variance analysis revealed that there was significant effect of cultivars (C) upon both the characteristics and of the populations (P) on the EF, VP and GV. The sites (L) only did not influence the GV. The triple interaction C*P*L was significant only in relation to the EF, while the double interactions C*P and P*L were significant in relation to the EF and VP. The C*L interaction was significant in the cases of EF, P100 and REND.

Independent of the site, the GV was inversely proportional to grain size, standing out cultivar Ouro Vermelho, that of smallest grain. Cultivar Novo Jalo outyielded cultivar Radiante as to the P100 at Mocambinho, but it did not differ as to this characteristic at Jaíba. In both places, cultivar Radiante was the one of highest grain yield (Table 1).

The increase of plant population in addition to raising EF, reduced in quadratic form the VP (Figures 1 and 2) and in a liner form the P100 (Figure 3), but it did not affect significantly the REND. These relations among the components of yield were also obtained by other authors, such as Valério et al. (1999), who worked on Carioca grain type grain cultivars.

**Table 1** Average values of the average weight of one hundred grains (kg ha$^{-1}$) of our bean cultivars at Jaíba and Mocambinho, Minas Gerais, Brazil

| Tratamento | P100 | REND |
|---|---|---|
| **Jaíba** | | |
| Radiante | 38.6 **a** | 3410 **a** |
| Novo Jalo | 38.7 **a** | 2492 **b** |
| Bolinha | 35.1 **b** | 2399 **b** |
| Ouro Vermelho | 21.3 **c** | 1962 **b** |
| **Mocambinho** | | |
| Radiante | 36.6 **b** | 2739**a** |
| Novo Jalo | 40.0**a** | 2311 **b** |
| Bolinha | 31.5 **c** | 2530**a** |
| Ouro Vermelho | 19.1 **d** | 2227 **b** |

Within each factor, means followed by different letters lie in different groups according to the Knott-Scott test at the 5% level of probability

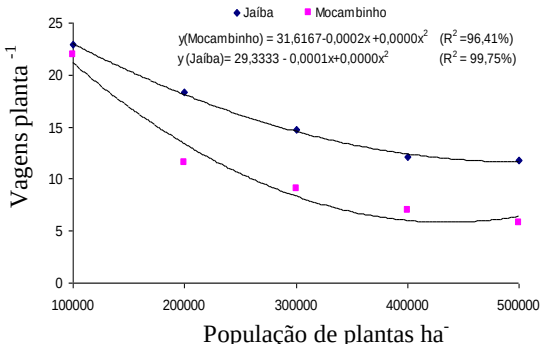


**Figure 1** Number of pods per bean plant (mean of four cultivars in two localities) on the basis of the population densities at Jaiba and Mucambinho, MG, winter of 2007

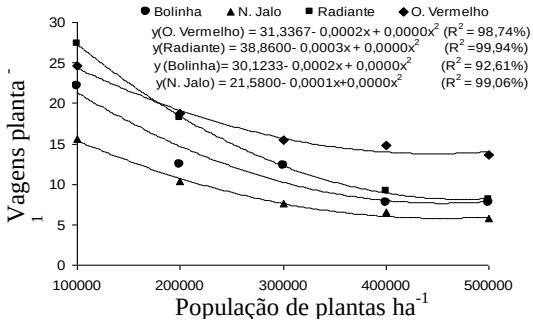


**Figure 2** Number of pods per bean plant (mean of two localities) on the basis of the population densities at Jaiba and Mucambinho, MG, winter of 2007

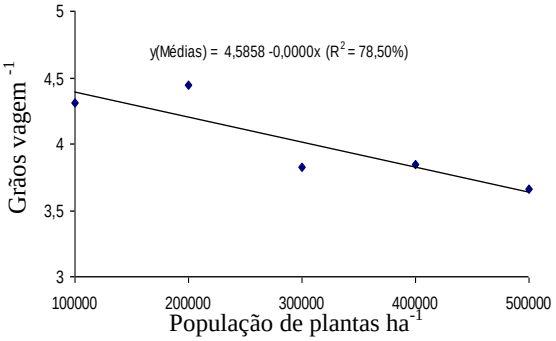


**Figure 3** Number of grains pr bean plant pod (mean of four cultivars in two localities) on the basis of the population densities at Jaiba and Mucambinho, MG, winter of 2007

## CONCLUSIONS

Increased plant population reduces the number of pods per plant and the number of grains pr pod, but it did not impact grain yield.

The usual populations around 240 thousand plants per hectare can be utilized also for the alternative cultivars without any yield loss.

Cultivars Radiante, Novo Jalo, Bolinha and Ouro Vermelho presents good performance in the irrigated winter crop and stand for new alternative for the cultivation in North region of Minas Gerais.


## ACKNOWLEDGEMENTS

The authors thank the financial support of CAPES, CNPq and FAPEMIG.


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# PRODUCTION PARAMETERS IN 14 GENOTYPES OF COMMON BEAN

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## INTRODUCTION

Common bean (*Phaseolus vulgaris*) is a species with economic, social and cultural importance to Brazil, and it exercises influence under commercial balance, promotes job generation, besides to serve as base in Brazilian alimentation (Lobato et al., 2009).

Aim of this investigation was to evaluate productive potential of 14 common bean (*Phaseolus vulgaris*) genotypes, being measured number of pod per plant, number of grain per pod, 100-seed weight, and grain yield.

## MATERIALS AND METHODS

Study was conducted in experimental area located in Centro de Treinamento em Irrigação (CTI) of Universidade Estadual de Maringá (UEM), during 2008 season. Precipitation index during period is presented in Figure 1, and soil conditions in Table 1. Fertilization was carried out based in soil analysis and crop exigency. Plants were grown in field conditions with minimum/maximum temperature of 15.2/32.7ºC, and relative humidity was 48/74%, respectively.

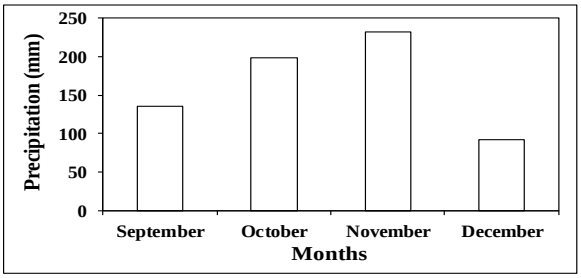


**Fig. 1:** Index precipitation during experimental period.

**Table 1.**Chemical and physical characteristics of Eutrophic Red Nitosol used during study.

| pH* | $Ca^{+2}$ | $Mg^{+2}$ | $K^{+}$ | $Al^{+3}$ | CTC | OM** | V | P*** |
|---|---|---|---|---|---|---|---|---|
| | ---------- $cmol_c.dm^{-3}$ ---------- | | | | | $g.kg^{-1}$ | % | $mg.dm^{-3}$ |
| 5.1 | 3.3 | 1.3 | 0.4 | 0.5 | 11.9 | 40.7 | 41.4 | 30.5 |

| sand ($g\ Kg^{-1}$) | silt ($g\ Kg^{-1}$) | clay ($g\ Kg^{-1}$) |
|---|---|---|
| 300 | 160 | 540 |

* $CaCl_2$; **Organic matter in soil; *** extractor Mehlich

Experimental design used was randomized blocks, with 14 genotypes (IAPAR 81, IPR Tangará, IPR Uirapuru, BRS Campeiro, CHC 9729, CHP 9859, GEN C2-1-1, GEN C2-1-3, SM 1007, SN 1207, CNFC 10408, CNCF 10429, LP 0403, and LP 0472). Experiment was composed of 4 blocks, and each plot with 5x5 m (diameter x width), being used spacing of 1 and 2 m among plots and blocks, respectively.

In this study were evaluated number of pod per plant, number of grain per pod, 100-seed weight, and grain yield. Data were analyzed employing a variance analysis, and using Scott-Knott test at 5% level of probability. Standard errors were also calculated in all treatments evaluated.

## RESULTS AND DISCUSSION

Results showed in number of pod reveal that there are 2 groups, in which group with higher performance oscillated between 5 and 7 pods (Figure 2A), being this composed by genotypes IAPAR 81, IPR Uirapuru, BRS Campeiro, CHC 9729, CHP 9859, SN1207, CNFC 10408, CNFC 10429, and LP 0472.

In number of grains per pod occurred not significant difference among genotypes, and this variable to remain between 3 and 2 grains per pod (Figure 2B). Results linked to 100-seed weight present statistically three groups (Figure 2C), being group with better performance composed by IAPAR 81, IPR Tangará, and SN1207 genotypes, and higher value was presented by IPR Tangará genotype with 27.7 g.

Results linked to grain yield revealed presence of three groups, being group with higher yield composed by IAPAR 81, IPR Tangará, IPR Uirapuru, BRS Campeiro, CHC 9729, CHP 9859, SN 1207, and CNFC 10408 genotypes (Figure 2D).

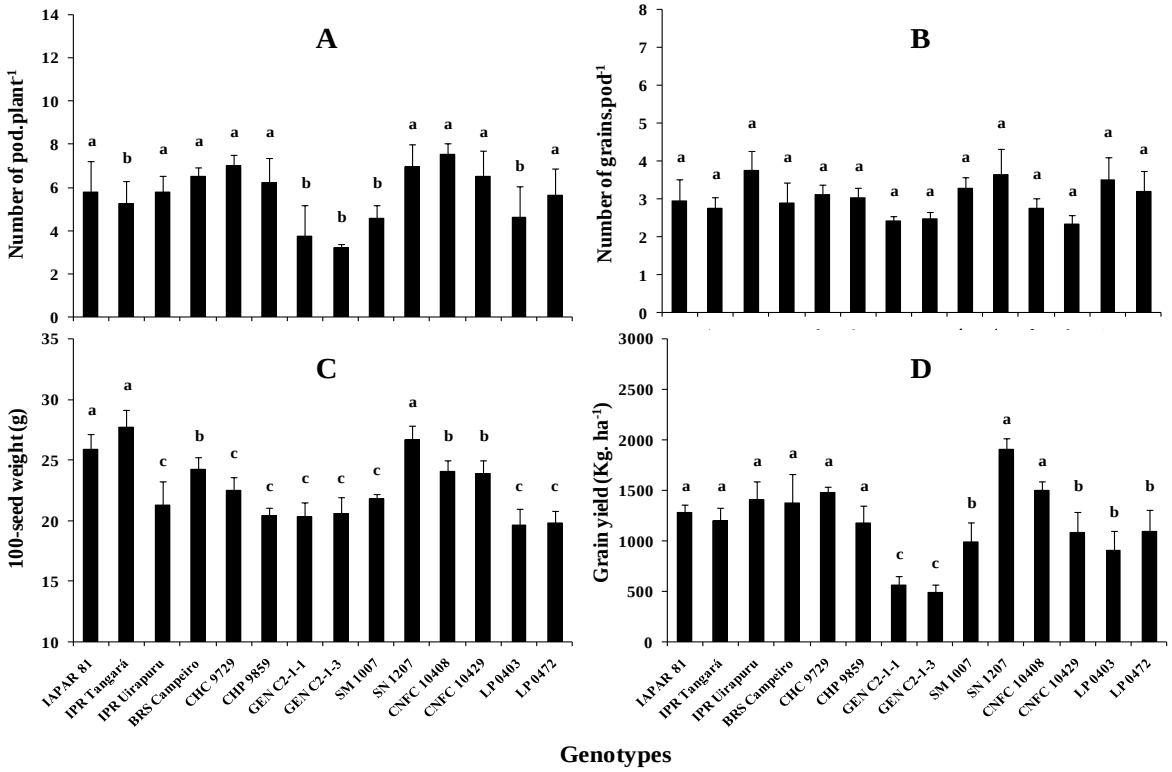


**Fig. 2:** (A) number of pod.plant$^{-1}$, (B) number of grains.pod$^{-1}$, (C) 100-seed weight, and (D) grain yield in 14 genotypes of *Phaseolus vulgaris*. Same letters do not show significant differences at Scott-Knott test ($P < 0.05$). Bars represent mean standard error.

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# CARBOHYDRATES IN 14 GENOTYPES OF COMMON BEAN TESTED TO BRAZILIAN ENVIRONMENT

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## INTRODUCTION

Carbon metabolism is responsible for regulation of compounds as sucrose, glucose, and starch, besides enzymes such as sucrose synthetase and invertase. In addition, sucrose is an important energy component exported from leaves to several plant organs (Kingston-Smith et al., 1999).

Nutritionally, common bean grain is frequently used as source proteins and carbohydrates by Brazilian population, and this crop presents also economic importance for this country (Vieira, 2005).

Aim of this investigation was to obtain responses linked to carbon metabolism of 14 genotypes of common bean favorable to Brazilian conditions, and it was evaluated total soluble carbohydrates, starch, and sucrose.

## MATERIALS AND METHODS

Study was conducted in experimental area located in Centro de Treinamento em Irrigação (CTI) of Universidade Estadual de Maringá (UEM), during 2008 season. Fertilization was carried out based in soil analysis and crop exigency. Plants were grown in field conditions with minimum/maximum temperature of 15.2/32.7ºC, and relative humidity was 48/74%, respectively.

Experimental design used was randomized blocks, with 14 genotypes (IAPAR 81, IPR Tangará, IPR Uirapuru, BRS Campeiro, CHC 9729, CHP 9859, GEN C2-1-1, GEN C2-1-3, SM 1007, SN 1207, CNFC 10408, CNCF 10429, LP 0403, and LP 0472). Experiment was composed of 4 blocks, and each plot with 5x5 m (diameter x width), being used spacing of 1 and 2 m among plots and blocks, respectively. Total soluble carbohydrates, starch, and sucrose were determined according to Dubois et al. (1956), Van Handel (1968), and Dubois et al. (1956), respectively.

In this study were evaluated total soluble carbohydrates, starch, and sucrose. Data were analyzed employing a variance analysis, and using Scott-Knott test at 5% level of probability. Standard errors were also calculated in all treatments evaluated.

## RESULTS AND DISCUSSION

Results showed in total soluble carbohydrates levels indicated that this parameter is significantly different in 14 genotypes studded (Figure 1A), as well as higher level was obtained in BRS Campeiro genotype. In intermediate group were showed values fluctuating between 1.25 and 1.16 mmol g $DM^{-1}$, and it was composed by IAPAR 81, CHP 9859, SM 1007, and LP 0403 genotypes. Group formed by IPR Tangará, IPR Uirapuru, CHC 9729, GEN C2-1-1, GEN C2-1-3, SN 1207, CNFC 10408, CNFC 10429, and LP 0472 genotypes oscillated between 0.97 and 1.12 mmol g $DM^{-1}$, and it presented lower amounts of carbohydrates, when compared with other group.

Starch amounts in common bean plants indicate that there are three groups (Figure 2A), and group with higher starch level fluctuated between 0.68 and 0.70 mmol g $DM^{-1}$, with this being formed by genotypes SM 1007, CNFC 10429, and LP 0472. Seven genotypes presented statistically equal results and lowers to other groups, in which was composed by IAPAR 81, IPR Tangará, IPR Uirapuru, CHC 9729, CHP 9859, GEN C2-1-1, and GEN C2-1-3 genotypes.

In relation sucrose genotypes evaluated were grouped in 4 types (Figure 2B), and group with greater sucrose level in leaf tissue were SM 1007, CNFC 10408, and LP 0472. Other group oscillated between 0.71 e 0.67 mmol g $DM^{-1}$, and was formed by CNFC 10429 and LP 0403 genotypes. Minor amounts of sucrose were showed in IPR Tangará, BRS Campeiro, and GEN C2-1-1 genotypes.

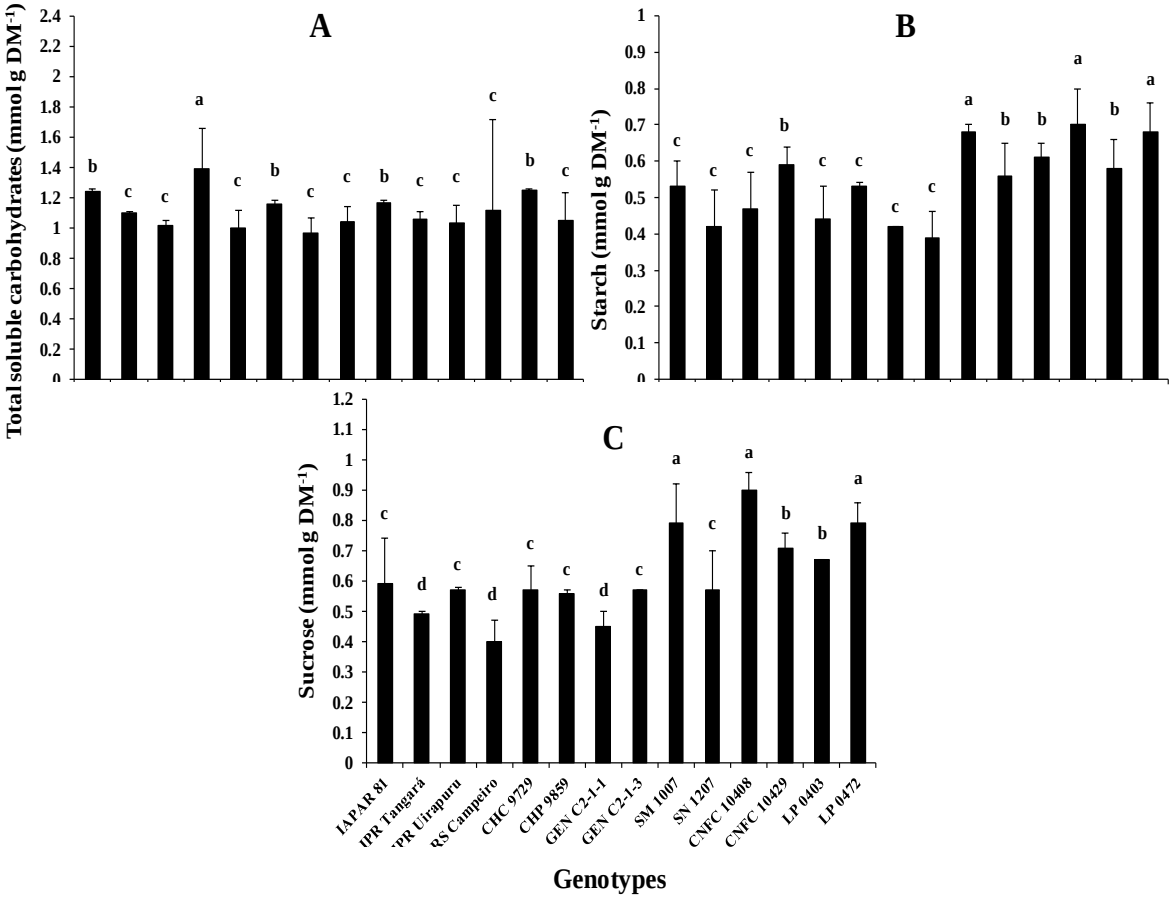


**Fig. 1:** (A) Total soluble carbohydrates, (B) starch, and (C) sucrose in 14 genotypes of *Phaseolus vulgaris*. Same letters do not show significant differences at Scott-Knott test ($P < 0.05$). Bars represent mean standard error.

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# AMINO ACIDS, PROLINE, AND PROTEINS BEHAVIORS IN GENOTYPES OF *PHASEOLUS VULGARIS* CULTIVATED BRAZILIAN CONDITIONS

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## INTRODUCTION

Nitrogen metabolism is important for growth, development and consequent accumulation biomass (Nejidat et al., 1997), in which proteins are formed by amino acids after nitrate and/or ammonium absorption.

Normally specie with agronomical potential when cultivated in field conditions can be affected by abiotic and biotic stresses (Lobato et al., 2009), in which are characterized by environment and biological factors, respectively. Therefore, proline by to be an osmoregulator can be used as tool to measure the ability of higher plants in to attenuate inadequate situations.

Study had aim to evaluate behavior of nitrogen compounds, and it were measured total soluble amino acids, proline, and total soluble proteins in leaf of 14 genotypes of common bean.

## MATERIALS AND METHODS

Study was conducted in experimental area located in Centro de Treinamento em Irrigação (CTI) of Universidade Estadual de Maringá (UEM), during 2008 season. Fertilization was carried out based in soil analysis and crop exigency. Plants were grown in field conditions with minimum/maximum temperature of 15.2/32.7ºC, and relative humidity was 48/74%, respectively.

Experimental design used was randomized blocks, with 14 genotypes (IAPAR 81, IPR Tangará, IPR Uirapuru, BRS Campeiro, CHC 9729, CHP 9859, GEN C2-1-1, GEN C2-1-3, SM 1007, SN 1207, CNFC 10408, CNCF 10429, LP 0403, and LP 0472). Experiment was composed of 4 blocks, and each plot with 5x5 m (diameter x width), being used spacing of 1 and 2 m among plots and blocks, respectively. Total soluble amino acids and proline were determined according to Peoples et al. (1989), Bates et al. (1973), and Bradford (1976), respectively.

In this study were evaluated total soluble amino acids, proline, and total soluble proteins. Data were analyzed employing a variance analysis, and using Scott-Knott test at 5% level of probability. Standard errors were also calculated in all treatments evaluated.

## RESULTS AND DISCUSSION

Genotypes of common bean based in total soluble amino acids were described by two groups, in which group with higher level in this variable oscillated between 41.78 and 42.76 µmol g $DM^{-1}$ (Figure 1A), being composed by genotypes IPR Uirapuru, CHC 9729, GEN C2-1-3, SM 1007, LP 0403, and LP 0472. In other group the genotypes IAPAR 81, IPR Tangará, BRS Campeiro, CHP 9859, GEN C2-1-1, CNFC 10408, and CNFC 10429 were statistically equals.

Values obtained in proline indicate occurrence of three groups, being that group with greater proline accumulation presented as extremes values of 9.15 and 7.71 µmol g $DM^{-1}$ (Figure 1B), and in this group the genotype that has higher proline level was IAPAR 81. However, genotypes that

presented proline medium levels, if compared with other, was composed by IPR Uirapuru, CHC 9729, GEN C2-1-3, SM 1007, SN 1207, and CNFC 10429 genotypes.

Total soluble proteins were statistically different among genotypes investigated (Figure 1C), and was possible to show 3 groups. Higher protein level was obtained in SM 1007 genotype with 15.1 mg g $DM^{-1}$. Additionally, CNFC 10408 genotype presented 12.8 mg g $DM^{-1}$, and this value is statistically intermediate. In general, genotypes evaluated oscillated between 9.4 and 11.3 mg g $DM^{-1}$.

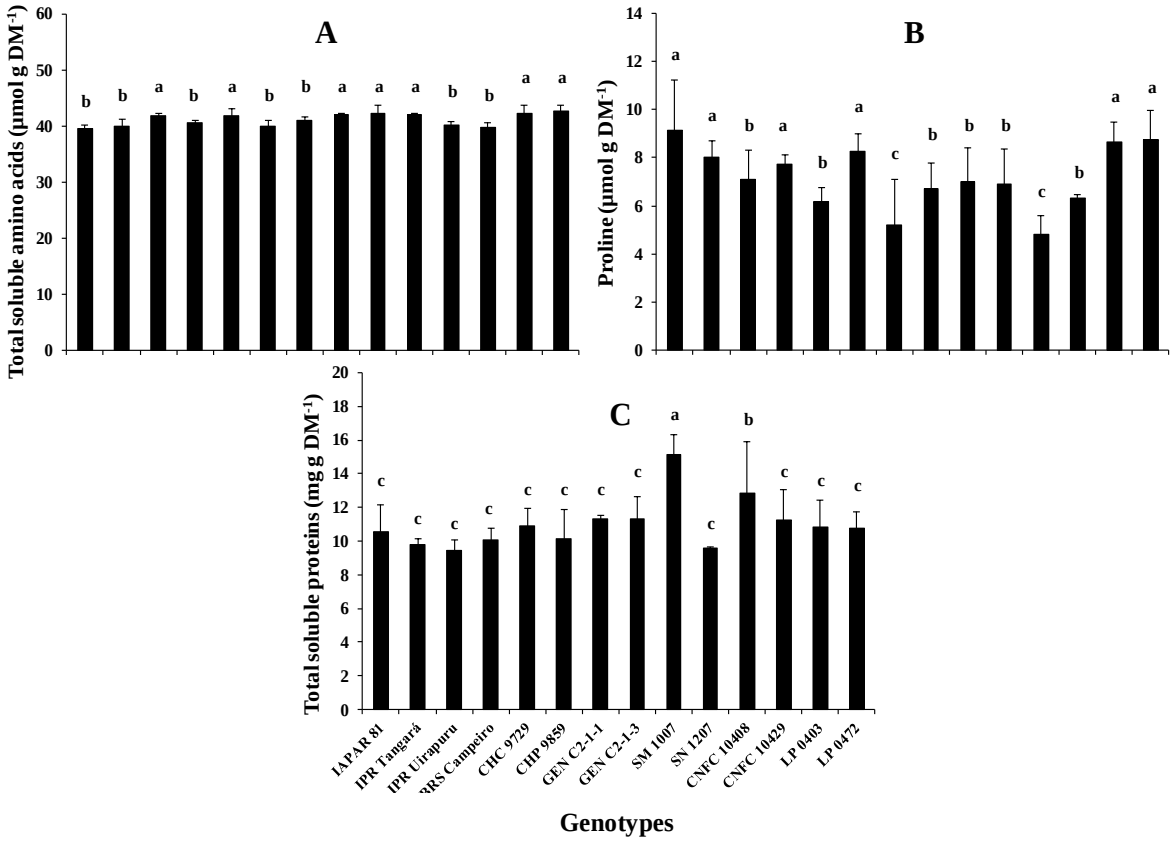


**Fig. 1:** (A) Total soluble amino acids, (B) proline, and (C) total soluble proteins in 14 genotypes of *Phaseolus vulgaris*. Same letters do not show significant differences at Scott-Knott test ($P < 0.05$). Bars represent mean standard error.

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# ASSIMILATED NITROGEN FORMS AND PLANT DEVELOPMENT IN GENOTYPES OF COMMON BEAN

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## INTRODUCTION

Nitrogen supply exercise fundamental role in protein formation (Bredemeier and Mundstock, 2000), and nitrate and ammonium by be two nitrogen forms that can be assimilated by higher plants, it can influence nitrogen compounds (Lobato et al., 2009) and consequently in biological processes such as plant growth, as well as filling and composition grain.

Aim of this study was to investigate assimilated nitrogen forms and plant development in 14 genotypes of *Phaseolus vulgaris*, using as parameters nitrate and free ammonium in leaf, and plant height.

## MATERIALS AND METHODS

Study was conducted in experimental area located in Centro de Treinamento em Irrigação (CTI) of Universidade Estadual de Maringá (UEM), during 2008 season. Fertilization was carried out based in soil analysis and crop exigency. Plants were grown in field conditions with minimum/maximum temperature of 15.2/32.7ºC, and relative humidity was 48/74%, respectively.

Experimental design used was randomized blocks, with 14 genotypes (IAPAR 81, IPR Tangará, IPR Uirapuru, BRS Campeiro, CHC 9729, CHP 9859, GEN C2-1-1, GEN C2-1-3, SM 1007, SN 1207, CNFC 10408, CNCF 10429, LP 0403, and LP 0472). Experiment was composed of 4 blocks, and each plot with 5x5 m (diameter x width), being used spacing of 1 and 2 m among plots and blocks, respectively. Nitrate and free ammonium were determined according to Cataldo et al. (1975) and Weatherburn (1967), respectively.

In this study were evaluated nitrate, free ammonium, and plant height. Data were analyzed employing a variance analysis, and using Scott-Knott test at 5% level of probability. Standard errors were also calculated in all treatments evaluated.

## RESULTS AND DISCUSSION

Results linked to nitrate level in leaf of 14 genotypes revealed that there are 3 groups (Figure 1A), being genotype CNFC 10429 presented higher nitrate amount. Other group is formed by 2 genotypes, CHC 9729 and LP 0403 that presented intermediate levels of nitrate in leaf, when compared with others. Group with lower nitrate level in leaf oscillated between 0.44 e 0.57 µmol $NO_3^-$ g $DM^{-1}$, and composed by other genotypes. Nitrate is main assimilated nitrogen form in several species with agronomical potential as *Phaseolus vulgaris*, and this compound can be reduced to nitrite ($NO_2^-$) in leaf and root aiming formation of other compounds such as amino acids and proteins.

In ammonium level in leaf the variance analysis indicated existence of 4 groups (Figure 1B), being that genotypes with higher ammonium assimilation were IPR Tangará, SM 1007, and CNFC

10408 with values of 25.09, 24.76, and 26.35 µmol $NH_4^+$ g $DM^{-1}$, respectively. In other group were showed genotypes GEN C2-1-1, GEN C2-1-3, and LP 0472. In addition, group with smaller level was composed by genotypes IAPAR 81, IPR Uirapuru, CHP 9859, and LP 0403.

Plant height oscillated between 56.9 and 39.7 cm (Figure 1C), as well as genotypes LP 0472 and GEN C2-1-1 presented higher and lower height, respectively. Statistically this variable can be divided in three groups, and genotypes with better performance is formed by IPR Uirapuru, SM 1007, SN 1207, CNFC 10429, LP 0403, and LP 0472 genotypes.

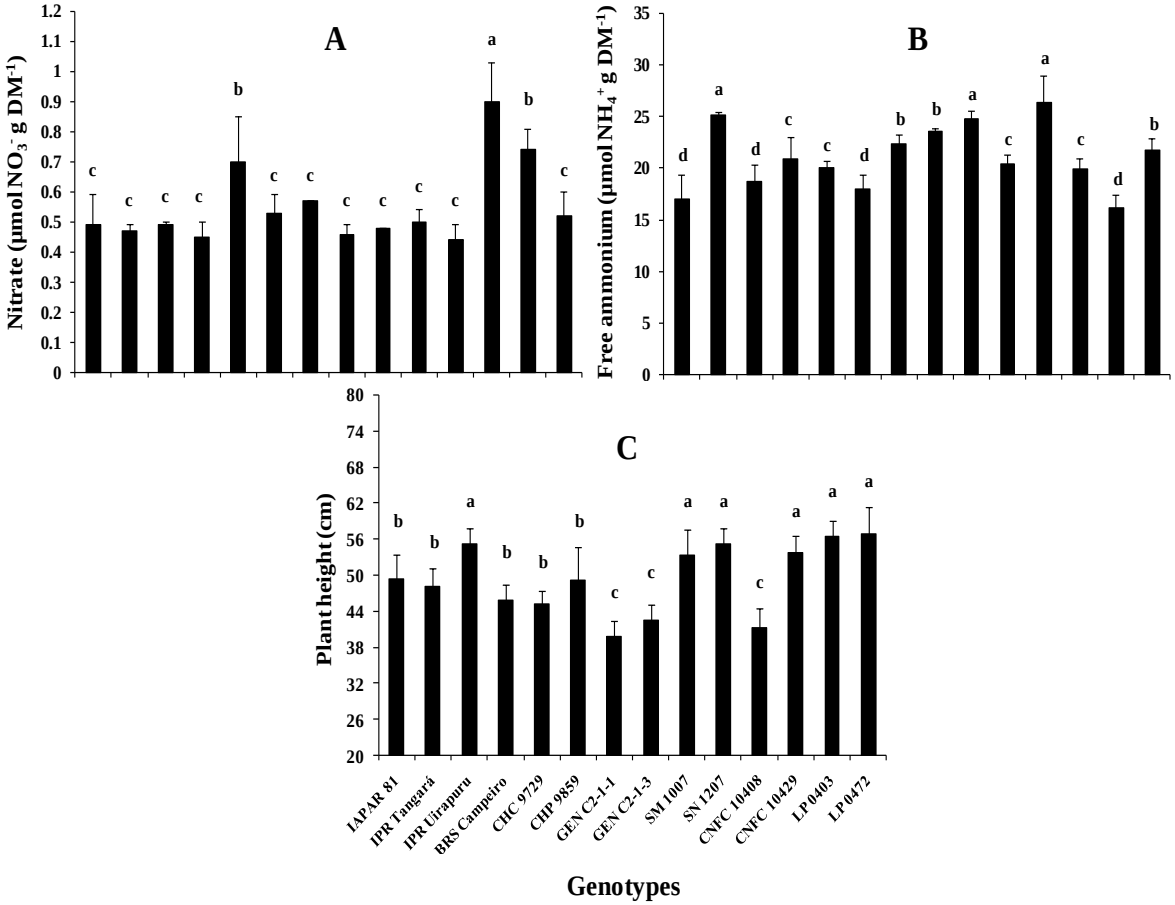


**Fig. 1:** (A) Nitrate, (B) free ammonium, and (C) plant height in 14 genotypes of *Phaseolus vulgaris*. Same letters do not show significant differences at Scott-Knott test ($P < 0.05$). Bars represent mean standard error.

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# GROWTH AND DEVELOPMENT IN THREE GENOTYPES OF LIMA BEAN

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## INTRODUCTION

Gender *Phaseolus* comprise cultivated and wild species, in which all are originated in American continent. Each cultivated species form a pool genic primary, with your wild ancestral forms (Wetzel et al., 2006). *Phaseolus lunatus* is commonly called as lima bean and it is consumed in Brazil in form of grain (Oliveira et al., 2004; Santos et la., 2002). This crop has economic and social importance for Brazil, and due to rusticity and high tolerance to drought is possible cultivation during dry period (Vieira, 1992). In other hand, it is source of food and vegetal protein to Brazilian population.

Objective of this research is to evaluate growth and development in UFPI 463, UFPI 465, and, UFPI 482 genotypes of *Phaseolus lunatus* with potential agronomical.

## MATERIALS AND METHODS

Study was carried out in experimental area of Departamento de Fitotecnia of the Universidade Federal do Piauí. Plant materials used in this investigation are three (UFPI 463, UFPI 465, and UFPI 482) genotypes of *Phaseolus lunatus* L. with higher agronomic characteristics coming from of germplasm bank of the Universidade Federal do Piauí.

Experimental design was entirely randomized with three genotypes (UFPI 463, UFPI 465, and UFPI 482) and 5 replicates. Plants Measurements were implemented in 9 vegetative stages (V1, V2, V3, V4, V5, V6, V7, V8, and V9), as well as in 5 reproductive stages (R1, R2, R3, R4, and R5).

Data were submitted to variance analysis, means were separated with Skott-Knott test at 5% level of error probability, and software used was ASSISTAT 7.5.

## RESULTS AND DISCUSSION

For vegetative stage were showed significant differences in V1, V2, V5, V7, and V9 (Table 1). Emergency or V1 stage presented significant difference, and UFPI 463, UFPI 465, and UFPI 482 genotypes reached in 10, 11, and 9 days, respectively. V4 stage presented not significant difference in genotypes evaluated. In addition, this stage is important in *P. lunatus* due to be moment that is showed second separated trifoliate leaf and this leaf is commonly used in phytopathology studies linked to inheritance and allelism of resistance genes in several species of *Phaseolus* gender. Precocity is an agronomical characteristic important, because for farmer is wanted a cycle more short and high yield.

In V7 stage were showed values of 29, 30, and 28 days in UFPI 463, 465, and 482, respectively. UFPI 482 completed the vegetative phase with 41 days, and it is considerable more

precocious. As well as the vegetative stage duration can be used to characterize genetic variability among genotypes studded.

In reproductive phase were showed significant difference in R2 and R3 stages. UFPI 482 had lower time to reach R2 and R3 stages (Table 2), which exhibited lower time to have first flower totally opened and mature pod. This study suggested that in research on flower biology or artificial pollinations must be carried out in different days.

R4 stage presented not significant difference among genotypes, in which values showed were 57.2, 57.2, and 55.8 days in UFPI 463, UFPI 465, and UFPI 482, respectively. Time medium for maturation in 90% of pod was equal in all genotypes (68 days). And this fact reveals adequate adaptation of this species in this condition, because reproductive development was reached in all genotypes evaluated during this study.

**Table 1:** Growth and development in vegetative period of three *Phaseolus lunatus* genotypes evaluated in Teresina, PI.

| **Genotypes** | **Stages (days)** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **V1** | **V2** | **V3** | **V4** | **V5** | **V6** | **V7** | **V8** | **V9** |
| **UFPI 463** | 10.6 a | 13.4 a | 17.8 a | 21.4 a | 24.4 a | 27.6 a | 29.4 b | 32.6 a | 41.2 a |
| **UFPI 465** | 11.2 a | 11.4 b | 17.8 a | 23.2 a | 25.6 a | 27.8 a | 30.60 a | 33.8 a | 37.6 b |
| **UFPI 482** | 9.4 b | 12.6 a | 17.0 a | 21.2 a | 22.2 b | 25.8 a | 28.8 b | 33.6 a | 37.4 b |
| **Medium value** | 10.4 | 12.4 | 17.5 | 21.9 | 24.0 | 27.0 | 29.6 | 33.3 | 38.7 |

Averages followed by the same lowercase letter in column do not differ among themselves by Skott-Knott test at 5% of probability.

**Table 2:** Growth and development in reproductive period of three *Phaseolus lunatus* genotypes evaluated in Teresina, PI.

| **Genotypes** | **Stages (days)** | | | | |
|---|---|---|---|---|---|
| | **R1** | **R2** | **R3** | **R4** | **R5** |
| **UFPI 463** | 39.6 a | 44.4 b | 50.0 b | 57.2 a | 67.8 a |
| **UFPI 465** | 37.8 a | 48.0 a | 53.6 a | 57.2 a | 68.0 a |
| **UFPI 482** | 37.8 a | 41.4 c | 47.6 b | 55.8 a | 68.0 a |
| **Medium value** | 38.4 | 44.6 | 50.4 | 56.7 | 67.9 |

Averages followed by the same lowercase letter in column do not differ among themselves by Skott-Knott test at 5% of probability.

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# GENETIC DIVERGENCE BY MORPHOAGRONOMIC TRAITS IN LIMA BEAN

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## INTRODUCTION

Lima bean (*Phaseolus lunatus* L.) is an important crop in Central and South America and Africa. In Brazil, mainly in the Northeast region, is an income and alternative food source for the population; however it is also cultivated in South and Southeast regions. Lima bean reveals high polymorphism and seed morphology has been one of the key traits in understanding the genetic diversity in this species. The aim of this research was to study the genetic dissimilarity among 166 lima bean samples from Embrapa's Collection.

## MATERIAL AND METHODS

A total of 166 samples of *P. lunatus* from Germoplasm Bank of the Embrapa Genetic Resources & Biotechnology (Cenargen, Brazil) and from Internacional Center for Tropical Agriculture (CIAT, Colombia) were evaluated with basis in eight continuous agronomic traits: days to flowering (DFL), length (LP) and width (WP) of the pod, number of locules per pod (NLP), number of seeds per pod (NSP), length (LS), width (WS) and thickness (TS) of the seed, included in descriptor list for lima bean published by International Plant Genetic Resources Institute (IPGRI, 2001). Mahalanobis' distance ($D^2_{ij}$) between genotypes and grouping Tocher´s were estimated, as well as the relative contribution of each trait (SINGH, 1981).

## RESULTS AND DISCUSSION

Significant differences ($P<0.01$), by F test, for all traits among the genotypes tested were observed (Table 1), indicating the existence of genetic variability for those traits. Weight of 100 seeds ranged from 17.52 g (BF197) to 147.03 g (BF1113). DFL trait ranged from 26 to 184 days, demonstrating the selection possibility for this trait. LP and WP presented 42.50 mm to 129.17 mm and 8.40 mm to 27.32 mm, respectively. While NLP showed average of 2.79 and and NSP average of 2.76. Length (LS), width (WS) and thickness (TS) of the seed ranged from 8.43 mm to 22.53 mm, 6.59 mm to 14.17 mm and 3.52 mm to 7.32 mm, respectively. However, there is samples includes in the three cultigroups defined by Mackie (1943) as well as comprises the genotypes of lima bean, from either the Andean or the Middle American Center of Domestication. In relationship to the size, most of the samples ranged from small the medium, in agreement with the criterion proposed by Mateo Box, cited by Vilhordo et al. (1996). Genetic distance among 166 samples ranged from 0.95 (BF84 and BF124) to 983.63 (BF113 and BF124). Three groups were formed by Tocher's method. 160 samples composed the group I; group II contains 5 genotypes; group III formed by only one sample (BF1113). The relative contribution of each trait (Table 1) indicated that the pod length (LP) (41.66%), days to flowering (DFL) (23.32%)

and number of locules per pod (NLP) were those who most contributed the total divergence (74.90%) among the samples of lima beans evaluated. These traits were the most efficient to explain the variability between samples and can be used in choose of parental aiming improving of lima bean. There wasn't correlation between genetic distance and origin of the samples.

**Table1**. Summary of the analysis of variance for days to flowering (DFL), length (LP) and width (WP) of the pod, number of locules per pod (NLP), number of seeds per pod (NSP), length (LS), width (WS) and thickness (TS) of the seed and relative contribution of each trait for genetic divergence among 166 lima bean samples, by method proposed by Singh – $S._j$ (1981).

| S.V | D.F. | MS | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | DFL (days) | LP (mm) | WP (mm) | NLP (un) | NSP (un) | LS (mm) | WS (mm) | TS (mm) |
| Samples | 165 | 1474.07** | 435.32** | 14.00** | 0.25** | 0.26** | 15.72** | 5.04** | 0.83** |
| Error | 166 | 63.19 | 12.49 | 1.05 | 0.05 | 0.07 | 1.02 | 0.39 | 0.19 |
| Average | - | 95.87 | 64.30 | 14.47 | 2.79 | 2.76 | 12.60 | 9.26 | 5.59 |
| UL[1/] | - | 26.00 | 42.50 | 8.40 | 1.60 | 1.30 | 8.43 | 6.59 | 3.52 |
| LL[2/] | - | 184.0 | 129.17 | 27.32 | 4.00 | 4.00 | 22.53 | 14.17 | 7.32 |
| C.V (%) | - | 8.29 | 5.49 | 7.10 | 8.41 | 9.29 | 8.17 | 6.79 | 7.86 |
| $S._j$ (%) | - | 23.32 | 41.66 | 4.13 | 9.92 | 2.94 | 6.12 | 6.70 | 5.21 |

**: F significant at 1% (P<0.01)
[1/]UP: Upper limit; [2/]LL: Lower limit.

## CONCLUSIONS

High divergence among samples occurred in lima bean, especially between BF113 and G25633 samples. The traits pod length, days to flowering and number of locules per pod were those who most contributed the total divergence.


## ACKNOWLEDGEMENTS

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# GENETIC DIVERSITY FOR LIMA BEAN SAMPLES

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**INTRODUCTION:** One of the main crops and income and alternative food source for the population from Northeast region of Brazil is the lima bean (*Phaseolus lunatus*), besides an important source of protein for the rural population from South America and Africa. It is consumed either as fresh or dried beans. The characterization of *Phaseolus lunatus* germplasm has a significant impact on the impovment of crop plants and could help to improve the knowledge about genetic variability and differentiation patterns. Thus, this work aimed to evaluate genetic diversity among 24 lima bean samples using quantitative and qualitative descriptors by multivariate procedures.

**MATERIAL AND METHODS:** 24 lima bean samples from Lima Bean Germplasm Active Bank from Federal University of Piauí were evaluated with basis in morphoagronomic descriptors: days to flowering (DFL), days to maturity (DM), number of pod per plant (NPP), length (LP) and width (WP) of the pod and number of seeds per pod (NSP). The experimental design was a lattice 5x5 with four replications, arranged in plots with four lines of five meters long, spaced 1.0 m x 1.0 m. Cultural practices were the commonly used for lima bean crop. The genetic divergence among the samples was estimated and the grouping by Tocher method, with the employment of the Mahalanobis distance, as measure of dissimilarity. The relative contribution of each trait for divergence (SINGH, 1981) was estimated.

**RESULTS AND DISCUSSION:** Significant differences ($p<0.01$) were observed among samples for number of pod per plant (NPP), length (LP) and width (WP) of the pod (Table 1). NPP ranged from 14.11 (UFPI-494) to 291.16 (UFPI-220), while LP and WP traits ranged from 49.84 (UFPI-220) to 99.85 (UFPI-276) and 12.20 (UFPI-251) to 17.62 (UFPI-468), respectively. DFL, DM and NSP traits presented average of 92.60 days, 150.18 days and 2.6 seeds per pod, respectively. It was observed that the sample UFPI-220 showed greater number of pod per plant and smaller length (LP) of the pod, indicating the existence of negative correlation among these traits. Genetic dissimilarity measures among 24 samples showed lower limit of 0.54 (UFPI-470 and UFPI-582) and upper limit of 35.60 (UFPI-468 and UFPI-220). Tocher's method allowed formation of three groups. It is important for parents' choice. Since the new hybrid to be established must be based on the magnitude of their dissimilarities and potential *per se* of the parents. Genotypes grouped into groups more distant genotypes collected in more distant groups give an indication they are dissimilar, as can be potential combinations. Group I contains 75% of the samples, with superior averages for WP trait; group II composed by samples UFPI-278, UFPI-251, UFPI-230, UFPI-243 and UFPI-220, with superior averages for NPP trait; group III formed by only one sample (BF1113). The relative contribution of each trait (Table 1) indicated that the width of the pod (35.23%), number of pod per plant (22.52%), and length (20.82%) were those who most contributed the total divergence (78.57%) among the samples of lima beans evaluated.

**CONCLUSIONS**: UFPI-468 and UFPI-220 samples were most divergent. Width of the pod was the trait that most important for genetic divergence.

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**Table 1.** Means and relative contribution of each trait for genetic divergence among 24 lima bean samples for traits follows: days to flowering (DFL), days to maturity (DM), number of pod per plant (NPP), length (LP) and width (WP) of the pod and number of seeds per pod (NSP).

| Samples | DFL | DM | NPP | LP | WP | NSP |
|---|---|---|---|---|---|---|
| UFPI-032 | 94.02 a[1/] | 149.77 a | 54.01 c | 82.97 a | 16.59 a | 2.95 a |
| UFPI-121 | 90.97 a | 151.30 a | 153.17 b | 65.40 c | 14.25 b | 2.80 a |
| UFPI-123 | 92.77 a | 136.18 a | 39.97 c | 66.57 c | 14.65 b | 2.17 a |
| UFPI-177 | 93.92 a | 154.10 a | 96.83 c | 70.91 b | 16.41 a | 2.55 a |
| UFPI-220 | 91.87 a | 144.15 a | 291.16 a | 49.48 c | 13.27 c | 2.77 a |
| UFPI-222 | 90.92 a | 162.25 a | 159.14 b | 60.80 c | 14.26 b | 2.37 a |
| UFPI-228 | 92.85 a | 157.07 a | 53.79 c | 56.34 c | 14.05 b | 2.95 a |
| UFPI-230 | 89.50 a | 150.82 a | 169.93 b | 56.61 c | 12.40 c | 2.55 a |
| UFPI-243 | 91.12 a | 155.85 a | 211.62 a | 61.56 c | 13.72 b | 2.62 a |
| UFPI-251 | 91.85 a | 149.25 a | 142.10 b | 59.49 c | 12.20 c | 2.50 a |
| UFPI-274 | 89.40 a | 156.67 a | 18.33 c | 70.83 b | 14.93 b | 2.50 a |
| UFPI-275 | 94.47 a | 157.62 a | 81.68 c | 77.90 b | 17.30 a | 2.82 a |
| UFPI-276 | 95.60 a | 130.87 a | 90.08 c | 99.85 a | 15.66 a | 2.55 a |
| UFPI-278 | 92.40 a | 143.07 a | 123.56 b | 67.83 c | 15.35 a | 2.60 a |
| UFPI-463 | 91.80 a | 159.14 a | 63.81 c | 78.15 b | 15.60 a | 2.60 a |
| UFPI-465 | 93.55 a | 143.22 a | 101.78 c | 62.21 c | 14.26 b | 2.95 a |
| UFPI-468 | 91.02 a | 150.97 a | 29.75 c | 86.20 a | 17.62 a | 2.58 a |
| UFPI-470 | 91.80 a | 152.87 a | 41.06 c | 74.35 b | 16.32 a | 2.80 a |
| UFPI-483 | 98.05 a | 145.10 a | 42.78 c | 71.73 b | 14.17 b | 2.52 a |
| UFPI-494 | 98.90 a | 155.47 a | 14.11 c | 65.51 c | 16.39 a | 2.72 a |
| UFPI-500 | 91.22 a | 146.10 a | 91.75 c | 73.58 b | 15.99 a | 2.62 a |
| UFPI-515 | 92.02 a | 143.07 a | 46.78 c | 72.18 b | 15.28 a | 2.30 a |
| UFPI-579 | 93.27 a | 150.55 a | 94.12 c | 58.88 c | 13.86 b | 2.52 a |
| UFPI-582 | 92.50 a | 154.64 a | 21.65 c | 78.69 b | 16.61 a | 2.70 a |
| Média | 92.60 | 150.18 | 95.09 | 69.43 | 15.04 | 2.60 |
| CV (%) | 5.94 | 11.05 | 71.31 | 16.00 | 11.37 | 7.79 |
| $S_{.j}$ (%) | 4.74 | 5.59 | 22.52 | 20.82 | 35.23 | 11.10 |

[1/] Values in the same column followed by the same letter are not different by Scott-Knott test ($p<0.05$).

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# BAYO AZTECA: MEXICAN IMPROVED BEAN VARIETY WITH RESISTANCE TO *APION GODMANI* WAGNER

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## INTRODUCTION

In Mexico, the bean pod weevil *Apion godmani* Wagner (*Coleoptera: Curculionidae*) is an insect pest exclusive of dry bean. It is present between 1,600 and 2,600 m above sea level. This pest causes losses up to 90% in dry bean production (Cardona, 1989). In some areas, bean pod weevil may have higher economic impact than any other insect. Early dry bean varieties could avoid defoliation caused by *E.varivestis*, but not the attack of *A. godmani* which causes damages at ovipositing in pods of early development stage and feeding on the grain in formation. Due to the small size of the puncture the damage is not easily visible until the dry pods are opened by the producers. The most useful and economical method of controlling this pest is developing resistant varieties. Now the first bean variety bred with resistance to *A. godmani* has been produced by the Bean Program of the Campo Experimental Valle de México of INIFAP through multiple crosses involving a long breeding process.

**Origin**: Bayo Azteca was derived from a multiple-cross population that included seven parents, among them some commercial Mexican dry beans. Population was generated at CIAT. In 1994 some populations were taken from CIAT to the Campo Experimental Valle de Mexico (INIFAP), where pedigree selection combined with mass selection was used. The first three years, selection was made for resistance to bean pod weevil, anthracnose, and common blight were selected. In the next three years they were selected with respect to grain production, adaptation, and resistance. The selection criteria used in the years 7 to 9 were grain production, short cooking time, high protein content, resistance to anthracnose and common blight. Subsequently, we continued assessing yield. Since the cross, came from several parents it was until generation F9 in 2001 when phenotypic characteristics and grain quality traits began to show uniformity. In the selection process the line was called M-93, the pedigree C93-1- 51SL-19SL-18SL-5SL-5SL-5SL-1SL-0SL, following Bayo Azteca.

**Agronomic Features:** Bayo Azteca exhibits an indeterminate semi-prostrate growth habit type III with short vines and white flowers; pods are medium-large containing 5 to 6 medium grains. In the high valleys, it matures in 102 to 118 days; it is resistant to bean pod weevil, anthracnose, and common blight; fast to cook and high protein content.

**Adaptation and Yield Performance:** Bayo Azteca, variety with wide adaptation, reaches its best yield potential at locations with deep soils (mijagon clayey-sandy) and rainfall over 300 mm during its biological cycle. When assessed for five years (2004-08) over six locations, Bayo Azteca mean yielded 1,796 kg ha$^{-1}$ and significantly exceeded the yield of commercial cultivars like Bayo Inifap and Flor de Mayo M-38, The highest yield, reached by Bayo Azteca was 2,770 kg ha$^{-1}$ under rainfall conditions in 2008 at Chapingo, Texcoco, State of Mexico.

Resistance to the bean pod weevil makes the difference to other varieties such as Bayo INIFAP, Bayomex, Canario 107, and Flor de Mayo M-38. This resistance of Bayo Azteca to bean pod weevil (Table 1), anthracnose, and common blight reduces the use of pesticides and production costs and causes less negative environmental impact.

**Table 1.** Response of Bayo Azteca and four commercial cultivars to the attack of the bean pod weevil (*Apion godmani* W.), in the states of México and Hidalgo 1996-2003.

| Site and evaluation year | Dry bean varieties | | | | | | | | Flor de Mayo M-38 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Bayo Azteca | | Canario 107 | | Bayomex | | Bayo INIFAP | | | |
| | Damaged grain (%) and qualification | | | | | | | | | |
| Sta Lucía, PV-96 | 21.5 | IR | 92.2 | S | | | | | | |
| Sta Lucía, PV-97 | 27.9 | IR | 72.8 | S | 82.1 | S | | | 34.6 | IR |
| Atotonilco Grande, PV-2001 | 26.8 | IR | | | 57.6 | S | | | 69.8 | S |
| Atotonilco Grande, P-V 2003 | 39.3 | IR | | | | | 84.4 | S | 47.5 | S |

Scale modified from Garza et al., 1996 where R (Resistant)=0-30% damaged grain, IR (Intermediate Resistance)=31- 50 %, S (Susceptible)= >51 %
Santa Lucía de Prías is located in State of México and Atotonilco El Grande in the state of Hidalgo

**Grain Quality.** Bayo Azteca is a preferential grain for the central region of Mexico. Weight and grain size of Bayo Azteca are the same as those of Bayo INIFAP and Flor de Mayo M-38. The mean weight of 100 seeds is 27.5 g and their volume 20.5 ml. Bayo Azteca shows excellent capacity of water uptake, approximately 100% of its dry weight after 18 hours o soaking; its cooking time is short as well (68 min), compared to Bayo INIFAP, which requires about 80 min. Bayo Azteca has higher protein content (25%) than Flor de Mayo M-38 (22.5%). Content of solids in broth is 0.35%, associated to moderately thick broth, which is preferred for watery preparations.

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# RELEASE OF "ALBICAMPO" BLACK BEAN VARIETY

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## INTRODUCTION

In the High Valleys o the Central Plateau of Mexico, mainly covering the states of Hidalgo, Mexico, Puebla and Tlaxcala, an approximate bean production of 85 thousand tons is obtained; nevertheless, the achievement of higher production is required in order to contribute to the demand of the Distrito Federal and the conurbation zone, consisting of 250 thousand tons, mostly of black bean.

The Campo Experimental Valle de Mexico of INIFAP informs the release of "Albicampo", variety of black bean *Phaseolus vulgaris*, which is distinguished from the rest of the black varieties of small grain by its high yield potential, excellent grain quality, and high phenological plasticity, which allow for being adapted to critic conditions at good abundant rainfalls in the High Valleys of the Central Plateau.

**Origin**: Albicampo is derived from a population obtained of simple crossing of lines S-12 with Negro 8025 conducted in 1996 at the Experimental Field Valle de Mexico (INIFAP). In the first four years (1997-2000) mass selection was employed mainly utilizing resistance to anthracnose and common blight as selection criteria; in $F_5$ (2001) individual selection was applied, and one of the selected lines was 91. Subsequently, this line was advanced using mass selection in order to test it later on in regional assays, where selection criteria were: resistance to anthracnose, common blight, and rust; high yield potential, being soft for cooking, and high protein content. From 2007 to 2009, it was included in validation plots by dry bean producers.

**Agronomic Features**: Albicampo is a variety possessing the following important characteristics: indeterminate growth habit of Type III, purple flower, short guide, resistance to anthracnose, common blight, rust, and root rot; it has an intermediate development cycle ( 105 to 115 days to maturity), small-sized pods with six small grains, is soft for cooking, and has good protein content. Albicampo is a widely adapted variety, but its highest yield potential is reached at altitudes of 2000-2300 m above sea level, deep soils (loamy, clayey-sandy) and with rainfalls about 300 mm during its biological cycle.

**Adaptation and Yield:** At comparing Albicampo with Negro 8025, one of the highest-yielding varieties recently recommended in the High Valleys of the Central Plateau, it exceeds the yield of

Negro 8025 by 214 kg $ha^{-1}$ on average, up to 3,552 kg $ha^{-1}$ being the highest yield under good rainfall conditions at Santa Lucía de Prías, Texcoco, State of Mexico; whereas in the most critical environment, yield was approximately 1,229 kg $ha^{-1}$.Yield efficiency of Albicampo is associated to its greater tolerance to diseases.

**Grain Quality:** Albicampo is a preferential grain, Jamapa type. The mean weight of 100 seeds is 18 g and their volume 12 ml. Albicampo shows excellent capacity of water uptake, approximately 100% of its dry weight after 18 hours of soaking; its cooking time is short as well (75 min) and has high protein content (24 %).

# BRSMG UNIÃO: A COMMON BEAN CULTIVAR WITH GRAIN TYPE "JALO" FOR THE STATE OF MINAS GERAIS, BRAZIL

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**INTRODUCTION:** Common beans 'Jalo' type has yellow and large grains, and usually reaches the higher market prices. However, most of the cultivars with this grain type present some problems as susceptibility to pathogens, especially *Erysiphe poligoni*, causal agent of powdery mildew disease, which can cause significant losses in grain yield. Availability of cultivars 'Jalo' grain type is limited. Thus institutions that work with common bean breeding in Minas Gerais, Brazil, Universidade Federal de Lavras (UFLA), Universidade Federal de Viçosa (UFV), Empresa de Pesquisa Agropecuária de Minas Gerais (Epamig) and Empresa Brasileira de Pesquisa Agropecuária (Embrapa), have evaluated lines with this grain type, aiming to obtain and recommend new cultivars that are superior to 'Jalo EEP 558' cultivar, which was indicated to Minas Gerais state in 1980. Result of this work, is recommendation of BRSMG União cultivar, a new option of common bean cultivar type 'Jalo', for the state of Minas Gerais.

**MATERIALS AND METHODS:** Cultivar BRSMG União was obtained by hybridization method, using as parents Jalo EEP 558 and ESAL 686 cultivars. After hybridization and obtained $F_1$, backcross (BC) was performed with Jalo EEP 558. The $F_1BC_1$ seeds were sown at the UFLA's experimental area, and were selected 64 progenies $F_{1:2}BC_1$. These progenies seeds were multiplied until the $F_{1:3}BC_1$ generation, when they were evaluated in experiments with repetitions. Thirty three progenies were selected considering especially the resistance to *E. poligoni*, and grain type 'Jalo'.

These 33 $F_{1:3}BC_1$ progenies were evaluated at the UFLA's experimental area. The parents Jalo EEP 558 and ESAL 686 and the Perola cultivar were used as cultivar control. The sowing was in July/2000. Data of mildew severity was obtained using a 1 – 9 grade scale, where 1 indicated absence of symptoms, and 9, 90% to 100% of leaf area infected. Grain yield, in $Kg.ha^{-1}$, was also obtained. Eighty four per cent of the progenies evaluated presented grain yield higher than Jalo EEP 558 cultivar. One of these lines stood out by grain type 'Jalo' and resistance to powdery mildew and originated BRSMG União cultivar.

From the dry season of 2005 to rainy season of 2006/2007, this line was assessed in experiments for determining the Value for Cultivation and Use (VCU), joint other 21 lines and BRS Radiante and Jalo EEP 558 cultivars as control. The experiments were carried out by UFLA, UFV, Embrapa Arroz e Feijão and Epamig in the state of Minas Gerais, Brazil, in different environments presented in Table 1.

**RESULTS AND DISCUSSION:**

**Plant architecture:** BRSMG União cultivar presented indeterminate grows habit, type III. Plant architecture and tolerance to lodging was similar to Jalo EEP 558 (Table 2)

**Disease reaction:** BRSMG União cultivar was tolerant and/or resistant to diseases that have occurred in field (powdery mildew, rust and angular leaf spot) (Table 2).

**Flowering and maturity:** flowering of BRSMG União cultivar occurred 35 days after sowing and crop cycle was completed in around 77 days, considering a semi-early cultivar (Table 2).

**Grain yield and type:** BRSMG União cultivar also showed mean grain yield higher than controls cultivars in most environments (Table 1). The grains were cream uniform, similar to Jalo EEP 558 cultivar. The average of 100 seeds weight was 39,6g.

BRSMG União cultivar, mainly by the productive potential and resistance to powdery mildew, is an excellent choice for producers interested in common beans type 'Jalo' in the state of Minas Gerais, Brazil.

**Table 1**. Mean grain yield ($Kg.ha^{-1}$) of BRSMG União cultivar and controls cultivars (BRS Radiante and Jalo EEP 558) by location, season and year of assessment, in the state of Minas Gerais, Brazil.

| Location | Season | Year | BRSMG União | Controls cultivars | | % mean controls |
|---|---|---|---|---|---|---|
| | | | | Radiante | Jalo | |
| Lavras | Dry | 2005 | 2575 | 2058 | 2242 | 119,8 |
| Lambari | Dry | 2005 | 2283 | 1142 | 1087 | 204,8 |
| Patos de Minas | Dry | 2005 | 2145 | 2433 | 2012 | 96,5 |
| Viçosa | Dry | 2005 | 2800 | 2695 | 2613 | 105,5 |
| Ponte Nova | Dry | 2005 | 1399 | 1061 | 1367 | 115,2 |
| Ijaci | Winter | 2005 | 2454 | 2531 | 2354 | 100,5 |
| Patos de Minas | Winter | 2005 | 1154 | 1425 | 1023 | 94,3 |
| Ibiá | Winter | 2005 | 2479 | 2215 | 2099 | 114,9 |
| Sete Lagoas | Winter | 2005 | 3183 | 3008 | 2150 | 123,4 |
| Ijaci | Rainy | 2005 | 2352 | 1938 | 2317 | 110,6 |
| Lavras | Rainy | 2005 | 1523 | 1823 | 1158 | 102,2 |
| Lambari | Rainy | 2005 | 1821 | 1533 | 1346 | 126,5 |
| Patos de Minas | Rainy | 2005 | 3075 | 2450 | 2604 | 121,7 |
| Lavras | Dry | 2006 | 2512 | 2323 | 2144 | 112,5 |
| Lambari | Dry | 2006 | 3092 | 3083 | 3379 | 95,7 |
| Patos de Minas | Dry | 2006 | 2104 | 2129 | 2062 | 100,4 |
| Viçosa | Dry | 2006 | 2908 | 3033 | 2547 | 104,2 |
| Coimbra | Dry | 2006 | 1875 | 1692 | 1638 | 112,6 |
| Lambari | Winter | 2006 | 2554 | 1820 | 2196 | 127,2 |
| Patos de Minas | Winter | 2006 | 1923 | 1660 | 1190 | 134,9 |
| Uberlândia | Winter | 2006 | 1155 | 1568 | 2112 | 62,8 |
| Coimbra | Winter | 2006 | 2683 | 2145 | 2073 | 127,2 |
| Sete Lagoas | Winter | 2006 | 2692 | 2967 | 3075 | 89,1 |
| Lavras | Rainy | 2006 | 1712 | 1483 | 1858 | 102,5 |
| Patos de Minas | Rainy | 2006 | 1921 | 2292 | 2017 | 89,2 |
| Viçosa | Rainy | 2006 | 1899 | 1056 | 1948 | 126,4 |
| Rainy season average | | | 2043 | 1796 | 1893 | 110,8 |
| Dry season average | | | 2369 | 2165 | 2109 | 110,9 |
| Autumn/winter season average | | | 2253 | 2149 | 2030 | 107,8 |
| Overall average | | | 2241 | 2060 | 2024 | 109,8 |

**Table 2.** Traits of BRSMG União cultivar and Jalo EEP 558 control cultivar obtained in the VCU experiments conducted in the state of Minas Gerais in 2005 and 2006

| **Traits** | **BRSMG União** | **Jalo EEP 558** |
|---|---|---|
| Architecture [1] | 5,8 | 6,2 |
| Lodging[2] | 5,3 | 5,9 |
| Days to flowering | 35 | 34 |
| Days to maturity | 77 | 82 |
| Powdery mildew [3] | 2,8 | 6,8 |
| Rust[3] | 1,5 | 1,0 |
| Angular leaf spot [3] | 2,0 | 1,1 |

[1]1, erect plants and 9, prostate plants; [2]1, absence of lodging and 9, lodged plants; [3]Disease severity: 1, resistant and 9, susceptible.

## ACKNOWLEDGEMENTS

CNPQ, CAPES and FAPEMIG for financial support.

# NOTICE OF RELEASE OF CRANBERRY DRYBEAN CULTIVAR 'KRIMSON'

United States Department of Agriculture
Agricultural Research Service
Washington, D.C.

Cranberry dry bean cultivar 'Krimson' was developed by USDA-ARS (Prosser, WA) for wide adaptation, increased yield potential, virus resistance, and lack of marsh spot. Participating scientist was Phil Miklas, Research Plant Geneticist.

Krimson (tested as BD1003) derives from a multiple parent cross Cardinal/PS98-302-5-5. Cardinal is a cranberry cultivar that is widely adapted with high yield potential. It has nice outward seed appearance; but on the inside, the cotyledons of opened seeds often reveal a black blemish called hollow heart, black heart, or marsh spot, which is commercially unacceptable. The cultivar is still used as a check because of its high yield potential and nice size, shape, and outer appearance of seeds prior to cooking. Cardinal possesses *I* gene for resistance to *Bean common mosaic virus* (BCMV) and *Bct* gene for resistance to *Beet curly top virus* (BCTV). Except for Cardinal, most cranberry bean cultivars are susceptible to BCTV. Susceptibility to BCMV and BCTV plagues seed and commercial cranberry production in the Western U.S. (CA, ID, OR, and WA).

PS98-302-5-5 is an early generation cranberry bean breeding line with high yield potential. This line derives from a cross between two breeding lines: Z9758-28, a cranberry obtained from the three-way cross Montcalm/K59-7//Cardinal, and Z9758-9, a pompadour bean from the cross C92161/Pompadour B. These breeding lines represent attempts to increase genetic diversity within the cranberry market class for enabling yield improvement. Montcalm and K59-7 are kidney beans and Pompadour B is a pompadour landrace market type from the Dominican Republic.

Krimson possesses the beneficial traits of Cardinal: virus resistance, nice outside seed appearance, and wide adaptation. The negative black heart trait in Cardinal is less pronounced in Krimson. The new cranberry Krimson possesses increased yield potential obtained from the breeding line PS98-302-5-5 with diverse parents. Krimson also possesses moderate resistance to bean rust which limits dry bean production east of the Rocky Mountains.

Yield and agronomic performance for Krimson in WA from 2004-2006 revealed a slight yield advantage (+150 lbs/A), slightly larger seed size, and similar maturity compared to Cardinal. Across 10 to 11 locations in N. America in 2007 and 2008, Krimson revealed a slight yield advantage and two-day earlier maturity compared to the check cultivar Capri.

Krimson was released through an exclusive license agreement (Lic. No. 1503-001) between ARS Office of Technology Transfer (OTT) and Basin Seed Company, LLC, in 2010. Certified Seed will be available for the 2011 planting season. For further information contact Phil Miklas (phil.miklas@ars.usda.gov) or Ron Riley (ron.riley@basinseed.com).

**2007 & 2008**
**CDBN**

| Line | Yield | | Seed weight | | Harvest maturity | | Rust | | Canning quality | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2007 | 2008 | 2007 | 2008 | 2007 | 2008 | 2007 | 2008 | 2007 | 2008 |
| | lbs/A | | g 100-1 | | DAP | | | | 1-7 | |
| Krimson | 2090 | 2364 | 45.8 | 54.7 | 91 | 97.6 | MR | MR | 3.1 | 3.3 |
| Capri | 1860 | 2285 | 46 | 54.4 | 93 | 102 | S | IR | 3.6 | 5.2 |
| locations | 11 | 10 | 10 | 9 | 8 | 9 | 2 | | 1 | 1 |

# RELEASE OF 'SHINY BLACK PEARL' BLACK BEAN

**Mark A. Brick, J. Barry Ogg, Howard F. Schwartz and Fred Judson**

Colorado State University, Fort Collins, CO

The Colorado Agricultural Experiment Station released 'Shiny Black Pearl' a black bean cultivar with shiny seed coat luster. Shiny Black Pearl was tested as CO29113. The variety was derived from a single $F_{4:5}$ plant selection in 2001 from the pedigree ARA 13/UI 906//H9652-5. ARA13 is an opaque black seeded line obtained from international nurseries distributed previously by Dr. Shree P. Singh at the International Center for Tropical Agriculture, Cali Colombia. The pedigree of ARA13 is unkown. UI 906 (PI 549091) is a black seeded cultivar released by the University of Idaho (Myers et al. 1991). UI 906 is an opaque black seeded line with medium maturity, high seed yield that possesses I gene for resistance to *Bean common mosaic virus* (BCMV) and *Bean common necrosis virus*. H9652-5 is a shiny black seeded experimental line developed by Dr. Phillip Miklas, USDA-ARS, Prosser, WA. The pedigree of H9652-2 has not been published. The plant selection that led to the development of Shiny Black Pearl was planted to a progeny row at Fort Collins CO, in 2002. During 2002, 20 single plants were selected from the progeny row to increase for testing and pure seed increase in Fruita, CO in 2003. Among the 20 progeny rows grown in Fruita in 2003, seven rows were selected and tested in 2004. After testing the seven derived lines at Fort Collins in 2004, one line C029113-14 was selected for increase and to release as the variety Shiny Black Pearl. The seed from this line was used in all subsequent seed increases and testing.

Shiny Black Pearl has shiny seed coat luster, similar to the cultivar 'Shiny Crow'. The shiny luster provides a unique black bean for some commercial markets for value added products such as black bean chips or canned black beans. This trait is controlled by a single dominant gene with shiny dominant to opaque. The gene has been designated Asper (Asp) by Lamprecht (1940) and single dominant gene inheritance was confirmed (Brick et al. 2000). Seed coat luster is known to influence water uptake by black beans. Seed of check cultivars Raven, Midnight, Shiny Crow, and T-39 were compared to Shiny Black Pearl for water uptake after 16 hours soaking for three years of production at Fort Collins, CO. Shiny Black Pearl had slightly higher water uptake than Shiny Crow (10%), but approximately 50% lower water uptake compared to the checks. Therefore, Shiny Black Pearl will require longer soak time to hydrate the seed prior to cooking than traditional opaque black bean cultivars.

Shiny Black Pearl has expressed type II growth habit (CIAT classification) in all environments tested. Seed yield and seed size of Shiny Black Pearl is similar to other commercial black bean varieties (Tables 1, 2). Shiny Black Pearl carries I gene resistance to BCMV and exhibits mid-season maturity (94 to 97 d) in Colorado.

Shiny Black Pearl is owned by Colorado State University and has been licensed for exclusive use by Lee Bean & Seed Inc, PO Box 37, Borup, MN 56519. Application for Plant Variety Protection (No. 201100048) under Title V has been submitted. Information regarding Shiny Black Pearl is available from Mark Brick, Department of Soil and Crop Sciences, Colorado State University, Fort Collins, CO 80523, 970-491-6551 or Mark.Brick@Colostate.edu.

**Table 1.** Seed yield of Shiny Black Pearl compared to four black bean cultivars grown in Fort Collins, Colorado during 2003, 2004, 2005, 2007 and 2008.

| | Year | | | | |
|---|---|---|---|---|---|
| | 2003 | 2004 | 2005 | 2007 | 2008 |
| Entry | ----------kg ha$^{-1}$---------- | | | | |
| Shiny Black Pearl | 2928 | 2858 | 1540 | 2985 | 2092 |
| Shiny Crow | 3058 | 2817 | 1566 | 2725 | 1494 |
| T-39 | NT | NT | NT | 2738 | 1622 |
| Raven | 2616 | 2616 | 1635 | 3316 | 1322 |
| Midnight | 2987 | 3153 | 1303 | 2726 | 1711 |
| LSD (0.05) | 529 | 484 | 620 | 370 | 529 |

NT = not tested in that year.

**Table 2.** Seed weight of Shiny Black Pearl and four black bean cultivars grown in Fort Collins, Colorado during 2005, 2007, and 2008.

| | Year | | |
|---|---|---|---|
| | 2005 | 2007 | 2008 |
| Entry | ----------g 100 seed$^{-1}$---------- | | |
| Shiny Black Pearl | 20.2 | 17.1 | 18.3 |
| Shiny Crow | 18.9 | 18.7 | 18.3 |
| T-39 | NT | 18.6 | 16.9 |
| Raven | 16.1 | 18.6 | 14.6 |
| Midnight | 17.3 | 18.6 | 17.7 |
| LSD (0.05) | 1.5 | 1.4 | 1.6 |

NT = not tested in that year.

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# 2010 FINANCIAL STATEMENT
# BEAN IMPROVEMENT COOPERATIVE

| | | | |
|---|---|---|---|
| **BALANCE AS OF January 1, 2010** | | | **$ 9,498.86** |
| **INCOME** | | | |
| | 2010 Dues | $ 4,208.00 | |
| | Extra CDs | $ 140.00 | |
| | Extra Books | $ 16.00 | |
| | 2011 Dues | $ 48.00 | |
| | Reimbursement for deposit for 2009 BIC Meeting | $ 500.00 | |
| | Back Issues | $ 78.00 | |
| | Bank Interest | $ 157.46 | |
| | **TOTAL INCOME** | $ 5,147.46 | |
| **EXPENSE** | | | |
| | Labor Charges | $ 1,987.50 | |
| | Deposit for 2011 Meeting | $ 3,750.00 | |
| | Postage, Copy Charges and Office Supplies | $ 2,472.21 | |
| | Printing – Volume 53 | $ 2,146.54 | |
| | Google Checkout and PayPal Fees | $ 83.56 | |
| | **TOTAL EXPENSE** | $ 10,439.81 | |
| **BALANCE AS OF December 31, 2010** | | | **$ 4,206.51** |