**ANNUAL REPORT OF THE** 

# BEAN IMPROVEMENT COOPERATIVE

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

> VOLUME 58 2015

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# THE LVIII

# Report of The

# **BEAN IMPROVEMENT COOPERATIVE**

No. 58

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# **Coordinating Committee**

Kirstin Bett Jim Kelly (Ex officio) Ken Kmiecik Phil Miklas (President) Jim Myers Juan Osorno Marcial 'Talo' Pastor-Corrales Peter Pauls Ron Riley Antonio de Ron Dan Wahlquist

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

Dr. Phillip N. Miklas USDA-ARS 24106 No. Bunn Road Prosser, WA 99350-9687 Phone: 509-786-9258 FAX: 509-786-9277 phil.miklas@ars.usda.gov

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

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Cover: phenotyping cart in Puerto Rico courtesy of Angela Linares

# THE 58<sup>th</sup> 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-eighth Biennial Meeting from November 1 through November 4, 2015, in Niagara Falls, Ontario, Canada. The primary local BIC meeting organizer is K. Peter Pauls <u>ppauls@uoguelph.ca</u>. In addition, the associated meetings with our colleagues in the North American Pulse Improvement Association will be from November 4 to November 6, 2015 [NAPIA host: contact Rebecca McGee (<u>rebecca.mcgee@ars.usda.gov</u>)]. The Phaseolus Crop Germplasm Committee, BIC Genetics Committee and the Regional W-3150 Committee are scheduled for November 4. A joint BIC/NAPIA - International Year of the Pulse (IYOP) event will occur the evening of November 4<sup>th</sup>. A field trip is also planned. Please refer to the information provided by the local organizing committee in the current report, and look for additional information and updates on the website <u>http://www.uoguelph.ca/hosted/bic2015/index.html</u> for the conference and or check for information and updates on the BIC web site <u>www.css.msu.edu/bic</u>.

Please review the call for nominations for the **BIC Meritorious Service Award**, **BIC Achievement Award**, and new **BIC Technical Merit Award**, and forward your nominations to the Awards Committee Chairperson, James Beaver (j\_beaver@hotmail.com) by May 31, 2015. We will continue to recognize our founding members through the **Frazier-Zaumeyer Distinguished Lectureship**. The Lectureship will be awarded at the meeting in Niagara Falls and nominations should be sent to James Beaver. A current membership list of BIC Committees and those who have received awards throughout the history of the BIC is provided in the current issue and on the BIC website to assist you in nominating colleagues for these awards. We want to make this a memorable meeting, so please share this information with interested colleagues who might like to attend these meetings and/or join the BIC.

The BIC website continues to be maintained at Michigan State University under the direction of Dr. James Kelly. The BIC recognizes this continued support of Dr. Kelly for maintaining the website. Note that some Research Technology sections on the website have been updated while others await new contributions. The goal for this Research section is to provide an overview of appropriate techniques for breeding common beans for a particular trait, identify cultivars and breeding lines that can be used as sources of resistance for a particular stress, and to provide references where researchers can obtain more detailed information. Please feel free to contact us with any new ideas, contributions, or updates for the BIC website.

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

## Dr. Phillip Miklas, BIC President

#### BIC COMMITTEE MEMBERSHIP - 1957 to 2014

**Coordinating Committee** (approximate year of appointment):

- Dean, Enzie, Frazier\* (BIC Coordinator/President), McCabe, Zaumeyer 1957
- 1960 Anderson, Atkin, Dean, Enzie, Frazier, McCabe, Zaumever
- 1962 Anderson, Atkin, Dean, Frazier, Pierce, Polzak, Zaumeyer
- 1968 Anderson, Coyne, Dean, Jorgensen, Polzak, Zaumeyer
- 1971 Briggs, Covne, Dean, Jorgensen, Polzak, Zaumever
- 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
- Dickson, Emery, Grafton, Magnuson, Schwartz, Singh, Stavely, Steadman, Uebersax 1992
- 1994 Antonius, Dickson, Grafton, Magnuson, Park, Schwartz, Singh, Stavely, Uebersax
- 1996 Antonius, Grafton, Park, Schwartz, Singh, Stavely, Myers, Kotch, Miklas, Riley
- 1998 Antonius, Park, Schwartz (ex officio), Singh, Myers, Kotch, Miklas, Riley, Beaver, Vandenberg, Kelly
- 2000 Antonius, Beaver, Kelly, Kotch, Miklas, Myers, Park, Riley, Schwartz, Singh, Vandenberg
- 2001 Antonius, Beaver, Kelly, Kotch, Miklas, Myers, Park, Riley, de Ron, Schwartz, Vandenberg
- 2003 Beaver, Kelly, Kmiecik, Kurowski, Miklas, Myers, Park, Riley, de Ron, Schwartz, Vandenberg
- 2007 Beaver, Kelly, Kmiecik, Miklas, Myers, Park, Riley, de Ron, Schwartz, Shellenberger, Vandenberg
- 2008 Beaver, Kelly, Kmiecik, Miklas, Myers, Pauls, Riley, de Ron, Schwartz, Shellenberger, Vandenberg
- 2010 Beaver, Kelly, Kmiecik, Miklas, Myers, Pauls, Riley, de Ron, Schwartz, Shellenberger, Vandenberg
- 2011 Bett, Kelly, Kmiecik, Miklas, Myers, Osorno, Pastor-Corrales, Pauls, Riley, de Ron, Wahlquist

#### **Awards Committee:**

- Baggett, Briggs, Burke, Dean, Wallace 1971
- 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 Covne, Silbernagel, Wallace
- 1995 Covne, Dickson, Stavely
- 1997 Covne, Schwartz, Stavely
- 2001 Hosfield, Magnuson, Schwartz
- 2004 Hosfield, Schwartz, Singh
- Hosfield, Schwartz, Singh 2008
- Noffsinger, Schwartz, Singh 2012
- 2014 Beaver, Noffsinger, Urrea

#### **Genetics Committee**

- 2004 Bassett (Chair), Beaver, Blair, Gepts, McClean, Miklas, Welsh (ex officio)
- 2005 Beaver (Acting Chair), Blair, Gepts, McClean, Miklas, Porch, Welsh (ex officio)
- 2007 Beaver, Blair, Gepts, McClean, Miklas, Porch (Chair), Welsh (ex officio)
- 2008 Bett, Blair, Gepts, McClean, Miklas, Porch (Chair), Urrea, Welsh (ex officio)
- 2010 Bett, Blair, Gepts, McClean, Miklas, Porch (Chair), Carlos Urrea, Welsh (ex officio)
- 2011 Bett, Blair, Gepts, Kelly, McClean, Porch (Chair), Carlos Urrea, Welsh (ex officio)
- 2014 Bett (Chair), Ferreira, Gepts, Goncalves-Vidigal, Kalavacharla, Kelly, Kisha (ex officio), McClean, Osorno, Porch, Urrea

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

#### Year Recipients

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

- 1991 Iver L. Jorgensen- Northrup King & Co., Plant Breeder
  John L. Morris- Rogers/NK Seed Co., Plant Breeder
  Rosario Provvidenti- Cornell University, Plant Pathologist
  Shree P. Singh- CIAT, Plant Breeder
  J. Rennie Stavely- ARS/USDA-Beltsville, Plant Pathologist
  Daniel Debouck- IBPGR, Rome, Plant Geneticist [Achievement Award]
  Paul L. Gepts- Univ. of Calif.-Davis, Plant Geneticist [Achievement Award]
  Pat Barnes-McConnell- Bean/Cowpea CRSP, Director [Achievement Award]
- 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]
- James R. Baggett Oregon State University, Plant Breeder
   James S. Beaver University of Puerto Rico, Plant Breeder
   Phillip McClean North Dakota State University, Geneticist [Achievement Award]
   James Myers Oregon State University, Plant Breeder [Achievement Award]
- 2001 Dermot P. Coyne University of Nebraska, Plant Breeder [Frazier-Zaumeyer Distinguished Lectureship] Mark J. Bassett – University of Florida, Plant Geneticist Soon J. Park – Agriculture and Agri-Food Canada, Plant Breeder Mark A. Brick – Colorado State University, Plant Breeder [Achievement Award] Ron Riley – Syngenta, Plant Breeder [Achievement Award] Juan Carlos Rosas – Escuela Agricola Panamericana, Honduras, Plant Breeder
- 2003 Fredrick A. Bliss Seminis Seeds, Plant Breeder [Frazier Zaumeyer Distinguished Lectureship] Steve Beebe – CIAT, Colombia, Plant Geneticist Paul Gepts – University of California, Plant Geneticist Marcial A. 'Talo' Pastor-Corrales – USDA-ARS, Beltsville, Plant Pathologist
- 2005 Perry B. Cregan USDA-ARS, Beltsville, Geneticist, Soybean Genomics [Frazier Zaumeyer Distinguished Lectureship]
   Jorge A. Acosta Gallegos, INIFAP, Mexico, Plant Breeder
   Phillip N. Miklas, USDA-ARS, Prosser, Plant Geneticist (BIC President, 2010-present)
   David M. Webster, Seminis Seeds, Plant Breeder
   A. 'Bert' Vandenberg, University of Saskatchewan, Plant Breeder [Achievement Award]

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

- 2009 Maurice Bennink, Michigan State University, Nutritionist [Frazier Zaumeyer Distinguished Lectureship] Henry Thompson, Colorado State University, Nutritionist [Frazier - Zaumeyer Distinguished Lectureship] Mark Brick, Colorado State University, Plant Breeder
- 2011 Phillip McClean, North Dakota State University, Geneticist [Frazier Zaumeyer Distinguished Lectureship] Kenneth F. Grafton, North Dakota State University, Plant Breeder and Dean, Director, & Vice President of Agriculture Juan Jose Ferreira Fernández, SERIDA Spain, Plant Breeder [Achievement Award] Timothy G. Porch, USDA-ARS, Mayaguez, Plant Geneticist [Achievement Award] Carlos A. Urrea Florez, University of Nebraska, Plant Breeder [Achievement Award]
- 2013 James D. Kelly, Michigan State University, Plant Breeder [Frazier Zaumeyer Distinguished Lectureship] James Nienhuis, University of Wisconsin, Plant Breeder K. Peter Pauls, University of Guelph, Plant Geneticist Kirstin E. Bett, University of Saskatchewan, Plant Geneticist [Achievement Award] Thomas Smith, University of Guelph, Research Technician [Technical Merit]

**Please consider nominating** your colleagues for the 2015 BIC Awards. Details on nominating colleagues are provided below

# 2015 BIC AWARDS - NOMINATION REQUEST

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

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

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

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

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

Nomination for these awards should be sent to the BIC Awards Committee Chair (see below). The awards will be presented at the next BIC Biennial Meeting to deserving candidates nominated by their peers and selected by the BIC Awards Committee. Award recipients will be acknowledged at the twenty-eighth Anniversary of the BIC Biennial Meeting in Niagara Falls, Ontario, Canada on the 3<sup>rd</sup> November 2015.

## **BIC AWARD NOMINATION**

Return by May 31, 2015 to:

James S. Beaver (Chair) Dept. of Agronomy and Soils Mayaguez, PR 00681-9030 <u>j beaver@hotmail.com</u>

The other Awards Committee members are Drs. Steve Noffsinger and Carlos Urrea.

Nominee:	Name:	
	Address:	
	Discipline:	
Nominated for:		Meritorious Service Award
		Achievement Award
		Frazier-Zaumeyer Distinguished Lectureship
		Technical Merit Award Nomination
Submitted by:		
Date of Submission:		

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

## Second announcement for the Biennial BIC/NAPIA 2015 meeting in Niagara Falls BIC = 1-4 November, 2015 and NAPIA = 4-6 Nov, 2015

Registration costs and forms have not been finalized yet; but will be provided online as soon as they become available. Abstract submission instructions and hotel information are currently available online at http://www.bic2015.com/

The meeting will be held at the Marriott Gateway on the Falls in beautiful Niagara Falls. A block of rooms has been set aside, and reservations can be made by contacting the hotel directly at **1-877-353-2557** and quoting the "**Bean Improvement Cooperative**".

Room rates are as follows: Fallsview Room: \$135.00 per night Cityview Room: \$115.00 per night Cityview Room (Students): \$99.00 per night

Please note that a fee of \$20 each will be applied for the 3<sup>rd</sup> or 4<sup>th</sup> person in a room. Student rooms are exempt from this charge.

Hotel Address: 6755 Fallsview Boulevard Niagara Falls, ON L2G 3W7

BIC hosts contacts: <u>BIC2015@uoguelph.ca</u> K. Peter Pauls <u>ppauls@uoguelph.ca</u>

NAPIA hosts contact: Rebecca McGee rebecca.mcgee@ars.usda.gov

Day	Date	Activity	Time				
Sunday	1-Nov	Welcome mixer	6-9P				
Monday	2-Nov	BIC talks and posters	8A-6P				
		Wine/ Regional Tour	evening				
Tuesday	3-Nov	BIC talks and poster	8A-5P				
		BIC banquet	6-8P				
Wednesday	4-Nov	W3150/PCGC	8A-12P				
		BIC Genetics Committee	1-3P				
		IYOP event BIC/NAPIA	7-9P				
Thursday	5-Nov	NAPIA	8A-5P				
Friday	6-Nov	NAPIA	8A-5P				

## **Tentative Schedule**

## **IN MEMORY OF ORLANDO TORO-CHICA**



Orlando Toro-Chica passed away in Cali on January 22, 2015. The Genetic Resources Program of CIAT lost a friend and a highly dedicated professional who spent forty years documenting the diversity of *Phaseolus* beans in view of introducing them into the genebank. Orlando was a recognized expert on bean genetic resources among his colleagues in CIAT, and was ever willing to dedicate his time to assist his colleagues in understanding the history or significance of different accessions.

With an education in agriculture from the "Instituto Politécnico Colombiano" of Medellín in Colombia, Orlando joined the Bean Program in 1974. From his contacts with Victor Manuel Patiño, who was then collecting cassava germplasm for CIAT, he quickly realized the importance of the appropriate documentation of genetic resources. And when the Genetic Resources Unit was established in 1977, he took responsibilities in the safe introduction of many large collections of *Phaseolus* beans, which would be essential for the identification of resistances to diseases and pests and eventually the success and lasting impact of so many elite varieties of the Bean Program.

Later on, his knowledge about bean genetic resources was critical for the development of the core collection. From a couple of thousands in 1977, thanks to his care, the number of bean accessions in the genebank has risen to 37,810 accessions. That increase was also the result of his germplasm explorations in Colombia and Ecuador in the 1980s and early 1990s. Beans of Colombia particularly triggered his curiosity, and he correctly recognized early on that they rightly deserved collection and study. Fascinated by diversity, he was always fond of any rare variant that eventually makes the CIAT bean collection so unique. It is largely the result of his efforts that CIAT has been able to distribute thousands of samples to more than one hundred countries, to make safety backups at CIMMT and in the Global Seed Vault at Svalbard, as well as to return collections that were lost.

He has been author or co-author of more than thirty widely cited publications, namely on aspects of taxonomy of new *Phaseolus* species, distribution and ecology of wild bean species, genetic diversity, gene flow, and protein quality. He was a source of information and reliable data, highly esteemed by the bean breeding and enhancement community all around the world. His outstanding knowledge of the diversity in the Phaseolus collection allowed him to develop *ad hoc* collections to investigate specific topics, such as determinacy in common bean. The discovery of the marked haplotype diversity at the *fin* locus would not have been possible without Orlando's knowledge (Kwak et al. 2012). The bean database available on the CIAT web site (<u>http://isa.ciat.cgiar.org/urg/beancollection.do</u>) is one of his most lasting contributions.

Hasta luego, paisa! Nos encontraremos de nuevo, amigo!

## RHIZOCTONIA SOLANI: UNDERSTANDING THE TERMINOLOGY

## Linda Hanson<sup>1</sup> and Doug Minier<sup>2</sup>

<sup>1</sup>USDA-ARS and <sup>2</sup>Plant, Soil and Microbial Sciences, Michigan State University, 1066 Bogue Street, East Lansing, MI 48824

*Rhizoctonia solani* can cause seedling damping-off and root rot (Fig. 1) in dry bean (*Phaseolus vulgaris*) (Hagedorn & Hanson 2005) and a number of other major crops including sugarbeet, soybean, cotton, potato, etc. (Sneh et al. 1991). There appears to be an increase in reported incidence in both temperate regions and in tropical areas. As well as a root rot, some strains can cause foliar blights while other strains are non-pathogenic (Sneh et al. 1991) or beneficial to plants (Sneh et al. 1991, Carling et al. 1999). Identification of the type of *R. solani* present in soil or plant tissue is important to determine the risk for a given crop and to monitor changes in where or on what crops different types occur. The type of *R. solani* is an important concern for breeders as resistance to the different forms can be independent (O'Brien et al. 2001).



*Rhizoctonia solani* is a filamentous fungus in the Basidomycota. It is characterized by production of brown hyphae with right angle branching and a septum near the point of origin in branches, dolipore septa, no clamp connections, and multiple haploid nuclei per cell (Anderson 1982, Ogoshi 1987, Sneh et al. 1991). If a sexual stage is produced, it is *Thanatephorus cucumerimus* (Sneh et al. 1991). It produces no asexual spores and thus a number of different methods have been used to characterize the strains within this species complex.

Figure 1. Root and crown symptoms of Rhizoctonia root rot on dry bean showing sunken, dark lesions.

The most accepted method for characterization of *R. solani* is hyphal anastomosis and vegetative compatibility (Sneh et al. 1991, Carling 1996, Cubeta & Vilgalys 1997). Using this method, isolates are paired on a medium and observed for their interaction. If the two isolates are not closely related, there will be little or no obvious interaction. If the two isolates are very closely related (basically clones), the hyphae will fuse and continue to grow. If isolates are closely related, but not essentially the same isolate, the hyphae will fuse but the fused cells, and often cells around them, will die (Carling 1996). This hyphal incompatibility is used to classify *R. solani* isolates into an anastomosis group (AG). It is a good indication of genetic relatedness in most cases (Sneh et al. 1991, Kuninaga et al. 1997). Different AGs can vary in factors such as host range and types of symptoms produced. Most AGs can be viewed as separate species as they are related but genetically isolated (Anderson 1982, Cubeta & Vilgalys 1997, Gonzalez et al. 2006). Separation of *R. solani* into different species has not been done.

While most AGs show the above characteristics, isolates in a few AGs can have more variable interactions. This is particularly true for "AG 2". Isolates of AG 2-1, AG 2-BI, and AG 2-2 are not closely related, but can undergo hyphal fusion. The rate of anastomosis is lower between than within the same AG (Ogoshi 1987, Sneh et al. 1991). In addition, isolates in some of these

can fuse at low rates with AGs outside of "AG 2". The above divisions within "AG 2" are genetically distinct (Liu et al. 1992, Carling et al. 2002) and are not necessarily closely related (e.g. Gonzalez et al. 2006) so it is important to include these distinctions.

The majority of AGs in *R. solani* have been further subdivided. The sub-classification has been done because of characteristics such as variable host range or differences in growth requirements, as well as on morphological characteristics. While these subgroups are not official taxonomic categories, several are phylogenetically supported. For example, within anastomosis group 1, three groups IA, IB, and IC have been identified, with others proposed (e.g. Priyatmojo et al. 2001). The first three were differentiated based on size and shape of the sclerotia, but also vary in that IA causes sheath and leaf blights, IB causes web blights, and IC can cause some damping-off in seedlings rather than damage to aerial parts of plants (Sneh et al. 1991). In AG 4, at least three subgroups have been proposed, including HG-I, HG-II, and HG-III (Sneh 1991). Both HG-I and HG-II occur on beans (Cebi Kilicoglu & Ozkoc 2013) and HG-I is virulent on bean roots (Nerey et al. 2010).

In *R. solani* AG 2-2, several subgroups have been proposed. The first divisions were AG 2-2 IIIB and AG 2-2 IV, based on host range. AG 2-2 IIIB was reported to cause disease on mat rush while AG 2-2 IV caused root rot of sugar beet (Sneh et al. 1991). Subsequently both were found to cause disease of sugar beet, dry bean, and soybean (Engelkes & Windels 1989), but AG 2-2 IIIB was determined to also be pathogenic on grass species like corn (Sumner & Bell 1982). Thus knowledge of which subgroup is present can be an important consideration either for selecting rotation crops or in understanding the potential impact of the existing crop rotation on disease. If the strains present are AG 2-2 IV, corn can be part of rotation for disease management but not if AG 2-2 IIIB is the primary type. Additional subgroups occur on other hosts, and one, AG 2-2 WB, has been proposed based on the ability to cause web blight on dry bean (Godoy-Lutz et al. 2008). The subgroups within AG 2-2 have not been as well supported phylogenetically as those in some other AG and work is ongoing to re-examine AG 2-2 subgroups (Martin et al. 2012).

The types of *R. solani* prevalent on bean can vary depending on the cropping system and/or the location. AG 2-2 is reported commonly associated with bean root rot in some areas of North America (Engelkes & Windels 1989, Muyolo et al. 1993), although more AG 4 is reported in other studies (Papavizas et al. 1975). Other AGs also can damage beans but are less prevalent (Galindo et al. 1982, Muyolo et al. 1993). From Japan, AG 5 was reported as the predominant type on bean, followed by AG 4 (Inoue & Ui, 1974, as reviewed by Ogoshi 1987). In Central and South America, Africa, and parts of the Middle East, AG 4 was reported as the predominant *R. solani* associated with bean root rot (Bolkan & Ribeiro 1985, Diaz & Herrera 2000 as cited by Nerey et al. 2010, Karaca et al. 2002), with AG 1, AG 2-2, and AG 5 also causing damage in some areas (Muyolo et al. 1993, Karace et al. 2002).

For web blight or foliar blight of bean, the most common cause is *R. solani* AG 1 (Bolkan & Ribeiro 1985, Godoy-Lutz et al. 2003). In addition, some strains of AG 2-2 cause web blight (Godoy-Lutz et al. 2008). As stated above, the subgroups within AG 2-2 are debated, and other authors report no web blight with isolates genetically similar to AG 2-2 WB strains (Nerey et al. 2010). This could be due in part to differences in growing conditions in the various experiments as well as variability within the subgroups.

Knowing which AG types are present in a field may be an important factor for selecting resistant material. Varieties can show differential response to AGs (Figure 2). For example, while the cultivar Red Hawk dark red kidney was susceptible to both AG 2-2 and AG 4 with both a seedling and adult plant inoculation, germplasms ADP-629 (H9659-27-10, light red kidney) and ADP-622 (UCD 0701, Jacob's cattle) showed some reduced severity with AG 4 at both the seedling and, for one, at the adult stage. In addition, they showed little resistance to AG 2-2 at the seedling stage but had reduced disease severity as adult plants.



Figure 2. Response of three dry bean lines to Rhizoctonia solani isolates, representing two anastomosis groups (AG). Plants were inoculated at planting ("seedling") or 2 weeks after emergence and rated for disease using a 0-6 scale where 0=no symptoms and 6= plant dead.

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## TO: THE BEAN IMPROVEMENT COOPERATIVE COMMUNITY

## FROM: James D. Kelly

#### **RE:** The Question of Original Identity of Bean Germplasm

When the bean genome was sequenced by Schmutz et al. (2014), I fallaciously asked the question which genotype was actually sequenced. Was it G19833 or Chaucha Chuga from Peru? No mention of the original identify or name of G19833 accession appears in the paper. We all recognize the valuable service that the National Plant Germplasm System in the U.S. and the worldwide CGIAR system has done in securing, maintaining and cataloguing plant germplasm as Plant Introduction (PI) or Germplasm (G) accession numbers. When these materials are used by researchers, however, the PI or G-numbers are commonly used to identify the material in the literature in lieu of using the original name or number of the genotype. For example the 12member anthracnose differential series includes PI 207262 and G2333 accessions instead of the original names Tlalnepantla 64 and Colorado de Teopisca, both landraces from Mexico. If materials prove to be unique the author feels that there is an inherent injustice to the originator, institution and/or country as that individual or institution should gain some recognition for having developed the materials. In those cases where PI or G accessions have no original identity other than the country where they were collected the only identifier is the PI or Gnumber. Such is the case for the rust differential accession PI 181996, a landrace from Guatemala that carries the Ur-11 gene for rust resistance. However this is a rarity as many accessions are cultivars, landraces, farmer selected varieties and the geographic region, country or institution from where they came is lost to the reader if the original identity is not provided. Other problems, I foresee, is if there is continued use of only the PI or G-numbers in genetic studies. It is common in the identification of OTL to use the initials of the parents of the mapping populations for ready identification of genotypes where QTL are identified (Soule et al. 2011). If original names are not used the bean community will be overrun with G codes or PI codes that really are not effective identifiers of the germplasm. The writer sees the problem increasing as researchers expand the use of genome wide association studies (GWAS) as these studies require a broad panel of genotypes or germplasm accessions to conduct these association mapping studies. Many panels are being developed and one example is the Andean Diversity Panel (ADP). As the ADP is used for GWAS, the authors need to use the original identity of germplasm rather than the convenience of the ADP-code when discussing individual genotypes. Researchers are very particularly careful to identify original articles and authors when citing literature in their publications, so every effort should be made to identify the original name and code of germplasm accessions when publishing literature on unique traits that are identified in genotypes being studied in GWAS and QTL studies. The absence of such information shows a disrespect to originator. Since many of us are in the business of developing unique cultivars and breeding materials we would like to see valuable materials getting the identity they deserve rather than being disguised as an incarceration number in a gene bank. When the originator is not recognized the reader is left with the false impression that the material originated with the collection agency (NPGS or CIAT). This writer has been guilty of this oversight in past publications and plans on charting a new and insightful course in the future.

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## TO: THE BEAN IMPROVEMENT COOPERATIVE COMMUNITY

## FROM: Phillip Miklas

## **RE:** Pending reduction in workforce

Within the next few years, due to retirements of university positions, three common bean (eg. black, kidney, navy, pinto) pathologists (George Abawi, Cornell University; Howard Schwartz, Colorado State University; James Steadman, University of Nebraska) and four dry bean breeder/geneticists (James Beaver, University of Puerto Rico; Mark Brick, Colorado State University; James Kelly, Michigan State University; Shree Singh, University of Idaho), which represents a significant portion (SYs and not to mention irreplaceable expertise and knowledge) of the Phaseolus bean research community in the U.S. could be lost, as many of the positions will likely remain unfilled or filled with a different non-legume mandate by the universities. Similar loss of SYs will occur in the international bean research community.

This loss of workforce comes at a critical time of worldwide interest and recognition of edible legumes as an important and critical food for improving health and food security. The positions, if unfilled, will significantly reduce the capacity for the U.S. to meet national needs for the industry, and will lead to a significantly reduced role in global research, at a time of increasing need. This situation is real and there should be proactive discussions now across agencies about how to minimize this pending gap in workforce and knowledge for our industry and the people who depend on it. Significant investments by NIFA, Pulse Health Initiative (if appropriated), and USAID, combined with 2016 as Year of the Pulses, suggests the need to act in order to keep the momentum going to meet the greater worldwide demands on the dry bean workforce at present levels of activity in breeding, genetics, pathology, training and international development.

# PHYTOHEMAGGLUTINATION ACTIVITY IN EXTRUDED DRY BEAN POWDER Berry, M<sup>1</sup>, Cichy, KA<sup>1,2</sup>, Ai, Y<sup>3</sup> and Ng, PKW<sup>3</sup>

<sup>1</sup>Department of Plant, Soil, and Microbial Sciences, Michigan State University, East Lansing, MI. <sup>2</sup>USDA-ARS, Sugarbeet and Bean Research Unit, East Lansing, MI. <sup>3</sup>Department of Food Science and Human Nutrition, Michigan State University, East Lansing, MI.

**INTRODUCTION:** Dry beans (*Phaseolus vulgaris* L.) are a highly nutritious food, high in iron and fiber among a multitude of other benefits. Besides making beans palatable, cooking is required to denature lectin, a protein found in beans. If consumed raw or undercooked, lectin poisoning can occur. Symptoms of lectin poisoning include vomiting, diarrhea, and abdominal pain, and occur within hours of ingestion (Rodhouse et al., 1990).

Commercial companies have begun to realize the value of beans as an ingredient and some are producing bean powder as a possible alternative to grain flour. A concern exists that in preparing the powder for consumption, it will not reach an appropriate temperature to break down lectin. This concern is well founded as outbreaks of lectin poisoning have occurred when food was cooked (i.e., in a slow cooker), but not at a high enough temperature to sufficiently break down lectin (Rodhouse et al., 1990).

**MATERIALS AND METHODS:** Four varieties representing different bean market classes (navy, fuji, black, and small red) were milled and extruded under different conditions to produce bean powder. Extrusion treatments included different screw speeds (100 or 200 rpm), dough moistures (25 or 30%), and barrel temperature profiles (40, 60, 80, 120, 120 or 40, 60, 80, 120, 140 C°). Raw milled beans were included as a positive control. Cooked navy and black beans were included as negative controls. Cooked beans (in boiling water) have already been proven to be safe for human consumption so by comparing the extruded samples to the cooked samples, the safety of consuming the extruded bean powders could be determined. A genotype devoid of lectin (LPA-280-10-3) was also included as a control (Campion et al., 2009).

**RESULTS:** The experiment conducted indirectly measures content lectin via phytohemagglutination activity. This experiment is commonly used to measure lectin activity and has been adapted from work by Campion et al. (2008). Briefly, hemagglutination is the process whereby blood cells clump together in response to a molecule. Serial 1:1 dilutions were used across 12 wells in a 96-well plate. By using these dilutions, any lectin present was diluted across a large range from 2x to 4096x. Lectin causes blood cells to bind together forming a large mass at the bottom of a curved well in a 96-well plate. If lectin is not present, however, the blood cells do not clump together and, through gravity, coalesce at the bottom of the well where they appear as a red dot or ring. In this work, the dilution at which the last positive result was recorded is what is reported in Fig. 1.

The highest PHA activity was observed in the raw powder of the black, navy and fuji beans. Extrusion reduced PHA activity to similar levels of what was present in the cooked samples. The cooked sample level was set as the threshold and is indicated by the red line (Fig 1.)

Previous works suggests that extrusion is a safe method to prepare bean powder (Alonso et al., 1999). Our results also suggest that extrusion of beans to prepare bean flour is a safe way

to prepare beans for human consumption. In addition, recipes that use bean powder involve additional cooking or dilution with other ingredients further reducing active lectin present.

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Figure 1 Phytohemagglutination (PHA) Activity in Different Preparations of Dry Beans. Four different commercially available beans were prepared through different methods of extrusion. These were compared to a cooked beans (red bar). The lectin free lines (milled raw seed) are in green. The dilution reported is the highest dilution where PHA activity (clumped blood cells) was still observed. The dilution ranges included are 2x up to 4096x.

# ANALYSIS OF GENETIC AND NUTRITIONAL DIVERSITY AMONG SELECTED ACCESSIONS OF DRY BEANS AND NUÑA BEANS (*PHASEOLUS VULGARIS* L.) FROM THE USDA-ARS NATIONAL PLANT GERMPLASM SYSTEM

# Theodore Kisha<sup>1</sup> and Giuliana Naratto<sup>2</sup>

<sup>1</sup>USDA-ARS Western Regional Plant Introduction Station, <sup>2</sup>Washington State University, Department of Food Science and Human Nutrition

Beans (*Phaseolus spp.*) are one of the most economically and nutritionally important crops world-wide, with a value of over \$17 billion harvested annually (FAO statistics, 2012). They are one of the most ancient crops of the New World, having been cultivated for thousands of years (Kaplan and Lynch, 1999). They are an environmentally diverse crop, growing in temperate and sub-tropical environments from sea level to more than 3000m above sea level (Broughton et al., 2003), and are consumed as either fresh pods or as a dry bean, making them an ideal nutritional food legume in areas where storage without refrigeration is necessary. Of the more than 20,000 *Phaseolus* accessions held at the Western Regional Plant Introduction Station (WRPIS), the most abundant species by far is *P. vulgaris* L. with over 17,000 accessions. Among the many accessions of *Phaseolus vulgaris* in the WRPIS, 90 of these are classified as nuña beans, or the Peruvian "popping" bean. These beans have been selected and raised among the Andean natives in the high mountains for millennia (Kaplan and Lynch, 1999), and have the unique characteristic of bursting when subjected to heat, making them a high protein food in conditions where boiling would consume scarce fuel. This property also makes these beans a potential nutritious snack food, both in and of itself, as well as in the form of an extruded product.

We analyzed the molecular diversity of 35 nuña and 8 common dry bean accessions and compared a range of nutritional factors, including protein, starch, phytate, phenolics (extractable and fiber-bound non-extractable), and antioxidant activity. Genetic analysis using AFLP markers showed nuñas were distinct from the common dry beans analyzed, and there were two distinct groups within the nuñas. There was a similar wide range of nutritional characteristics within both the common dry beans and the nuñas. Values for nuñas and common bean respectively were: protein (18-25 and 17-27%), extractable polyphenols (50-350 and 50-450 mg GAE/100g), non-extractable polyphenols (50-220 and 70-175 mg GAE/100g), phytate (0.45-1.2 and 0.6-1.0%), and total antioxidant activity (8-52 and 7-48 mgTE). Introgression of the popping ability into temperate adapted common bean has been successful (Pearson et al., 2012; Vorwald and Nienhuis, 2009). There is enough genetic variation in both nuña and common dry beans to breed popping beans both adapted to a temperate, long-day environment and to develop a highly nutritious snack food for America.



Figure 1. Phylogenetic tree showing three distinct populations given by the program STRUCTURE. Black boxes indicate common dry beans.



Figure 2. Distribution of A) Protein B) Phytate C) Total polyphenols D) Total antioxidant activity among nuña beans and selected common dry beans (in black).

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## **BEAN SEED DRYING AND ITS PHYSIOLOGICAL QUALITY**

# García-Urióstegui, A<sup>1</sup>., J.R. García-Nava<sup>1</sup>, E. Uscanga–Mortera<sup>1</sup>, A. García-Esteva<sup>1</sup>, J. Kohashi-Shibata<sup>1\*</sup> and G. García-De los Santos<sup>2</sup>

<sup>1</sup>Postgrado en Botánica y <sup>2</sup> Postgrado en Semillas, Colegio de Postgraduados, Montecillo, Edo. de México. 56230. México <sup>\*</sup>jkohashi@colpos.mx

**INTRODUCTION:** Common bean (*Phaseolus vulgaris*, L.) is a basic component of the diet of the people of Mexico and other Latin American countries. It is the world's most important leguminous crop for human consumption and is produced under different systems, regions and climates (Jones, 1999). Bean seed is an orthodox seed. At physiological maturity the seeds have 50 % water content which it loses to reach 10-12 %, being able to keep in storage without losing its viability; at this point it presents the maximum germination and vigor (González *et al.*, 2008). Consequently, seed drying is an important process to preserve the germination and vigor, which are the main physiological qualities of the seed (Delouche, 2002; Bewley *et al.*, 2013). A common practice in maize seed is to harvest with moisture content as a high as 40 % and subject it to artificial drying to reach 13 to 14 % moisture content to keep its physiological quality (Arisnabarreta *et al.*, 2012). The objective of the present work was to asses in bean seed, the influence of artificial drying and compare it with the natural type on its physiological quality.

MATERIALS AND METHODS: The research was conducted under greenhouse conditions. Seven bean genotypes were employed: Wild (W13) and domesticated Negro Tacaná (NT) which were employed to obtain the inbred lines 118b, 53b, 51b, 3.3 and 11.1\*. A complete randomized factorial design with four replications and two factors was used: 1) genotype with seven levels (genotypes) and 2) type of drying: *artificial*, in temperature chambers, and *natural*, left to dry on plant under greenhouse conditions. The artificial type had two levels (corresponding to 15 and 35 °C) and the natural, one level (about 25 °C). The seeds were sowed (May 13, 2014) in plastic pots containing 19 kg of red "tezontle" (inert volcanic cinder). one plant per pot, (which constituted the experimental unit) and watered with complete nutrient solution. The seeds for the artificial drying were harvested at 50 % moisture content and were left to dry in chambers under 15 °C or 35 °C until they reached 10 % humidity. Afterward, the seeds of each genotype were placed in polyethylene bags and left at 5 °C to be used for the evaluation of germination. The seeds of the natural-type drying were harvested at maturity just before the dehiscence of the pods. Other seeds of the genotypes were employed to assess the seed vigor by the accelerated aging test (42±1 °C and 100 % relative humidity) (Delouche, 1996). Statistical analysis of data was accomplished with the SAS program (SAS Institute, 2012).

**RESULTS:** Average germination in two types of drying, without and with accelerated aging. Seed germination after drying on average was about 97 % (53b, W13, 3.3 y NT) and the lowest percentage was of 49 % (118b) due to an increase of hardseededness (Table 1). In contrast, seeds of genotype 118b increased seed germination to be about 87 % due to seed softness after the accelerated aging test. After the accelerated aging test the seed germination of the wild bean (W13) was about 66 % and the domesticated bean (NT) was about 55 %, mainly due to the growth of fungi on the seed testa (Table 1).

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Table 1. Average	seed germination after	er arti	ficial (15 and	35°C)	and natural	drying	(25°C)
and germin	and germination after drying plus accelerated aging test.						
Genotype	<sup>#</sup> SG (%)		Genotype	e	<sup>#</sup> SC	GA (%)	
53b	99.7 a		53b	9	3.7 a		
W13	97.3 a		3.3		9	0.3 a	
3.3	95.3 a		11.1		89	9.0 ab	
NT	95.0 a		118b		87	7.3 ab	
11.1	82.3 b		51b		8	2.7 b	
51b	73.4 c		W13		6	4.7 c	
118b	49.0 d		NT		5	2.0 d	

Mean values with the same letters are statistically similar with Tukey test by column,  $\alpha = 0.05$  <sup>#</sup>SG= standard germination after drying; <sup>#</sup>SGA= standard germination after drying plus accelerated aging test.

Average germination at several drying temperatures without and with accelerated aging test. The best drying temperature to maintain seed germination was at 15 °C. Dried seeds and accelerated aging diminished seed germination to the lowest values due to proliferation of fungi. Seeds of wild and common bean were susceptible to fungi compared to the inbreed lines. In general artificial drying showed higher seed germination percentage than natural drying (Table 2).

Table 2. Average seed germination at several drying temperature without and with accelerated					
aging test.					
Temperature (°C)	<sup>#</sup> SG (%)		Temperature (°C)	<sup>#</sup> SGA (%)	
15 (artificial)	95.3 a		35 (artificial)	90.1 a	
35 (artificial)	89.4 b		25 (natural)	88.1 a	
25 (natural)	69.0 c		15 (artificial)	61.6 b	

Mean values with the same letters are statistically similar with Tukey test by column,  $\alpha = 0.05$  <sup>#</sup>SG= seed germination after drying, <sup>#</sup>SGA= seed germination after drying plus accelerated aging test.

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## ENZYME activity IN SLOW DARKENING PINTO COMMON BEAN CULTIVARS UNDER ACCELERATED AGING TREATMENT

# Janet A. Gutiérrez-Uribe<sup>1</sup>, Cintya G. Soria-Hernández<sup>1</sup>, Rigoberto Rosales-Serna<sup>2</sup>, Silverio García-Lara<sup>1</sup>, Sergio R. O. Serna-Saldívar<sup>1</sup>, Eleazar Reyes-Barraza<sup>1</sup>

<sup>1</sup>Tecnológico de Monterrey. ITESM. School of Engineering and Sciences. Av. Eugenio Garza Sada 2501 Sur. Col. Tecnológico. Monterrey, N. L. Méx. C. P. 64849. <sup>2</sup>INIFAP-Durango. km 4.5 Carretera Durango-El Mezquital. Durango, Dgo., México. C. P. 34170

**INTRODUCTION:** In Durango, problems were registered for common bean (*Phaseolus vulgaris*) grain marketing due to accelerated seed darkening phenomenon. Seed darkening is an undesirable trait because the consumer associates this condition with aging (old seeds) and prolonged cooking time (Siqueira *et al.*, 2014). In recent years (2006-2014), Pinto Saltillo became the most popular common bean cultivar planted in the Mexican Highlands due to the higher yield and slow darkening seeds. Significant advances were obtained in previous works implemented to understand slow darkening phenomenon in common beans (Junk-Knievel *et al.*, 2008). Chemical composition of common bean coat need to be analyzed in order to understand the browning effects related to storage under high values for temperature, moisture and light. Polyphenol oxidase (PPO) and Peroxidase (POD) had been related to enzymatic browning observed in fruits and vegetables (Gacche *et al.*, 2006; Marles *et al.*, 2008; Arnnok *et al.*, 2010). The objective was to understand the influence of polyphenol oxidase and peroxidase enzymes on common bean darkening kinetics in seeds exposed to accelerated aging conditions.

MATERIALS AND METHODS: Seeds were harvested in 2012 for three common bean cultivars: Pinto Saltillo (slow darkening), Pinto Villa (accelerated darkening) and Pinto Coloso (intermediate response). After harvest, plants were threshed and seeds cleaned removing debris by hand. After three months under storage room conditions, seed samples were evaluated, by ten replicates, in the Hunter Lab portable colorimeter (Minolta CR-300). After initial color profile readings one set, consisting in one kilogram of seeds for each cultivar was exposed to a 120 h process under accelerated aging conditions. Aging conditions included UVC radiation in the 254 nm light spectrum (Junk-Knievel et al., 2007) and room temperature (20 °C) and relative humidity (35 %). Sequential readings for Hunter Lab were registered, by ten replicates per cultivar, using 100 g seed samples. After Hunter Lab readings samples were milled, homogeneizated and extracted. Each sample was accurately weighed (2.5 g) and extracted with 25 mL of 80 % methanol according to Guajardo et al., (2012). Flavonols, saponins and isoflavones were quantified using an HPLC-DAD-ELSD (Agilent Technologies, Santa Clara, CA) system. Activity of polyphenol oxidase (PPO) (Marles et al., 2008) and peroxidase (POD) (Elez-Martínez et al., 2006) was also measured. Data obtained at four sampling times (0, 36, 72 and 108 h) were analyzed under a completely randomized design and to calculate average and standard deviation values (PPO and POD activity).

**RESULTS AND DISCUSSION:** Pinto Villa showed statistically significant lower values for clarity (L= 50.1), but higher values for red-green (a= 11.5) tones (Table 1). Tolerant and intermediate seed darkening cultivars (Pinto Saltillo and Pinto Coloso) registered higher values for clarity (61.1-62.2) and significant lower values for a (6.9 and 7.7) and similar levels for b (24.7 and 25.4). Seed darkening process in pinto beans was related to lower values for seed coat clarity combined with higher values for red-green tones. Pinto Villa showed higher values for Quercetin (71.3  $\mu$ g g<sup>-1</sup>) and significant higher levels for Kaempferol (2.6  $\mu$ g g<sup>-1</sup>) compared to

Pinto Saltillo (Quercetin= 0.0  $\mu$ g g<sup>-1</sup> and Kaempferol= 0.9  $\mu$ g g<sup>-1</sup>). Intermediate cultivar (Pinto Coloso) also showed intermediate values for Quercetin= 20.4  $\mu$ g g<sup>-1</sup> and Kaempferol= 1.0  $\mu$ g g<sup>-1</sup>.

Cultivar	a*	b	L	Quer	Kaemp	Soy Af	Soy V	Soy ag	Soy βg
							$\mu g g^{-1}$		
P. Villa	11.5 <sup>a</sup>	30.0	50.1 <sup>b</sup>	71.3	2.6 <sup>a</sup>	38.5 <sup>b</sup>	2.9	1.1	11.5 <sup>a</sup>
P. Saltillo	7.7 <sup>b</sup>	25.4	61.1 <sup>a</sup>	0.0	$0.9^{b}$	53.6 <sup>a</sup>	1.7	1.2	$10.0^{ab}$
P. Coloso	6.9 <sup>b</sup>	24.7	62.2 <sup>a</sup>	20.4	$1.0^{b}$	36.1 <sup>b</sup>	2.4	1.8	5.8 <sup>b</sup>
*	. 1		1		. 1 0 1			6	

Table 1. Color traits and bioactive compounds content in seeds of three pinto bean cultivars.

\*a= red-green, b= yellow-blue, L= clarity; Quer= quercetin 4-*O*-galactoside, Kaemp= kaempferol 3-*O*-glucoside and Soy= Soyasaponins. <sup>a-b</sup>means in each column followed by a different letter indicate a statistically significant ( $p\leq0.05$ ) or highly significant ( $p\leq0.01$ ) difference.

Pinto Saltillo registered highest values for peroxidase (POD) in all sampling times (Figure 1a), with fluctuations from 459 UPOD g-1 to 856 UPOD g-1. In contrast Pinto Villa showed lower and more stable values (121 to 187 UPOD g-1). Higher PPO values were also observed in Pinto Villa, at 0 h (4.2 Uppo g-1) and 12 h (3.6 Uppo g-1) sampling times (Figure 1b). Reduction for enzyme activity was observed at 24 h sampling time and the lowest value was observed in Pinto Villa after 120 h (0.18 Uppo g-1) of the accelerated aging treatment. Seed darkening in common bean was related to higher POD content and its interaction with bioactive products such as Quercetin (r=-0.56\*), Kaempferol (r=-0.69\*), Soyasaponin Af (r= 0.76\*\*) and Soyasaponin V (r=-0.58\*). Susceptible cultivars also showed high values for PPO at initial stages of seed darkening process.



Figure 1. Peroxidase (a) and Polyphenol oxidase (b) enzyme activity in three pinto common bean cultivars at different time periods under aging treatments.

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## ANTHRACNOSE RESISTANCE IN ANDEAN BEANS

## Grady H. Zuiderveen, Kelvin Kamfwa and James D. Kelly

Plant, Soil, & Microbial Sciences, Michigan State University, E. Lansing, MI 48824

Anthracnose is a seed-borne disease of common bean (*Phaseolus vulgaris* L.) caused by the fungus *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib, and is cosmopolitan in distribution. It is one of the most economically important diseases of common bean (Melotto et al., 2000), and can cause devastation to farmers' fields, resulting in yield losses as high as 95% in susceptible bean cultivars. The objectives of this study were: (i) identify new sources of anthracnose resistance in a diverse panel of Andean beans comprised of multiple seed types and market classes from the Americas, Africa, and Europe, and (ii) explore the genetic basis of this resistance using Genome-wide association analysis (GWAS).

**MATERIALS AND METHODS:** A subset of 230 bean lines from the Andean Diversity Panel (ADP) developed by Cichy et al. (2015), was screened with eight different races of anthracnose in the greenhouse during 2014. The choice of races was made to include common races such as races 7 and 73, highly virulent Andean races 39, 55, and 109 and also highly virulent races 2047 and 3481 across both gene pools (Ferriera et al., 2013). Inoculations were done by spraying a suspension of  $10^6$  *C. lindemuthianum* conidia ml<sup>-1</sup> onto the leaves and stems of seedling plants. Plants were then maintained under high humidity (>80%) in a mist chamber for a minimum of three days. Symptoms of anthracnose were observed 8-10 days after initial inoculation using a 1-5 scale developed by Drijfhout and Davis (1989). DNA for each sample was collected from young leaf tissue using a modified CTAB extraction protocol, quantified using a spectrophotometer, and its quality was checked on an agarose gel. The Andean panel was previously genotyped using an Illumina BARCBEAN6K\_3 with 5398 SNPs (Hyten et al., 2010). GWAS was conducted to improve understanding of the genetic architecture of anthracnose resistance within Andean beans.

**RESULTS AND DISCUSSION:** Numerous resistant lines were identified within the 230 bean lines screened for all eight races of anthracnose included in the study (races 7, 39, 55, 65, 73, 109, 2047, and 3481). Outputs from the GWAS indicated major QTL for resistance within Andean beans on four linkage groups: Pv01, Pv02, Pv04, and Pv11 for races 65 and 73, 7, and 55, respectively (Figure 1). The data suggest a correspondence with the major resistance genes Co-1 on Pv01, Co-3 on Pv04 and Co-2 on Pv11 (Ferreira et al., 2013) and with the ANT02.1<sup>UC</sup> QTL or the Co-u gene on Pv02 (Oblessuc et al., 2014). The Co-1 gene is known to condition resistance to races 65 and 73; both Co-3 and Co-11 genes condition resistance to race 7; and it would appear that the new locus on Pv02 conditions resistance to race 55. A new locus on Pv02 was recently reported to condition resistance to races 3, 19 and 449 in the Andean cultivar Xana (Campa et al., 2014). A lack of information on specific SNP markers linked to these major genes prevents a final determination of co-localization between GWAS and the published literature. Those Andean bean lines with different resistance genes will be useful in future breeding efforts to develop anthracnose resistant Andean beans.



Figure 1: Manhattan Plot for anthracnose resistance in 230 Andean Diversity Panel accessions using 5326 SNP markers. Clockwise from top left include results for anthracnose race 7, race 55, race 73, and race 65.

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# SCREENING FIELD RESISTANCE TO THE ROOT ROT COMPLEX WITHIN THE ANDEAN DIVERSITY PANEL (ADP)

## Jose Vasquez, Kiran Ghising, Albert Jody VanderWal, Michael Kloberdanz, and Juan M. Osorno

North Dakota State University, Dept. of Plant Sciences, 7670 Fargo, ND 58102

## **INTRODUCTION**

Dry bean root rot is caused by a fungal complex mostly including *Fusarium solani* f. sp. *phaseoli*, in association with *Rhizoctonia solani*, *F. oxysporum*, *Phythium* spp., among others. Root rots are an increasing problem in Minnesota, the largest producer of kidney beans in the U.S. Root rot can reduce seed yield up to 100% under severe disease pressure (Schwartz, 2014). Large seeded-cultivars planted in the area are especially susceptible to the Fusarium root rot complex when conditions are favorable. *F. solani* is the primary pathogen involved in bean root rot in Minnesota (Estevez de Jensen, 1998), and few sources of resistance exist, especially within the Andean Gene Pool. The objective of this study was to evaluate the reaction of a set of Andean genotypes to the root rot complex in the field.

## **MATERIALS AND METHODS**

A total of 310 genotypes from the Andean Diversity Panel (ADP) (Cichy et al., 2015) were screened at Perham, MN in 2013. From those, 45 genotypes were photoperiod-sensitive or did not complete the production cycle. Remnant 265 genotypes were screened again in 2014 in two trials separated by days to maturity. The early maturity trial consisted of 144 genotypes, and the late maturity trial of 121 genotypes. Six common checks, one resistant, two intermediate, and three susceptible were used. The early and late maturity trials were planted in a 12 x 12 and 11 x 11 alpha-lattice design with two replications per trial. Root rot disease severity was determined on a scale 1-9 (1= healthy, 9= dead plant) at flowering stage (R6) using four plants per plot, evaluating individually and computing the average. In addition to root rot, seed size and seed yield were also measured. Resistant genotypes were considered those ranging from 1 to 3, intermediate from 4 to 6, and susceptible from 1 to 9.

## RESULTS

Plant samples collected in the field allowed to confirm that the most abundant pathogen was *F. solani*. In the early trial there were significant differences (P < 0.05) among genotypes for Fusarium root rot. Using Least Square Means, 23 genotypes are in the range 1 to 3, 102 in the range 4 to 6, and 19 in the range 7 to 9. VAX 3 was resistant, Talon and Dynasty were intermediate, Montcalm, Cabernet, GTS-104 were susceptible as expected. The average 5 and the reaction of the checks suggest high disease pressure in the field. Table 1 shows the top 10 resistant and high seed yield early and late genotypes in addition to the checks. In the late trial there were also significant differences (P < 0.1) among genotypes for Fusarium root rot. Using Least Square Means, 21 genotypes are in the range 1 to 3, 62 in the range 4 to 6, and 38 in the range 7 to 9. The average 5 and similar reaction of the checks (compared to early trial) suggest high disease pressure in the field. A subset of contrasting genotypes will be evaluated again in the field in order to confirm results.

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Table 1. Root rot reaction to 16 early-maturity and 16 late-maturity ADP genotypes at Perham, MN, 2014.

Genotype	Seed type	Root rot (1-9)	100 seed weight (g)	Seed yield kg ha <sup>-1</sup>
	Early-Maturity	(1)	() eight (g)	ing ina
VAX 3 (resistant check)	Small Red	1	29.9	2856
ADP462 PI527540B*	Yellow	2	32.1	1897
ADP608 UI 51*	Cranberry	3	54.8	1111
ADP640 Beluga*	White kidney	3	48.2	1500
ADP172*	Dark red	3	25.7	1632
ADP624 Dolly*	Cranberry	3	60.7	1579
ADP12 W6 16489	Dark red kidney	3	52.2	1398
ADP467 PI209808	Pink spotted	3	43.4	1121
ADP680 Clouseau	Light red kidney	3	59.1	1540
ADP477 PI527512*	Pink mottled	3	44.6	1332
ADP438 46 1	Red mottled	3	31.8	1308
Talon (intermediate check)	Dark red kidney	5	49.2	1152
Dynasty (intermediate check)	Dark red kidney	5	56.8	1495
ADP636 Montcalm(susceptible check)	Dark red kidney	5	47.7	927
Cabernet (susceptible check)	Dark red kidney	7	41.0	453
GTS-104 (susceptible check)	Dark red kidney	7	50.9	1195
Mean	-	5	43.4	892
Coefficient of variation (%)		28.8	7.0	33.9
	Late Maturity			
ADP48 W6_6534*	Dark red	1	28.0	1716
ADP465 PI321094D	Cream	2	28.1	1001
VAX3 (resistant check)	Small red	2	29.1	2772
ADP621 JaloEEP558	Yellow	2	35.7	1309
ADP93 Moro*	Yellow	2	29.5	977
ADP84 Kablanketi ndefu*	Black spotted	2	37.5	1311
ADP628 H9659_27_7*	Light red kidney	3	43.7	1459
ADP105 Sewani_97	Dark red	3	40.7	1102
ADP454 Iniap429*	Red mottled	3	45.1	1886
ADP122 Kranskop*	Cranberry	3	44.3	1278
ADP474 PI527519	Red mottled	3	33.7	1258
Talon (intermediate check)	Dark red kidney	5	51.5	1433
Dynasty (intermediate check)	Dark red kidney	6	55.1	1305
Cabernet (susceptible check)	Dark red kidney	7	43.6	759
ADP636 Montcalm (susceptible check)	Dark red kidney	7	51.0	1198
GTS-104 (susceptible check)	Dark red kidney	7	46.3	666
Mean		5	39.2	1001
Coefficient of variation (%)		36.3	5.6	31.3

\* Resistant in 2013 and 2014 seasons
### ANDEAN BEAN PERFORMANCE UNDER TERMINAL DROUGHT IN WASHINGTON

### Eninka Mndolwa and Phillip Miklas

### USDA-ARS, Prosser, WA

**INTRODUCTION:** The effects of drought on common bean are dependent on the intensity, type, and duration of the stress (Munoz-Perea et al., 2006). In Africa, an estimated 682,000 hectares of beans are cultivated in semi-arid environments, with annual yield losses to drought of 781,000 tons across all environments (Wortmann et al., 1998). Highland Mexico, Central America, Northeast Brazil, and much of Eastern and Southern Africa are bean producing areas where drought is endemic. Using climate models the effect of global climate change is predicted to be more severe in the future (Williams et al., 2007). Identification of dry beans that require less water or have better water use efficiency will enhance management options for maintaining profitability. Our objective was to screen lines from the Andean Diversity Panel (ADP) (Cichy et al., 2015) for reaction to terminal drought stress.

**MATERIALS AND METHODS:** An experiment was conducted in Othello Washington 2014 with two treatments: drought stress (DS) and non-stress (NS). Seventy-four ADP lines and 6 checks: G44445, G51495A, G51495, G44445A, Buster and Roza were evaluated for emergence, flowering date, harvest maturity, 100 seed weight (g) and yield kgha<sup>-1</sup>. The experimental design was RCB with two replications for each treatment. Experiments were furrow irrigated until flowering when frequency of irrigation in drought stressed plots was terminated.

Drought Intensity Index (DII) was calculated as (DII = 1 - Mean yield average DS / Mean yield averaged NS). To predict the performance of a line under DS and NS conditions we calculated Drought susceptibility index (S) (where S = (1 - Mean yield of line under DS/Mean yield of line under NS) / DII). Geometric mean was also calculated. Data were analysed using PROC GLM (SAS 9.4).

**RESULTS AND DISCUSSION:** A drought intensity index (DII) value of 0.42 indicated moderate drought was observed. Data from the two treatments (DS and NS) were combined. The ANOVA shows a significant difference among lines and treatments for all the traits measured. Also there is interaction effect between treatments and lines for harvest maturity. Plants were three days later under DS than NS because partitioning was adversely affected by the drought. Average 100 seed weight for NS was 48 g while for DS was 44 g.

Yields ranged from 463 to 3499 kg ha<sup>-1</sup> under DS while the range was from 1292 to 5165 kg ha<sup>-1</sup> under NS (Figure 1).Three lines ADP-63 (Kablanketi type), ADP-57 (large red), and ADP-41 (large red) combined high yield potential based on geometric means combined with low DSI values similar to the drought tolerant check Roza (Table 1). These ADP lines represent landraces from Tanzania that are present in the PI collection. All three had indeterminate vine growth habit and outperformed North American bred materials. The best performing lines with determinate bush growth habits were ADP-37 (brown seed) and Krimson cranberry bean from the USDA-ARS Prosser breeding program.

**CONCLUSION:** The level of drought exposure DII = 0.42 was enough to separate 74 Andean lines into tolerant and susceptible groups. The experiment will be repeated in 2015.



#### Figure 1: Frequency distribution for yield

#### Table 1. Mean Performance of 10 best and 10 worse yielding lines

ADP	Genotype	Emer LSM	gence EAN	Flow matu LSM	ering trity IEAN	Harv g mat LSM	estin turity EAN	100 s weig LSM	eeds ht EAN	Yield LSM	kgha <sup>-1</sup> EAN	% Yield Reduction kgha <sup>-1</sup>	GM Kgha <sup>-1</sup>	DSI Kgha <sup>-1</sup>
		NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	0		
ADP-63	Soya	4	4	53	52	117	113	48	44	4649	3499	25	4033	0.6
Roza	Roza	2	3	51	50	103	94	40	35	4489	3496	22	3962	0.6
ADP-590	SEQ11	3	3	51	53	106	109	32	30	4695	2781	41	3613	1.0
ADP-41	Mrondo	4	4	52	51	110	106	42	37	4383	2797	36	3501	0.9
ADP-549	RWR10	5	4	63	62	118	116	50	39	4445	2588	42	3392	1.0
ADP-57	Kijivu	4	5	61	51	115	118	51	47	4062	2749	32	3341	0.8
ADP-37	W616488	3	4	50	57	109	116	54	51	4570	2412	47	3320	1.2
ADP-660	Krimson	5	6	43	42	106	112	61	53	4701	2293	51	3283	1.3
ADP-80	Kablanke	3	4	61	62	114	116	38	36	5165	2030	61	3238	1.5
ADP-546	REDCANAD	4	4	50	50	106	106	43	34	4484	2327	48	3230	1.2
ADP-367	G23086	4	4	69	61	120	115	48	46	1292	1908	-48	1570	-1.2
ADP-111	Uyole98	3	3	45	49	106	109	43	39	1970	1224	38	1553	0.9
ADP-443	Vazon7	3	3	40	40	106	112	34	29	2437	924	62	1501	1.6
ADP-436	JB-178	3	4	50	60	114	119	49	43	2178	993	54	1471	1.4
ADP-741	PI638823	4	6	46	45	102	111	34	33	2006	1024	49	1433	1.2
ADP-681	Belagio	4	4	50	50	109	116	53	53	2248	874	61	1402	1.5
ADP-513	Canario	3	5	53	50	103	112	40	39	1596	998	37	1262	0.9
ADP-670	AC-Calmo	4	4	45	47	100	118	53	52	1658	892	46	1217	1.2
ADP-636	Montcalm	4	4	47	50	106	114	53	54	2228	639	71	1194	1.8
ADP-618	ACElk	4	3	40	40	99	113	51	44	1553	463	70	848	1.8
Overa	l Means	4	4	49	51	109	112	48	44	3188	1854	40	2399	1

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### COOKING TIME OF THE ANDEAN BEANCAP LINES EXPOSED TO TERMINAL DROUGHT IN WESTERN NEBRASKA

### Carlos A. Urrea

University of Nebraska, Panhandle Res. & Ext. Center, Scottsbluff, NE

**INTRODUCTION:** Worldwide drought is the most limiting abiotic stress affecting dry bean production. It may also affect cooking time. Cooking time is a major concern in Africa because longer cooking time requires use of more energy resources. This is particularly an issue in countries where firewood is scarce as rural households depend on firewood for cooking and must spend time searching for it. This study explored the effect of drought on cooking time by comparing the cooking time of the Andean BeanCAP lines grown under irrigated and terminal drought conditions.

**MATERIALS AND METHODS:** In 2013, 49 Andean Bean Coordinated Agricultural Project (Bean-CAP) lines were evaluated in replicated trials in adjacent irrigated (non-stressed, NS) and non-irrigated (drought-stressed, DS) plots at Mitchell, NE ( $41^{\circ}56.6'$  N,  $103^{\circ}41.9'$  W, 1240 m elevation). Within each block, the Andean lines were assigned to experimental units using a 7 x 7 lattice design with 2 replicates. Each plot consisted of two 7.6-m rows spaced 0.6 m apart. Targeted plant density was 200,000 plants ha<sup>-1</sup>. Both NS and DS blocks were irrigated until flowering to ensure good plant establishment and normal vegetative growth. Thereafter, the stressed block was not irrigated. Data recorded included daily rainfall during the growing season (mm), seed yield (kg ha<sup>-1</sup>), 100-seed weight (g), and number of days to flowering and maturity.

We used a Matson Bean Cooker to evaluate the effect of drought stress on cooking time. This cooker had 24 weighted plungers (metal rods) that pierced the seed when fully cooked. Seed from each plot was processed separately as follows. A 60-seed sample was soaked in distilled water overnight (16 hours). Distilled water was added to the cooker and heated to 98 °C then 24 of the pre-soaked seeds were placed in the template in the cooker to align the seeds with the plungers. An observer recorded the time when the beans were placed in the cooker and when 80% were cooked (indicated by the plungers dropping).

**RESULTS AND DISCUSSION:** The NS and DS plots received 453.0 and 248.2 mm, of total water, respectively. A total of 63.2 mm of precipitation occurred after flowering, resulting in moderate drought stress (DII = 0.47) for the DS plots. Beans produced under NS conditions had greater minimum and maximum values for yield (1402-4011 kg ha<sup>-1</sup> vs 682-2847 kg ha<sup>-1</sup>), 100-seed weight (33.4-60.8 g vs 28.8-51.0 g), and days to maturity (81-100 days vs 71-96 days) than those produced under DS conditions.

In general, dry beans grown under DS took 10 minutes longer to cook. However, cooking time was 33 and 29 minutes longer, respectively, for K-59 and Pompadour B grown under DS and 22 minutes longer for Fiero, Monclam, Litekid, and USDK-CBB-15 grown under DS. In contrast, Myasi, Red Rider, UCD 0801, Etna, and USCR-CB-20 grown under DS cooked more rapidly than those grown under NS conditions. Cardinal and K-42 had the longest cooking time under both DS and NS conditions, whereas, Jalo EEP, a yellow bean, had the shortest.

AndeanBeanCAP Trial tested to t	erminal drought	at Michell, NE during 2	2013.	
			Cookin	g Time
Entry	ADP Code	African Code	DS	NS
			min	min
Charlevoix	ADP-598	BC-57	55	50
Montcalm		BC-58	75	53
Isabella		BC 64	53	40
Pad Hawk		BC 81	76	61
Chine als 2000		DC-01	56	46
Clillook 2000		DC-02	50	54
Conri		DC-05	08 82	67
	ADD (25	DC-93	82	70
Cuelicul	ADP-025	BC-499	/4	/0
		BC-108	90	83
Fox Fire		BC-117	66	50
Lassen		BC-118	52	41
Sacramento			67	59
Myasi		BC-144	59	67
Red Kanner		BC-147	72	52
Red Kloud		BC-148	55	51
Wallace 773-V98	ADP-603	BC-149	56	43
Red Kote			69	52
K-42		BC-246	82	82
K-59		BC-247	80	48
USDK-CBB-15		BC-250	79	57
Fiero		BC-252	72	51
Royal Red		BC-253	56	45
Kardinal Kidney		BC-254	71	70
Blush		BC-255	68	68
USCR-9		BC-263	83	75
USCR-CBB-20		BC-264	64	65
G-122	ADP-610	BC-265	66	50
Silver Cloud		BC-274	64	43
USWK-CBB-17		BC-275	60	54
VA-19		BC-277	62	50
Pompadour B	ADP-611	BC-283	73	45
02-385-14	ADP-613	BC-313	78	62
ND061106	ADP-614	BC-315	78	57
Litekid	ADP-615	BC-338	72	10
Red Rider	7101-015	BC-556	61	65
CDRK		BC-350	60	46
LIC Nichols		BC 360	64	64
UC Conorio 707		DC-300	60	52
		DC-300	60	55
	ADD (21	BC-370	03	20
Jalo EEP558	ADP-621	BC-3/6	41	39
		BC-3//	65	62
Etna		BC-396	63	12
Hooter		BC-39/	80	61
Pink Panther		BC-398	82	65
Drake	ADP-623	BC-399	72	58
Dolly	ADP-624	BC-401	73	57
Bellagio			79	70
Krimson			77	65
GRAND MEAN	N		68	58
LSD 5 %	6		18	14
CV %	6		13	12

## SCREENING FIELD RESISTANCE TO HALO BLIGHT WITHIN THE ANDEAN DIVERSITY PANEL

### Jose Vasquez, Kiran Ghising, Albert Jody VanderWal, Michael Kloberdanz, and Juan M. Osorno

North Dakota State University, Dept. of Plant Sciences, Fargo, ND 58102

### **INTRODUCTION**

During the evaluation of the ADP genotypes for root rot complex in the field, the leaf canopy also was attacked by Halo Blight disease (caused by Pseudomonas syringae pv. phaseolicola or Psp) during 2013 and 2014 planting cycles. This provided an opportunity to also screen the ADP genotypes for their reaction to this disease under field conditions. Psp is a seed-borne bacterial disease that cause yield losses in dry bean ranging from 10 to 40% depending on disease pressure, environmental conditions, and cultivar (Asencio and Singh, 2005).

### MATERIALS AND METHODS

A total of 310 genotypes from the Andean Diversity Panel (ADP) (Cichy et al., 2015) were planted at Perham, MN in 2013. From those, 51 genotypes were photoperiod sensitive or did not complete the production cycle. Remnant 259 genotypes were screened again in 2014 in two trials separated by days to maturity. The early maturity trial consisting of 144 genotypes, and the late maturity trial consisting of 121 genotypes. Six common checks, one resistant, four intermediate, and one susceptible were used. The early and late maturity trials were planted in a 12 x 12 and 11 x 11 alpha-lattice design respectively, with two replications per trial. Bacterial disease severity was determined on a scale 1-9 (1= healthy, 9= death plant) in the whole plot at pod-filling stage (R8). In addition to Psp, seed size and seed yield were also measured. Resistant genotypes were considered those ranging from 1 to 3, intermediate from 4 to 6, and susceptible from 7 to 9.

### RESULTS

In 2013, from the selected 259 genotypes, 137 were in the range 1 to 3, 115 in the range 4 to 6, and 7 in the range 7 to 9 (data not shown). Table 1 shows the top 20 high resistant genotypes in 2014 early and late trials in addition to the checks. From the top 20, 17 were also resistant in 2013. Using the analysis of variance, in the early trial there were significant differences (P < 0.01) among genotypes. Using Least Square Means, 5 genotypes are in the range 1 to 3, 74 in the range 4 to 6, and 65 in the range 7 to 9. In the late trial also there were significant differences (P < 0.01) among genotypes. Using Least Square Means, 42 genotypes are in the range 1 to 3, 64 in the range 4 to 6, and 15 in the range 7 to 9. The mean 6 for early and 4 for late trials and the extreme values of the checks and genotypes suggest the high disease pressure in the field during the 2014 season. If the environmental conditions are favorable in the next cycle, a set of contrasting genotypes for root rot also will be evaluated for *Psp* in order to confirm results.

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Genotype	Seed type	Halo	100 seed	Seed yield
		blight(1-9)	weight (g)	kg ha-1
	<u>2014 Early matu</u>	<u>rity</u>		
VAX3 (resistant check)	Small red	1	29.9	2856
ADP43 Bwana shamba	Dark red kidney	3	38.4	1436
ADP678 Hooter*	Cranberry	3	50.1	1107
ADP2 W6_16444*	Red mottled	4	47.9	1444
ADP647 Red Kanner*	Light red kidney	4	50.5	1753
ADP614 Rosie*	Light red kidney	4	53.4	1952
ADP624 Dolly*	Cranberry	4	60.7	1579
ADP477 PI527512*	Pink spotted	4	44.6	1332
ADP649 Kamiakin*	Light red kidney	4	59.8	1329
ADP112 Uyole96*	Red	4	47.4	1314
ADP685 Chianti*	Cranberry	4	51.5	1233
ADP636 Montcalm(inter. check)	Dark red kidney	5	47.7	927
Dynasty (intermediate check)	Dark red kidney	5	56.8	1495
Talon (intermediate check)	Dark red kidney	6	49.2	1152
GTS104 (intermediate check)	Dark red kidney	6	50.9	1195
Cabernet (susceptible check)	Dark red kidney	9	41.0	453
Mean		6	43.4	892
Coefficient of variation (%)		16.8	7.0	33.9
	2014 Late matur	<u>rity</u>		
ADP50 Salunde*	Yellow	2	47.0	1313
ADP71 Njano dolea*	Yellow	2	45.0	1616
ADP122 Kranskop*	Cranberry	2	44.3	1278
VAX3 (resistant check)	Small red	3	29.1	2772
ADP57 Kijivu*	Dark red kidney	3	34.0	1337
ADP84 Kablanketi ndefu*	Black spotted	3	37.5	1311
ADP454 Iniap429*	Red spotted	3	45.1	1886
ADP626 Badillo*	Light red kidney	3	48.0	1289
ADP621 JaloEEP558*	Yellow	3	35.7	1309
ADP523 Canario cela*	Yellow	3	39.1	1267
ADP37 W6 16488	Brown	3	49.1	1252
ADP636 Montcalm(inter. check)	Dark red kidney	6	51.0	1198
Talon (intermediate check)	Dark red kidney	6	51.5	1433
Dynasty (intermediate check)	Dark red kidney	6	55.1	1305
GTS104 (intermediate check)	Dark red kidney	7	46.3	666
Cabernet (susceptible check)	Dark red kidney	9	43.6	759
Mean		4	39.2	1001
Coefficient of variation (%)		22.0	5.6	31.3

**Table 1.** Halo blight reaction to 16 early-maturity and to 16 late-maturity ADP genotypes at Perham, MN, 2014.

\*Resistant in 2013 and 2014 seasons

### POTENTIAL USE OF THE BARCBEAN6K\_3 GENOTYPING PLATFORM IN THE DOMESTICATED SPECIES OF THE *PHASEOLUS* GENUS

### Jorge Carlos Berny<sup>1</sup>, Eneas Ricardo Konzen<sup>2</sup>, Siu Mui Tsai<sup>2</sup>, and Paul Gepts<sup>1</sup>

<sup>1</sup>Department of Plant Sciences, University of California, Davis, CA. <sup>2</sup>Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, SP, Brazil

**INTRODUCTION**: The Common Bean Coordinated Agricultural Project (BeanCap) has developed the BARCBEAN6K\_3 genechip for genotyping common bean (*Phaseolus vulgaris* L.). This tool has been of enormous value to the scientific community to perform diversity and mapping analyses that are high-throughput, fast, convenient and relatively inexpensive. However breeding programs and researchers working with *Phaseolus* species that are not common bean are limited by the lack of high-throughput resources for genotyping their germplasm. This hinders the advance in basic and applied research and breeding within those species. As a result, we analyzed the common bean genechip with diverse accessions of the other domesticated species within the genus *Phaseolus*, to assess its usefulness to detect polymorphism within and between species.

**MATERIALS AND METHODS:** Fifteen accessions of wild and domesticated forms within the *Phaseolus* genus were genotyped with the Illumina Infinium Genechip BARCBEAN6K\_3 platform (Table 1). This chip was developed by Dr. Perry Cregan (Soybean Genomics and Improvement Laboratory, ARS/USDA in Beltsville, Maryland) and the BeanCAP. The analysis was conducted at the Soybean Genomics and Improvement Laboratory, ARS/USDA, in Beltsville, Maryland. The assay scores 5,398 SNPs selected from polymorphisms between Jalo EEP558, BAT94, and 17 cultivars, all *P. vulgaris* dry beans, and positioned within the whole genome sequence assembly (Hyten et al., 2010; Cregan & Song, 2013). Allele calls were made with GenomeStudio<sup>TM</sup> with a no-call threshold of 0.15 and further visual clustering refinement using known heterozygotes as reference. Polymorphisms between lines were quantified using JMP<sup>®</sup>, excluding heterozygotes.

**RESULTS AND DISCUSSION:** The number of amplified and polymorphic markers was higher within the P. vulgaris accessions (Table 1). After quality control, common bean amplified 92% of the 5,398 assay markers, while P. coccineus, P. dumosus, P. acutifolius and P. lunatus amplified 73%, 81%, 61% and 51%, respectively. As expected, Jalo and BAT 93 showed the higher number of polymorphic markers (3,209 markers), since they belong to different gene pools and the chip was mainly developed with sequences of these two lines. Polymorphism between domesticated and wild accessions within each gene pool in P. vulgaris was lower in the Andean (Jalo vs PI 638868; 466 markers) than in the Mesoamerican gene pool (BAT 93 vs PI 417653: 1727 markers). Within the other species, the number of polymorphic markers was low in three of them, with an average of 33.3, 51.6 and 20 makers for P. coccineus, P. acutifolius and P. lunatus, respectively. P. dumosus, showed higher polymorphism (1,318). However, the polymorphism pattern of PI 311859 suggests it might be misclassified or that it is an interspecific hybrid. There was an average of 1.322 polymorphic markers when P. vulgaris was compared with the other species. An assessment of usefulness of the genotyping platform for an interspecific cross was carried out with a comparison of one P. vulgaris, one P. coccineus, and their hybrid. There were 1,631 polymorphic markers between the two parents (Table 2), of which 1,386 were confirmed with heterozygotes in the  $F_1$ . The markers were scattered across the genome, with a higher density near the telomeres (Figure 1).

				Q \ 83	0	W-V 659	865 v-W	210 c-D	0-5 <b>6</b> 0	608 C-W	W-3 1610	0-9 858	242 d-W	0-811	06 a-W	06a-W		0-126	
				BAT	a	1.417	89		Ĩ	141		1311	8	ŝ	6603	6603	<b>Heat</b>	9	
Species	Form	Pool	Accession	I		a.	- a.			6.	<b>6</b> .		a.					_	
P. vulgaris	Domesticated	Mesoamarican	BAT 93			-	-												
P. vulgaris	Domesticated	Andean	Jalo	3209			-	-					_						
P. vulgaris	Wild	Mesoamerican	PI 417653	1727	2309			-											
P. vulgaris	Wild	Andean	PI 638865	2894	466	2021							_	-		_			1
P. coccineus	Domesticated		PI 311210	1505	1519	1163	1286												1
P. coccineus	Domesticated		Hestia	1517	1491	1168	1273	26					-						2
P. coccineus	Wild		PI 417608	1533	1508	1162	1287	23	47										- 2
P. coccineus	Wild		PI 430191	1514	1530	1177	1301	31	60	13			-						- 3
P. dumosus	Domesticated		PI 311859	1376	2729	1345	2445	1296	1309	1292	1318			-	_	_			3
P. dumosus	Wild .		PI 653242	1504	1521	1162	1299	96	148	114	116	1274							
P. acutifolius	Domesticated		G40111	1330	1289	963	1127	248	287	256	271	1117	295						
P. acutifolius	Wild	var. acutifolius	G40206	1334	1285	1025	1123	245	293	252	273	1127	294	43					
P. acutifolius	Wild	var. tanifolius	G40204	1320	1295	1013	1135	236	279	241	256	1119	276	53	59				
P. lunatus	Domesticated	Mesoamerican	UC Haskel	1077	1042	832	892	243	272	239	256	894	282	147	164	160			
P. lunatus	Domesticated	Andean	UC 92	1032	2061	820	885	267	289	260	276	882	<b>29</b> 9	159	171	170	20		
			AA	2271	2345	2051	2207	1395	1404	1409	1403	2110	1424	1292	1280	1236	998	970	
		Conchen	_ A6	4	4	15	6	121	67	158	115	208	77	85	65	65	169	164	
		Genocyp	e 68	2723	2700	2875	2801	2441	2410	2433	2462	2662	2371	1973	1979	2005	1623	1553	
			NC	400	349	457	384	1441	1517	1398	1418	418	1526	2048	2074	2092	2608	2711	

Table 1. Number of polymorphic markers between 15 accessions of wild and domesticated *Phaseolus* spp. beans using the BARCBEAN6K-3 genotyping platform. The table lists the different accessions included in the comparison. The triangle below the diagonal shows the number of polymorphic SNP markers shared in each two-way comparisons. The graph above the diagonal is a heat map of the same. Abbreviations: v: *vulgaris*; c: *coccineus*; d: *dumosus*; a: *acutifolius*; l: *lunatus*; D: domesticated; W: wild.



Table 2. Genotype comparison of UCD 9634 (*P. vulgaris*) and Scarlet runner (*P. coccineus*).

Figure 1. Marker coverage of UCD 9634 (*P. vulgaris*) and Scarlet runner (*P. coccineus*) and their  $F_1$  cross on chromosome 1.

Taken together, the low number of polymorphic markers within non-*P. vulgaris* species suggests – perhaps not surprisingly - that the BARCBEAN6K\_3 platform should not be used in other species, as a minimum of several hundreds of markers are needed for linkage mapping analysis, and several thousands of polymorphic markers are needed for family mapping and/or certain types of diversity analysis. For interspecific crosses, the platform could be adequate. However, when new chips are developed with a higher number of markers and sequences from other species become available, it will be possible to develop a new chip that will minimize ascertainment bias and be useful for high-throughput genotyping in other species besides *P. vulgaris*.

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# USING SNP GENETIC MARKERS TO ELUCIDATE THE LINKAGE OF THE *Co-3<sup>4</sup>/Phg-3* ANTRHRACNOSE AND ANGULAR LEAF SPOT RESISTANCE GENE CLUSTER WITH THE *Ur-14* RUST RESISTANCE GENE

### G. Valentini<sup>1</sup>, M.C. Gonçalves-Vidigal<sup>1</sup>, P.B. Cregan<sup>2</sup>, Q. Song<sup>2</sup>, M.A. Pastor-Corrales<sup>2</sup>

<sup>1</sup> Dep. Agronomia, Universidade Estadual de Maringá, Av. Colombo, 5790, Maringá, PR, Brazil
 <sup>2</sup> Soybean Genomics Improvement Lab, USDA-ARS, BARC-West, Beltsville, MD 20705, USA

**INTRODUCTION:** The common bean is a very important human food in many countries of the Americas and eastern and southern Africa (Broughton et al. 2003). The rust disease caused by Uromyces appendiculatus (Pers.) Unger., anthracnose (ANT) caused by Colletotrichum lindemuthianum and angular leaf spot (ALS), caused by Pseudocercospora griseola (Sacc.) are some of the most widespread diseases of common bean in the tropics (Pastor-Corrales and Tu, 1989; Singh and Schwartz 2010). Genetic mapping of genes and the use of molecular markers allow a better understanding of these genes and increases the efficiency of plant breeding programs. A previous study on Ouro Negro revealed the presence of a resistance gene cluster named  $Co-3^4/Phg-3$  that confers resistance to ANT and ALS mapped on Pv04 and that these genes were tightly linked at 0.0 cM (Gonçalves-Vidigal et al. 2013). Ouro Negro also carries the dominant Ur-14 gene that confers resistance to the rust disease (Souza et al. 2011). Another study reported that the SF10 marker was linked to the Ur-14 rust and Co-10 (renamed Co- $3^4$ ) ANT-resistance genes on chromosome Pv04 at a distance of  $6.0 \pm 1.3$  cM (Corrêa et al. 2000). However, the relationship between the  $Co-3^4/Phg-3$  cluster and Ur-14 has not been investigated. The main objective of this study was to elucidate the linkage of the  $Co-3^4/Phg-3$  resistance gene cluster and the Ur-14 rust resistance gene using single nucleotide polymorphism (SNP) markers.

MATERIAL AND METHODS: The co-segregation analysis between the Ur-14 rust and the ANT and ALS resistance genes  $Co-3^4/Phg-3^4$  was performed on 107 F<sub>2:3</sub> families derived from the Rudá  $\times$  Ouro Negro cross. The seeds of the F<sub>2:3</sub> families were obtained at the Núcleo de Pesquisa Aplicada à Agricultura (Nupagri) of the Universidade Estadual de Maringá (UEM), state of Paraná, Brazil. Evaluation for rust and anthracnose diseases on the F2:3 families and the molecular analysis were conducted at the Soybean Genomics and Improvement Laboratory, ARS-USDA, Beltsville. Twenty parental plants (Rudá and Ouro Negro) and 12 F<sub>2.3</sub> plants from each F<sub>2.3</sub> family were inoculated with races 41, 47, 53 and 58 of the rust pathogen and 10 plants of the parents as well as 10 F<sub>2.3</sub> plants from each of the 107 F<sub>2.3</sub> families were inoculated with race 73 of the ANT pathogen as described by Gonçalves-Vidigal et al. (2001). The DNA previously isolated from each  $F_{2,3}$  plants was used for genotyping with 5,399 SNP markers on the BARCBean6K 3 Illumina BeadChip following the Infinium HD Assay Ultra Protocol. The automatic allele calling for each locus available in the Genome Studio software was manually checked and positive markers for Ur-14 and Co- $3^4$ /Phg- $3^4$  were recorded. The sequence scaffold on which SNPs associated with rust and ANT resistance were located, were interrogated for the presence of Simple Sequence Repeats (SSRs). New SSR markers were designed and those that were polymorphic between the Rudá vs. Ouro Negro parents were validated on the set of 107 F<sub>2:3</sub> families. Linkage analyses positioned the SSRs, the Ur-14 and  $Co-3^4/Phg-3^4$  into linkage group Pv04 using the JoinMap 4.1 software based on the regression mapping method and the Kosambi map function.

**RESULTS AND DISCUSSION:** Of the 107  $F_{2:3}$  families derived from the Rudá × Ouro Negro cross, 106 families that were inoculated with races 41, 47, 53 and 58 of *U. appendiculatus* and race 73 of *C. lindemuthianum* exhibited a similar co-segregation of resistance/susceptibility to both pathogens (Table 1). Except for one family, all families that were resistant to ANT were also resistant to the rust pathogen, and the families that were susceptible to ANT were susceptible to

rust. The 107  $F_{2:3}$  families segregated into classes as follows: for rust 30RR:52Rr:25rr (P = 0.76) and the ANT/ALS resistance gene cluster  $Co-3^4/Phg-3$  showed a segregation 30RR:51Rr:26rr (P = 0.77). These results revealed a co-segregation between the Ur-14 and Co- $3^4$ /Phg- $3^4$  genes that fit a 1R:2Rr:1S ratio for a single dominant gene. We observed only one recombinant in the F<sub>2.3</sub> families, revealing a tight linkage (1.4 cM) of the Ur-14 and Co-3<sup>4</sup>/Phg-3 loci. The combined genetic linkage analysis of selected SSR markers resulted in a good fit to the expected ratio of 1RR:2Rr:1rr. All of the 81 families with resistance to anthracnose had the Ouro Negro allele at SSR loci BARCPVSSR04574, BARCPVSSR04558, BARCPVSSR04569, BARCPVSSR04561, BARCPVSSR04562 and BARCPVSSR04570, and the 26 susceptible F<sub>2</sub> plants were homozygous for the Rudá allele at these SSR loci, revealing close linkage between  $Co-3^4/Phg-3$ locus and the molecular markers (0.0 cM). Furthermore, it was observed that Ur-14 is located at 0.0 cM from the SSR markers BARCPVSSR04590, BARCPVSSR04605, BARCPVSSR04592, BARCPVSSR04599, BARCPVSSR04595 and BARCPVSSR04596. The lack of recombinants between the molecular markers and the Ur-14 and  $Co-3^4/Phg-3$  genes indicated that these markers are tightly linked to these genes. These results also will reduce the time and cost of pyramiding the  $Co-3^4/Phg-3$  and Ur-14 genes into commercial common bean cultivars. In addition, the many more effective SSR molecular markers were found linked to the abovementioned gene cluster ( $Co-3^4/Phg-3/Ur-14$ ) and these should be recommended for use in breeding programs.

Pv04

0.0

0.5

0.9

19

BARCPVSSR04557 Co-34/Phg-3 BARCPVSSR04561 BARCPVSSR04570 BARCPVSSR04558 BARCPVSSR04569 BARCPVSSR04574 BARCPVSSR04562 BARCPVSSR04581

BARCPVSSR04590 BARCPVSSR04605 BARCPVSSR04592 BARCPVSSR04599

Table 1 Observed and expected reaction of the cultivars Rudá (susceptible) and Ouro Negro (resistant), and the F2.3 lines from Rudá × Ouro Negro cross inoculated with races 73 of Colletotrichum lindemuthianum and 41, 47, 53 and 58 of Uromyces appendiculats for characterization of the resistant genes Co-14 and  $Co-3^4/Phg-3$  in the cultivar Ouro Negro



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### EVALUATION OF CREOLES AND ANDINOS MARKET TYPES OF COMMON BEAN TO PATHOTYPES OF *PSEUDOCERCOSPORA GRISEOLA*

### Batista, M. S.<sup>1</sup>; Machado, M. A. M.<sup>1</sup>; Lopes, E. M. G.<sup>1</sup>; Vieira, A. F.<sup>1</sup>; Santos, J. M. C.<sup>1</sup>; Carneiro, A. R. T.<sup>1</sup>; Campos, R. G. C.<sup>1</sup>; Santos, L. P.<sup>1</sup>; Rocha, F. S.<sup>1,2</sup> and Sanglard, D. A.<sup>1,3\*</sup>

<sup>1</sup>Instituto de Ciências Agrárias (ICA); <sup>2</sup>Lab. de Fitopatologia; <sup>3</sup>Lab. de Biotecnologia; Universidade Federal de Minas Gerais (UFMG), 39.404-547, Montes Claros, MG, Brazil; \*E-mail: demerson.ufmg@gmail.com

**INTRODUCTION -** One of the major limiting factors of common bean (*Phaseolus vulgaris* L.) productivity and performance are diseases. Among the most important diseases, angular leaf spot caused by *Pseudocercospora griseola* (Sacc.) Crous & U. Braun deserves attention. As primary sources of inoculum include the seeds and contaminated debris of infected plants. In both cases, the produced conidia are disseminated to the leaves by wind and rain splash (Pria et al., 1999). The disease early may be due to: *i*) planting crops in a new season (autumn, winter and spring) under favorable temperatures with infected crop plants or contaminated crop tissues in the field throughout the year; *ii*) crop growing under center pivot, which provides high humidity condition and favorable for disease development; *iii*) use of contaminated seeds, introducing pathogen into new areas or regions; *iv*) use of cultivars with genetic to specified disease resistance; and *v*) possible changes in the context of pathogen races over the years (Paula & Zambolim Jr., 1998). The constant work of identification and characterization of potential sources of resistance is essential for breeding programs. In this context, the aim of this study was to evaluate the reaction of Creoles and Andinos access of common beans to pathotypes of *P. griseola*.

**MATERIAL AND METHODS** - The screening of germplasm (access) for reaction to angular leaf spot are from microregion of Montes Claros, North of Minas Gerais State ('Japanese', 'Big Blue', 'Early Carioca', 'Cone', 'Criangu' and 'Pardo'). In addition, materials of grain type "Jalo" resulting from the Bean Breeding Program of the Federal University of Lavras ('BJ-1', 'BJ-2', 'BJ-3', 'BJ-4', 'BJ-5 "and" BJ-6 ') were evaluated. Seeds of each line were sowed directly in 2.5 L plastic pots containing a mixture of soil and dung cured (4: 1), fertilized (4-14-8) with 5 kg per m<sup>3</sup>. The pots of each genotype were maintained in greenhouse until inoculation. The inoculum of each pathotype, classified in the races 31.4, 31.7, 47.39, 63.23, 63.31 and 63.63 (Balbi et al., 2009), were obtained from petri dishes containing the culture medium (Sanglard et al., 2009) and incubated for 10 days at 24 °C. Fifteen days after sowing, inoculations were done in both surfaces of the first trifoliate leaf using a suspension containing 2.0 x 10<sup>4</sup> conidia/ml and brushes. Disease severity was visually assessed at 15, 18 and 21 days after inoculation using a scale of nine degrees of severity proposed by Pastor-Corrales & Jara (1995). For each genotype were inoculated 12 plants in the number of three plants per pot. Lines received notes 1 to 3 were considered resistant; between 3 and 6, intermediate resistance; and between 6 and 9 susceptible.

**RESULTS AND DISCUSSION** - In this work were used admittedly virulent and high aggressiveness isolates. The results confirm the great difficulty in identifying genotypes with broad resistance to angular leaf spot (Balbi et al., 2009). Five genotypes were susceptible to at least three races of *P. griseola* (Table 1). However, Creole genotype 'Japanese' was resistant to all six races tested. In general, we observed that Andinos genotype showed lower average severity. Orozco & Araya (2005) showed that in places where they are grown exclusively of Mesoamerican type beans, selection pressure often occur on populations of *P. griseola*, leading to the increase of races corresponding to the genetic pool of the host. In Brazil, this is due to the

fact that most planted cultivars belong to the Mesoamerican gene pool. Consequently, selection pressure favored races of Mesoamerican origin, which are more adapted to the Mesoamerican genotypes, although inciting disease in both groups. Genotype 'Japanese', showed resistance reaction to six *P. griseola* isolates tested. In summary, we showed that adinos were those that showed higher resistance levels (lower levels of disease severity) in comparison with other genotypes.

	Ra	ices (isolat	tes) of <i>Psa</i>	eudocerco	spora grise	ola
Access	31.4	31.7	47.39	63.23	63.31	63.63
	(B <sub>4</sub> 6)	(Cb21)	$(B_{4}4)$	(SM32)	$(CM_{3}11)$	(Vic7)
BJ-1	6.0*	6.6	5.0	5.7	5.3	7.8
BJ-2	3.5	3.9	3.7	7.0	2.9	1.7
BJ-3	2.8	7.5	8.2	2.2	6.4	8.0
BJ-4	3.0	4.2	7.6	8.0	8.9	2.2
BJ-5	1.9	1.2	4.0	1.2	1.8	6.2
BJ-6	3.4	6.9	2.5	1.5	4.0	1.2
Japonês	1.9	1.4	2.5	3.5	2.5	2.7
Azulão	7.9	6.4	8.6	7.2	4.2	9.0
Carioca Precoce	6.5	4.1	8.0	1.5	7.2	7.5
Casquinha	9.0	8.7	7.0	4.6	9.0	8.2
Criangu	6.0	6.6	5.0	5.7	5.3	7.8
Pardo	3.5	3.9	3.7	7.0	2.9	1.7

**Table 1.** Reaction of creoles and andinos lines of common bean to the six isolates of *P. griseola* collected in Minas Gerais State, Brazil.

\*Average severity was taken base on twelve plants for each genotype in each replication.

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### RECURRENT SELECTION PROGRAM FOR ANGULAR LEAF SPOT RESISTANCE IN CARIOCA SEEDED COMMON BEAN

## Adélia C. F. Silva<sup>1</sup>, Adriane Wendland<sup>1</sup>, Helton S. Pereira<sup>1</sup>, Luís C. Faria<sup>1</sup>, Maurício Martins<sup>2</sup>, Thiago L. P. O. Souza<sup>1</sup> and Leonardo C. Melo<sup>1\*</sup>

 <sup>1</sup>Embrapa Arroz e Feijão (Embrapa Rice and Beans), Santo Antônio de Goiás, GO 75375-000, Brazil;
 <sup>2</sup>Universidade Federal de Uberlândia (UFU), Uberlândia, MG 38408-100, Brazil.
 \*Corresponding author: +55 (62) 3533.2158 – leonardo.melo@embrapa.br

The common bean (*Phaseolus vulgaris* L.) crop is susceptible to different diseases. The majority of them causes significant yield losses and drastically affects grain quality. One of this diseases is the angular leaf spot (ALS), caused by the fungus *Pseudocercospora griseola* (Sacc. Ferr). Losses caused by ALS can affect up to 80% of grain yield when bean plants are early infected. A desirable method to control this disease is the use of resistant cultivars. However, the development of cultivars with effective resistance is being a challenge for the common bean programs worldwide. The reason for that is because ALS resistance is a complex trait, controlled by major and minor genes (Caixeta *et al.* 2005; Borel *et al.* 2010). Thus, a breeding method proposed and used in Brazil by the Embrapa common bean breeding program aiming to develop resistant cultivars is the recurrent selection (RS). RS is a dynamic and continuous breeding process involving the development of individual genotypes and progenies, their evaluation for different traits, followed by the selection and intercrossing of the superior ones. The objective of this breeding strategy is to increase the frequency of favorable alleles associated with the expression of the traits under selection. The main goal of this work was to develop and evaluate carioca seeded common bean SR populations and progenies resistant to ALS.

The initial population was formed during the years 2008 and 2009 using a conical crossing design between eight different ALS resistance sources previously identified. The initial parents were the lines: MA-1-15-13, 2003200396, CNFC10755, MA-1-2-10-1, CNFN10284, MA-1-8-9, 203200330, and CF220249. The  $C_0S_0$  population formed by about 5,000 plants was evaluated at Embrapa Research Center, in Santo Antônio de Goiás, GO, during the winter season of 2009. Then, the resulting  $C_0S_1$  and  $C_0S_2$  generation were evaluated during the fall and summer seasons of 2010, respectively, in Ponta Grossa, PR. Aiming a better field screening of the  $C_0S_2$  generation for ALS resistance, it was also evaluated during the fall season of 2011, in Santo Antônio de Goiás and Ponta Grossa.

Using the results obtained from the multisite evaluation accomplished in three different environments, 613 individual  $C_0S_2$  plants were selected based on grain yield and resistance to ALS. The resulting  $C_0S_{2:3}$  seeds were grown for generation advancement and seed increase during the winter season of 2011, in Santo Antônio de Goiás, GO. The subsequent  $C_0S_{2:4}$ progenies were grown during the fall season of 2012, in Santo Antônio de Goiás and Ponta Grossa. Out of these  $C_0S_{2:5}$  progenies, 55 were selected as superiors based on their agronomic performance in both environments, mainly considering grain yield, aspect of carioca grains, and ALS resistance. These selected 55  $C_0S_{2:5}$  progenies and two carioca seeded control cultivars (BRS Sublime - resistant to ALS, and BRS Horizonte - susceptible) were evaluated during the fall season of 2013 in Ponta Grossa, PR. This field trial used a randomized complete block design with two replications, being each plot formed by two rows three meters long. All treatments were evaluated for ALS reaction and for the reaction to other diseases, in addition to grain yield, architecture of plants and resistance to lodging according to Melo (2009). The results obtained during the fall season trial in Ponta Grossa, PR were used to select 10  $C_0S_{2:5}$  progenies as superiors based on grain yield, ALS resistance and other important agronomic traits (Table 1). Out of these progenies, four (SRCMA.127, SRCMA.23, SRCMA.26 and SRCMA.320) presented ALS mean severity scores lower than the score presented by the cultivar BRS Sublime, the resistant control (Table 1). SRCMA.23 and SRCMA.26 were among those presenting high yield. Based on these results, these 10 selected progenies are being used to develop elite lines and for recombination aiming to obtain a new cycle of recurrent selection (C<sub>1</sub>S<sub>0</sub> generation).

Drogony	AY		Mean s	core (1-to-9 sca	ule) <sup>a</sup>	
Progeny	(Kg/ha) <sup>a</sup>	AN	ALS	CBB	AP	RL
SRCMA.26	3,226.4	5.5	2.5	3.0	6.0	5.5
SRCMA.23	2,449.4	3.5	2.5	3.0	6.0	3.5
SRCMA.73	2,375.4	2.5	4.0	5.0	5.0	4.5
SRCMA.34	2,197.8	2.0	3.5	6.0	5.5	4.0
SRCMA.320	2,153.4	6.0	2.5	4.5	5.5	4.0
SRCMA.75	1,909.2	3.0	3.5	3.0	5.0	4.0
SRCMA.28	1,779.7	4.5	3.5	4.5	6.5	7.5
SRCMA.127	1,391.2	4.0	2.0	2.5	5.0	4.5
SRCMA.29	1,391.2	6.5	3.5	4.0	7.0	7.0
SRCMA.147	1,328.3	3.5	3.0	3.0	5.0	3.0
BRS Sublime	1,180.3	8.0	3.0	5.0	5.5	4.5
BRS Horizonte	799.2	1.0	9.0	6.0	5.0	3.0
Mean	2,020.2	4.2	3.5	4.1	5.6	4.6
CV (%)	13.4	36.9	22.4	22.2	11.0	26.0
Tukey (5%)	1,073.3	6.5	4.8	5.0	2.7	5.4
F-value	8.4	3.3	3.7	2.1	2.4	2.5

**Table 1.** Average yield, ALS mean severity score and other important agronomic traits presented by the 10 selected  $C_0S_{2:5}$  carioca seeded progenies derived from the Embrapa common bean recurrent selection program for ALS resistance. Fall season of 2013, Ponta Grossa, PR, Brazil.

<sup>a</sup>AY: average yield; AN: anthracnose severity; ALS: angular leaf spot severity; CBB: common bacterial blight severity;

AP: architecture of plants; RL: resistance to lodging.

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### SEVERITY LEVELS OF ANGULAR LEAF SPOT IN COMMON BEAN CULTIVARS AND ELITE LINES

### Sanglard, D. A.<sup>1\*</sup>; Souza, T. L. P. O.<sup>2</sup>; Machado, M. A. M.<sup>1</sup>; Rocha, F. S.<sup>1</sup>; Sanglard, N. A.<sup>3</sup>; Barros, E. G.<sup>4</sup> and Moreira, M. A.<sup>5</sup>

<sup>1</sup>ICA/UFMG, Montes Claros, MG 39.404-547, Brazil; <sup>2</sup>Embrapa Arroz e Feijão (CNPAF), Santo Antônio de Goiás, GO 75375-000, Brazil; <sup>3</sup>PPGM/UFES, Alegre, ES 29.500-000, Brazil; <sup>4</sup>UCB, Brasília, DF 70.790-160, Brazil; <sup>5</sup>BIOAGRO/UFV, Viçosa, MG 36.571-000, Brazil. \*E-mail: demerson.ufmg@gmail.com

In Brazil, among the most important economic disease of bean crop is the angular leaf spot incited by Pseudocercospora griseola (Sacc.) Crous and U. Braun. The objective of this study was to assess the reaction of various cultivars and elite lines bean to P. griseola isolates obtained by Balbi et al. (2009). Twenty-five genotypes including cultivars and strains from Common Bean Breeding Program of the Federal University of Viçosa (UFV) were evaluated for resistance to 24 isolates of P. griseola. Fifteen days after planting, these genotypes were inoculated with a suspension containing 2.0 x  $10^4$  conidia mL<sup>-1</sup>. Twelve plants of each genotype were inoculated. The symptoms were evaluated at 15, 18 and 21 days after inoculation using a nine-point scale (notes of 1 to 3 were considered resistant; notes of 3 to 6 intermediate resistance; and notes of 6 to 9 susceptible). Eight Mesoamerican genotypes were susceptible to at least 12 isolated from fourteen genotypes tested (Table 1). However, genotypes 'Ouro Negro', 'Diamante Negro' and 'BRSMG Majestoso' were resistant to 17, 15 and 17 isolates, respectively, among the twentyfour isolates tested. These genotypes also showed the lowest levels of severity. The cultivar 'Ouro Negro' (black group) has high productivity and good cooking qualities, which was recommended in 1991 (Araújo et al., 1991), and introduced as Honduras 35 in Brazil. Alzate-Marin et al. (2004) reported that the cultivar 'Ouro Negro' was tested with 24 isolates of U. appendiculatus from ninety-four isolates retained by USDA ("Beltsville United States Department of Agriculture"), showing resistance reaction to 22 races, intermediate resistance to one race, and susceptibility only to race 108. Ouro Negro cultivar has also good combining ability in crosses with cultivars like "carioca" (Faleiro et al., 2002). In addition, it has a resistance to anthracnose gene designated Co-10 (Alzate-Marin et al., 2003). Sartorato (2006) evaluated the reaction of 28 bean genotypes for resistance to eight races of P. griseola, and noted that 'Ouro Negro' showed higher degree of resistance. In summary, we showed greater resistance of 'Ouro Negro' cultivar to isolates of *P. Griseola* tested, than previously reported. These results confirm the great difficulty in the identification of genotypes with broad resistance to angular leaf spot.

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Table 1. Reaction of twenty-five genotypes of common bean to 24 isolates of *P. griseola* collected in the state of Minas Gerais, Brazil.

	63.63 (AJ12)	4.2	3.6	7.0	4.2	3.I	3.5	1.5	5.I	3.4	3.5	2.8	9.0	6.8	5.0	5.9	8.8	5.0	8.0	7.0	I.I	6.6	6.0	7.7	7.3	8.2	
	63.23 (Ma14)	3.8	4.6	5.8	6.0	6.2	2.7	5.1	4.9	3.8	5.0	7.7	6.6	7.0	4.4	I.7	2.9	2.0	I.I	2.4	2.0	3.0	4.7	5.4	7.0	6.0	
	63.63 (Vic7)	5.2	5.0	4.0	3.8	3.1	6.0	3.5	3.5	6.0	4.1	I.I	8.9	4.6	I.I	7.1	7.3	5.1	7.2	I.9	3.5	2.6	5.0	7.1	2.9	6.4	
	63.23 (Vic3)	2.5	7.9	3.7	5.6	4.0	4.3	4.3	8.0	3.0	6.3	4.0	8.I	8.7	6.2	8.6	7.I	6.0	7.I	3.2	6.7	I.7	7.7	4.4	8.6	8.4	
	63.23 (SM32)	4.0	I.4	3.7	7.0	I.I	5.0	4.5	4.1	4.9	2.2	3.7	7.5	7.6	7.9	3.0	1.5	I.4	3.5	6.5	5.0	6.1	8.0	6.0	9.0	8.5	
	63.63 (SM28)	3.1	2.5	1.7	2.6	2.6	4.2	1.6	2.4	5.5	3.5	2.3	5.5	7.4	6.6	5.8	5.2	7.0	6.4	1.8	6.5	2.0	5.8	7.3	5.6	7.1	
	63.63 (SM20)	<i>T.T</i>	5.5	7.1	3.6	3.6	8.6	3.6	4.5	5.8	6.6	4.3	8.8	8.6	2.5	5.0	3.3	4.3	4.7	1.2	2.8	3.0	7.5	5.5	8.2	8.8	
	63.63 (SM <sub>2</sub> 11)	4.6	6.2	7.2	4.0	4.2	3.5	4.4	8.4	7.5	7.0	3.6	6.0	7.8	3.6	7.6	3.5	8.0	8.7	1.2	1.1	1.8	7.8	5.3	7.2	9.0	
-	31.7 (Cb21)	3.0	4.9	5.9	4.0	3.8	5.5	3.1	5.5	3.2	3.4	6.0	8.9	4.8	3.2	7.0	4.3	8.0	3.4	I.I	2.0	4.4	7.6	I.2	5.0	9.0	
seola	63.7 (Cb20)	4.2	5.2	1.1	9.7	5.1	0.0	1.7	4.5	6.1	2.0	4.0	6.4	6.8	4.2	5.0	2.7	3.9	2.6	I.7	6.0	I.2	3.1	5.0	6.6	9.0	
o. gri	63.31 (CM <sub>3</sub> 11)	3.2	3.5	5.5	3.0	4.7	1.9	2.9	3.1	5.4	6.1	3.9	5.0	7.5	6.2	5.3	7.7	7.0	7.6	3.5	2.1	3.4	5.9	6.6	7.7	8.1	
/ Jo (	63.63 (CM <sub>1</sub> 2)	2.6	4.0	9.4	<i>t</i> .7	3.3	9.4	4.2	3.6	2.0	5.2	4.6	8.0	8.0	5.4	2.6	4.4	4.1	3.0	2.0	4.3	2.5	5.5	4.1	9.0	7.7	
lates	23.23 (C <sub>2</sub> 10)	7.2	4.1	0.0	5.5	5.1	3.1	5.2	2.5	3.8	3.7	I.2	7.8	6.9	5.3	6.3	6.1	6.1	8.2	I.I	3.0	2.9	8.5	3.3	9.0	2.8	
i (iso	63.6 (C <sub>1</sub> 28)	6.0	3.5	3.1	3.4	1.7	3.4	3.7	3.8	2.1	3.9	4.5	6.7	8.2	7.0	4.5	2.8	7.2	7.0	3.1	2.1	1.3	2.0	8.0	8.6	4.4	
laces	3.23 (C <sub>1</sub> 17)	6.0	5.0	2.1	3.8	3.0	2.2	4.3	0.8	4.7	4.5	2.3	7.5	$I^{\cdot}L$	3.1	4.0	3.0	6.8	4.2	2.0	2.2	6.8	2.8	7.3	9.0	9.0	otype.
¥	63.63 (C <sub>1</sub> 1)	4.8	6.1	3.2	6'I	4.0	1.7	5.4	3.3	9.9	5.0	4.1	8.6	2.7	2.9	6.7	5.5	3.1	6.5	I.7	4.0	2.7	6.7	2.9	7.0	8.6	h gen
	63.63 (B <sub>7</sub> 50)	5.3	5.4	5.0	1.1	3.1	5.0	5.6	5.7	2.5	6.0	5.9	5.6	6.5	5.3	5.1	3.0	4.9	6.1	I.0	2.7	I.2	6.0	6.2	8.7	7.7	of eac
	31.4 (B <sub>4</sub> 6)	2.5	3.8	4.0	6'I	3.9	2.7	3.3	2.9	3.0	2.2	0.7	8.2	2.7	6.2	4.1	4.2	6.7	3.5	2.9	I.9	5.5	8.2	3.0	7.6	9.0	lants
	47.39 (B <sub>4</sub> 4)	6.6	4.0	5.5	3.5	7.9	5.5	4.7	4.6	7.5	3.0	4.0	7.5	8.7	4.3	7.8	I.7	8.0	2.2	6.2	I.2	2.7	9.0	7.5	8.2	7.6	elve p
	63.47 (B <sub>3</sub> 8)	4.0	2.8	7.1	5.4	3.1	4.8	8.5	2.0	5.0	6.5	4.0	8.3	8.3	3.6	5.3	2.9	6.4	8.9	I.8	4.0	2.5	4.2	7.2	9.0	6.7	ing tw
	63.63 (B <sub>1</sub> 46)	5.0	5.6	3.4	6.7	3.5	7.0	3.8	4.4	2.8	5.0	3.3	7.9	7.2	4.4	5.7	7.0	2.2	8.0	1.2	1.5	3.5	7.2	1.5	4.6	8.2	/aluat
	63.63 (A <sub>2</sub> 12)	5.5	5.9	3.6	4.9	6.0	6.0	4.2	3.9	4.1	5.3	5.5	7.2	6.9	6.4	5.0	3.7	8.2	7.6	4.0	2.5	2.5	8.6	8.0	7.0	7.9	l by e
	63.7 (A <sub>2</sub> 4)	5.8	7.3	2.5	3.8	6.1	5.0	4.5	7.0	4.5	5.4	2.5	9.0	7.0	4.0	6.6	3.9	7.5	4.2	I.2	6.9	I.4	6.4	4.1	8.7	8.4	ulated
	15.7 (A <sub>1</sub> 13)	3.4*	2.5	6.0	3.7	5.5	3.1	4.2	3.0	1.5	3.5	4.3	8.0	8.6	4.2	6.0	3.5	2.8	3.0	I.9	3.4	I.9	7.9	6.5	9.0	8.2	re calc
	Genotypes	BJ-1	BJ-2	BJ-3	BJ-4	BJ-5	BJ-6	BJ-7	BJ-8	Jalo EEP 558	Jalo MG 65	CAL 143	Rudá	Rudá R	Pérola	Pérola R	BRSMG Talismã	VC-6	BRSMG Madrepérola	BRSMG Majestoso	Diamante Negro	Ouro Negro	Meia Noite	Valente	Ouro Vermelho	Vermelhinho	* Severity averages we

## EFFECTS OF SILICON ON THE SEVERITY OF THE ANGULAR LEAF SPOT IN COMMON BEAN

### **Rafael Pereira<sup>1</sup> and Elaine A. de Souza<sup>1\*</sup>**

<sup>1</sup>Universidade Federal de Lavras (UFLA), CEP 37200-000, Lavras, MG – Brazil E-mail: <u>\*easouza@dbi.ufla.br</u>

### **INTRODUCTION**

Pseudocercospora griseola (Sacc) Crus & U. Brown is the causal agent of angular leaf spot in common bean, one of the most severe diseases on crop. The disease control can be performed chemically by the use of fungicides, resulting in decreased yield and promote damage to the environment (Miklas et al., 2006). Use of resistant cultivars to the pathogen, associated with good management practices, is the best strategy for the control of angular leaf spot due to its practicality and economy. However, the proper management of diseases requires not only the development of resistant cultivars, but also to find alternatives to increase the durability and stability of this resistance (Casela, 2002). The losses can be minimized with the use of management strategies, such as mineral nutrition. Correct use of certain nutrients can help in reduce of the disease intensity during the crop development. Silicon has been reported as one of the elements associated with the induction of resistance in plants. The absorption of silicon (Si) can increase resistance, particularly for the crops that accumulate this element (Savant et al. 1999). Research carried out in other crops confirmed the silicon potential in reducing the intensity and severity of diseases. However, studies showing efficiency of Si to reduce the severity of angular leaf spot in bean were yet not done. The aim of this study was to evaluate the effect of silicon in controlling angular leaf spot in common bean lines.

### **MATERIAL AND METHODS**

We used four common bean lines, Cornell, Pérola, Esal 686 and MAVIII- 94, and a strain of *P. griseola* (race 63-63). To evaluate the effect of Si in the induction of resistance, four lines and two doses of Si (1.00 g.kg<sup>-1</sup> CaSiO<sub>3</sub>, 2.00 g.kg<sup>-1</sup> CaSiO<sub>3</sub>) and the control (1,00g.kg<sup>-1</sup>CaCO<sub>3</sub>) were used. Calcium carbonate (CaCO<sub>3</sub>) was added to the soil of control aiming the balance of the calcium amount (Ca) among treatments and amount provided by the calcium silicate (CaSiO<sub>3</sub>). The experiment was conducted in randomized blocks design in factorial scheme 4 x 3 with four replications. Each plot consisted of a pot in which four seeds were sown. *P. griseola* strain was cultivated in leaf dextrose agar medium and incubated at 24°C for 14 days with 12 hours of photoperiod. The spores concentration was adjusted to  $2 \times 10^4$  conidia/mL. Plants of common bean plants in V3 stage were inoculated. After the inoculations, the plants were kept in a greenhouse with a relative humidity of 80%, temperature at 24°C. Lines reaction to *P. griseola* was evaluated by disease severity 13 to 14 days after inoculation according to the descriptive scale 1-9, developed by Pastor-Corrales and Jara (1995).The severity data were submitted to analysis of variance using the GENES software.

### **RESULTS AND DISCUSSION**

From the results obtained in the analysis of variance (Table 1), it was found that only the source of variation lines was significant (P < 0.01). There was no significant difference in disease severity in common lines to Si doses (Table 1). Therefore, Si was not effcient in decrease angular leaf spot severity compared to the control. In contrast to the results obtained in this study, Moraes et al. (2005) conducted a study on the pathossystem of *Colletotrichum lindemuthianum* - common bean and observed decrease in disease severity using sodium silicate that provided reduction of 62.4 % in AUDPC (area under the disease progress curve). These authors did not observe in common bean leaves the formation of a physical barrier and silicon accumulation externally with the application of calcium silicate, although the element has contributed to reduce the anthracnose. Similarly, Cross (2012) investigated the effect of the monosilicic acid (Si) in the infectious process of *Phakopsora pachyrhizi* in soybean and observed a reduction of 27, 23 and 60 % in number of lesions, closed and opened uredinia, respectively. Therefore, the presence of silicon salts in common bean did not increase angular leaf spot resistance in common bean.

**Table 1** Summary of the analysis of variance for angular leaf spot severity in bean common lines in different doses of calcium silicate.

Source	DF	Mean Square
Rep	3	0.29354
Lines	3	8.26354**
Doses	2	0.73937 <sup>ns</sup>
Lines x Doses	6	1.47604 <sup>ns</sup>
Error	33	1.06278

\*\* significant at 1 % probability by the F test

ns- no significant at 5% probability, by the F test

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### EXCISED LEAF INOCULATION METHOD FOR COMMON BEAN ANTHRACNOSE TO AID BREEDING AND MONITORING OF NEW ISOLATES

## Sanglard, D. A.<sup>1,2\*</sup>; Machado, M. A. M.<sup>1</sup>; Carneiro, A. R. T.<sup>1</sup>; Santos, J. M. C.<sup>1</sup>; Campos, R. G. C.<sup>1</sup>; Vieira, A. F.<sup>1</sup>; Santos, L. P.<sup>1</sup>; Batista, M. S.<sup>1</sup>; Lopes, E. M. G.<sup>1</sup> and Rocha, F. S.<sup>1,3</sup>

<sup>1</sup>Instituto de Ciências Agrárias (ICA); <sup>2</sup>Lab. de Biotecnologia; <sup>3</sup>Lab. de Fitopatologia; Universidade Federal de Minas Gerais (UFMG), 39.404-547, Montes Claros, MG, Brazil; \*E-mail: demerson.ufmg@gmail.com

**INTRODUCTION** - The currently used methods (for genotype identification of carriers of different R genes) for the inoculation of the fungus *Colletotrichum lindemuthianum* do not allow an accurate detection of specific pathotypes of different urged symptoms (Faleiro et al., 2003). It is quite costly and requires expensive infrastructure, greenhouse and fog chambers. Some breeding programs using molecular markers, especially DNA, to monitor the pyramiding of genes "R", in elite breeding lines (Kelly et al. 2003; Ragagnin et al., 2009). However, in many cases, the alleles of interest have not yet been identified tags. Preliminary tests indicate that the use of detached leaves of the host plants for the inoculation of pathogens could be a viable alternative (Höfte & Bigirimana, 2001). The purpose of this study was to test a new methodology of inoculation to assess the bean resistance to the fungus *C. lindemuthinaum* using primary detached leaves of host plants and the maintenance of these in vitro.

MATERIAL AND METHODS - Used the segregating population composed of 356 RILs (Recombinant Inbreed Lines) of common bean, originated from crosses between varieties 'AND 277' e 'Rudá' (Sanglard et al., 2013). 'Rudá' is susceptible to various physiological races of Colletotrichum lindenmuthianum. All RILs and their parents were evaluated for resistance to race 89 (Balardin et al., 1997) using the new methodology of inoculation, proposed in this paper, and the conventional method described by Pastor-Corrales (1992). The inoculum was grown in test tubes containing sterile pods and partially immersed in agar-water 3.0 % for 10 days at 23 °C. In the inoculations using a new methodology, one of the primary leaves (2/3 of full development) of the tested plants was excised at ten days of age. Subsequently, it was immersed in a suspension of inoculum at concentration of  $1.2 \times 10^6$  conidia/mL. So, the leaves were transferred to Petri dishes containing moistened filter paper previously with 3.0 ml distilled water and incubated at 20 °C, photoperiod of 12 hours (Phillips® TLT 20W/75RS), and an incidence of 28 µmoles m<sup>-2</sup> s<sup>-1</sup>. After inoculation, was added (space of three-day) 1.5 mL of distilled water on the filter paper to maintain high humidity inside Petri dishes. Primary leaves inoculated using the conventional method were considered as control. Ten days after planting, inoculated were also made (electric-DeVilbiss atomizer no.15) in plants (on both surfaces of the primary leaves) using the same concentration of suspension described above. After inoculation, the plants were incubated for seven days in a mist chamber ( $20 \pm 1$  ° C and relative humidity > 95%) under 12 h photoperiod. Plants were maintained in a greenhouse until evaluation. In both methods, anthracnose disease was evaluated at 10 days after inoculation, based on the scale from 1 to 9 described by Pastor-Corrales (1992). Plants with 1 to 3 degrees were considered resistant, and those with 4 or more susceptible. The data obtained in the evaluations were submitted to the chisquare test ( $\alpha = 5\%$ ) by the GENES program (Cruz, 2006).

**RESULTS AND DISCUSSION** - The monogenic segregation hypothesis of resistance to isolate of race 89 of *C. lindemuthinaum* in the population was confirmed by results obtained with the two methods of inoculation. The coincidence index of RILs plants with same reaction inoculated using the two methods was 98.90 % for resistant plants, and 98.86 % for susceptible plants (Table 1). Therefore, the new method of inoculation of *C. lindemuthinaum* proposed in this work was efficient to identify RILs plants containing resistance alleles for locus *Co-1* of 'AND 277' (Alzate-Marin et al., 2003). Although several methods of inoculation of pathogenic fungi are known this new method of inoculation of *C. lindemuthinaum* has following characteristics: practical, easy adoption, the dynamism of the method, availability of adequate basic infrastructure in most laboratories and the reliability of results. Faleiro et al. (2003) evaluated the response of 'Ouro Negro' cultivar to isolates of *C. lindemuthinaum*, and demonstrated that simultaneous or sequential inoculations by the conventional method can lead to errors in the evaluation of specific symptoms caused by different pathotypes of the fungus. In this case, using the new method allows more reliable results. In addition, the proposed method still has the advantage of allowing a single plant to be tested simultaneously against different isolates or races of the pathogen, or even by different pathogens and yet can produce seeds in health with adequate quantity and quality. However, future studies are needed to evaluate the efficiency of this method for other combinations of race-cultivar and/or other resistance genes that can be displayed as a standard method of inoculation in this study.

Table 1. Response of RILs plants, derived from a cross between the varieties of bean 'AND 27	77'
(gene Co-1) and 'Rudá', to race 89 of C. lindenmuthianum race and inoculated by the	wo
different methods.	
	-

Deastion	Method of	of inoculation	$(\mathbf{N} + \mathbf{C})^{a}$	$\mathbf{IC} (0)^{\mathbf{b}}$
Reaction	New (N)	Conventional (C)	(N+C)	IC (%)
Resistant (R)	180	182	180	98.90
Susceptible (S)	176	174	174	98.86
Expected frequency	$1(R) : 1(S)^{c}$	1(R) : 1(S)		
$\chi^2$	0.0449	0.1797		
$P(\%)^d$	83.21	67.16		

<sup>a</sup>Number of  $F_2$  plants that showed the same response for both inoculation methods; <sup>b</sup>Index of coincidence in percentage: IC =  $[(N+C)/\text{total}_{R/S}]x100$ ; <sup>c</sup>Expected frequency for the segregation of a single locus, with intra-allelic ratio of complete dominance in an  $F_2$  population derived from the cross between homozygous parents; <sup>d</sup>Probability in porcentagem.

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### RESISTANCE TO Collectrichum lindemuthianum IN INBRED COMMON BEAN LINES FROM THE OURO NEGRO X MEIA NOITE CROSS

## B.M. Olivera<sup>1</sup>, E.F. Celin<sup>1</sup>, A.C. Pereira<sup>1</sup>, P.C.S. Carneiro<sup>1</sup>, J.E.S. Carneiro<sup>1\*,</sup> R.F. Vieira<sup>2</sup>, T.J. Paula Júnior<sup>2</sup>

<sup>1</sup>Departamento de Fitotecnia, Universidade Federal de Viçosa, 36570-900, Viçosa, MG-Brasil. <sup>2</sup>Empresa de Pesquisa Agropecuária de Minas Gerais, 36570-000, Viçosa, MG-Brasil <sup>\*</sup>Corresponding author: jesc@ufv.br

### **INTRODUCTION**

Anthracnose, caused by the fungus *Colletotrichum lindemuthianum*, is one of the most serious diseases of common bean (*Phaseolus vulgaris* L.). The main anthracnose control strategies include the use of healthy seeds, fungicides and resistant cultivars. The use of resistant cultivars is the most efficient strategy, because it is environmentally safe and cost-effective in controlling the disease. However, this fungus has a large number of physiological races (Barcelos et al. 2011). Thus, resistant cultivars often become susceptible in a few years after being released, leading breeders to a constant search for new breeding lines with broad resistance to the fungus. The aim of this study was to characterize the reaction of inbred lines from the Ouro Negro x Meia Noite cross derived from the common bean breeding program of the Federal University of Viçosa to races of *C. lindemuthianum* frequently found in the state of Minas Gerais, Brazil.

### MATERIAL AND METHODS

We evaluated 93 inbred lines of common bean derived from the population of the black cultivars Ouro Negro x Meia Noite. These lines were tested for their reaction to the C. lindemuthianum races 65, 73, 81, 87, 89, 453 that occur in the state of Minas Gerais (Alzate-Marin & Sartorato, 2004; Damasceno & Silva et al., 2007). The parents of these inbred lines (Ouro Negro and Meia Noite) along with the Rudá (susceptible to all races) and Rudá-R (resistant to all races) cultivars were used as controls. Common bean seeds were pre-germinated in germitex paper for 24 hours at 25°C. Seedlings were transplanted to trays with 128 cells containing the commercial substrate Topstrato HT<sup>©</sup>. The inoculum of each physiological race was multiplied in partially immersed sterile pods on agar medium in test tubes. The tubes were maintained for 10 days in a growth chamber at 24°C for the production of spores. These spores were inoculated on eight seedlings from each treatment. The inoculation was performed seven days after sowing, atomizing a suspension containing  $1.2 \times 10^6$  spores/mL on both surfaces of the primary leaves, with a DeVilbiss atomizer activated by an electric compressor. During seven days the plants were incubated in a mist chamber at  $20 \pm 1^{\circ}$ C using a 12 hour photoperiod at temperature and relative humidity > 95%. Disease reactions were scored seven days after inoculation based on the scale described by Pastor-Corrales (1992), ranging from 1 to 9, where scores of 1-3 plants are considered resistant and scores of 4-9 susceptible.

### **RESULTS AND DISCUSSION**

The inbred lines 50 and 92 exhibited resistance to all *C. lindemuthianum* races (Table 1). Race 65 was the most virulent, since approximately 66% of the genotypes were susceptible to this race. For over three decades race 65 has been reported to be widely distributed in Brazil. This explains why race 65 is the target race of the majority common bean breeding programs (Davide & Souza, 2009). Reaction phenotypes S<sup>65</sup>R<sup>73</sup>S<sup>81</sup>S<sup>87</sup>S<sup>89</sup>S<sup>453</sup> and S<sup>65</sup>S<sup>73</sup>S<sup>81</sup>R<sup>87</sup>S<sup>89</sup>S<sup>453</sup> were not expected, since the parents of these inbred lines are susceptible only to the race 65 (Ouro Negro) or 453 (Meia Noite). We believe that gene flow among genotypes present in the field occurred during the

trials. Among all genotypes, 87% were resistant to five out of six races evaluated. These results emphasize the potential of these lines, especially the lines 50 and 92, as sources of resistance to anthracnose.

**Table 1.** Reaction of inbred lines from the Ouro Negro x Meia Noite cross to *Colletotrichum lindemuthianum* races 65, 73, 81, 87, 89 and 453.

Reaction phenotypes	Inbred lines or cultivars
$R^{65} R^{73} R^{81} R^{87} R^{89} R^{453}$	50, 92, Rudá-R
$S^{65} R^{73} R^{81} R^{87} R^{89} R^{453}$	1, 2, 4, 5, 6, 7, 10, 12, 13, 14, 15, 16, 19, 21, 22, 23, 25, 26, 28, 29, 32, 33, 34, 35, 37, 39, 44, 46, 47, 52, 54, 56, 59, 60, 63, 65, 68, 70, 75, 76, 78, 79, 80, 81, 82, 83, 86, 87, 88, 89, 91, Ouro Negro
$R^{65} R^{73} R^{81} R^{87} R^{89} S^{453}$	3, 8, 9, 11, 17, 18, 20, 24, 30, 36, 38, 40, 41, 42, 43, 45, 48, 49, 51, 53, 55, 58, 62, 66, 67, 69, 73, 74, 85, Meia Noite
$S^{65} R^{73} R^{81} R^{87} R^{89} S^{453}$	27, 57, 61, 64, 72, 90, 93
$S^{65} R^{73} S^{81} S^{87} S^{89} S^{453}$	31, 71, 84
$S^{65} S^{73} S^{81} R^{87} S^{89} S^{453}$	77
$S^{65} S^{73} S^{81} S^{87} S^{89} S^{453}$	Rudá

R: resistant; S: susceptible.

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## REACTION OF COMMON BEAN AND SOYBEAN CULTIVARS TO *Colletotrichum* sp. and *Glomerella* sp. STRAINS FROM COMMON BEAN LESIONS

### Suellen F. Mota<sup>1</sup>, Elaine A. de Souza<sup>1\*</sup> and Mariana A. Dias<sup>1</sup>

<sup>1</sup>Universidade Federal de Lavras (UFLA), CEP 37200-000, Lavras, MG - Brazil <u>\*easouza@dbi.ufla.br</u>

### **INTRODUCTION**

Common bean anthracnose is a fungal disease that stands out causing large losses in yield and grain quality. Another disease that has caused damage to the common bean is scab. Both diseases are caused by species of the genus Colletotrichum, anamorphic form, and Glomerella in teleomorphic form. Anthracnose is caused by the fungus Colletotrichum lindemuthianum and has a wide variability in the pathogen population (Pinto et al., 2012). However, informations about common bean scab are scarce and Colletotrichum truncatum is usually reported as the causal agent of the disease. In recent years, Glomerella cingulata f. sp. phaseoli strains have been obtained from anthracnose lesions and these strains have been investigated by morphological, cytological, molecular and pathogenic characterization (Barcelos et al., 2014). The authors identified two distinct groups of strains Glomerella sp. collected from anthracnose lesions on common bean, called *Glomerella* sp. group I and II, since the group I did not cause symptoms in common bean and the II group had mild symptoms at 10 days after inoculation. Due to the simultaneous occurrence of the symptoms of scab and anthracnose common bean, and the similarity of symptoms in the common bean plant organs on the field, the proposal of study was compare the pathogenicity of strains of *Glomerella* sp. group II and scab in common bean and soybean cultivars. This information is important for breeding programs aiming to obtain resistant cultivars.

### **MATERIAL AND METHODS**

Ten strains were used, four obtained from scab lesions (S1, S2, S5 and S6) and six strains of anthracnose lesions belonging *Glomerella* sp. group II (G85-1, G86-1, G89-1, G92-1, G93-1, G99-1) for pathogenic evaluation. The pathogenicity test was carried out from the inoculation of ten strains in three common bean cultivars (Majestoso, Pérola and Talismã) and three soybean cultivars (CD202, CD237 and Favorita). The strains were grown in Petri dishes with PDA for 15 days at 22°C and the spores suspension was obtained by the method described by Pinto et al., 2012. An experiment was conducted for each strain in a CRD (completely random design) with six treatments (cultivars) and two replications. Each plot consisted of nine plants. The six cultivars were sown in trays containing MultiPlanta substrate. The seedlings were inoculated according to the methodology described by Pinto et al. 2012. The severity of the disease was evaluated at 7, 10, 14 and 17 days after inoculation by scored according to the scale proposed by Schoonhoven and Pastor Corrales (1987). Analysis of variance and means test (Scott Knott test) were carried out using the R software.

### **RESULTS AND DISCUSSION**

The statistical analysis performed for disease severity indicated that all factors were significantly, except cultivars x time and cultivar x strains x times interactions. Significant strains x time interaction indicated that the ranking of strains was not coincident in different days of evaluation of disease severity. Scab symptoms were more severe at 17 days of evaluation showing that the symptoms of disease should be evaluated later (Table 1). Anthracnose symptoms in common bean were similar to the 10 days evaluation. As for the soybean plants, the symptoms were

typical of soybean anthracnose. Disease symptoms evaluated in common bean pods were typical lesions of scab. The symptoms caused by strains of group *Glomerella* sp. II and *Colletotrichum* spp. expressed later compared to the strains of *C. lindemuthianum* in present study. Evaluation of the common bean anthracnose can be assessed as early as the 7 days (Pinto et al., 2012). Symptoms of scab presented as described in the literature, it was noted that as the disease develops the tissue becomes necrotic showing a brown colour. These lesions grow stem in the longitudinal direction and increase in size and they can taking its entire diameter. When these symptoms occur, the plants wilt and die. Small black spots appear on the pods in which may contain acervula (Sartorato, 2002). The results of this study indicate that more studies are needed to elucidate the species of these strains and the disease caused by them.

			Time	
Strain				
	7 days	10 days	14 days	17 days
G85-1	$1.904 \pm 0.77 A$	2.331±0.91 A	$2.454 \pm 0.87 A$	$2.454{\pm}0.87D$
G86-1	$1.516 \pm 0.40 A$	$2.089 \pm 0.54 A$	$2.810 \pm 1.27 A$	$3.832 \pm 1.21 \text{A}$
G89-1	$1.699 \pm 0.71 A$	$2.334 \pm 0.71 A$	$2.739 \pm 0.78 A$	$3.157 \pm 0.78B$
G92-1	$1.242 \pm 0.23 A$	$1.445 \pm 0.38B$	$2.037{\pm}0.76\mathrm{B}$	$2.210 \pm 0.71$ D
G93-1	$1.486 \pm 0.52 A$	$1.963 \pm 0.56 A$	$2.482 \pm 0.98 \text{A}$	$3.202 \pm 0.97B$
G99-1	$1.426 \pm 0.38 A$	$1.882 \pm 0.56B$	$2.782 \pm 0.62 A$	$3.730 \pm 0.58 A$
S1	$1.263 \pm 0.39 A$	$1.784{\pm}0.60\mathrm{B}$	$2.141 \pm 0.52B$	$2.265 \pm 0.56D$
S2	$1.172 \pm 0.19 A$	$1.662 \pm 0.28 B$	$2.461 \pm 0.57 A$	$2.764 \pm 0.69C$
S5	$1.463 \pm 0.31 \text{A}$	$1.645{\pm}0.28\mathrm{B}$	$2.510 \pm 0.58 A$	$2.750 \pm 0.66C$
S6	$1.405 \pm 0.29 A$	$1.887{\pm}0.40\mathrm{B}$	$2.521 \pm 0.46 A$	$3.239 \pm 1.05B$

Table 1Average score of disease severity of six cultivars for ten strains (Colletotrichum spp.<br/>and Glomerella sp.) evaluated at 7, 10, 14 and 17 days after inoculation.

\* Means followed by the same letter belong the same group (P <0.05) according to the test of Scott-Knott.

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### Variability within race 65 of *Colletotrichum lindemuthianum* collected in different regions from Brazil through sequencing of ITS regions

### Marcela Coelho<sup>1</sup>, Maria Celeste Gonçalves-Vidigal<sup>1</sup>, Lorenna L. Sousa<sup>1</sup>, Marta Z. Galván<sup>2</sup>

<sup>1</sup> Núcleo de Pesquisa Aplicada a Agricultura, Universidade Estadual de Maringá, Maringá, Brazil; <sup>2</sup> CONICET, Biotecnología, INTA EEA Salta, Argentina.

**INTRODUCTION:** Anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc & Magnus) Briosi and Cavara, is one of the most serious diseases of common bean in the worldwide (Pastor-Corrales and Tu 1994). The disease is managed through deployment of resistant cultivars, but new pathotypes present a challenge to the successful implementation of this strategy (Singh and Schwartz2010). Among the races of *C. lindemuthianum*, the pathotype 65 stands out for being confirmed its presence in various regions of Brazil and the world (Balardin et al. 1997; Gonçalves-Vidigal et al. 2008; Davide and Souza 2009). One way to detect genetic variability in plant pathogens is by amplifying the ITS region (Internal Transcribed Spacer) ribosomal DNA (rDNA) by PCR. Thus, the objective of this study was to characterize isolates of *C. lindemuthianum* race 65 from different regions of Brazil through sequencing of ITS regions.

MATERIALS AND METHODS: The experiments were conducted at Laboratório de Melhoramento do Feijoeiro Comum e Biologia Molecular do Núcleo de Pesquisa Aplicada a Agricultura (Nupagri), Universidade Estadual de Maringá and at the Centro de Estudos do Genoma Humano, Universidade de São Paulo. Seventeen isolates of the race 65 were used for ITS regions analyses. Twelve isolates were obtained from the mycology collection of NUPAGRI Laboratory and five were kindly provided by Dr. Tamires Ribeiro of the Instituto Agronômico de Campinas, Campinas, São Paulo state. The genomic DNA extraction from mycelial mass where performed according to Cárdenas et al. (2012). The PCR conditions for the primers iniciadores ITS1F (5' CTTGGTCATTTAGAGGAAGTAA 3') (Gardes and Bruns, 1993) and ITS4 (5' TCCTCCGCTTATTGATATGC 3') (White et al., 1990). The PCR products were analyzed on 1.2% agarose gels stained with SYBR Safe (0.02%). The purification of the PCR products where made using the Kit PureLink PCR Purification Kit (Invitrogen®) and the sequencing was performed using the ABI 3730 DNA Analyser with BigDye® Terminator v 3.1 Cvcle Sequencing Kit). For the sequence analyses BioEdit (version 7.0) and MEGA 5.2 softwares where used.

**RESULTS AND DISCUSSION:** DNA sequences of 17 analyzed isolates in this study were compared with the sequence of race 23 (Table 1). Variability of *C. lindemuthianum* isolates was inferred from the sequence comprising ITS 1 and ITS 2 regions and the  $5\pm8S$  rRNA gene. Differences among sequences of isolates due to single nucleotide substitutions were observed in the ITS 1 and ITS 2 regions. The results revealed the presence of a SNP at position 79 of ITS 1 region, occurring substitution of **C** by **T** in the sequence of the isolate 3, from Mato Grosso. In turn, the isolate 4 from Parana State presented a SNP at position 120, where there was an exchange of **G** by **C**. It was observed wide variability in the sequences from the isolate 8 collected in Santa Catarina, which presented three SNPs, verifying the following substitutions: **C** by **A** at position 83, **G** by **T** at position 119 and **G** by **A** at position 199. Interesting results were observed in isolates 9 and 12 from Santa Catarina; 14 and 15 from São Paulo which showed similar SNPs at positions 119 and 199 where there was an exchange of **G** by **T** and **G** by **A**, respectively. Additionally, isolate 14 also presented a SNP at position 156 enabling the substitution of **A** by **G**. The sequences of the isolates 13 and 17 from São Paulo, resulted in the

following substitutions: **G** by **A** at position 199 and **G** by **T** at position 119, respectively. Davide and Souza (2009) obtained similar results of the existence of pathogenic variation within the race 65. Furthermore, at ITS 2 region, the sequences of 8, 9, 12, 14, 15 and 17 isolates showed the substitution of **C** by **A** at position 501. The sequence of the isolate 4 at position 480 presented a substitution of **G** by **A**. Moreover, the sequence of the isolate 16 revealed the presence of three SNPs at positions 435, 436 and 470 with the following substitutions, respectively: **C** by **G**, **G** by **A** and **A** by **C**. Similar results were obtained by Balardin et al. (1999) who found variability at ITS 2 region of *C*. *lindemuthianum*. The greatest genetic divergence was observed among isolates 10 (Santa Catarina) and 3 (Mato Grosso), which magnitude was 0.772. However, the most similar isolates were 2 and 7, with genetic distance value of 0.002; these ones are from Mato Grosso and Santa Catarina, respectively. Most of the variability observed in the sequence analysis of our 17 isolates from race 65 of *C*. *lindemuthianum* was in the ITS 1 region. The results obtained in this study revealed the existence of high genetic variability among and within the race 65 through analysis of ITS regions.

**Table 1.** Single nucleotide polymorphisms (SNP) on sequences of the isolates from race 65 of

 *Colletotrichum lindemuthianum* at the ITS 1 and ITS 2 regions

Isolates	Position											
	79	83	119	120	156	199	435	436	470	480	501	
Race 23	Т	С	G	G	А	G	С	G	А	G	С	
3	С	-	-	-	-	-	-	-	-	-	-	
4	-	-	-	С	-	-	-	-	-	А	-	
8	-	А	Т	-	-	А	-	-	-	-	А	
9	-	-	Т	-	-	А	-	-	-	-	А	
12	-	-	Т	-	-	А	-	-	-	-	А	
13	-	-	-	-	-	А	-	-	-	-	-	
14	-	-	Т	-	G	А	-	-	-	-	А	
15	-	-	Т	-	-	А	-	-	-	-	А	
16	-	-	-	-	-	-	G	А	С	-	-	
17	-	-	Т	-	-	-	-	-	-	-	А	
SNP	T/C	C/A	G/T	G/C	A/G	G/A	C/G	G/A	A/C	G/A	C/A	

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### SNP DISCOVERY IN GENOMIC REGIONS FLANKING THE COMMON BEAN ANTHRACNOSE RESISTANCE LOCUS *Co-4*

### Jorge F. Cieslak<sup>1</sup>, Gesimária R. C. Coelho<sup>2</sup>, Thiago L. P. O. Souza<sup>2</sup> and Rosana P. Vianello<sup>2\*</sup>

<sup>1</sup>Universidade Federal de Goiás (UFG), Goiânia, GO 74001-970, Brazil; <sup>2</sup>Embrapa Arroz e Feijão (Embrapa Rice and Beans), Santo Antônio de Goiás, GO 75375-000, Brazil. \*Corresponding author: rosana.vianello@embrapa.br

### INTRODUCTION

Anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara, is one of the major fungal diseases that affect the common bean (*Phaseolus vulgaris* L.) crop in tropical and subtropical areas. Long periods of moderate temperatures (13-27°C) and high humidity during the crop cycle may cause losses up to 100%. A efficient and cost-effective strategy available for the bean anthracnose control is the use of resistant cultivars. In Brazil as well as in other parts of the world, an important resistant allele explored by the common bean breeding programs is the  $Co-4^2$  (Souza *et al.* 2014). Molecular markers linked to resistance genes have been used as an important auxiliary tool by common bean breeding programs worldwide. The main goal of this work was to characterize the genetic variability of genomic regions flanking the anthracnose resistance locus Co-4 aiming to develop SNP markers for specific identification of the different alleles presented by this locus, mainly of the  $Co-4^2$ .

### **MATERIAL AND METHODS**

DNA sequences from amplified products obtained using the SCAR marker SAS13, linked to the locus *Co-4*, and DNA samples from 15 common bean genotypes were used to mining conserved domains of plant resistance genes (putative genes) using the BLAST tool and the *P. vulgaris* reference genome sequence (http://phytozome.jgi.doe.gov/pz/portal.html#). Among those 15 bean genotypes were 12 anthracnose resistant lines, including TO (*Co-4*), SEL1308 (*Co-4*<sup>2</sup>) and PI207262 (*Co-4*<sup>3</sup>), and three susceptible lines. For each identified putative gene, PCR primers were designed and used to amplify DNA samples from the same 15 common bean genotypes used before. After sequencing, all DNA fragment sequences were aligned and used for SNP discovery. The total nucleotide diversity ( $\theta$ ) in these genomic regions associated with the anthracnose resistance locus *Co-4* was calculated according to Halushka *et al.* (1999).

### **RESULTS AND DISCUSSION**

Five putative genes were identified and signed as PARG (Putative Anthracnose Resistance Gene) 1-to-5. All these genes were annotated as harboring the domain STK (Serine Threonine Kinase), a conserved domain in plant disease resistance genes. Three putative genes were selected based on their DNA sequence quality for SNP discovery. A total of 60 SNPs were identified in CDS (Coding DNA Sequence) regions. Out of these SNPs, 20 were classified as transitions and 40 as transversions. The total nucleotide diversity was  $\theta = 0.006151$ . Twenty-two SNPs were classified as synonymous mutations and 38 as non-synonymous. Eleven SNPs were selected as potential markers for specific identification of the locus *Co-4* and of the different alleles presented by this locus (Figure 1 and Table 1). These selected SNP markers are being tested in co-segregation studies using an  $F_{2:3}$  population derived from a test cross that used SEL 1308 (*Co-4*<sup>2</sup>) as the resistant parent.



**Fig. 1.** Representation of three putative genes identified in genomic regions flanking the common bean anthracnose resistance locus *Co-4*. Exons (dark bars), introns (lines), and their respective lengths in bp, in addition to the position of each one of the 11 selected SNPs, are showed.

Table 1.	SNPs	selected	as	potential	markers	for	specific	identificatio	n of	the	common	bean
anthracno	ose resi	istance lo	cus	<i>Co-4</i> and	of the dif	ffere	nt alleles	presented b	y thi	s loc	us.	

Putativa Cana	Co-4 Allele	-	<i>Co-4</i>	<i>Co-4</i> <sup>2</sup>	<i>Co-4</i> <sup>3</sup>
rutative Gene	SNP Position	REF*	ТО	SEL1308	PI207262
PARG 1	817	G	А	А	А
	990	G	Т	Т	Т
PARG 4	157	А	G	G	А
	178	G	С	С	G
	192	G	С	С	G
	194	G	Т	Т	G
PARG 5	269	А	Т	А	А
	315	G	С	G	G
	340	G	Т	G	G
	357	А	Т	А	А
	934	А	А	G	А

\**P. vulgaris* reference genome sequence.

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### NEW RACES OF *COLLETOTRICHUM LINDEMUTHIANUM* IN COMMON BEAN FROM PARANA STATE, BRAZIL

### E.B. Uchôa<sup>1</sup>, M.C. Gonçalves-Vidigal<sup>1</sup>, M.C.M. Souza<sup>1</sup>, P.S. Vidigal Filho<sup>1</sup>, S.A.L. Castro<sup>1</sup>, J.P. Poletine<sup>2</sup>

<sup>1</sup>Departamento de Agronomia, Universidade Estadual de Maringá; <sup>2</sup>Departamento de Ciências Agronômicas, Universidade Estadual de Maringá, *Campus* Regional de Umuarama, Paraná, Brazil

### **INTRODUCTION**

Anthracnose is one of the most widespread and economically important worldwide common disease (Pastor-Corrales et al. 1995). This disease, which is caused by the fungus *Colletotrichum lindemuthianum*, is particularly important and recurrent in sub-tropical and temperate bean production regions (Singh and Schwartz 2010). This pathogen is highly variable with many different races occurring in different locations of the world. Previous studies have identified 246 races of *C. lindemuthianum*, among them 73 were found in Brazil (Nunes et al. 2013). Therefore, for an effective breeding program, there is a need to continuously monitoring the distribution and the variability of the pathogen as a matter of guiding breeders on which races to target. The present study had as objective to characterize isolates collected in common bean growing crops of Parana State.

### MATERIAL AND METHODS

Twenty-six isolates were tested on a set of 12 international bean differential cultivars for anthracnose. Monosporic cultures of each isolate of C. *lindemuthianum* used in this study were prepared in young green common bean pod medium and incubated at  $20 \pm 2^{\circ}$ C for 14 days (Cárdenas et al. 1964) and adjusted to a concentration of  $1.2 \times 10^{6}$  conidia mL<sup>-1</sup>. The inoculation was performed on sets of 12 common bean differential cultivars to characterize the virulence spectra of anthracnose. Seedlings were grown under natural light in greenhouse supplemented by 400 w high-pressure sodium lamps giving total light intensity of 115 µmoles m<sup>-2</sup> s<sup>-1</sup> for 7 to 10 days until it reached the first trifoliate leaf stage. Ten seedlings of each differential cultivar were inoculated with the spore suspension by using De Vilbiss air compression. The inoculation was performed in 12 differential cultivars and plants were moved to a mist chamber, remained for 72 h at 20°C, 12 h light 12 h dark<sup>-1</sup> and relative humidity nearly to 100%. Anthracnose disease reaction were rated visually using a scale from 1 to 9 (Pastor-Corrales et al. 1995). Plants with disease reaction scores between 1 and 3 were considered resistant, whereas plants that rated 4-9 were considered susceptible.

### **RESULTS AND DISCUSSION**

Twenty-one races were identified 2, 3, 10, 27, 31, 64, 73, 75, 79, 81, 82, 83, 90, 91, 93, 259, 283, 287, 339, 346 and 351 (Table 1). This was the first report of races 82, 90, 259, 283, 287, 346 e 351 in Parana State; it is worth mentioning that this is the first report of race 3 in Brazil. This race presented the highest frequency of occurrence (11.5%), followed by race 64 with 7.7%. Races 64, 73 and 75 presented compatibility reactions only with cultivars of Mesoamerican origin. On other hand, races 3, 10, 27, 31, 79, 81, 82, 91, 93, 259, 283, 287, 346 and 351 showed compatibility reactions with both Andean and Mesoamerican cultivars (Table 1). All Andean cultivars presented reaction of compatibility with isolates, except Kaboon cultivar. All isolates were incompatible with PI 207262, TO, TU, AB 136 and G 2333 cultivars, thus becoming

important sources of resistance for use in common bean breeding programs aiming anthracnose control in Parana State.

Icolata	Differential cultivars <sup>1/2</sup>												Dago	
Isolate	Counties	Α	В	С	D	Ε	F	G	Η	Ι	J	K	L	Race
CL01	Cascavel	S	S	R	R	R	R	R	R	R	R	R	R	3
CL02	Cascavel	S	S	R	S	S	R	R	R	R	R	R	R	27
CL03	Cascavel	S	S	R	S	S	R	R	R	S	R	R	R	283
CL04	Guarapuava	R	S	R	S	R	R	R	R	R	R	R	R	10
CL05	Guarapuava	S	R	R	S	R	R	S	R	R	R	R	R	73
CL06	Guarapuava	S	R	R	R	S	R	S	R	R	R	R	R	81
CL07	Guarapuava	R	S	R	R	S	R	S	R	R	R	R	R	82
CL08	Guarapuava	S	R	S	S	S	R	S	R	R	R	R	R	93
CL09	Irati	R	R	R	R	R	R	S	R	R	R	R	R	64
CL10	Irati	S	S	R	R	S	R	S	R	R	R	R	R	83
CL11	Irati	S	S	S	S	S	R	S	R	S	R	R	R	351
CL12	Maringá	R	S	R	R	R	R	R	R	R	R	R	R	2
CL13	Maringá	S	S	R	R	R	R	R	R	R	R	R	R	3
CL14	Maringá	S	S	R	R	R	R	R	R	R	R	R	R	3
CL15	Maringá	R	R	R	R	R	R	S	R	R	R	R	R	64
CL16	Maringá	S	S	R	S	R	R	S	R	R	R	R	R	75
CL17	Maringá	S	S	S	S	R	R	S	R	R	R	R	R	79
CL18	Maringá	R	S	R	S	S	R	S	R	R	R	R	R	90
CL19	Maringá	S	S	R	S	S	R	S	R	R	R	R	R	91
CL20	Maringá	S	S	R	R	R	R	R	R	S	R	R	R	259
CL21	Maringá	S	S	S	S	S	R	R	R	S	R	R	R	287
CL22	Maringá	S	S	R	R	S	R	S	R	S	R	R	R	339
CL23	Maringá	S	S	S	S	S	R	S	R	S	R	R	R	351
CL24	Prudentópolis	S	S	S	S	S	R	R	R	R	R	R	R	31
CL25	Prudentópolis	S	S	R	S	S	R	S	R	R	R	R	R	91
CL26	Prudentópolis	R	S	R	S	S	R	S	R	S	R	R	R	346

**Table 1** Reaction of differential cultivars to isolates of C. lindemuthianum collected in Parana State, Brazil

<sup>1</sup>: A- Michelite (1); B- Michigan Dark Red Kidney (2); C- Perry Marrow (4); D- Cornell 49-242 (8); E- Widusa (16); F- Kaboon (32); G- Mexico 222 (64); H- PI 207262 (128); I- TO (256); J- TU (512); K- AB 136 (1024); L- G 2333 (2048). <sup>2</sup>: R = Resistant; S = Susceptible.

### **ACKNOWLEDGEMENTS**

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### LOCALIZATION OF A GENE FOR PARTIAL RESISTANCE TO POWDERY MILDEW IN X2776 BREEDING LINE

#### Campa A., B. Mateos, J.J. Ferreira

Área de cultivos Hortofrutícolas y Forestales, SERIDA, Apdo. 13, 33300, Villaviciosa, Spain

Powdery mildew (PM) is a widespread plant disease which incidence has significantly increased in common bean crops of northern Spain. The disease is caused by Ascomycete fungi, including a broad range of genera and species within order *Eryshiphales*. Qualitative resistance have been described in common bean, being indirectly located two genes on linkage groups (LG) Pv04 and Pv11 (Trabanco et al. 2012; Pérez-Vega et al. 2013). Previous analyses, suggested the presence of a single gene conferring partial resistance against PM in the X2776 breeding line, although its location in the genetic map was not established. The main objective of this work was to map the gene conferring resistance against PM in the X2776 line.

#### **MATERIAL AND METHODS**

A population of 90  $F_{2:3}$  families derived from the cross X2776 x G122 was used in this analysis. Line X2776 showed an infection type 3 (IT3), with moderate mycelial development on leaves, and line G122 showed an infection type 4 (IT4), with abundant mycelial development on leaves and profuse sporulation. Resistance tests were developed according to Trabanco et al. (2012) using a local isolate of PM. The reaction in each  $F_2$  plant was evaluated using 10-20  $F_3$  seedlings per  $F_{2:3}$  family.

Fourteen markers tagging the chromosome regions involved in the PM resistance (LG Pv04 and Pv11) were analyzed according to respective authors (Pérez-Vega et al. 2013; Moghaddam et al. 2013). Linkage relationships between loci were established using Mapmaker 2.0 software.

#### **RESULTS AND DISCUSSION**

Observed segregation of PM resistance in the  $F_{2:3}$  population X2776 x G122 was: 26 families having all seedlings with IT3 response, 37 families having seedlings with IT3 and IT4 response, and 27 families having all seedlings susceptible with IT4 response. This ratio fitted the expected for one gene ( $\chi^2_{1:2:1}$ = 2.87, *p*= 0.24) indicating that IT3 response in the X2776 genotype is controlled by one gene.

Only five of the fourteen markers analyzed were polymorphic between the parental lines. Table 1 shows segregation ratio and linkage relationships between each marker and the gene conferring IT3 response. A good fit to the expected ratio for a single locus was obtained in all cases. PM resistance was significantly linked to markers located on chromosome 11 and showed an independent segregation of the molecular markers located on chromosome 4. Figure 1 shows relative positions on LG Pv11 of the gene conferring IT3 response and three molecular markers.

In this position of LG Pv11, the Co-2 cluster involved in the response to specific *Colletotrichum lindemuthianum* races (causal agent of anthracnose) was mapped. The X2776 line has resistance genes to anthracnose in the Co-2 cluster. At a genomic level, a cluster of sequences encoding NBS (nucleotide-binding site) -LRR (leucine-rich repeat) proteins were annotated in this region (www.phytozome.net). The involvement of genes encoding LRR proteins in the resistance to PM has been suggested in other legumes. Therefore, the location of a PM resistance gene in this region was not unexpected, although more accurate genetic approach

to this gene should be established and its relationship with the anthracnose resistance genes of the Co-2 cluster.

**Table 1.** Observed ratios, recombination fractions (RF), and LODs obtained in the X2776 x G122 population for the gene conferring IT3 response and different molecular markers located on chromosomes 4 or 11. The physical position assigned for each marker, according to the common bean reference genome sequence, is indicated in base pairs (bp). RF, recombination fraction

		PM response in F <sub>2:3</sub> X2776 x G122 families												
	Physical	]	IT3/IT3		]	IT3/IT4			IT4/IT4					
Marker	position	XX	XG	GG	XX	XG	GG	XX	XG	GG	RF	LOD	$\chi^{2a}$	p
Chromosome 4														
IND4_0.2324	232.391	7	13	6	7	16	14	5	14	8	0.46	0.12	1.98	0.37
IND4_2.0349	2.034.932	7	11	8	9	14	14	4	13	10	0.46	0.18	5.38	0.07
Chromosome 11														
IND11_47.7684	47.768.390	19	7	0	1	35	1	0	1	26	0.06	26.93	1.27	0.53
SH13b	48.598.836	19	6	1	1	34	1	3	2	21	0.11	17.72	0.22	0.89
IND11_48.6909	48.690.876	19	7	0	1	34	2	0	2	25	0.07	24.88	1.27	0.53

<sup>a</sup>: All markers were codominant, being expected ratio for one locus 1:2:1

X/X homozygous for the X2776 alleles of the corresponding marker, X/G heterozygous, G/G homozygous for the G122 alleles



**Figure 1**. Relative positions on LG Pv11 of the resistance gene conferring IT3 response to PM and three molecular markers. Map distances are indicated in centiMorgans.

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### NEW SOURCES OF RESISTANCE AGAINST POWDERY MILDEW IN BREEDING LINES OF COMMON BEAN

### Ferreira J. J., B. Mateos, A. Campa

Área de cultivos Hortofrutícolas y Forestales, SERIDA, Apdo. 13, 33300, Villaviciosa, Spain

Powdery mildew can cause severe yield losses in bean crops. This fungus produces small, round, greyish or whitish spots on leaves and/or stems which can finally cover the whole upper leaf, even causing plant death. The use of resistant cultivars can be the most efficient method to control of diseases. The development of new resistant cultivars through breeding programs requires the availability of resistance sources. However, only five of the 245 genotypes evaluated by Trabanco et al. (2012) showed a total resistance to this pathogen (Amanda, Belneb, Cornell 49242, Negro San Luis, and Porrillo Sintetico). In this work, we investigated the reaction to powdery mildew in a set of 45 breeding lines. Results can be of interest for plant breeding programs focused on the introgression of genetic resistance to different diseases in bean genotypes.

#### **MATERIAL AND METHODS**

Forty-five breeding lines maintained in the bean genetic stock of SERIDA (Table 1) were selected in order to evaluate the reaction against a local isolate of powdery mildew (*Erysiphe* spp).

Resistance tests were carried out according to Trabanco et al. (2012). A local isolate of powdery mildew obtained from a single spot and maintained on the Xana bean genotype was used. The inoculation procedure consisted in blowing conidia on seedlings with totally developed primary leaves, in a density of about 5-10 spores/cm<sup>2</sup>. Inoculations were performed in sets of 25 pots with 4-5 plants placed in a box (80 x 80 x 80 cm). Plants were maintained in greenhouse at moderate temperature (18-24 °C) and humidity (60-70%).

Plant response was recorded 10- 12 days after inoculation following a 0-4 scale of infection types (IT) adapted from Mains and Dietz (1930): IT0, no visible symptoms; IT1, necrotic reaction on leaves with little or no mycelial development; IT2, necrotic reaction and moderate mycelial development; IT3, moderate mycelial development on leaves; IT4, abundant mycelial development on leaves and profuse sporulation (see Trabanco et al. 2012). Lines Xana (IT4), X2776 (IT3), A195 (IT2) and Porrillo Sintetico (IT0) were included in each resistance test as control of different infection types.

#### **RESULTS AND DISCUSSION**

Results presented in Table 1 show the different powdery mildew reactions observed in this set of breeding lines. Genotypes used as control showed the expected reaction. Eleven lines exhibited complete resistance with no symptoms (IT0). Resistance in these eleven genotypes were confirmed in a second test. Resistant genotypes included eleven different seed phenotypes classified in five market classes according to Voysest (2000): Caballero, Carioca, Jalinho, Ojo de tigre, and Small Red.

Breeding lines A483 and SEL1308 displayed high levels of resistance based on macroscopically visible hypersensitive response (IT1 or IT2). Eleven genotypes exhibited intermediate resistance based on a diffuse pathogen growth on the leaf (IT3). Nineteen remaining

genotypes were very susceptible showing symptoms similar to those of the susceptible control Xana (IT4). Lines A475 and ARA18 showed mixture in their respective reactions.

Not many resistance sources to powdery mildew had been described in common bean, particularly from resistance tests carried out in controlled conditions (Trabanco et al. 2012). These eleven resistant genotypes supply to bean breeders an additional genetic variation which can be interesting for the implementation of breeding programs focused on the development of new resistant cultivars.

**Table 1** Reaction of 43 common bean genotypes against a local isolate of powdery mildew observed in greenhouse tests.

Reaction	Genotypes
IT0	A774, A801, BillZ, Black Turtle Soup, BRB57, DOR364, Frijol Negro, G19833, NY211412, Rojo Chiquito, VAX3
IT1	-
IT2	A483, SEL1308
IT3	BeryL, Blanco Laran, Cardinal, G22263, MAM 13, MO 162, Red Mexican 35, Redlands Green Leaf C, SEA13, SEA5, UI906
IT4	A193, A429, AND279, ARA17, BRB173, Canadian Wonder, Chase, COS 16, DICTA 17, IVT 7214, Lamon, Montcalm, Montcau, PVA 773, Topcrop, VAX 2, WAF 53, WAF 9, ZAA 2

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### INTERACTION BETWEEN THE Ur-4 AND Ur-5 BEAN RUST RESISTANCE GENES

### G. Valentini<sup>1</sup>, P.B. Cregan<sup>2</sup>, Q. Song<sup>2</sup>, M.A. Pastor-Corrales<sup>2</sup>

Departamento de Agronomia, Universidade Estadual de Maringá, Av. Colombo, 5790, Maringá, PR 87020-900, Brazil; 2Soybean Genomics and Improvement Laboratory, USDA-ARS, BARC-West, Beltsville, MD 20705, USA

#### **INTRODUCTION**

Using specific races of the rust pathogen to detect single and even multiple resistance genes in common bean is laborious but rather effective. However, using specific races to detect epistasis, in which one gene masks the expression of the other, is not always feasible. Alternatively, molecular markers can be useful to detect resistance genes in the presence of epistasis. Epistasis between the *Ur-3* and *Ur-11*, *Ur-6* and *Ur-11* and between other rust resistance genes in common bean has been mentioned in the literature; however, little is known about the dynamics of the epistatic interactions among most rust resistance genes. The resistant reaction of the *Ur-4* gene described in the Andean bean Early Gallatin, is characterized by a hypersensitive reaction (HR) visualized as necrotic spots with no sporulation. The resistant reaction (R) of the *Ur-5* gene present in the Mesoamerican bean Mexico 309 is characterized by tiny uredinia less than 0.3 mm in diameter. In this study we aim to elucidate the interaction between the *Ur-4* and *Ur-5* genes.

### **MATERIAL AND METHODS**

This experiment was conducted at the Soybean Genomics and Improvement Laboratory of USDA-ARS, Beltsville, Maryland. A total of six F<sub>1</sub> plants and 182 F<sub>2</sub> plants from a Mexico 309 (Ur-5) x Early Gallatin (Ur-4) cross, 20 plants of each of the two parents, and eight plants each of the check cultivars Pinto 114, Aurora, Golden Gate Wax, PI 181996, BARC-RR-2, -3, -4, -7, -23 and BelMiDak-RR-3 were inoculated with races 40, 44, 53 and 108 of Uromyces appendiculatus (Table 1). Races 53 and 108 were used to detect the presence of Ur-5 and Ur-4, respectively, and races 40 and 44 to determine the plausible epistatic interaction between these two genes. To this end, simple sequence repeated (SSR) genetic markers linked to Ur-4 and Ur-5 were developed at the Soybean Genomics and Improvement Laboratory of USDA-ARS. These SSR markers were discovered using single nucleotide Polymorphism (SNP) genotyping with bulk segregant analyses (unpublished data). The presence of Ur-4 and Ur-5 in each  $F_2$  plant was corroborated through analysis with SSR markers; BARCPVSSR07452 and BARCPVSSR07454 linked to Ur-4 and BARCPVSSR04582 and BARCPVSSR04600 linked to Ur-5. SSR markers BARCPVSSR07452 and BARCPVSSR07454 were linked to Ur-4 at a distance of 0.2 cM on Pv06 and markers BARCPVSSR04582 and BARCPVSSR04600 were linked to Ur-5 at a distance 0.0 cM from on Pv04 (unpublished data).

### **RESULTS AND DISCUSSION**

Inoculation of the 182  $F_2$  plants with the races 53 and 108 revealed that the dominant *Ur-4* gene from Early Gallatin confers resistance to race 108 (*p-value* 0.6687) and the dominant *Ur-5* gene from Mexico 309 confers resistance to the race 53 (*p-value* 0.9318). The inoculation of the 182  $F_2$  plants with the races 40 and 44 of revealed that the resistant reaction of *Ur-5* is epistatic to the hypersensitive reaction of *Ur-4*; that is, the resistant phenotype of *Ur-5* (R) masks the resistant phenotype of *Ur-4* (HR) when the two genes are ccombined. A segregation ratio of 12:3:1 (12 R: 3 HR: 1 S) was observed (*p-value* 0.964), suggesting complete dominance at booth gene pairs, but one gene, when dominant is epistatic to the other (Table 2). Epistasis causes deviations from the common phenotypic ratio in  $F_2$  of 9:3:3:1.  $F_2$  plants having the *Ur-4* gene alone exhibited HR resistance phenotype (genotype aaB\_) to race 108 but were susceptible to race 53. Conversely,  $F_2$  plants with the *Ur-5* gene alone exhibited the tiny pustule R resistance phenotype (genotype A-BB) to race 53 but were susceptibility to race 108.  $F_2$  Plants combining *Ur-4* and Ur-5 also exhibited the R tiny pustule resistant phenotype (genotype A\_B\_) to races 53 and 108. The molecular analysis carried out on the 182  $F_2$  plants using the SSR markers previously identified as linked to the *Ur-4* and *Ur-5* resistance genes, gave the same results as the phenotypic evaluation, except for one plant (Table 3). In summary, results from this study indicate that the Mesoamerican *Ur-5* rust resistance gene is epistatic to the *Ur-4* Andean gene.

Cultivar	Gene	Gene pool	Races of U. appendiculatus						
Cultival	Och	Gene poor	40	44	53	108			
Early Gallatin	Ur-4	A/MA	2	2	4,5	2			
Mexico 309	Ur-5	MA	3,f2	3,f2	f2,3	5,6			
Pinto 114	-	MA	5,4	5,4	5,4	5,4			
Aurora	Ur-3	MA	2	4,5	2	2			
Golden Gate Wax	Ur-5	A/MA	2	2	4,5	4,5			
PI 181996	Ur-11	MA	f2	f2, 3	f2	5,6			
BARC-RR-2,3,4,7,23	Ur-4; Ur-5	MA	f2, 3	f2, 3	f2, 3	f2, 3			
BelMiDak-RR-3	Ur-4; Ur-5	MA	f2, 3	f2, 3	f2, 3	f2, 3			

**Table 1.** Reaction of the parents Early Gallatin and Mexico 309 and checks inoculated with races 40, 44, 53 and 108 of *Uromyces appendiculatus* 

Standard bean rust grading scale: 1 = no visible symptoms; 2, 2+ = Necrotic spots without sporulation; 3 = Tiny uredinia (sporulating pustules) less than 0.3mm in diameter; f2 = faint and tiny chlorotic spots; 4 = Medium uredinia, 0.3-0.5mm in diameter; 5 = Large uredinia, 0.5-0.8 mm in diameter, 6 = Very large uredinia, larger than 0.8mm in diameter. Reactions 2, 3, f2 are considered Resistant, and 4, 5, 6 are considered Susceptible.

**Table 2,** Observed and expected reaction of the  $F_1$  and the  $F_2$  population of Mexico 309 x Early Gallatin inoculated with races 40 and 44 of *Uromyces appendiculatus* for a hypothesis of dominant epistasis 12:3:1 between Ur-4 and Ur-5

Canatama	Concration		Obs. No	).	$E_{\rm res}$ (12D.211D.1C)	2	n valua			
Genotype	Generation	R	HR	S	Ехр. (12К.ЭПК.15)	χ <sup>2</sup> 0.073	p-value			
Mexico 309 x Early Gallatin	$F_1$	6	0	0						
Mexico 309 x Early Gallatin	$F_2$	137	33	12	136.5:34.125:11.375	0.073	0.964			

RP = resistant parent, R resistant; HR hypersensitive; S susceptible

**Table 3,** Results of the genotyping of the  $F_2$  population Mexico 309 x Early Gallatin using SSR markers relative to *Ur-4* (allele A) and *Ur-5* (allele B)

Fenotype	Genotype	Obs. No.	Exp. (12R:3HR:1S)	χ2	P value
Resistant (R)	A_B_ and aa_B_	137	136.5		
Hypersensitivity (HR)	A_bb	32	34.125	0.3660	0.8326
Susceptible (S)	aabb	13	11.375		
# AN ISOLATE OF *BEAN COMMON MOSAIC VIRUS* OVERCOMES THE *bc-3* ALLELE IN COMMON BEAN

## Xue Feng<sup>1</sup>, James R. Myers<sup>2</sup>, and Alexander V. Karasev<sup>1</sup>

<sup>1</sup>Department of PSES, University of Idaho, Moscow, ID; <sup>2</sup>Department of Horticulture, Oregon State University, Corvallis, OR

**INTRODUCTION:** Seven alleles have been implicated in the resistance of common bean (Phaseolus vulgaris L.) to Bean common mosaic virus (BCMV) and some other potyviruses, one dominant I allele, and six recessive alleles, bc-u, bc-1,  $bc-1^2$ , bc-2,  $bc-2^2$ , and bc-3 (1). Based on interactions with these resistance alleles, BCMV strains and isolates are classified into seven pathotypes (numbered I to VII) differentiated in 12-14 reference bean lines carrying these resistance genes in different combinations. The dominant *I*-allele conditions immunity against all BCMV strains known so far, but is linked to a necrotic reaction against a closely related potyvirus, Bean common mosaic necrosis virus (BCMNV), and additional, "protective" recessive alleles need to be present to prevent this necrotic reaction against BCMNV. The recessive bc-3 allele, when combined with the strain non-specific *bc-u* allele, conditions immunity against all known strains of BCMV, BCMNV, and of Clover yellow vein virus (CYVV) (2). Bc-3 allele, thus, represents the most advanced source of resistance against BCMV and other legume potyviruses. No BCMV isolate have been described so far, able to overcome the bc-3 allele when present as a homozygous combination with bc-u. In 2013, a field sample was collected in the Willamette Valley, OR, suspected of being infected with BCMV, and subjected to biological typing on bean differentials, to serological typing, and to molecular characterization.

**MATERIALS AND METHODS:** Two virus isolates, initially named 1755a and 1755b, were collected near Corvallis, OR, from the field-grown common bean accession 91-1755 exhibiting symptoms of mosaic and leaf deformation. This line was collected by Mike Dickson (Cornell University) in China in 1991 and was stored without being entered into the system at the Western Region Plant Introduction Station. Both isolates originally came in a single sample, representing a mixed infection, and were biologically separated and subsequently typed on a set of 11 bean differentials according to Drijfhout (1). Three BCMV and one BCMNV isolates from the laboratory collection were used as controls, US1 (BCMV, pathotype I), NY15P (BCMV, pathotype V), TN1 (BCMNV, pathotype VI), and US10 (BCMV, pathotype VII). Serological typing was conducted using a set of strain-specific polyclonal antibodies against control isolates NY15P, TN1, and US10 in TAS-ELISA.

**RESULTS:** When the original sample was subjected to an initial serological typing, a mixture of the two 1755a and 1755b isolates was suspected, due to the exhibited mixed A and B serotype (3). Specifically, we suspected a mixed BCMNV (A-serotype) and BCMV (B-serotype) infection. When tested on bean differentials, an unusual feature – an ability to replicate in IVT7214 was noted, with the virus replicating in IVT7214 exhibiting only B-serotype. This permissiveness of IVT7214 was used to biologically separate BCMV-1755a: it was back inoculated from IVT7214 into Dubbele Witte (DW) for subsequent propagation and typing on bean differentials and serologically. In addition to DW and IVT7214, BCMV-1755a was able to infect Stringless Green Refugee (SGR) and Sanilac only, thus displaying a novel and unusual pathogenicity profile (Table 1). Given this pathogenicity profile, we concluded that this isolate, BCMV-1755a, is able to overcome bc-u, bc-2 and bc-3 alleles. Following the established convention, we propose to name this new pathogenicity group as pathogroup VIII. The second

component of the original mixed infection, 1755b, was biologically separated from RGLB where it exhibited only A-serotype: it was back inoculated from RGLB into DW for subsequent propagation and typing on bean differentials and serologically. In addition to DW and RGLB, BCMV-1755b was able to infect SGR, RGLC, and Sanilac only (Table 1), thus displaying a pathogenicity profile typical of pathogroup VI, identical to TN1 (BCMNV, pathotype VI). It also induced a systemic vein necrosis in Jubila characteristic of other BCMNV isolates, but unlike TN1, the necrosis was more severe and inoculated Jubila plants eventually died by 4-6 weeks post-inoculation. We concluded that the second virus present in the original mixed infection is an isolate of BCMNV, and named it BCMNV-1755b.

Table 1. [	Table 1. Disease and ELISA reactions of bean differentials inoculated with BCMV isolates. <sup>1)</sup>													
<b>.</b>		<b>Bean cultivar</b> (resistance genes)												
ID	Dubbele Witte (none)	<b>SGR</b> ( <i>bc</i> - <i>u</i> )	RGLC (bc-u, bc-1)	$\begin{array}{c} \textbf{RGLB} \\ (bc-u, \\ bc-l^2) \end{array}$	Sanilac (bc-u, bc-2)	UI35 (bc-u, bc-12, bc-22)	<b>IVT7214</b> ( <i>bc-u</i> , <i>bc-</i> 2, <i>bc-3</i> )	<b>Jubila</b> ( <i>I</i> , <i>bc</i> -1)	<b>Amanda</b> ( <i>I</i> , <i>bc</i> - <i>1</i> <sup>2</sup> )	<b>US1006</b> ( <i>I</i> , <i>bc</i> -2 <sup>2</sup> )	<b>IVT7233</b> ( <i>I</i> , <i>bc-u</i> , <i>bc1</i> <sup>2</sup> , <i>bc</i> - 2 <sup>2</sup> )			
1755a	+/+	+/+	-/-	-/-	+/+	-/-	+/+	-/-	-/-	-/-	_/_			
1755b	+/+	+/+	+/+	+/+	+/+	-/-	-/-	BRS/NA	-/-	-/-	_/_			
US1 (PG-I)	+/+	+/+	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	_/_			
NY15P (PG-V)	+/+	+/+	+/+	-/-	+/+	-/-	-/-	-/-	-/-	-/-	-/-			
TN1 (PG-VI)	+/+	+/+	+/+	+/+	+/+	-/-	_/_	nec/-	-/-	-/-	_/_			

<sup>1)</sup> Disease reaction is shown first as a numerator followed by ELISA reaction as a denominator. Three plants were inoculated for each BCMV isolate per an experiment;

<sup>2)</sup> Numerator: + = symptoms on inoculated beans; - = no symptoms on inoculated beans;

nec = systemic necrosic reaction on some uninoculated leaves;

<sup>3)</sup> Denominator: + designates ELISA signal ( $A_{405}$ ) in an infected plant exceeding healthy control 10-fold or more; - designates ELISA signal in an infected plant equal to that of a healthy control.

**CONCLUSIONS:** Our data suggests that isolate BCMV-1755a appears to exhibit a novel pathogenicity profile. This isolate may be able to overcome bc-2 and bc-3 alleles in common bean. Presence of BCMV isolates of this novel pathotype in the field should be taken into account, and corresponding efforts to incorporate them into resistance screening for bean breeding programs would be desirable.

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## IN SILICO VALIDATION OF NEW SNP MARKER LINKED TO BCMV RESISTANCE ACROSS MULTIPLE RIL POPULATIONS

## M. H. Bello<sup>1</sup>, J. Myers<sup>2</sup>, P. Cregan<sup>3</sup>, and P.N Miklas<sup>1</sup>

<sup>1</sup>USDA-ARS, Prosser, WA 99350; <sup>2</sup>Dep. of Hort., OSU, Corvallis, OR 97330; <sup>3</sup>USDA-ARS, Beltsville, MD 20705

**INTRODUCTION:** Resistance to *Bean common mosaic virus* (BCMV) is conditioned by a single dominant (*I*), conferring immune or hypersensitive resistance to all known strains of BCMV and four recessive (*bc-u, bc-1, bc-2, bc-3*) genes, conferring strain-specific resistance (1). The *I* gene locates in chromosome 2 and encompasses a ~81.7 kb region containing seven putative NBS-LRR-type disease resistance genes (Phvul.002G323000 to Phvul.002G323500, and Phvul.002G323800) (2). Recently, our group conducted *in silico* bulked segregant approach using the BeanCAP panel, genotyped on the Illumina BARCBEAN6K\_3 Infinium SNP BeadChip, and identified SNP markers surrounding the *I* gene regions that cosegregated with BCMV resistance. The closest SNP (ss715641188) to the *I* gene was validated on a subset of the BeanCAP and Andean diversity panel genotypes, and a recombinant inbred line (RIL) population derived from G122 (susceptible) × Montcalm (resistant), using KASP and CAPS marker assays. The objectives of this work were 1) to further validate *in silico* the SNP marker (ss715641188) across multiple RIL populations of different genetic background, and 2) to identify potential recombinants to use in fine mapping of *I* gene.

MATERIALS AND METHODS: Four RIL populations known to be segregating for resistance to BCMV, based on previous inoculation and/or genotypic assays of their parental lines, were utilized in this study (2). The RILs were derived from the following crosses, between resistant (R) and susceptible (S) lines: Buster (R, Middle American, pinto) × Roza (S, Middle American, pink), [BR]; OSU5446 (R, Middle American/Andean, Blue Lake snap bean) × RR6950 (Middle American, black-seeded snap bean), [RR138]; Rojo (R, Andean, large red) × CAL 143 (S, Andean, red mottle), [RC]; Aztec (S, Middle American, pinto) × ND88-106-04 (R, Middle American, navy) [AN]. The primary leaves of four 10-old day plants from each RIL population (BR= 137, RR138= 167, RC= 147; AN= 82) and their parents, including BCMV differential cultivars used as controls, were rub-inoculated with strain NL-3 of BCMNV under greenhouse conditions (3). From one to two weeks after inoculation, the parents and RIL lines were evaluated, and only those showing symptoms of systemic necrosis (I-) or vein necrosis (I-  $bc-l^2$  $bc-1^2$ ) were scored as resistant. Parental and RIL lines (except the AN RILs and their ND88-106-04 parent), were previously genotyped with the BARCBEAN6K 3 SNP BeadChip. A total of six SNP that cosegregated consistently with BCMV resistance (2) were manually inspected across each RIL population SNP data set.

**RESULTS:** Parental lines Buster, OSU5446 and ND88-106-04, expressed symptoms of top necrosis, whereas Rojo expressed vein necrosis. The resistant parents were homozygous for the presence of SNP allele (A), at marker ss715641188, linked to BCMV resistance conditioned by *I* gene (Table 1). The other parental lines had the alternate SNP allele (G) associated with susceptibility. Only BR and AN RILs segregated for resistance and susceptibility in a Mendelian fashion (BR= 66 R : 69 S; AN= 39 R : 41 S). In contrast, RC and RR138 RILs showed distorted segregation favouring the presence of *I* gene (RC= 103 R : 44 S; RR138= 118 R : 41 S). There were RILs still segregating for reaction to BCMNV NL-3 strain (BR= 2; RR138= 8; AN= 2). *In silico* analysis of marker data from the RIL populations revealed strong marker-trait associations

(P<0.05), except for the most distal markers (ss715648451, ss715648452, ss715639908) that are homozygous in the BR population and parents (Table 2). A few RILs were identified as putative recombinants (i.e., homozygous for both phenotype and each marker). Particularly, for SNP ss715641188 there were resistant RILs classified as genotypically susceptible (BR= 1, RR138= 1, RC= 3), and vice versa, susceptible RILs classified as genotypically resistant (RR138=1, RC= 2). This work demonstrates that the new SNP marker alone is able to predict the resistant/susceptible phenotype across populations, and its utility in marker-assisted selection. The putative recombinant RILs from RC will be used to fine map the *I* gene with a genotypingby-sequencing approach.

<b>SNP</b> <sup>a</sup>	Resistant SNP allele <sup>b</sup>	Susceptible SNP allele <sup>b</sup>	Physical position	Buster (R)/ Roza (S) <sup>c</sup>	OSU5446 (R)/ RR6950 (S) <sup>c</sup>	Rojo (R)/ CAL143 (S) <sup>c</sup>	ND88-106-04 (R)/ Aztec (S) <sup>c</sup>
ss715641188	AA (A)	BB (G)	48,289,722	AA / BB	AA / BB	AA / BB	NA / BB
ss715648456	AA (T)	BB (C)	48,447,134	AA / BB	AA / BB	AA / BB	NA / BB
ss715639906	BB(G)	AA (T)	48,512,722	BB / AA	BB / AA	BB / AA	NA / AA
ss715648451	BB (G)	AA (A)	48,606,577	AA / AA	BB / AA	BB / AA	NA / AA
ss715648452	BB(G)	AA (A)	48,617,402	AA / AA	BB / AA	BB / AA	NA / AA
ss715639908	AA (A)	BB (G)	48,620,266	BB / BB	AA / BB	AA / BB	NA / BB

Table 1. SNP	associated	with th	e I gene	for resistance	to BCMV

<sup>a</sup>NCBI Assay ID (ss#) at dbSNP Short Genetics Variation database (<u>http://www.ncbi.nlm.nih.gov/projects/SNP/</u>) containing the target SNP and flanking sequence information. SNP ss715641188 was converted into KASP/CAPS marker (2).

<sup>b</sup>Genotype calls obtained from data of BARCBEAN6K 3 SNP Chip. The corresponding SNP allele at each locus is in parenthesis.

Parental lines and their disease reaction to BCMV. R = resistant, S = susceptible (S). NA = Data not available.

Table 2. Marker cosegregation with resistance to BCMV in RIL popula	ations
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	0 0			
	Genotype call	Buster (R)/ Roza (S) <sup>a</sup>	OSU5446 (R)/ RR6950 (S) <sup>a</sup>	Rojo (R)/ CAL143 (S) <sup>a</sup>
	Resistant /	Resistant /	Resistant /	Resistant /
SNP	Susceptible	Susceptible	Susceptible	Susceptible
ss715641188	AA /	65 AA (1 BB) /	113 AA (1 BB, 5 AB) /	99 AA (3 BB, 1 AB) /
	BB	69 BB	39 BB (1 AA, 1AB)	38 BB (2 AA, 4 AB)
ss715648456	AA /	65 AA (1 BB) /	109 AA (6 BB, 4 AB) /	99 AA (3 BB, 1 AB) /
	BB	68 BB (1 AA)	36 BB (3 AA, 2 AB)	38 BB (2 AA, 4 AB)
ss715639906	BB /	65 BB (1 AA) /	108 BB (6 AA, 5 AB) /	99 BB (3 AA, 1 AB) /
	AA	68 AA (1 BB)	36 AA (3 BB, 2 AB)	37 AA (3 BB, 4 AB)
ss715648451	BB /	0 BB (66 AA) /	107 BB (6 AA, 6 AB) /	99 BB (3 AA, 1 AB) /
	AA	69 AA	35 AA (4 BB, 2AB)	35 AA (5 BB, 4 AB)
ss715648452	BB /	0 BB (66 AA) /	107 BB (6 AA, 6 AB) /	99 BB (3 AA, 1 AB) /
	AA	69 AA	35 AA (4 BB, 2AB)	35 AA (5 BB, 4 AB)
ss715639908	AA /	0 AA(66 BB) /	107 AA (6 AA, 6 AB) /	99 AA (3 BB, 1 AB) /
	BB	69 BB	35 BB (4 AA, 2 AB)	35 BB (5 AA, 4 AB)

<sup>a</sup>Number of genotypes corresponding to resistant (shaded rows) or susceptible (non shaded rows) RILs. The number of RILs not matching the expected genotypic class is indicated in parenthesis. Segregating RILs are not included in the table.

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## EVALUATION OF SNAP BEAN GERMPLASM FOR COMMON BACTERIAL BLIGHT RESISTANCE

## Felipe Aranha de Andrade\*; Édison Miglioranza; Leandro S. A. Gonçalves; Anderson Y. S. Fukuji

<sup>1</sup>Department of Agronomy, Universidade Estadual de Londrina (UEL), Rodovia Celso Garcia Cid Km 380, Londrina, PR, 86051-980, Brazil. <sup>\*</sup>Email: felipearanhaa@hotmail.com

**INTRODUCTION:** Snap bean (*Phaseolus vulgaris* L) is a vegetable rich in fiber, protein, potassium, iron and vitamins (Silva et al., 2004). One of the most important diseases that affect *P. vulgaris* L. is common bacterial blight (CBB) caused by *Xanthomonas axonopodis* pv. *Phaseoli* (Liu et al., 2008). It occurs in all cultivation environments, proliferating mainly in high temperature and humidity conditions. The loss of yield and pod quality in *P.* vulgaris depends on the intensity of the disease, environmental conditions and the susceptibility of cultivars. Thus, the aim of this research was to evaluate the level of resistance of snap bean genotypes determinate growth habit in relation to bacterial blight.

**MATERIAL AND METHODS:** The experiment was conducted in a greenhouse at the State University of Londrina, Londrina-Paraná-Brazil from December, 2013 to February, 2014. Evaluations were performed for 25 snap bean genotypes of determinate growth habit. The bacterial isolates (*Xanthomonas axonopodis* pv. *phaseoli* – XAP and *Xanthomonas axonopodis* pv. *phaseoli* var. Fuscans - XAV) were grown on Petri dishes containing solid DYGS (Rodrigues Neto et al., 1986) at 28°C. Plants were grown in pots containing two plants per pot. Two leaflets from each trifoliate were previously identified by wool-yarn strings of different colours that were tied to the leaflet and indicated the strain that would be used. Leaves were inoculated 35 days after planting. Each leaflets received the bacterial suspension. To determine the severity of the bacterial blight daily evaluations were carried out for 30 days using the diagrammatic scale of Pastor-Corrales et al. (1981): 1 = no symptoms; 2 = 1 to 5% of necrosis; 3 = 6 to 25% of necrosis; 4 = 26 to 50% of necrosis; 5 => 50% of necrosis. The results of daily evaluations were used to calculate the area under disease progress curve (AUDPC). The experimental design was a randomized complete with ten replications of two plants per pot.

**RESULTS AND DISCUSSION:** The analyses of variance showed significant differences (P < 0,01) for the source of genotype variation for AUDPC in both evaluated isolates (Table 1), indicating the existence of a wide variability among genotypes of snap bean. The average values of AUDPC were 82,09 and 101,17 for *Xanthomonas axonopodis* pv. *phaseoli* (Xap) and *Xanthomonas axonopodis* pv. *phaseoli* vr. *Fuscans* (Xav), respectively, indicating more aggressive Xav in advance of symptoms. These results confirm those reported by Mutlu et al. (2008). After 30 days of evaluation, all genotypes were susceptible to both isolates, however, by two dimensional graph, it was found that HAB 409 and HAB 407 genotypes had lower values for AUDPC for Xap (68 and 64,78, respectively) and Xav (90,50 and 87,28, respectively) (Figure 1). Based on the commercial genotypes, UEL 2 cultivar had the lowest value for Xap (44,20), on the other hand had the highest value for Xav (117,20), showing that the genes that controlling the resistance in Xap are differents for isolated Xav. For UEL 1 and Alessa Xap values were 84 and 104,25, respectively, while for Xav were 98,67 and 114,6, respectively. In this context, the search for new sources of resistance and/or the introgression of resistance genes found in common bean becomes very important for the sustainable production of snap bean.

**Table 1.** Analysis of variance for AUDPC and severity score in snap beans genotypes inoculated with *Xanthomonas axonopodis* pv. *phaseoli* (Xap) and *Xanthomonas axonopodis* pv. *phaseoli* vr. *Fuscans* (Xav)

		Mean S	quare	-	Mean Square		
$\mathbf{SV}$	DF	Xa	DF	Xav			
		AUDPC SCORE			AUDPC	SCORE	
Treatment	24	1113.00**	1.19**	24	399.49**	0.4233**	
Error	163	471.96	0.51	164	199.39	0.2119	
Mean		82.09	2.73		101.17	3.36	
CV(%)		26.46	26.01		13.95	13.7	

\*\*Significant at 1% probability by F test.



Xanthomonas axonopodis pv. phaseoli

Figure 1. Dimensional graph for AUDPC values between 25 snap bean genotypes inoculated with *Xanthomonas axonopodis* pv. *phaseoli* (Xap) and *Xanthomonas axonopodis* pv. *phaseoli* var. Fuscans (Xav).

**CONCLUSION:** All snap bean genotypes were susceptible to *Xanthomonas axonopodis* pv. *phaseoli* and *Xanthomonas axonopodis* pv. *phaseoli* var. Fuscans. The genotypes HAB 409 and HAB 407 were more tolerant to both isolates.

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## USE of Muti Site Screening to identify and VERIFY Partial Resistance to White Mold in Common Bean in 2014

## R. Jhala, B. Higgins, K. Eskridge and J.R. Steadman

Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln, NE 68583 Data also from H. Schwartz (CO), S. Singh (ID), J. Kelly (MI), M. Wunch (ND), J. Myers (OR), P. Miklas (WA), K. Kmiecik (WI), and Ellen Withofs (BEL)

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

Breeders sent seed of 11 bean lines with putative sources of resistance to our laboratory where the seeds were divided in equal amounts for field (400g/line) and/or greenhouse (25 seeds/ line) tests and then sent to nine locations to be evaluated by standardized greenhouse and/or field screening methods. Three bean lines were included in both tests as controls: partially resistant G122, Bunsi with mostly field avoidance and susceptible GN Beryl.

The field tests consisted of two rows of each of the 7 entries and one row of a local semi vine WM susceptible genotype, resulting in a three-row plot 4.6 m (15 ft.) long replicated three times in a randomized complete block design. There were six field tests conducted in six locations. The field nurseries were all evaluated using a CIAT 1 to 9 scale (1 = no visible symptoms to 9 = death) (Van Schoonhoven et al., 1987). Nebraska and Wisconsin did not have results due to unfavorable weather. These problems that resulted in no data were unfortunate, but demonstrate the importance of testing in multiple locations. In the field tests, all 7 lines were significantly more resistant than Beryl (Table 1). The results of the four field tests reported were that 3 bean lines, 031-A-11, USPT-WM-12 and ASR 1002 were similar to G122 with intermediate resistance while B10244 and R12859 were similar to Bunsi. N11283 and G12901 were less suceptible than Beryl.

The greenhouse trials tested 11 entries, plus 3 controls, using the straw test on 21- to 28-day-old G122 beanplants. The plants were inoculated 2.5 cm above the fourth node with a plug of PDA media containing advancing margin *S. sclerotiorum* mycelia pressed into a 2.5 cm clear drinking straw sealed at one end and fitted over the cut internode. The inoculated plants were scored 8 days later using the modified Petzoldt and Dickson scale (Teran et al, 2006). The greenhouse results (Table 2) indicate that three bean lines had ratings similar to G122 including 031-A-11 and USPT WM-12 while seven bean lines had ratings similar to Bunsi; however, greenhouse conditions are more favorable and allow the fungus to grow in optimal conditions which is less likely to be encountered in the field. For example, bean line ASR 1002 had a field rating lower than G122 but was similar to Beryl in the greenhouse test and was likely exhibiting escape or avoidance mechanisms. All field entries including pinto, great northern, black, navy and cranberry seed classes were rated lower than Beryl. Progress in incorporating WM resistance into dry bean lines with commercial potential validates use of multisite screening and National Sclerotinia Initiative support over the last 10 years.

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

ENTRY	SEED CLASS	COLLABORATOR	OR	WA	ND	MI	Mean	t Grouping
BERYL	G. NORTHERN	Susceptible Check	8.3	7.6	4.7	9.0	7.4	А
G 12901	G. NORTHERN	J. Kelly- MI	6.3	6.3	4.6	5.0	5.6	В
N 11283	NAVY	J. Kelly- MI	4.3	5.3	5.4	4.4	4.9	BC
R 12859	Red	J. Kelly- MI	4.0	4.2	4.4	5.5	4.5	B C D
B 10244	BLACK	J. Kelly- MI	3.0	4.5	3.8	3.3	3.7	C D E
EX RICO (BUNSI)	NAVY	Intermediate Check	4.3	4.6	2.2	2.0	3.3	DE
G122	CRAN	Resistant Check	2.3	2.5	3.0	4.4	3.1	Е
031-A-11	G. NORTHERN	P. Miklas- WA	2.7	3.8	2.7	3.0	3.1	Е
USPT-WM-12	PINTO	P. Miklas- WA	2.3	3.6	2.8	2.0	2.7	Е
ASR 1002	SNAP BEAN	J. Theuws- BEL	2.3	1.8	1.5	4.4	2.5	Е

\*CIAT Scale: 1 = no disease, 9 = plants dead \*\*Alpha = 0.05, LSD = 1.5 WI and NE had no data from field due to weather

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

ENTRY	SEED CLASS	COLLABORATOR	СО	BEL	NE	WI	WA	ID	Mean	t Grouping
G 12901	G. NORTHERN	J. Kelly- MI	7.3	6.8	8.0	9.0	7.5	7.4	7.7	А
BERYL	G. NORTHERN	Susceptible Check	7.7	6.7	6.6	7.3	7.4		7.1	A B
B 10244	BLACK	J. Kelly- MI	2.8	4.9	6.9	8.3	7.0	7.9	6.3	ВC
ASR 1002	SNAP BEAN	J. Theuws- BEL	6.0	2.3	6.7	9.0	6.1	7.6	6.3	ВC
N 11283	NAVY	J. Kelly- MI	4.4	5.0	5.5	9.0	7.1		6.2	ВC
PT13-17	PINTO	P. Miklas- WA	5.5	5.2	6.5	7.2	6.9	5.8	6.2	ВC
PT13-18	PINTO	P. Miklas-WA	6.2	5.6	5.9	6.8	6.0	5.0	5.9	С
EX RICO (BUNSI)	NAVY	Intermediate Check	3.6	4.8	7.2	6.5	6.7		5.8	С
R 12859	Red	J. Kelly- MI	5.4	4.5	5.8	6.3	5.7	4.7	5.4	C D
039-A-5	PINTO	P. Miklas- WA	5.7	4.6	6.0	5.0	6.0	4.8	5.4	C D
031-A-11	G. NORTHERN	P. Miklas-WA	3.1	4.6	4.3	4.0	5.6	5.0	4.4	DE
G122	CRAN	Resistant Check	3.7	2.9	4.9	4.9	4.2	5.9	4.4	DE
A195	LGCREAM	S. Singh- ID	3.6	3.8	4.4	3.6	4.0	5.1	4.1	Е
USPT-WM-12	PINTO	P. Miklas- WA	3.3	3.4	4.8	5.2	3.5	3.9	4.0	Е

\*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 = 1.2

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## PARTIAL RESISTANCE OF COMMON BEAN ELITE LINES DEVELOPED IN BRAZIL TO WHITE MOLD

## Lima, R.C.<sup>1</sup>; Teixeira, P.H.<sup>1</sup>; Souza, A.F.F.<sup>1</sup>; Silva, R.A.<sup>1</sup>; Bonicontro, B.F.<sup>1</sup>; Vieira<sup>2</sup>, R.F.<sup>2</sup>; Paula Júnior, T.J.<sup>2</sup>; Lehner, M.S.<sup>1</sup>; Carneiro, J.E.S.<sup>1</sup>

<sup>1</sup>Univ. Federal de Viçosa, 36570-000 Viçosa (MG), Brazil (renan.lima@ufv.br); <sup>2</sup>Epamig, CP 216, 36570-000 Viçosa (MG), Brazil

## INTRODUCTION

One of the most important diseases affecting common bean during the fall-winter season in the State of Minas Gerais, Brazil, is white mold (WM). The most commonly used control measure is the application of fungicides. However, the high cost and the potentially deleterious environmental and human effects have motivated the search for new alternatives of WM management. Genetic resistance is a key component of WM management, because it is easier for farmers to adopt and environmentally safe. Common bean lines developed by Universidade Federal de Lavras (UFLA), Universidade Federal de Viçosa (UFV), and Embrapa Arroz e Feijão are tested every year at several locations in the State of Minas Gerais through the "cultivation and use value" (CUV) experiments. We selected lines from these trials under WM pressure. In four experiments, we compared the performance of the lines with the current cultivars used by farmers, also under WM pressure. Our objective was to select lines with resistance to WM associated with high yield potential.

## **MATERIAL AND METHODS**

Genotypes tested in the CUVs conducted from 2008 to 2011 were assessed for their reaction to WM and yield in an area naturally infested with sclerotia. The experiments were carried out between April and July and were sprinkler irrigated. Based on the results, 14 genotypes were selected with partial resistance to WM. With these genotypes, four experiments were conducted during the fall-winter season of 2012 and 2013 in Viçosa and Oratórios, Zona da Mata region, State of Minas Gerais, Brazil. The reactions of these genotypes to WM were compared with those of the following cultivars: Pérola, Majestoso, Ouro Negro, Ouro Vermelho, and Estilo. A 195 was also included in the experiments for its known WM partial resistance and good adaptability to Brazilian conditions (Lehner et al., 2015). A randomized complete block design with five replications was used. Plots were 2 rows x 3 m long, with 0.50 m between rows. The final stand was approximately 10 plants per meter of row. White mold intensity (incidence + severity) was evaluated visually, using a 1-to-9 scale (Miklas et al., 2001).

## **RESULTS AND DISCUSSION**

WM intensity was low in 2012 and high in 2013. In 2013, but not in 2012, yield correlated significantly with WM intensity in Oratórios (r = -0.72, p = 0.001) and Viçosa (r = -0.64, p = 0.001). In general, the cultivars, especially Majestoso, Ouro Vermelho, and Ouro Negro, were among the most susceptible genotypes to WM. In 2013, in Oratórios, the early maturity genotypes, CAL 96 and Ouro Branco were more susceptible to WM than these three cultivars, probably because rains coincided with their flowering time. Unexpectedly, the early maturity and resistant line A 195 was as susceptible to WM in that experiment as the cultivar Ouro Negro. The carioca line VC 17 (Type III) belonged to the group of the most productive genotypes in the four experiments. This line yielded 3694 kg/ha when the WM pressure was high in Oratórios. In this experiment, VC 17 was among the genotypes with the lowest WM intensity. The carioca lines

CNFC 10432 and CNFC 10720 and the black lines CNFP 11990, and CNFP 10798 also showed partial resistance to WM and high yield.

Genotype		Yield (k		WM intensity <sup>3,4</sup>				
(commercial class or	Vi	Or	Vi	Or	Vi	Or	Vi	Or
group <sup>1</sup> / plant Type <sup>2</sup> )	2012	2012	2013	2013	2012	2012	2013	2013
VC 17 (C /III )	3056 A	2756 A	3174 A	3694 A	2.4 A	2.8 B	4.4 B	4.6 D
CNFC 10432 (C/II)	2896 A	2500 B	3236 A	3733 A	2.0 A	1.4 C	4.4 B	4.9 D
CNFP 11990 (B/II)	2826 A	2626 B	2963 A	3420 A	1.6 A	2.2 B	4.1 B	4.9 D
CNFC 10720 (C/II)	2756 A	2290 B	3350 A	3480 A	1.6 A	2.6 B	3.2 C	5.0 D
CNFP 10798 (B/II)	2646 A	2743 A	3037 A	3090 B	1.6 A	2.2 B	2.9 C	4.7 D
CNFP 11980 (B/II)	2623 A	2023 C	2620 B	2670 B	1.8 A	1.2 C	3.4 C	5.7 C
Estilo (C/II)	2510 A	2460 B	2967 A	2257 C	2.6 A	1.8 C	4.8 B	7.0 B
Majestoso (C/III)	2470 B	2390 B	2303 B	2676 B	2.8 A	4.4 A	6.7 A	5.5 C
RP-1 (C/II)	2436 B	3183 A	2970 A	2223 C	2.0 A	1.9 C	4.2 B	6.2 C
A 195 (A/I)	2400 B	2583 B	2800 B	2113 C	1.2 A	2.8 B	5.3 B	6.5 B
O. Vermelho (R/III)	2380 B	1450 D	1790 C	2930 B	2.0 A	4.6 A	7.4 A	6.1 C
CNFC 10722 (C/II)	2333 B	2126 C	2990 A	2553 B	1.6 A	1.0 C	3.1 C	5.3 D
Pérola (C/III)	2303 B	2046 C	2683 B	2760 B	2.0 A	2.4 B	5.3 B	6.0 C
VP 21 (B/II)	2280 B	2080 C	2487 B	2716 B	1.8 A	3.6 A	5.0 B	5.6 C
Ouro Negro (B/III)	2276 B	1756 D	1360 C	2127 C	3.0 A	4.9 A	7.9 A	7.0 B
BRS Executivo (A/IIb)	2083 C	2313 B	2530 B	1456 D	1.6 A	2.8 B	4.2 B	7.0 B
Ouro Branco (A/I)	2013 C	2110 C	2303 B	1620 D	1.0 A	4.7 A	3.8 C	7.9A
CAL 96 (A/I)	1990 C	2343 B	2627 B	1386 D	1.2 A	4.5 A	4.7 B	8.4 A
BRS Vereda (R/III)	1906 C	1726 D	2500 B	2830 B	2.2 A	1.3 C	2.3 C	4.5 D
CNFC 11965 (C/II)	1876 C	1720 D	2657 B	3240 A	2.0 A	2.2 B	3.2 C	5.8 C
Mean	2403	2261	2665	2649	1.9	2.8	4.5	5.9
CV(%)	16	16	12	25	41	36	18	16

**Table 1**. Yield and white mold (WM) intensity of lines/cultivars selected for partial resistance to WM and five current cultivars (in bold) in 2012 and 2013 in Viçosa (Vi) and Oratórios (Or), State of Minas Gerais, Brazil.

<sup>1</sup> C = carioca, B = black, A = Andean gene pool, R = red. <sup>2</sup> I – determinate growth habit; II – indeterminate growth habit, upright plants; III – indeterminate growth habit, semi-prostrate or prostrate plants. <sup>3</sup> Means followed by the same letters belong to the same group (Scott-Knott test, p = 0.05). <sup>4</sup> 1 = no diseased plants; 9 = 80 to 100% diseased plants and/or 60 to 100% infected tissue.

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## **EFFICACY OF FIVE FUNGICIDES ON Sclerotinia sclerotiorum ISOLATES**

#### R. Jhala, B. Higgins and J.R. Steadman

Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln, NE 68583

In the characterization criteria that we have been using on 366 isolates of *Sclerotinia sclerotiorum* we have added fungicide sensitivity. One of the management options for white mold (WM) of common bean is use of fungicide applications at first bloom or first bloom and 7-10 days later. Thus, in addition to our isolate characterization information, the possibility that isolates exposed to fungicides over time may lose sensitivity to chemical applications was also investigated. In an earlier study of fungicide efficacy for WM on soybean only thiophanate methyl is used today and like our data had less efficacy (Mueller *et al.*, 2002).

The objectives of this study were to (i) determine the efficacy of a range of concentrations of five fungicides on mycelial growth of *S. sclerotiorum*; (ii) determine if field collected isolates vary in their growth response to field rates of the five fungicides.

Potato dextrose agar (PDA) autoclaved at  $122^{\circ}$ C for 20 min and cooled to 55 to 60°C was the fungal growth medium. Thiophanate methyl, prothioconazole, pyraclostrobin, iprodione and metconazole technical grade fungicides were diluted in acetone and added to PDA to yield concentrations of 0.1, 1, 10, 50, 100, and 500 µg a.i./ml of PDA of each fungicide. Nonamended PDA was used as a control and initial cultures of *S. sclerotiorum* also were grown on nonamended PDA (20 ml/plate). Plugs (6-mm-diameter) were taken from the actively growing margins of the colony, and one plug was transferred to the center of each of three replicated plates of each treatment. Plates were placed in a growth chamber at 25C for 3 days, when the diameter of the radial growth was measured. Percentage of inhibition of mycelial growth was calculated from radial growth measurements. Comparison of mycelial growth inhibition by five fungicides at field rate are presented for two clusters (groups) of *S. sclerotiorum* isolates using 2-D bar charts (Figs. 1 & 2). Clusters were determined by haplotypes from the 366 isolates characterized (Jhala *et al.*, 2014).

Using 1  $\mu$ g a.i./ml PDA fungicide, pyraclostrobin, iprodione and metconazole were more effective than thiophanate methyl and prothioconazole in inhibiting *S. sclerotiorum* mycelial growth of 64 isolates (data not shown). At field fungicide rates, prothioconazole, pyraclostrobin, iprodione and metconazole were more effective than thiophanate methyl in inhibiting *S. sclerotiorum* mycelial growth (Figs. 1 & 2). The isolates in cluster 3 (Group 3) were grower field collections and exhibited a wide range of aggressiveness. The isolates in cluster 4 (Group 4) were collected from WM screening nurseries and grower fields. Isolate sensitivity variation was detected for all fungicides except iprodione, for the rates tested.

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Fig. 1. Percent inhibition of mycelial growth of *S. sclerotiorum* isolates of high and low aggressiveness from ND, CO, NE, WA and MI by five fungicides at field rate.



Fig. 2. Percent inhibition of mycelial growth of *S. sclerotiorum* isolates from Washington collected in 2003, 2004, 2005, 2007 and 2008 by five fungicides at field rate.

## WITHIN-ROW DISTANCE BETWEEN TYPE II PLANTS CAN BE INCREASED IN COMMON BEAN FIELDS WITH HISTORY OF WHITE MOLD

# Lima, R.C.<sup>1</sup>; Soares, B.A.<sup>1</sup>; Rodrigues, L.B.<sup>1</sup>; Vieira; R.F.<sup>2</sup>; Paula Júnior, T.J.<sup>2</sup>; Carneiro, J.E.S.<sup>1</sup>

<sup>1</sup>Univ. Federal de Viçosa, 36570-000 Viçosa (MG), Brazil (renan.lima@ufv.br); <sup>2</sup>Epamig, CP 216, 36570-000 Viçosa (MG), Brazil

**INTRODUCTION:** In Brazil, Type II indeterminate growth habit bean cultivars are generally sown at row spacing of 0.4 to 0.5 m with 12 to 15 plants/m. However, these within-row densities (WRD) could not be the most appropriate plant population in fields with history of white mold (WM) as demonstrated by Vieira et al. (2010) for Type III cultivars. These authors showed that four or five plants/m can decrease WM intensity and increase yield compared to 10 to 12 plants/m. Our objective was to test whether the increasing of within-row distance between plants could help to manage WM in Type II cultivars of common bean.

**MATERIAL AND METHODS:** Two trials were conducted in Viçosa and Oratórios, Zona da Mata region, State of Minas Gerais, Brazil, during the fall-winter season of 2013 in fields naturally infested with sclerotia of *Sclerotinia sclerotiorum*. Treatments were arranged as  $4 \times 2 \times 2$  factorial combination of within-row densities (4, 7, 10 or 13 plants/m), Type II lines (CNFC 10720 or VC 6), and fungicide (with or without). The line CNFC 10720 exhibits partial resistance to WM in the field and VC 6, susceptibility. Fluazinam was applied twice at 0.625 kg/ha for WM control at beginning of flowering and 10 days later. Each plot was five rows 0.5 m apart and 3 m long. Trials were irrigated with overhead sprinklers. A randomized complete block design with four replications was used. Mature plants in each plot ( $4.5 \text{ m}^2$ ) were rated for WM incidence, disease severity index (DSI), and seed yield. DSI was assessed by mean of a "quarter scale" (Hall and Phillips, 1996), in which plants were rated from 0 (no symptoms) to 4 (76 to 100% of the plants with symptoms). Based on this scale we calculated DSI (Vieira et al., 2010).

**RESULTS AND DISCUSSION:** Yields achieved over 3000 kg ha<sup>-1</sup> in both trials (Table 1). WM intensity (incidence + severity) was higher in Oratórios than in Viçosa. Both fungicide applications and resistant line decreased disease significantly in the trials, but only fungicide increased yield in both trials. The resistant line CNFC 10720 yielded 11% more than VC 6 (susceptible) in Viçosa, but their yields were similar in Oratórios. In Viçosa, L x WRD interaction was significant for DSI. The line VC 6 had higher DSI with 13 plants/m compared with the other WRDs, but DSI of the line CNFC 10720 was not affected by WRD. In Viçosa, L x F interaction was significant for WM intensity and yield. With fungicide, yield (around 3350 kg/ha) was not affected by common bean line; without fungicide, CNFC 10720 yielded more than VC 6 (2952 vs. 2442 kg ha<sup>-1</sup>) (data not shown). In Oratórios, L x WRD x F interaction was significant for yield (Table 2). Without fungicide, WRD did not affect yield of both lines, which means that organic farmers could use 4 plants/m under high pressure of WM. With fungicide, yield did not differ from 7 to 13 plants/m for the resistant line CNFC 10720, and from 4 to 10 plants/m for the susceptible line VC 6. Our results suggest that decreasing within-row density for Type II plants from 12-15 to 7-10 plants/m might help to manage WM in common bean fields under high pressure of WM. Cultivars resistant to WM can tolerate higher within-row densities than susceptible cultivars without decreasing yield, especially when fungicide is applied.

Factor		Viçosa Oratórios				
_	INC	DSI	Yield	INC	DSI	Yield
	(%)	(%)	$(\text{kg ha}^{-1})$	(%)	(%)	$(\text{kg ha}^{-1})$
Lines (L)						
CNFC 10720	17.0	8.6	3192	47.7	33.1	3066
VC 6	47.4	28.5	2870	62.1	48.4	2917
WRD						
(plants/m)						
4	30.6	15.2	2908	54.1	36.5	2869
7	27.8	14.5	3094	56.5	40.8	3065
10	31.8	19.3	3057	56.3	43.2	3084
13	38.4	25.2	3065	52.8	42.5	2947
Fungicide (F)						
without	45.9	29.1	2697	71.0	54.4	2319
with	18.5	8.0	3365	38.8	27.0	3663
L	**	**	**	**	**	ns
WRD	**	**	ns	ns	ns	ns
F	**	**	**	**	**	**
L x WRD	ns	**	ns	ns	ns	ns
L x F	**	**	**	ns	ns	**
WRD x F	ns	ns	ns	ns	ns	ns
L x WRD x F	ns	ns	ns	ns	ns	*

**Table 1**. Incidence (INC), disease severity index (DSI) and yield in response to common bean lines, within-row densities (WRD) and fungicide levels in Viçosa and Oratórios, State of Minas Gerais, Brazil.

\* = significant at 5% level, \*\* = significant at 1% level, ns = not significant at 5% level.

**Table 2.** Yield (kg/ha) in response to within-row densities, lines and fungicide (Fung. = fungicide applied twice, No fung. = fungicide not applied) in Oratórios, State of Minas Gerais, Brazil.

Withih-row density (plants/m)	CNFC 10720		VC 6		
	Fung.	No fung.	Fung.	No fung.	
4	3017 b	2681 a	3803 ab	1978 a	
7	3747 a	2611 a	3753 ab	2147 a	
10	3528 a	2628 a	4114 a	2067 a	
13	3836 a	2481 a	3508 b	1964 a	

\* Means in the column followed by the same letters indicate significant difference by Duncan's multiple range test (P < 0.05).

ACKNOWLEDGMENTS: CNPq, CAPES and FAPEMIG for financial support.

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## ATTRACTIVENESS AND INJURY OF COMMON BEAN LEAVES BY Heliothis virescens (FABRICIUS, 1781) (LEPIDOPTERA: NOCTUIDAE)

## W.I. Eduardo<sup>1</sup>, A.L. Boiça Júnior<sup>1</sup>, R.F.O. Moraes<sup>1</sup>, Z.A. Ribeiro<sup>1</sup> and B.H.S. Souza<sup>1</sup>

<sup>1</sup>FCAV/UNESP, Laboratório de Resistência de Plantas a Insetos, Via de Acesso Prof. Paulo Donato Castellane, s/n, 14884-900, Jaboticabal, SP, Brasil. E-mail: wellington ie@hotmail.com

## **INTRODUCTION**

Common beans, *Phaseolus vulgaris* L., is a protein-rich leguminous plant with great importance for Brazilian people's diet. Brazil is the largest producer and consumer of common beans worldwide, despite the recent low yield rates of 1044 kg.ha<sup>-1</sup> (CONAB, 2015). Injuries caused by pest insects are factors directly responsible for the low yields in common bean crops. Among the pest species infesting common bean plants, *Heliothis virescens* (Fabricius, 1781) (Lepidoptera: Noctuidae) is a species of difficult control due to its wide distribution, migratory behavior, high reproductive potential, and broad range of host plant species and plant phonological stages the insect can infest, including common beans (FITT, 1989).

The use of resistant genotypes may be an alternative for controlling *H. virescens* as it does not contaminate the environment, reduces the costs associated with insecticide applications, does not require specific knowledge by the farmer, and can be integrated with other control tactics (BOIÇA JÚNIOR et al., 2013). However, studies involving *H. virescens* and common beans are lacking in the literature. Therefore, in this study we aimed to evaluate attractiveness and injury of common bean leaves to *H. virescens* larvae.

## **MATERIALS AND METHODS**

The experiment was conducted under controlled conditions of temperature  $(25 \pm 1^{\circ}C)$ , relative humidity (60 ± 10%), and photophase (12 h). The experiment consisted of a no-choice feeding assay setup in completely randomized design with 10 genotypes, namely Pérola, IAC Harmonia, IAPAR 81, IAC Una, and IAC Carioca Eté, following methodology of Souza et al. (2012). For the no-choice assay, 9-cm-diameter Petri dishes were lined with moist filter paper where one leaf disc (2.5 cm diameter) prepared from the genotypes was placed individually. Third-instar *H. virescens* larvae were collected from a colony maintained in laboratory and fed artificial diet and were released in the Petri dishes in the proportion of one larva per Petri dish.

Assessments were made on the number of *H. virescens* larvae attracted to leaf discs at 1, 3, 5, 10, 15, 30 min and 1, 2, 6, 12, 24, and 48 h after the larvae were released in the Petri dishes. Leaf injury was assessed after the end of the assay by scoring visually the amount of injury done by the larvae on the leaf discs. The injury score in each leaf disc was an average of individual scores of three evaluators and ranged from 0-100%.

All data were analyzed for normality (Levene's test) and variance homogeneity (Cramer von Mises's test). Data with normal distribution and homogenous variance were subjected to analysis of variance (ANOVA), and means were compared by Tukey's test (P < 0.05). Otherwise, data were analyzed by the non-parametric Kruskal-Wallis's test.

## **RESULTS AND DISCUSSION**

The mean number of *H. virescens* larvae attracted to bean leaf discs at the 12-48 h time interval was lower on leaf discs of genotypes BRS Supremo and IAC Harmonia as compared to leaf discs of IAC Carioca Tybatã and IAC Diplomata (Table 1). Number of larvae attracted to leaf discs

increased over time in all genotypes due to larvae need to feed, hence a clearer differentiation was observed among genotypes relative to the first time intervals. The genotype IAC Harmonia was less injured by *H. virescens* larvae relative to Raz 49 (Table 1). This may have occurred due to allomones released by leaf disc of this genotype what may have altered larvae behavior, making them to reduce leaf intake. Based on our results, we conclude that genotype IAC Harmonia is less preferred by *H. virescens* larvae.

	Nui	nber of larv	of discs	T •	
Genotypes	Min	utes	Но	urs	Injury scores
	1-5	10-30	1-6	12-48	(70)
Pérola	0.3±0.15	0.3±0.13	0.5±0.11	0.7±0.06 ab	31.1±6.48 ab
<b>Raz 49</b>	$0.3 \pm 0.13$	$0.4 \pm 0.09$	0.5±0.14	0.7±0,09 ab	54.6±9.49 b
<b>BRS Supremo</b>	$0.1 \pm 0.07$	$0.2\pm 0.07$	$0.2 \pm 0.07$	0.4±0.08 a	39.6±11.64 ab
IAC Carioca Tybatã	$0.4{\pm}0.15$	0.3±0.15	0.5±0.12	0.9±0.05 b	31.8±7.74 ab
IAC Galante	$0.0{\pm}0.00$	0.1±0.05	0.4±0.09	0.6±0.07 ab	21.7±5.24 ab
IAC Diplomata	$0.3 \pm 0.13$	$0.1 \pm 0.07$	0.3±0.09	0.9±0.07 b	33.7±7.61 ab
IAC Harmonia	0.3±0.12	0.2±0.05	0.4±0.11	0.4±0.11 a	9.7±2.52 a
IAPAR 81	$0.5\pm0.13$	$0.3 \pm 0.08$	0.3±0.09	0.6±0.10 ab	28.7±4.44 ab
IAC Una	$0.4 \pm 0.14$	0.3±0.10	0.6±0.11	0.6±0.11 ab	20.4±5.13 ab
IAC Carioca Eté	$0.2\pm0.12$	0.1±0.10	0.3±0.11	0.6±0.11 ab	32.7±10.60 ab
F	-	-	-	3.24**	2.44*
Н	14.87 <sup>ns</sup>	17.40 <sup>ns</sup>	10.27 <sup>ns</sup>	-	-

**Table 1.** Mean number of *Heliothis virescens* larvae attracted and injury scores (%) on leaf discs of common bean genotypes in no-choice test.

Means followed by different letters in columns differed significantly by Tukey's test (P < 0.05). \*\*(P < 0.01), \*(P < 0.05), <sup>ns</sup>non-significant by Kruskal-Wallis's test (P < 0.05).

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## ATTRACTIVENESS AND LEAF CONSUMPTION OF COMMON BEAN GENOTYPES BY THE SOYBEAN LOOPER

## Z. A. Ribeiro<sup>1</sup>, L. Nogueira<sup>1</sup>, M. M. Di Bello<sup>1</sup>, A. L. Boiça Júnior<sup>1</sup>, B. H. S. Souza<sup>1</sup>

<sup>1</sup>Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, Departamento de Fitossanidade, Laboratório de Resistência de Plantas a Insetos, Via de Acesso Prof. Paulo Donato Castellane s/n°, CEP 14884-900, Jaboticabal, SP, Brazil. E-mail: zribeiro@fcav.unesp.br

## **INTRODUCTION**

The soybean looper, *Chrysodeixis includens* (Walker, 1857) (Lepidoptera: Noctuidae) is an insect pest that has caused concern to farmers in recent years. Failure in using the integrated pest management combined with disorderly pesticide applications, especially fungicides (Sosa-Gómez, 2014), has contributed to *C. includens* population increase and migration to other crops, such as common beans. In an attempt to diminish pesticide use in cropping systems, alternative control methods have been investigated, and host plant resistance is one of them (Boiça Júnior et al., 2014). Searching genotypes that express tolerance and/or resistance to pest insects represents an important step for plant breeding. Thus, the aim of this work were to evaluate attractiveness and leaf consumption of common bean genotypes to the soybean looper.

## **MATERIALS AND METHODS**

The study was performed in the Laboratório de Resistência de Plantas a Insetos of FCAV/UNESP, Jaboticabal, SP, Brazil, under environmentally controlled conditions. The following common bean genotypes were tested: Pérola, Raz 49, BRS-Supremo, BRS-Horizonte, IAC-Galante, IAC-Diplomata, IAC-Harmonia, IAPAR-81, IAC-Una, and IPR-Eldorado.

The experiment consisted of free-choice and no-choice assays setup in completely randomized design with 10 replications each. For the free-choice test, leaf disks (3.0 cm diameter) prepared from the respective genotypes were arranged in glass arenas (26.2 cm diameter x 5.0 cm height) lined with moistened filter paper, where one third-instar *C. includens* larva was released per genotype, totaling 10 larvae per arena. In the no-choice test, one leaf disk was placed in each Petri dish (9.0 cm diameter x 1.5 cm height) with one third-instar *C. includens* larva. In both tests, leaf disk attractiveness was recorded at several time points by counting the number of larvae feeding on the leaf disks. Additionally, leaf intake by *C. includens* larvae were recorded in leaf disks at the end of the assays after 12h of larvae release. For this, two leaf disks was offered to the larvae in the assays and the other leaf disk was used as aliquot. Leaf material was dried in the oven at 60°C during 48 h, and the dry weight consumed was determined by difference between the aliquot and the consumed leaf disk.

Data obtained from both feeding preference assays were transformed into  $(x + 0.5)^{1/2}$  and then were subjected to analysis of variance. Means of treatments were compared by Tukey test at 5% probability whenever significant.

## **RESULTS AND DISCUSSION**

In both tests there were no significant differences in leaf disk attractiveness to third-instar C. *includens* larvae at the time points assessed and in the average of the times, except at 1-5 min in the no-choice test (Table 1). At this time point, the genotypes IAC-Una and IPR-Eldorado were less attractive to the larvae compared to IAC-Harmonia. The highest dry weight consumed by C.

*includens* larvae was observed in genotypes Pérola and Raz 49 in the free-choice test, whereas IPR Eldorado had the lowest dry weight consumed. In the no-choice test, Pérola, IAPAR-81, IAC-Una, IAC-Galante, IPR-Eldorado, and Raz 49 exhibited the least dry weight consumed by the larvae, and BRS-Supremo and BRS-Horizonte had the highest dry weight consumed.

Low leaf consumption recorded in IPR-Eldorado in the free-choice test and in IAPAR-81, IAC-Una, IAC-Harmonia, and IPR-Eldorado in the no-choice test suggests these common bean genotypes are less preferred by third-instar *C. includens* larvae.

	FREE-CHOICE TEST						
Times							
Genotypes	1-5min	10-30min	1-3h	6-12h	Average	Dry weight consumed (mg)	
Pérola	0.30	0.20	1.06	0.60	0.66	3.44 b	
IAPAR-81	0.40	0.26	0.13	0.30	0.20	1.26 ab	
IAC-Una	0.00	0.00	0.26	0.30	0.22	1.52 ab	
IAC-Harmonia	0.70	0.46	0.46	0.50	0.58	1.30 ab	
BRS-Supremo	0.60	0.39	0.13	0.10	0.36	1.70 ab	
IAC-Diplomata	0.50	0.33	0.33	0.30	0.36	1.74 ab	
<b>BRS-Horizonte</b>	0.40	0.26	0.13	0.40	0.42	1.80 ab	
IAC-Galante	0.60	0.40	0.13	0.30	0.34	1.8 0ab	
IPR-Eldorado	0.10	0.06	0.53	0.40	0.34	0.68 a	
Raz 49	0.30	0.20	1.06	0.6	0.66	3.48 b	
<i>F-value</i>	1.32 <sup>NS</sup>	1.30 <sup>NS</sup>	2.05 <sup>NS</sup>	0.98 <sup>NS</sup>	0.89 <sup>NS</sup>	1.32***	
P-value	0.2853	0.2683	0.0616	0.4727	0.5428	0.0065	
NO-CHOICE TEST							
Genotypes	1-5min	10-30min	1-3h	6-12h	Average	Dry weight consumed (mg)	
Pérola	0.30 ab	0.46	0.33	0.50	0.44	2.64 a	
IAPAR-81	0.10 ab	0.26	0.53	0.30	0.34	2.84 a	
IAC-Una	0.00 a	0.13	0.26	0.30	0.18	3.92 a	
IAC-Harmonia	0.80 b	0.60	0.53	0.40	0.66	3.52 a	
BRS-Supremo	0.60 ab	0.20	0.26	0.40	0.38	8.30 c	
IAC-Diplomata	0.20 ab	0.60	0.66	0.30	0.50	5.32 abc	
<b>BRS-Horizonte</b>	0.20 ab	0.13	0.46	0.60	0.36	8.52 c	
IAC-Galante	0.30 ab	0.40	0.40	0.50	0.44	2.58 a	
IPR-Eldorado	0.00 a	0.13	0.33	0.50	0.24	4.78 a	
Raz 49	0.20 ab	0.53	0.33	0.40	0.40	6.00 bc	
<i>F-value</i>	2.28*	1.49 <sup>NS</sup>	0.42 <sup>NS</sup>	0.29 <sup>NS</sup>	0.91 <sup>NS</sup>	6.09***	
P-value	0.0355	0.1831	0.9167	0.9759	0.5242	< 0.0001	

**Table 1.** Number of third-instar *Chrysodeixis includens* larvae attracted to leaf disks and dry weight consumed (mg) of bean genotypes in free-choice and no-choice tests.

Means followed by different letters in columns differ significantly by Tukey test. \*Significant at 5% probability. \*\*\*Significant at 0.1% probability. <sup>NS</sup> Non- significant.

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## BIOLOGICAL ASPECTS OF Spodoptera frugiperda (J. E. SMITH) ON COMMON BEANS

## R.F.O. Moraes<sup>1</sup>, W.I. Eduardo<sup>1</sup>, S.A.M. Carbonell<sup>2</sup>, B.H.S. Souza<sup>1</sup>, and A.L. Boiça Júnior<sup>1</sup>

<sup>1</sup>FCAV/UNESP, Laboratório de Resistência de Plantas a Insetos, Via de Acesso Prof. Paulo Donato Castellane, s/n, 14884-900, Jaboticabal, SP, Brazil. E-mail: renatomoraes2@hotmail.com.
<sup>2</sup>Instituto Agronômico de Campinas, IAC, Campinas, SP, Brazil.

## **INTRODUCTION**

Common bean crop faces various challenges over development of plants, and among them there are the defoliating pests such as larvae of *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae). This species is native from the tropical and subtropical zones of the American continent, and is widely distributed through Americas (Souza et al., 2012). In Brazil, *S. frugiperda* is considered the main pest of corn (Cruz, 2009), but infestation of this herbivore in common beans can occur sporadically. This work aimed to evaluate development of *S. frugiperda* fed on different common bean genotypes, attempting to find an antibiosis-resistant genotype against this pest.

## **MATERIALS AND METHODS**

The experiment was carried out at Faculdade de Ciências Agrárias e Veterinárias – UNESP, Jaboticabal, SP, Brazil, Departamento de Fitossanidade, Laboratório de Resistência de Plantas a Insetos, under controlled conditions of temperature  $(25 \pm 1^{\circ}C)$ , relative humidity  $(60 \pm 10\%)$ , and photophase (12 h).

The experiment was setup in a completely randomized design with 30 replications per treatment. The following genotypes of common beans were evaluated for antibiosis against S. frugiperda: Raz 49, BRS Supremo, IAC Carioca Tybatã, IAC Galante, IAC Diplomata, IAC Harmonia, Iapar 81, IAC Una, and IAC Carioca Eté. Thirty neonate S. frugiperda larvae per genotype were individualized in Petri dishes lined with moistened filter paper containing leaves of common beans. Each Petri dish represented one replication. Every day, leaves were exchanged and excrements were eliminated, avoiding potential contamination and reduction in food quality. When the larvae ceased feeding, what indicates beginning of the pre-pupal stage, leaves were no longer offered to the larvae. Insects remained in the Petri dishes until adult emergence. The biological parameters recorded from S. frugiperda were duration and viability of the larval stage, weight of 10-d-old larvae, duration and viability of the pupal stage, weight of 24-h-old pupae, adult longevity, and duration of the entire cycle (larvae eclosion to adult death). Data obtained from the assay were submitted to the analysis of variance (ANOVA) and means were compared by Tukey test at 5% probability. For statistical analysis, data of larval and pupal viability were transformed into accosine  $(x/100)^{\frac{1}{2}}$ , and data recorded from the other biological parameters were transformed into  $(x + 0.5)^{1/2}$ .

## **RESULTS AND DISCUSSION**

Genotype IAC Harmonia affected negatively *S. frugiperda* larval development (Table 1). The larvae of *S. frugiperda* that had fed leaves of this genotype had longer duration of larval period (34.66 d), lower larval viability (10%), and lower larval weight (26.3 mg) as compared to the other genotypes. These effects on larval development characterize expression of antibiosis in IAC Harmonia genotype. In addition, IAC Harmonia significantly affected *S. frugiperda* total cycle; this biological parameter was longer in *S. frugiperda* that had fed this genotype. A longer

period of development would involve fewer pest generations per year, thus acting as a regulatory factor in population growth in the field. Pupal duration, pupal viability, pupal weight, and adult longevity of *S. frugiperda* were not influenced by the common bean genotypes. Based on the results herein reported, we conclude that IAC Harmonia is less suitable for *S. frugiperda* development, and that this genotype expresses antibiosis-type resistance.

**Table 1**. Duration of larval and pupal period (d), larval and pupal viability (%), weight of 10-d-old larvae and 24-h-old pupae (mg), adult longevity, and total cycle (d) of *Spodoptera frugiperda* fed on common bean genotypes.

Constrans	Larvae						
Genotypes	Period Viability		Weight				
RAZ 49	27.66 bc	50	).0 bcd	69.5 abc			
BRS Supremo	25.12 abc	53	3.3 bcd	103.8 bcd			
IAC Carioca Tybatã	28.76 c	43	3.3 abc	42.9 ab			
IAC Galante	26.08 abc	4	0.0 ab	134.2 cd			
IAC Diplomata	22.12 a	53	3.3 bcd	16	161.1 d		
IAC Harmonia	34.66 d	1	0.0 a	26	5.3 a		
IAPAR 81	23.91 abc	8	0.0 cd	140	140.2 cd		
IAC Una	26.38 abc	70	).0 bcd	95.0	95.0 abcd		
IAC Carioca Eté	23.42 ab	8	86.0 d	150.2 d			
F (Genotypes)	6.81**	** 7.36**		8.96**			
C.V. (%)	7.32	7.32 86.13		5	5.48		
Ganatunas	Pupae			Ac	lults		
Genotypes	Period	Viability	Weight	Longevity	Total cycle		
RAZ 49	11.92 a	81.2 a	199.4 a	4.46 a	44,23 b		
BRS Supremo	12.29 a	100.0 a	221.7 a	4.25 a	41,62 ab		
IAC Carioca Tybatã	11.60 a	83.3 a	216.2 a	4.72 a	44,08 b		
IAC Galante	12.30 a	83.3 a	204.4 a	4.40 a	43,52 ab		
IAC Diplomata	12.00 a	87.5 a	216.7 a	3.78 a	37,71 a		
IAC Harmonia	11.33 a	100.0 a	181.5 a	4.66 a	50,66 c		
IAPAR 81	11.80 a	83.3 a	210.9 a	4.55 a	40,50 ab		
IAC Una	12.38 a	90.0 a	224.2 a	4.42 a	43,52 b		
IAC Carioca Eté	12.47 a	88.4 a	207.4 a	3.95 a	40,21 ab		
F (Genotypes)	1.27 <sup>ns</sup>	0.56 <sup>ns</sup>	0.89 <sup>ns</sup>	1.49 <sup>ns</sup>	5.91**		
C.V. (%)	4.38	36.52	2.86	11.60	4.70		

Means followed by same letter in columns did not differ significantly by Tukey test at 5% probability.  $n^{s} = non-significant$ , \*\* = significant at 1%.

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## FEEDING NON-PREFERENCE OF BEAN GENOTYPES TO NEONATE Chrysodeixis includens (LEPIDOPTERA: NOCTUIDAE) LARVAE

## L. Nogueira<sup>1</sup>, M. M. Di Bello<sup>1</sup>, Z. A. Ribeiro<sup>1</sup>, B. H. S. Souza<sup>1</sup>, A. L. Boiça Júnior<sup>1</sup>

<sup>1</sup>Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, Departamento de Fitossanidade, Laboratório de Resistência de Plantas a Insetos, Via de Acesso Prof. Paulo Donato Castellane s/n°, CEP 14884-900, Jaboticabal, SP, Brazil. E-mail: lucianonogueiraagro@gmail.com

## **INTRODUCTION**

Defoliating caterpillars are one of the groups of pests of common beans. Among the species of caterpillars infesting this crop in Brazil, *Chrysodeixis includens* (Walker, 1858) (Lepidoptera: Noctuidae) stands out due to population outbreaks and damages caused in the plants, factors that are associated with uncontrolled pesticide use (Bueno et al., 2007). Alternative control methods have been developed for *C. includens*, such as host plant resistance. This control measure holds some advantages over the other methods as it does not require sophisticated technology by farmers for use, does not interfere with the environment, and has persistent, cumulative, and non-polluting effects (Boiça Júnior et al., 2013). The objective of this study was to evaluate feeding non-preference of common bean genotypes to neonate *C. includens* larvae.

## **MATERIALS AND METHODS**

The experiment was conducted in the Laboratório de Resistência de Plantas a Insetos of the Departamento de Fitossanidade, FCAV/UNESP, São Paulo, Brazil, under controlled conditions of  $25 \pm 2$  °C temperature,  $70 \pm 10\%$  relative humidity, and 12 h photophase. The following common bean genotypes were tested: BRS-Supremo, IAC-Diplomata, IAC-Tibatã, IAC-Carioca Eté, IAC-Una, IAC-Harmonia, IAC-Galante, Pérola, Raz 49, and IAPAR-81.

Seeds of the common bean genotypes were sown in 5-L-pots containing a mixture of soil, manure, and sand at 2:1:1 ratio, and were kept in a greenhouse until use. Plants of common beans were used at the vegetative stage V3-V4. The free-choice feeding assay was designed in randomized blocks, with 10 replications. Each replication consisted of a glass arena (26 cm diameter x 5 cm height) containing the leaf discs (2.5 cm diameter) of each genotype distributed equidistantly on moistened filter paper. Thirty neonate *C. includens* larvae were released in each glass arena, and leaf disc attractiveness to *C. includens* larvae was recorded 48 h after the larvae were released. To evaluate leaf injury caused by the larvae, percentage of estimated injury ranging from 0 to 100% was assigned for uninjured leaf discs and fully injured leaf discs, respectively.

Data recorded from leaf disc attractiveness were transformed into  $(x+0.5)^{\frac{1}{2}}$  and data recorded from leaf injury percentage were transformed into arcsine  $(x/100)^{\frac{1}{2}}$ . Next, data were subjected to analysis of variance, and means were compared by Tukey test at 5% probability when significant.

## **RESULTS AND DISCUSSION**

Percentage of neonate *C. includens* larvae attracted to leaf discs of common bean genotypes 48 h after the larvae were released shows that genotypes Pérola and Raz 49 were the most preferred for larvae feeding (Figure 1), with no significant differences between both genotypes. IAC-Diplomata genotype showed intermediate attractiveness to *C. includens* larvae. The other

common bean genotypes were less attractive to *C. includens* larvae, with attractiveness ranging from 4.95% to 9.90%, but did not differ from IAC-Diplomata.



**Figure 1.** Percentage attractiveness of common bean genotypes to neonate *Chrysodeixis includens* larvae after 48 h in free-choice assay. \*\*sectors with same letter are not significantly different by Tukey test at 5% probability.

Figure 2. Injury caused by neonate *Chrysodeixis includens* larvae in bean genotypes after 48 h in free-choice assay. \*\*Bars with same letter are not significantly different by Tukey test at 1% probability.

The lowest injury percentages were verified in leaf discs of the genotypes IAC-Galante (8.8%) and IAC Harmonia (9.6%), and the highest injury percentages were found in leaf discs of the genotypes Pérola (29%) and Raz 49 (26%) (Figure 2). According to Vendramim and Guzzo (2009), the repellent effect of a certain plant toward an insect may occur due to volatilization of chemicals released from its leaves, which negatively affect insect feeding preference. The result obtained for the IAC-Harmonia genotype corroborates the results obtained by Morando (2014), who evaluated consumption of *C. includens* larvae in bean genotypes and also found lower consumption in this genotype.

By comparing leaf disc attractiveness and injury of *C. includens* larvae, it is noted that there was a direct relationship between the highest attractiveness of Pérola and Raz 49 genotypes to the larvae after 48 h and the highest injury percentages recorded in these genotypes (Figures 1 and 2), evidencing their susceptibility to *C. includens*.

In summary, we conclude the genotypes IAC-Harmonia and IAC-Galante are the least preferred by neonate *C. includens* larvae in free-choice condition, and should be further investigated for resistance against this pest.

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# ARE THERE FOOD PREFERENCES OF *Epilachna varivestis* Mulsant BY SNAP BEAN CULTIVARS?

## María Teresa Rodríguez-González<sup>1</sup>; José Alberto Salvador Escalante-Estrada<sup>1</sup>; Lucero Roldán-Serrato<sup>2</sup>; Ildefonso Ronquillo Cedillo<sup>3</sup>. <sup>1</sup>Postgrado en Botánica, <sup>3</sup>Postgrado en Fitosanidad

<sup>1,3</sup>Colegio de Postgraduados. Campus Montecillo. Km 36.5 Carretera México-Texcoco, 56230. Teléfono 01(595) 952 02 00 ext. 1330. Montecillo, Texcoco, Estado de México, México. E-mail: mate@colpos.mx, jasee@colpos.mx. <sup>2</sup>Centro de Ciencias aplicadas y desarrollo tecnológico. Universidad Nacional Autónoma de México, Circuito Exterior S/N, Ciudad Universitaria, AP 70-186, CP 04510, México, D.F. México

## **INTRODUCTION**

In Mexico there is great phenotypic diversity in bean (*Phaseolus vulgaris* L.), manifesting in both, on the plant and in the grain, from morphological to physiological, physicochemical, biochemical, etc. In plant, the most important difference is in growth habits, with consequent differences in the canopy. For grain there are differences in color, seed size, content of carbohydrates, proteins, lipids, minerals, etc., (Salinas *et al.*, 2012; Guzmán *et al.*, 2002). It has been observed that such differences are related to food preferences by humans. By other hand, during cultivation of these genotypes in field, pests that feed on the different structures of the bean plant are presented. The beetle (*Epilachna varivestis* Mulsant) is a major pest, as both larvae and adults, feed on the leaves, flowers and pods in formation, although the most important damage is on the leaves; normally it feeds on the surface of tissue the underside of the leaf, leaving only the veins and part of the epidermis; the tissue quickly turns brown and dies. The aim of this study is to record if *E. varivestes* have food preference for a particular genotype of snap bean.

## **MATERIALS AND METHOD**

The study was established in a glasshouse of Colegio de Postgraduados, located in Montecillo, Campus Montecillo, Texcoco, Mexico, Mexico (19  $^{\circ}$  29 ' N, 98  $^{\circ}$  53' W, 2250 m of altitude). Seeds of snap bean, Strike (SK) (white seed) and Black Valentine (BV) (black seed) were planted in pots with five kilograms of soil (forest soil mixed with sand, in the ratio 3: 1). Five seeds per pot were seeded for each cultivar, for leaving two plants per pot, when both cultivars were established. At 20 days after sowing, each pot was covered with a sheath of transparent cloth and four adults of *E. varvestis* were placed per pot. Were allowed to be fed for four days, after which they were removed and then the leaf area per pot was measured, as well as the area consumed by the beetles. In the same way as the experiment was established in the vegetative stage for both cultivars, it was made for the reproductive stage (flowering), which occurred at 35 days after sowing.

## **RESULTS AND DISCUSSION**

In the vegetative stage of Black Valentine, the leaf area average per pot (two plants) was 136  $cm^2$ , and the area consumed in four days was of 50  $cm^2$ , representing an average percentage of total consumption of 36%. For the same vegetative stage, Strike showed an average leaf area per pot (2 plants) of 338  $cm^2$ , with an area consumed of 50  $cm^2$  by four beetles, in four days, representing an average percentage of total consumption of 15%.

For the reproductive stage, Black Valentine shows an average leaf area per pot (2 plants) of 1161 cm<sup>2</sup>, with an area consumed of 103 cm<sup>2</sup> by the four beetles, in four days, representing an average percentage of total consumption 10%. Strike showed (for the same period), on average leaf area per pot (2 plants) of 866 cm<sup>2</sup>, with an area consumed of 88.7 cm<sup>2</sup> by four beetles in four days, representing an average percentage of total consumption of 10%.

Although or the vegetative stage it was observed a higher percentage of consumption by the beetle for Black Valentine with respect to Strike, the BV has less leaf area and because of this, the leaf area consumed by the beetle for both cultivars is the same ( $50 \text{ cm}^2$ ). For the reproductive stage, despite differences between leaf area per pot and leaf area consumed observed between both cultivars, the percentage of consumption by the beetles is the same (10%).

## CONCLUSION

Epilachna varivestis adults don't evince food preferences about the snap beans studied.

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## SCREENING OF COMMON BEAN (*PHASEOLUS VULGARIS*) FOR RESISTANCE AGAINST ROOT-KNOT NEMATODES

# Vieira, A. F.<sup>1</sup>; Machado, M. A. M.<sup>1</sup>; Carneiro, A. R. T.<sup>1</sup>; Campos, R. G. C.<sup>1</sup>; Santos, J. M. C.<sup>1</sup>; Santos, L. P.<sup>1</sup>; Batista, M. S.<sup>1</sup>; Lopes, E. M. G.<sup>1</sup>; Sanglard, D. A.<sup>1,2</sup> and Rocha, F. S.<sup>1,3\*</sup>

<sup>1</sup>Instituto de Ciências Agrárias (ICA); <sup>2</sup>Lab. de Biotecnologia; <sup>3</sup>Lab. de Fitopatologia; Universidade Federal de Minas Gerais (UFMG), 39.404-547, Montes Claros, MG, Brazil; \*E-mail: rochafsplant@yahoo.com.br

**INTRODUCTION -** Several species of plant parasitic nematodes that survive in the soil need a free film of water in the soil solution to move toward the roots and start the process of parasitism (Chitwood 1998). The genus *Meloidogyne* (root-knot nematodes) is a typical sedentary endoparasites that induce formation of galls in roots, and are generally considered to be the most important nematodes in the production of common bean around the world (Abawi & Agudelo 1994). The main species found parasitizing common bean are *M. incognita* and *M. javanica* (Silva, 1998). The most common symptoms in common bean are thickening of the root, inhibition of root growth, cracks, detachment of the cortex, and symptoms reflexes as leaf yellowing, stunting, leaf drop, leaf wilting or even plant death. Host plants growing in heavily infested soils with *Meloidogyne* species have few roots few roots and absorb little water and nutrients (Vale et al., 2004), and its leads to large economic losses due to reduced productivity. The chemical control of *Meloidogyne* in soil with high population density is inefficient and charges the cost of production. In this context, management strategies such as the selection of resistant genotypes are essential to minimize damage and increase productivity.

MATERIAL AND METHODS - Genotypes 'BRS Vereda', 'BRS Itaim', 'BRS Pitanga' and Branco (crioulo) were tested. Briefly, pots (4 L) were filled with mixed soil in the proportion 1:1 (soil:sand) and genotypes were grown in the pots. Suspension eggs were obtained of tomato roots (Lvcopersicon esculentum Mill.) var. Santa Cruz Kada infected with M. javanica and M. incognita (race 3) from pure cultures maintained in the greenhouse. Then, roots were cut into pieces, followed by grinding in NaOCl 0.5% for 40 s with a kitchen blender (Hussey & Barker, 1973) and suspension were obtained (Coolen & D'Herde, 1972). Two weeks after sowing, 1500 eggs were inoculated into roots of each genotypes grown in pots and maintained in greenhouse. Inoculation in tomato cv. Kada was used as control. Forty days after the inoculation, the aerial part was cut and the roots removed from the soil. Then, the weight of fresh and dry matter, the shoot length, the weight of the root system, the numbers of galls, egg masses and eggs per g of roots g of each genotype were quantified. Plants with FR value higher than or equal to 1.0 were classified as host plants and those that showed FR lower than 1.0, as resistant to the nematode studied (Oostenbrink, 1966). Analyses of variance (ANOVA) were done with the program SISVAR (Ferreira, 2000). The data were analyzed using Skott-Knott's test at 5% probability to compare the means with the program SISVAR.

**RESULTS AND DISCUSSION** - All genotypes were susceptible to *M. javanica* and *M. incognita* race 3, with FR varying from 7.44 to 29.06 for *M. javanica*, and 8.39 to 24.46 for *M. incognita* race 3 (Table 1). The 'BRS Pitanga'genotype had the highest infectivity (galls and egg masses) and reproduction (eggs) for both species of *Meloidogyne*, compared with control. Consequently, higher FR was observed in this genotype, despite showed good growth of the aerial part. Lower FR occurred in the 'Branco' genotype. The parameters of the fresh and dry weight, varied between genotypes, and the shoot length was not significant between genotypes inoculated with the tested species. Moura & Moura (1994) evaluated the 'Branco' genotype and observed lower values for egg masses and galls, compared to 'BR-IPA' and '512 722 NA' of Phaseolus vulgaris species inoculated with M. javanica and M. incognita race 1. Peixoto et al.

(2011) evaluated three P. vulgaris accessions and found that the cultivar 'Pearl' did not reduced shoot when inoculated with M. incognita. Evaluation of plants resistant to Meloidogyne is influenced by the inoculum density, the source of inoculum (juveniles or eggs), the period of evaluation and the genetics of genotype resistance (Dong et al., 2007).

Table 1. Number of galls (NG), number of egg masses (EM) and number of eggs (NE) per gram of root and reproduction factor (RF) of *Meloidogyne javanica* (MJ) and *Meloidogyne incognita* race 3 (MI3) to different common bean accessions after inoculation were determined

Access	Species	NG	EM	NE	RF	WFM	WDM	SL
Drongo (grigula)	MI3	5 c	8 c	2.251 c	8,39 c	71,70 a	18,74 b	97 a
Dialico (crioulo)	MJ	5 c	6 c	1.677 c	7,44 c	76,77 a	22,53 a	102 a
DDS Varada	MI3	9 c	11 c	3.208 c	11,86 c	52,13 b	16,69 b	92 a
DKS Veleua	MJ	10 c	15 c	6.017 b	26,25 b	53,42 b	19,27 a	89 a
DDS Ditongo	MI3	33 a	42 a	10.653 a	24,46 b	59,64 b	17,97 b	83 a
DKS Fitaliga	MJ	21 b	27 b	8.998 a	29,06 a	51,78 b	16,42 b	102 a
DDS Itaim	MI3	12 c	14 c	5.732 b	18,54 b	50,55 b	20,45 a	153 a
DK5 Italili	MJ	10 c	13 c	2.729 c	8,83 c	54,04 b	20,39 a	149 a
Control	MI3	140	137	17.500	40.12	-	-	-
Control	MJ	156	140	17.800	41,05	-	-	-
CV (%)		85,65	73,03	58,23	34,51	14,96	19,47	24,76

WFM: weight of fresh matter (g); WDM: weight of dry matter (g); SL: shoot length (cm). Means followed by the same letter are not significantly different according to Scott and Knott's test at 5% probability.

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## PERSPECTIVES OF *DREB* GENES STUDIES TOWARD IMPROVEMENT OF DROUGHT TOLERANCE IN COMMON BEAN VARIETIES

## Enéas Ricardo Konzen<sup>1</sup>, Ana Carolina Vieira Zakir Pereira<sup>1</sup>, Danielle Gregorio Gomes Caldas<sup>1</sup>, Jorge Carlos Berny<sup>2</sup>, Andrea Ariani<sup>2</sup>, Gustavo Henrique Recchia<sup>1</sup>, Paul Gepts<sup>2</sup>, Siu Mui Tsai<sup>1</sup>

<sup>1</sup>Center of Nuclear Energy for Agriculture, University of Sao Paulo, SP, Brazil. <sup>2</sup>Department of Plant Sciences, University of California, Davis, CA, USA

## INTRODUCTION

The DREB (Dehydration Responsive Element-Binding) gene family has been extensively studied in several crop species, but little has been done with the common bean genes. DREB transcription factors regulate several other genes when plants are under abiotic stresses, such as water deficit. They participate in a cascade of processes trying to circumvent the stress condition. Drought is one of the major constraints affecting common bean cultivation, so it is essential to understand genetic factors and gene pathways involved in tolerance mechanisms to such stress. Cortés et al. (2012) first studied PvDREB genes by examining nucleotide diversity patterns in a collection of wild and domesticated genotypes. We have categorized 54 PvDREB genes based on Phytozome and Genbank databases, considering several in silico criteria (Konzen et al., 2014). Some representatives were selected, based on orthologs to Arabidopsis and soybean. Our work has been driven toward the analysis of possible associations between DREB genes and drought tolerance traits in common bean under two main approaches. First, we have been examining expression profiles and SNP polymorphic sites within genes, trying to find correlations with phenotypic data obtained through greenhouse drought screenings. Second, one specific candidate, PvDREB6A, has been extensively studied on the functional basis, with heterologous expression in Arabidopsis to verify improvement in tolerance under dehydration conditions.

## MATERIAL AND METHODS

Many *PvDREB* genes have been isolated so far, including *PvDREB1*, *PvDREB2A*, *PvDREB5*, *PvDREB6A* and *PvDREB6B*. Expression profiles under dehydration conditions have been performed with qPCR analysis. The open reading frame and/or partial promoter region of each gene have been isolated. Primers flanking those regions were designed and after amplification, products were purified and sequenced. Resequencing was performed in a series of common bean genotypes. Sequences were aligned and SNP polymorphic sites were traced by means of sequence alignment. SNP sites close to all the 54 *DREB* genes originally categorized (Konzen et al., 2014) were traced based on Illumina BARCBEAN6K\_3 Infinium SNP BeadChip, which allows the detection up to 5,398 SNP sites throughout common bean genome. Phenotypic evaluations of a wild Mesoamerican common bean collection were performed and analyses to determine possible associations between *DREB*-specific SNP and phenotypic data are in progress (Figure 1A).

*PvDREB6A*, ortholog to the Arabidopsis *AtRAP2.4*, was cloned and analyzed on the functional basis. The molecular features of this gene were unraveled with its subcellular localization and an Electro Mobility Shift Assay (EMSA) to corroborate its role as a transcription factor. Arabidopsis wild type and null mutants were transformed for overexpression of *PvDREB6A*. Transgenic plants and respective controls were subjected to abiotic stress treatments including drought, and their potential tolerance was evaluated based on survival, dehydration rate and ions leakage.

## **RESULTS AND DISCUSSION**

Among all genes, *PvDREB6B* expression profiles revealed increased relative amount of *PvDREB6B* transcripts under dehydration conditions, especially in leaves and roots (Figure 1B). The analysis of nucleotide variation in the wild bean collection has shown considerable number of polymorphic sites along its ORF, counted as 37 in a coding region spanning up to 957 bp. Other SNP markers were identified along a selected group of bean genotypes for the genes *PvDREB1*, *PvDREB2A* and *PvDREB5* and those have been converted to allele-specific markers. The closer SNP markers to each member of the 54 *PvDREB* were traced at the Illumina SNP BeadChip. All these markers are being considered for the next step of the study, aimed at the association study with phenotypic variation in wild beans (Figure 1A).



Figure 1 - A – Candidate-genes association mapping prospects involving *PvDREB* genes, highlighting *PvDREB6B*, candidate explored through nucleotide diversity; and B - Gene expression analysis under exposition to dehydration during several time intervals. C – Functional analysis of *PvDREB2A* in Arabidopsis. Columbia is the wild type, non-transgenic. Transgenic plants showed higher survival rate under drought.

On the other hand, the functional analysis of *PvDREB6A* revealed important prospects. Proved to be a transcription factor located at the nucleus (Pereira et al., in preparation), *PvDREB6A* was successfully transferred and expressed in Arabidopsis. Four transgenic events with better expression of this gene were selected: Col-0/pFEC2.1 #1, Salk\_020767C/pFEC2.1 #13.1, Salk\_020767C/pFEC2.1 #19.7 and Salk\_020767C/pFEC2.1 #23.7. Plants overexpressing *PvDREB6A* presented higher survival rates that non-transgenic ones under drought conditions (Figure 1C). Engineering this and others genes and transforming common bean plants should be encouraged to improve drought tolerance in common bean.

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## IDENTIFICATION OF OPAQUE BLACK BEAN GERMPLASM TOLERANT TO TERMINAL DROUGHT IN VERACRUZ, MEXICO

## Ernesto López-Salinas, Oscar Hugo Tosquy-Valle, Néstor Francisco-Nicolás, Francisco Javier Ibarra-Pérez

INIFAP, Campo Experimental Cotaxtla. Km 34.5 carr. Federal Veracruz-Córdoba, Medellín de Bravo, Ver., México <u>salinaser@hotmail.com</u>

**INTRODUCTION:** In Veracruz, Mexico around 36,000 hectares are annually planted with common beans (*Phaseolus vulgaris* L.), and 70% of this area is managed under residual soil moisture conditions either in the fall-winter or winter-spring growing seasons. Under these environments, terminal drought is the most important abiotic factor which highly limits dry bean production. Terminal drought occurs right after flowering, during pod and seed filling and can be prolonged up to physiological maturity. The objectives of this study were to classify black common bean genotypes as drought tolerant, and identify those of higher seed yield efficiency when grown under both, irrigated and terminal drought conditions.

**MATERIALS AND METHODS:** In 2013 winter-spring growing season two field experiments were conducted in a farmer's field located in La Colonia Ejidal, Cotaxtla, Veracruz, Mexico; one field trial was irrigated during the entire growing cycle, in the other, in contrast, irrigation was withheld right after the beginning of plant flowering to induce the terminal drought condition. Samples from 0-15, 15-30 and 30-45 cm of the soil profile were taken weekly during the entire growing cycle in both soil moisture treatments, to determine soil water content and the soil moisture available for plant growth and development (Aguilera and Martínez, 1980). 22 bean breeding lines were tested and compared to three commercial cultivars used as checks: Negro INIFAP, Jamapa and Negro Tacaná (Salinas *et al.*, 1997). The experimental design was RCBD with three replications, plots were composed of three 5.0 m long rows 0.72 m apart. Seed yield was quantified and two estimates of efficiency were used, the drought susceptibility index (DSI) proposed by Fisher and Maurer (1978), and the relative yield efficiency index (RYEI) proposed by Graham (1984).

**RESULTS AND DISCUSSION:** Rainfall did not occur during the entire plant reproductive phase that might have interrupted the terminal drought condition of the field experiment, a situation commonly taken place in the winter-spring growing season at this particular location. According to results, soil moisture balance at the terminal phase of growth and development of the bean plants indicates that the average available soil moisture was 69.4% and 37.4% for the irrigated and non-irrigated plots, respectively suggesting that plants of bean genotypes tested in the yield trial under irrigation grew without soil moisture stress, in contrast genotypes were certainly exposed to terminal drought under the non-irrigated yield trial. The average seed yield reduction of bean genotypes due to terminal drought was 778 kg ha<sup>-1</sup> and a DSI that averaged 0.41. NCB-229. SCN-2, Jamapa Plus and SEN-70 with DSI values lower than 0.81 represent the drought tolerant genotypes; in contrast, the drought susceptible genotypes were X02-33-159-2, X02-33-147-2, B-98311, MBSF-14729 and Negro Jamapa. The highest relative yield efficiency with and without irrigation (RYEI = 1.35) was obtained by CIAT-103-25, SCN-2, SEN-70, NGO 17-99 and NCB-229, while X02-33-159-2, B-98311, MBSF-14729 and Negro Jamapa showed the lowest values (RYEI = 0.56).

Genotypes	Irrigated	Drought	Average	Reduction (%)	$\mathrm{DSI}^\dagger$	RYEI <sup>††</sup>
NGO-17-99	2194.33 *	1329.67 *	1762.00 *	39.40	0.95	1.41
ELS-9-27	1680.67	1091.33	1386.00	35.07	0.85	0.88
ELS-15-55	1668.67	915.67	1292.17	45.13	1.09	0.74
Jamapa Plus	1686.00	1150.00	1418.00	31.79	0.77	0.93
NGO-07022	1855.67	1214.33	1535.00	34.56	0.83	1.09
DOR-448	1763.00	976.67	1369.83	44.60	1.08	0.83
CIAT-103-25	2227.67 *	1365.67 *	1796.67 *	38.69	0.93	1.47
SEQ-344-21	1799.00	1058.00	1428.50	41.19	0.99	0.92
B-98311	1647.33	790.67	1219.00	52.00	1.26	0.63
MBSF-14729	1660.33	850.67	1255.50	48.76	1.18	0.68
SCN-2	2103.67 *	1443.67 *	1773.67 *	31.37	0.76	1.46
SCN-3	1780.67	1024.67	1402.67	42.46	1.03	0.88
SCN-4	1979.67 *	1123.00	1551.33	43.27	1.04	1.07
SCN-6	2041.67 *	1192.33	1617.00 *	41.60	1.00	1.17
SEN-26	1670.33	1097.00	1383.67	34.32	0.83	0.88
SEN-56	2040.67 *	1245.33 *	1643.00 *	38.97	0.94	1.22
<b>SEN-70</b>	2118.67 *	1420.33 *	1769.50 *	32.96	0.80	1.45
NCB-229	1936.00 *	1460.33 *	1698.17 *	24.57	0.59	1.36
TLP-19	1878.00 *	982.33	1430.17	47.69	1.15	0.89
X02-33-159-2	1805.67	649.00	1227.33	64.06	1.55	0.56
X02-33-153	2016.67 *	1279.33 *	1648.00 *	36.56	0.88	1.24
X02-33-147-2	2068.33 *	861.33	1464.83	58.36	1.41	0.86
Negro INIFAP	1710.33	961.00	1335.67	43.81	1.06	0.79
Negro Tacaná	1984.33 *	1192.33	1588.33 *	39.91	0.96	1.14
Negro Jamapa	1672.00	866.67	1269.33	48.17	1.16	0.70
Average	1879.57 *	1101.65	1490.61	41.39	1.00	1.00
LSD (0.05)	383.69	242.27	239.63			

**Table 1.** Seed yield (kg ha<sup>-1</sup>) of 25 black common bean genotypes grown under irrigated and terminal drought conditions, yield reduction (%) and estimates of the drought susceptibility (DSI) and relative yield efficiency index (RYEI).

\*Genotypes statistically superior according to LSD (0.05). <sup>†</sup>Drought Susceptibility Index.

<sup>††</sup>Relative yield efficiency index

**CONCLUSIONS:** NCB-229, SCN-2, SEN-70 and Jamapa Plus, bean genotypes are considered tolerant to terminal drought based on high average seed yield in both soil moisture conditions and a low DSI value; the same bean genotypes together with CIAT-103-25 y NGO 17-99 also showed the highest relative seed yield efficiency in both, irrigated and terminal drought conditions.

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## **Evaluation of the Durango Diversity Panel under Drought Stress**

## Jennifer Trapp and Phillip Miklas

USDA-ARS, Prosser, WA 99350

The development of diversity panels for characterizing different drought tolerant traits is needed to facilitate identification of linked markers with utility for marker assisted breeding. In this study, the diversity panel formed is made up of race Durango germplasm from the semiarid highlands of Mexico and selected for its known high level of drought tolerance (Terán and Singh, 2002). One objective of our study was to evaluate the Durango diversity panel (DDP) for drought related traits.

## MATERIALS AND METHODS

The Durango diversity panel (DDP) consists of 160 race Durango lines from within the Middle American gene pool origin of domestication and developed from the BeanCAP diversity panel (http://beancap.org). The race Durango represents medium seed-sized (~30-40 g 100 seed<sup>-1</sup>) market types and for the DDP included 79 pinto, 19 pink, 37 great northern, 20 small red, and 5 'other' market classes. The majority were cultivars and germplasm releases from North America.

Experimental design included two replications and two treatments, drought stress (DS) and nonstress (NS) in a lattice, 8 x 20, incomplete block design planted in early June 2014. Lines were planted in four-row plots, with 3 m length and 0.6 m row spacing. Traits measured include days to flowering (DF), harvest maturity (HM), days to seed fill (DSF), biomass (BM), pod wall ratio (PW), seed weight (SW), seed yield (SY), average basal root angle (BRA), basal root number (BRN), basal root whorl number (BRWN), number of adventitious roots (ADV), disease rating, nodule rating and tertiary branching score. Least squared means were determined for all traits using Proc Mixed (SAS Institute, 2008). Replication and blocks were fit as random effects and genotype and treatment as fixed effects. Phenotypic correlations between pairs of traits were calculated with means using the PROC CORR Spearman procedure of SAS. Principle component analysis (PCA) was performed using PROC FACTOR and the Rotate option in SAS.

## **RESULTS AND DISCUSSION**

A moderate level of drought stress was achieved as indicated by a drought intensity index (DII) of 0.4. The highest yielding varieties under NS were 'Monterrey', 'Tarus', and 'LaPaz' and under DS breeding lines PT9-5-6, PT9-17, and PT7-2, from USDA-ARS Prosser and 'Monterey' all had average yields above 3000 kg ha-1. PCA was conducted using 13 traits under DS. The results indicate that under NS the first three components explained 91% of the variation while the first component explained 50% of the observed variation. DF and SY produced large loading values for PC1 and DSF and HM had the largest loading values on PC2 (Figure not shown). Under DS, three factors explained 92% of the variation and PC1 alone explained 42% of the observed phenotypic variance. Traits DSF, HM, and PW had the largest loading values on PC1, respectively (Figure 1). Root traits grouped together in the third PC under NS and DS. Pearson's correlation coefficient showed greater BM and more efficient partitioning from pod wall to seed (PW) associated with increased SY under DS and NS; however, the importance of the association was reversed (DS: PW – r = -0.64\*\*\*; BM – r = -0.37\*\*\* and NS: PW – r = -0.24\*\*; BM – r = -0.46\*\*\*).

PCA is tool that can be used to further evaluate the importance of observed traits and allow the breeder to focus on the most relevant traits thereby increasing selection efficiency. The

Durango diversity panel indicates that by selecting lines with more efficient partitioning, fewer days to seed fill, and fewer days to maturity progress toward identifying more drought tolerant lines is enhanced.

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## Figure 1.



PC1 vs PC2 under DS

## BEHAVIOR OF THREE COMMON BEAN SEEDLINGS UNDER WATER DEFICIT CONDITIONS

# E.S. Pereira<sup>1</sup>, K.N.N. Coelho<sup>1</sup>, C.L.F.C. Souza<sup>1</sup>, A.P. Martins Filho<sup>1</sup>, T.R. Ferreira<sup>1</sup>, R.S. Okumura<sup>1</sup>, C.A.B. Andrade<sup>2</sup>, A.K.S. Lobato<sup>1</sup>

<sup>1</sup>Núcleo de Pesquisa Vegetal Básica e Aplicada; Universidade Federal Rural da Amazônia <sup>2</sup>Departamento de Agronomia; Universidade Estadual de Maringá E-mail: <u>allanllobato@yahoo.com.br</u>

## **INTRODUCTION**

The water deficit is an abiotic factor that affects the agricultural production with major frequency and intensity, influencing the aspects related with plant development, such as decrease in photosynthesis rate, reduction in leaf area, and stomatal closing (Barbosa et al ., 2013). Aim of this study was to describe the interferences related to water limitations in three seedlings of common bean largely cultivated in Brazil.

## **MATERIALS AND METHODS**

Study was implemented in Núcleo de Pesquisa Vegetal Básica e Aplicada of the Universidade Federal Rural da Amazônia, Brazil with seeds of *Phaseolus vulgaris* cvs. IPR-Siriri, IPR-Uirapuru and IAPAR 81. Experiment was organized in a factorial with four concentrations of - 0.6, -0.4, -0.2 and 0.0 (control) MPa of polyethylene glycol 6000 (PEG 6000) combined with three cultivars (IPR-Siriri, IPR-Uirapuru and IAPAR 81), being used five repetitions, and each repetition with 100 seeds. The seeds were placed in germitest paper with dimensions (length×width;  $38\times30$  cm), being prepared rolls, and it were kept in plastic container. These seeds were soaked with distilled water and PEG 6000 solutions in concentrations previously described. The Nine days after experiment implantation (Brazil, 2009), the parameters evaluated were hypocotyl length and root length, being expressed in cm. The images were obtained with *Olympus* digital camera with eight megapixels. An analysis of variance was performed, and when significant differences were present, a Scott-Knott test with a 5% level of error probability was used.

## **RESULTS AND DISCUSSION**

In relation to hypocotyl length, the treatments under -0.4 and -0.6 MPa of PEG, the better results were showed in IAPAR 81(Fig. 1 A). The concentration of concentration of -0.2 MPa the IPR-Uirapuru have better performance, while in 0.0 MPa of PEG promoted higher values linked to IPR-Siriri cultivar. The water deficit affected of negative form the hypocotyl length (Blue boxes), being this effect linked to lower water availability and the reduction in plant growth, caused by the decrease in cell expansion from decrease of the turgescence cell (Ávila et al., 2007). Similar results were described by Costa et al., 2004 studying *Glycine max* seeds submitted to water deficit.

The water restriction occasioned decrease in root length to all cultivars. In IPR-Uirapuru and IPR-Siriri cultivars presented significant reduction to concentration -0.2 and -0.6 MPa of PEG, respectively (Fig. 1 B). For IAPAR 81, occurred not significant differences, and in concentrations -0.2 and -0.4 MPa of PEG were showed better performances. The decrease in root length (Blue boxes) was promoted by the increase in concentrations of PEG, being this fact related to lower osmotic potentials induced restriction in water absorption by the seed and consequent inhibition in root elongation rate. These results are corroborated by Queiroz et al. (1998) evaluating the effects of the different osmotic conditions on *Phaseolus vulgaris* seedlings.



**Fig. 1:** Hypocotyl length (A) and root length (B) of three common bean cultivars exposed to concentrations -0.6, -0.4, -0.2 and 0.0 (control) MPa of PEG 6000. Means followed by the same letter in equal concentrations are not significantly different by the Scott-Knott test at 5% of probability. Blue boxes are presented the symptoms linked to water restriction in seedlings of IPR-Siriri (above), IPR-Uirapuru (center) and IAPAR 81 (below) submitted to concentrations - 0.6, -0.4, -0.2 and 0.0 (control) MPa of PEG 6000. conclusão

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## BIOMETRIC ANALYSIS OF COMMON BEAN GENOTYPES UNDER SEMI-ARID CONDITIONS IN PARAÍBA, BRAZIL

# Sanglard, D. A.<sup>1,2\*</sup>; Machado, M. A. M.<sup>1</sup>; Campos, R. G. C.<sup>1</sup>; Vieira, A. F.<sup>1</sup>; Santos, J. M. C.<sup>1</sup>; Carneiro, A. R. T.<sup>1</sup>; Santos, L. P.<sup>1</sup>; Batista, M. S.<sup>1</sup>; Lopes, E. M. G.<sup>1</sup> and Rocha, F. S.<sup>1,3</sup>

<sup>1</sup>Instituto de Ciências Agrárias (ICA); <sup>2</sup>Lab. de Biotecnologia; <sup>3</sup>Lab. de Fitopatologia; Universidade Federal de Minas Gerais (UFMG), 39.404-547, Montes Claros, MG, Brazil; \*Email: demerson.ufmg@gmail.com

**INTRODUCTION** - The semi-arid regions are limited in agricultural activities due to high salinity in soil and irregular rainfall that cause the phenomenon of drought. Adapting to these stresses is a multigenic and dependent on various physiological and morphological characteristics function. Therefore, salinity tolerant genotypes should provide qualitative and quantitative differences in gene expression (Pimentel et al., 1990). Changes in enzymes activity is based on its specific susceptibility to stress-causing agent, or the result of a single event such as the activation of certain proteases that affect the function of various enzymes (Vieira et al., 2000). The hydrolytic enzyme  $\alpha$ -amylase is produced by the aleurone layer in response to the action of gibberellins and released into the endosperm which causes the conversion of starch into sugars, used for embryo growth (Arteca 1995). Similarly, the acid phosphatase is an enzyme of the hydrolase type that acts in the metabolism of carbohydrates and phosphates, participating in the mobilization of storage proteins, especially during germination and seedling growth. This work aimed to perform biometric tests under field conditions with five bean genotypes, which were previously selected in the laboratory for salt tolerance (Brazil, 1992).

MATERIAL AND METHODS - The experiment was carried out on June 6, 2012, in the municipality of Sumé, Paraíba State, Brazil. The genotypes 'CNFC 1635', 'CNFC 9504', 'Jalo 08', 'PI-40-356-77' and 'PI-40-356-60' were evaluated. The genotypes 'Pérola' and 'Ouro Negro' were used as control. The experimental design was completely randomized blocks, with three replications of plots of three lines of 4.0 m long, spaced 0.5 m, with a density of 15 seeds/m. The emergence rate was expressed as percentage of germination. A soil analysis to confirm the saline edaphic condition was performed. The irrigation system was dripping with average flow of 12 mm.h<sup>-1</sup>. The weeds were controlled by manual weeding in pre-plant and postemergence. The analysis of variance considered the effects of treatment and the mean as fixed. We also evaluated the following quantitative traits: i) The number of days to flowering (BLW) number of days after planting until at least 50 % of plants showing a flower; ii) Number of days to maturity (MAT) - number of days after planting up to 90 % of dry pods; iii) "Stand" end (SE) total number of plants present in the portion of the harvest date; iv) Number of pods per plot (PODP) - total number of pods per plot; v) Weight of 100 seeds (WS100) - weight of 100 seeds sampled from each plot; vi) Grain production (PRODT) - total weight of seeds of each portion in grams; vii) Average number of pods per plant (ANPP) - it was calculated by dividing PODP by SE; viii) Average number of seeds per plant (ASPP) - it was calculated by the formula [(PRODT x 100) / (WS100 x SE)]; ix) Average number of seeds per pod (ASPOD) - it was calculated by dividing ASPP by ANPP; x) Average production per plant (APROP) - it was calculated by dividing PRODT by SE; xi) Average production per pod (APRODPOD) - it was calculated by dividing PRODT by PODP. Statistical analyzes were performed by the GENES program (Cruz, 2006).

**RESULTS AND DISCUSSION** - The soil analysis showed high salinity and electrical conductivity (EC) of 11.82 dSm<sup>-1</sup> (EC = 4.00, maximum value for saline soils). The results of germination percentage for each plot and average productivity are shown in Table 1. Note that

the selected lines exceeded 80%, highlighting the genotypes 'CNFC 1635' e 'CNFC 9504' which showed yield above 2400 kg/ha.

Constras		Weighted			
Genotypes	Portion 1	Portion 2	Portion 3	Average	productivity (kg/ha)
CNFC 1635	96.7	90.0	85.6	90.7	2678.70 a
CNFC 9504	84.4	72.2	84.4	80.4	2456.80 a
Jalo 08	82.2	83.3	82.2	82.6	1925.10 ab
PI-40-356-77	88.9	72.2	82.2	81.1	1737.26 b
PI-40-356-60	81.1	74.4	93.3	83.0	1150.40 c
Ouro Negro	6.6	0.0	0.0	2.2	unexpressive
Pérola	0.0	10.0	10.0	5.0	unexpressive

 Table 1. Percentage of germination of five genotypes and two control and productivity weighted according to the percentage of germination

.\*Genotypes used as control due to worse results in previous testing laboratory.

The results of the analysis of variance and mean square for each quantitative trait are shown in Table 2. There were significant F values at 1% probability level among genotypes for all characters, indicating the existence of considerable genetic variability. This genetic variability is crucial for identification of QTL's associated with each quantitative characteristics.

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Quantitative	Averages						
characteristics	CNFC 1635	CNFC 9504	Jalo 08	PI-40-356-77	PI-40-356-60	QMg	
BLW	40.50	41.25	43.24	45.75	42.15	12.19**	
MAT	92.75	89.60	95.78	99.50	95.64	17.23**	
ANPP	14.41	17.56	14.87	18.09	16.48	28.82**	
WS100	20.61	21.00	20.04	18.47	19.06	13.59**	
ASPP	70.14	81.75	77.92	94.73	80.54	787.52**	
ASPOD	4.85	4.16	4.90	5.22	4.90	0.35**	
APROD	14.51	16.45	17.12	17.45	15.31	27.33**	
APRODPOD	1.00	0.92	0.94	0.96	0.93	0.03**	

**Table 2.** Analysis of variance and mean quantitative characteristics of common bean genotypes tolerant to salinity

\*\*Significant at 1% probability by F test; BLW (number of days to flowering); MAT (number of days until maturity); ANPP (average number of pods per plant); WS100 (weight of 100 seeds); ASPP (average number of seeds per plant); ASPOD (average number of seeds per pod); APROP (average production per plant) and APRODPOD (average production per pod).

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# SEED YIELD INCREMENTS USING DIFFERENT IN-FIELD RAINWATER HARVESTING TECHNIQUES UNDER RAINFED CONDITIONS IN DURANGO, MÉXICO

# Hilario Flores-Gallardo<sup>1\*</sup>, Reynaldo Alfredo Domínguez-Gándara<sup>1</sup>, Rigoberto Rosales-Serna<sup>1</sup>, Héctor Flores-Magdaleno<sup>2</sup>

 <sup>1</sup>INIFAP-Campo Experimental Valle del Guadiana. Carretera Durango - El Mezquital, km 4.5, Durango, México. C. P. 34170. Tel. +52 (618) 826-04-26, ext. 207.
 \*flores.hilario@inifap.gob.mx
 <sup>2</sup>Colegio de Postgraduados. Programa de Hidrociencias. Carretera México – Texcoco, km 36.5, Montecillo, Estado de México. C. P. 56230.

# INTRODUCTION

Water shortage is the most limiting factor producing common bean under rainfed conditions in northern México. The traditional cropping system in Durango including straight furrows and high slope rocky-soils lead to significant water and soil losses during rainy years. Sustainable agriculture requires an efficient soil management technique in order to increase in-field rainwater harvesting and to enhance soil conservation. In Durango, significant yield increments were observed for common bean using tie ridging and contour furrows (Cuéllar, 2007; Flores *et al.*, 2014). The results obtained by using in-field rainwater harvesting techniques need to be corroborated at several environmental conditions in order to establish profitability under local production systems. The objective of this research was to evaluate increments in seed yield obtained by using different rainwater harvesting techniques under rainfed conditions in Durango, México.

#### MATERIALS AND METHODS

In 2013 and 2014, four rainwater harvesting techniques were evaluated at several locations of Durango, Mexico. Treatments included traditional method with straight furrows (SF), compared to contour furrows (CF), SF + tie ridging (TR) and CF + TR. Semi-commercial plots (1 hectare: 2,500 m<sup>2</sup> per each technique) were sown at five environments (Durango 2013, Francisco I. Madero 2013, Canatlán 2013, Durango 2014 and Peñón Blanco 2014). Early maturity 'Pinto Centauro' common bean cultivar (Rosales *et al.*, 2012) was sowed in delayed planting dates (late July and early August in 2013 and mid-July and early August in 2014) due to late precipitation registered under rainfed conditions. Foliar fertilizer was sprayed at the rate of 2.5 liter/hectare during reproductive period in 2013 and 2014. At maturity, five plant samples consisting in two furrows of 5 m in length by 0.81 m in width (8.1 m<sup>2</sup>) were taken in each plot for yield determinations. Analysis of variance was obtained under completely randomized design combined over locations and five replications. Mean comparison tests were performed by Tukey's significant difference test (HSD, P  $\leq$  0.05).

# **RESULTS AND DISCUSSION**

Highly significant (P  $\leq$  0.01) differences for seed yield were observed among rainwater harvesting techniques across locations (except for Peñón Blanco). Higher yield average values were observed for the combination of straight furrows plus tie ridging (1,527.8 kg ha<sup>-1</sup>) and contour furrows plus tie ridging (1,524.5 kg ha<sup>-1</sup>). Similar results were obtained in previous experiments testing different rainwater harvesting techniques (Cuéllar, 2007). Lower average seed yield across locations were registered for traditional (SF) sowing method (1,270.4 kg ha<sup>-1</sup>) and contour furrows (1,220.7 kg ha<sup>-1</sup>). Compared to traditional method (SF), average yield

increment was observed using SF + TR (20 %) and CF + TR (20 %). Adoption encouraging programs need to be implemented in order to use in-field rainwater harvesting techniques and to improve common bean seed yield in the Durango state. Higher average seed yield was obtained in Durango (1,835.8 kg ha<sup>-1</sup>) and Canatlán (1,881.0 kg ha<sup>-1</sup>) and this response was related to the high amount of rainfall, low soil slope (0.89-1.56 %) and greater soil depth (> 50 cm). On average, lowest seed yield was registered in Peñón Blanco in despite of the higher seasonal amount of rainfall (341 mm), compared to Canatlán 2013 (331 mm) and Durango 2014 (337). Results were related to delay sowing, soil slope of 1.92 %, soil traits (sandy and rocky) and rainfall with bad distribution through growing season. Soil traits are relevant for an efficient selection of an appropriate in-field rainwater harvesting technique. Tie ridging combined with straight or contour furrows was identified as an important agricultural practice that increases by 20 % the seed yield under rainfed conditions. Incentive programs need to be implemented in order to encourage farmer's adoption of the in-field rainwater harvesting techniques leading significant advances for seed yield, soil conservation and the development of sustainable agriculture.

**Table 1.** Yield registered in common bean cultivar 'Pinto Centauro' grown in different rainwater harvesting techniques at five environments in Durango, México (2013-2014).

Rainwater Harvesting	Durango	Francisco I.	Canatlán	Durango	Peñón	Average
Technique	2013	Madero	2013	2014	Blanco	
-		2013			2014	
		Seed Y	ield (kg ha	1)		
Straight Furrows (SF)	1,348.0 <sup>c</sup>	967.9 <sup>d</sup>	1,464.2 <sup>c</sup>	1,874.1 <sup>b</sup>	698.0	1,270.4
Contour Furrows (CF)	1,363.0 <sup>b</sup>	1,138.3 <sup>b</sup>	1,328.3 <sup>d</sup>	1,604.9 <sup>c</sup>	669.1	1,220.7
SF + Tie Ridging (TR)	1,229.6 <sup>d</sup>	1,281.5 <sup>a</sup>	1,967.9 <sup>b</sup>	2,269.1 <sup>a</sup>	891.1	1,527.8
CF + TR	1,404.9 <sup>a</sup>	1,116.0 <sup>c</sup>	2,765.4 <sup>a</sup>	1,595.1°	741.0	1,524.5
Average <sup>1</sup>	1,336.4 <sup>b</sup>	1,125.9 <sup>c</sup>	1,881.5 <sup>a</sup>	1,835.8 <sup>a</sup>	749.8 <sup>d</sup>	
Accumulated rain (mm)	283	318	331	337	341	
100 seed weight (g)	32.2	31.5	32.4	32.1	31.2	

 $^{a-d}$  = Letters in each column indicate significant differences according to Tukey's Significant Difference test (P  $\leq 0.05$ ). Average<sup>1</sup>: obtained for comparison between locations.

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# ELECTROLYTE LEAKAGE AND OXIDANT COMPOUNDS IN COMMON BEAN SEEDLINGS INDUCED TO SALT STRESS

# G.D.M. Viana<sup>1</sup>, M.A.M. Barbosa<sup>1</sup>, J.R.S. Barbosa<sup>1</sup>, T.S. Pereira<sup>1</sup>, O.N. Silva<sup>1</sup>, G.A.R. Alves<sup>1</sup>, E.M.S. Guedes<sup>1</sup>, A.K.S. Lobato<sup>1</sup>

<sup>1</sup>Núcleo de Pesquisa Vegetal Básica e Aplicada; Universidade Federal Rural da Amazônia E-mail: <u>allanllobato@yahoo.com.br</u>

#### **INTRODUCTION**

The higher salinity in soil is a problem serious that occurs in several Brazilian regions, caused mainly by the sodium salts, in particular by the sodium chloride (NaCl), that induces changes in morphological, physiological and biochemical levels in higher plants. In plant cells, the physical and chemical proprieties of the cell membranes can be modified by the salt stress (Esteves and Suzuki, 2008). The aim of this research was to evaluate the electrolyte leakage and oxidant compounds in common bean seedlings submitted to salt stress.

#### MATERIALS AND METHODS

Study was implemented in Núcleo de Pesquisa Vegetal Básica e Aplicada of the Universidade Federal Rural da Amazônia, Brazil with seeds of *Phaseolus vulgaris* cvs. IPR-Siriri, IPR-Uirapuru and IPR-139. Experiment was organized in a factorial with four concentrations of 0, 50, 100 and 150 mM NaCl combined with three cultivars (IPR-Siriri, IPR-Uirapuru and IPR-139), being used five repetitions, and each repetition with 100 seeds. The seeds were placed in germitest paper with dimensions (length×width; 38×30 cm), being prepared rolls, and it were kept in plastic container. These seeds were soaked with distilled water and NaCl solutions in concentrations previously described. The Nine days after experiment implantation (Brazil, 2009), the parameters evaluated were electrolyte leakage, glutathione and hydrogen peroxide, being measured in root tissue. An analysis of variance was performed, and when significant differences were present, a Scott-Knott test with a 5% level of error probability was used.

# **RESULTS AND DISCUSSION**

In electrolyte leakage, the IPR-Uirapuru presented lower accumulation in concentrations 50, 100 and 150 Mm NaCl (Fig. 1 A), when compared to other cultivars. This fact reveals that the IPR-Uirapuru cultivar is more tolerant to salt stress. The increase showed in all cultivars related to electrolyte leakage can be explained due to salinity to induce higher damages in cell membranes (Cao et al, 2007). Similar results were reported by Houimli et al. (2010) evaluating the salt stress in *Capsicum annuum* cultivars.

All cultivars analyzed presented increases in glutathione (GHS) levels when submitted to concentrations of 150 mM NaCl (Fig. 1 B). The IPR-139 had larger sensibility to salt stress. The increase in GSH to *Phaseolus vulgaris* seedlings is an indicator that occurred changes in oxidant metabolism, such as glutathione-ascorbate cycle (Nagesh Babu and Devaraj, 2008). Kaymakanova et al. (2009) also showed increase in glutathione of *Phaseolus vulgaris* plants submitted to salt conditions.

The salt stress provoked increase in hydrogen peroxide in all cultivars evaluated, more the IPR-Siriri cultivar presented higher sensibility, mainly in 150 mM NaCl (Fig. 1 C). The increase in hydrogen peroxide can be associated with lower enzyme activity responsible by the attenuation of oxidant compounds that generally are accumulated during salt stress (Weisany et al, 2012). Shahid et al. (2012) working with different *Pisum sativum* genotypes under salt stress also showed increase in hydrogen peroxide.



**Fig. 1:** Electrolyte leakage (A) glutathione (B) and hydrogen peroxide (C) in IPR-Siriri, IPR-Uirapuru and IPR-139 cultivars exposed to concentrations of 0, 50, 100 and 150 mM NaCl. Means followed by the same letter into each cultivar are not significantly different by the Scott-Knott test at 5% of probability.

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# SELECTION OF COMMON BEAN GENOTYPES WITH POTENTIAL FOR SALINITY TOLERANCE

# Sanglard, D. A.<sup>1,2\*</sup>; Machado, M. A. M.<sup>1</sup>; Santos, J. M. C.<sup>1</sup>; Carneiro, A. R. T.<sup>1</sup>; Campos, R. G. C.<sup>1</sup>; Vieira, A. F.<sup>1</sup>; Santos, L. P.<sup>1</sup>; Batista, M. S.<sup>1</sup>; Lopes, E. M. G.<sup>1</sup> and Rocha, F. S.<sup>1,3</sup>

<sup>1</sup>Instituto de Ciências Agrárias (ICA); <sup>2</sup>Lab. de Biotecnologia; <sup>3</sup>Lab. de Fitopatologia; Universidade Federal de Minas Gerais (UFMG), 39.404-547, Montes Claros, MG, Brazil; \*Email: demerson.ufmg@gmail.com

**INTRODUCTION** - Beans are the most important legume for human consumption in the world, is important in the primary diet of protein, carbohydrates and other minerals. It is an extremely diverse culture in terms of cultivation methods, uses, adapting to different environments and morphological variety. However, due to abiotic stresses during the development of bean such as salinity, have many losses in grain production. Despite successful efforts in Brazil and other countries, focusing on breeding cultivars susceptible to different adversities, still need to identify promising genotypes for inclusion in breeding programs (Vieira et al., 2000). This study aimed to evaluate bean genotypes tolerance to salt stress.

MATERIAL AND METHODS - One hundred and fifty genotypes were evaluated for germination and emergence capacity in saline substrate, provided by the Brazilian Agricultural Research Corporation - EMBRAPA Rice and Beans; Federal University of Vicosa - UFV; Federal University of Uberlândia - UFU and Federal University of Piauí - UFPI. In plastic pots (4 liter) with substrate (soil and dung 4:1) and containing saline (100 mMol.L<sup>-1</sup> of sodium chloride) at 60% of its mass, the seeds of each genotype to be tested were seeded in 20 mm deep. Six seeds were placed in each pot and six replications were adopted. The fertilizer was applied according to soil analysis and recommendations for culture. The emergence percentage was calculated based on the number of seedlings to the fifteenth day after sowing. Were considered emerged seedlings that showed larger than 20 mm of shoot. For seed analysis was used genotypes above 50% germination. The following tests were performed: i) Germination - carried out with four replications of 50 seeds germitest distributed over two sheets, moistened 2.5 times its weight of sodium chloride solutions at concentrations of 0, 25, 50 and 100 mMol.L<sup>-1</sup>. The seeds were placed to the pre-germination chamber at 25 ° C and the normal seedlings counts performed as Rules for Seed Analysis (RAS) (Brazil, 1992); ii) seedling length - Ten seedlings of each replication of the germination test were used to determine the shoot height and length of the root system; and iii) Electrical conductivity - were four replicates of 25 seeds previously counted, weighed and immersed in 75 ml of sodium chloride solutions in the previously described concentrations for one hour. After this period, the seeds were transferred to deionized water, left for 24 hours at 20 °C and reading (umhos. cm<sup>-1</sup>.g<sup>-1</sup> of seed) in conductivity (Digimed). The experimental design was completely randomized, with three replications. The data of the treatments and means were fixed and the analysis of variance performed by GENES program (Cruz, 2006).

**RESULTS AND DISCUSSION** - From 150 genotypes tested in greenhouse only five have reached 50% of emergency: 'CNFC 1635', 'CNFC 9504', 'Jalo 08', 'PI-40-356-77' and 'PI-40-356-60' (Table 1). We observed that all treatments affect germination and reduced the percentage of normal seedlings. Greater reduction in germination occurred in the treatment 3. The treatments 0 and 1 did not differ in the length of root and shoot length but were higher than the treatments 2 and 3. The electrical conductivity showed that as the salt concentration increased there was an

increase in the leach exudates and reducing the vigor. The increase in electrical conductivity favor the action of the salt in the organization of cell membranes, causing larger amount of electrolyte was released. The germination in broad terms is defined as a manifestation of force, which depends, among other factors, the environmental conditions encountered at the site where they were seeded (Khan 1976). In the field saline soils can be found and the seed should be vigorous to be viable. This is a pre-breeding and study possibly lead new hybridizations in programs that have this focus.

Genotypes	Treatments	NS (%)	RL (cm)	SL (cm)	EC
CNFC 1635	0	95a	8.0a	3.9a	34a
CNFC 1635	1	87ab	7.3a	3.6a	50b
CNFC 1635	2	76bc	4.8b	1.8b	60c
CNFC 1635	3	55d	4.0b	1.4b	74d
CNFC 9504	0	97a	9.1a	4.1a	30a
CNFC 9504	1	82b	8.3a	3.7a	48b
CNFC 9504	2	78bc	5.0b	1.6b	56c
CNFC 9504	3	60d	4.7b	1.4b	69d
Jalo 08	0	92a	8.6a	4.2a	28a
Jalo 08	1	87ab	8.6a	3.5a	55b
Jalo 08	2	85ab	5.4b	1.5b	70c
Jalo 08	3	70c	4.0b	1.2b	74c
PI-40-356-77	0	94a	9.7a	4.6a	31a
PI-40-356-77	1	90a	9.3a	4.0a	52b
PI-40-356-77	2	84b	4.6b	1.9b	77c
PI-40-356-77	3	46c	3.8b	1.0b	80c
PI-40-356-60	0	100a	10.1a	4.7a	26a
PI-40-356-60	1	96a	9.5a	4.1a	49b
PI-40-356-60	2	80b	5.0b	2.0b	75c
PI-40-356-60	3	68c	4.2b	1.4b	81d

**Table 1.** Means of normal seedlings (NS), root length (RL), shoot length (SL) and electrical conductivity (EC) measured in µmhos.cm<sup>-1</sup>. g<sup>-1</sup> of seeds

Means followed by the same letter are not significantly different according to Tukey at 5%.

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#### HEIRLOOM BEAN OBSERVATIONAL NURSERIES AND YIELD TRIALS

### Antonia Palkovic, Travis Parker, and Paul Gepts

Department of Plant Sciences / MS1, University of California, Davis, CA 95616

In 2013, we began evaluating heirloom bean varieties that may be of interest to organic and specialty crop growers in CA as a rotational crop, for direct market sales, or for inclusion in value added products. In our two years of field trials, we have observed that some heirloom lines yield comparably to modern varieties of common beans such as UCD Pink 9634 and Canario 707. So far, the yield of each variety has been significantly affected by location and year. We hypothesize that this is because the growth habit and disease resistance in many of the heirloom lines is less than ideal.

#### Table 2

	Summary Table of Observational Nurseries								
year	locations	total number of entries	number of heirlooms	UCD lines as checks	Entries by species	management type	irrigation method	soil type	
	Richvale	30	25	5		Organic	buried drip	clay	
2013	Meridian	30	25	25 5 2 lun ungu 27 v		Organic	furrow	silty clay loam	
	Davis	30	25	5	27 Vulgaris	Conventional	furrow	silt loam	
	Richvale	36	29	4	3 lunatus,	Organic	buried drip	clay	
2014	Davis	36	29	4	3 Glycine max, 27 vulgaris	Conventional	furrow	silt loam	

In June 2013 and 2014 we planted multiple observational nurseries, each with a randomized complete block design in three replicates. Heirloom varieties were selected and obtained by Lundberg Family Farms from various seed companies. UCD varieties Canario 707 and Pink 9634 were used as checks in both years. Pink 9634 is known to be high yielding in the area and Canario 707 has a novel yellow seed coat, with novel seed coats being one quality that makes heirlooms popular with growers and consumers. The nurseries were planted in three different locations in northern California. All plots were planted on 30" beds with one row per bed and two beds per plot in 20'plot lengths using a cone planter.

Location and year all had significant interactive effects on yield, indicating the need for further field testing and supporting the idea of a need for making location-specific breeding selections, even within Northern California (see figures 1 and 2). The inconsistency of seed stocks may also be influencing our yield data. Seed sourced from seed companies sometimes yielded poorly in 2013 because of low seed viability. The seed saved from the 2013 plots germinated better in 2014, but buildup of disease in saved seed stocks may then be a factor in reduced yields from some lines in 2014 (see Table 2).

Future activities include continued field testing coupled with the ongoing complimentary improvement of heirloom dry beans for organic and specialty market production systems.









Variety	Visual estimate of the percent of BCMV affected plants per plot at Davis location
Anasazi*	50 to 100%
Dapple Grey	20 to 100%
Jacob's Cattle*	20 to 100%
Appaloosa	10 to 50%
Zuni Gold Beans*	10 to 50%
Spanish Tolosna Beans	5 to 50%
Brockton	10 to 20%
Vermont Appaloosa Bean	5 to 20%
Rebosero	0 to 25%
Raquel Beans	0 to 20%
Red Calypso	0 to 5%

\* noted to have the worst symptoms of BCMV at Richvale location \*\* entries not listed here did not show BCMV symptoms

#### IMPROVING DISEASE RESISTANCE AND GROWTH HABIT IN HEIRLOOM BEANS

#### Travis Parker, Antonia Palkovic, and Paul Gepts

Department of Plant Sciences / MS1, University of California, Davis, CA 95616

There are several production constraints facing heirloom crops today. Many heirloom varieties of common bean, for example, lack resistance to common plant pathogens and have a poor growth habit. One particularly widespread pathogen is Bean Common Mosaic Virus (BCMV). As a seed-and aphid-transmitted pathogen, BCMV is problematic in both organic and conventional cultivation systems. As a result, it is now a stated or implicit requirement that all common bean varieties in North America carry the I gene for BCMV resistance. Similarly, the sprawling growth habit of many heirloom beans is challenging for weed management, direct harvesting, and other field practices.

In 2014, nine lines of heirloom bean were identified that required BCMV resistance and could greatly benefit from an improved growth habit. To avoid the issue of hybrid weakness between gene pools (Shii et al. 1980; Gepts and Bliss 1985; Hanna et al. 2007), it would be preferable to identify a BCMV resistance source (I gene) that belonged to the same gene pool as each heirloom variety. All nine of our varieties are Mesoamerican in origin based on the phaseolin PCR test (Kami et al. 1995) (Fig. 1). Hence, we decided to use Matterhorn, an elite Mesoamerican variety of the Great Northern market class developed by Kelly et al. (1999) at Michigan State. Matterhorn has excellent yield, disease resistance, and a superior upright growth habit. Crosses were initiated in December 2014 and will continue throughout the winter, followed by planting the F1 hybrid progeny. The first of five generations of repeated backcrosses to the heirloom parent will be carried out in spring 2015. Progeny showing the SW13 marker, which is linked to the I gene (Melotto et al. 1996), will be selected after each generation. By 2017, our improved heirloom varieties should trace approximately 98% of their ancestry to the original heirloom parent, but should be resistant to BCMV. Viral resistance will be verified by inoculations.

Many heirloom varieties have a prostrate, spreading growth habit (Fig. 2; Type III according to the CIAT classification, Singh 1982). These varieties rapidly grow into furrows, making it extremely difficult for field equipment to mechanically remove weeds without damaging the crop. Prostrate growth habit in common bean can also cause pods to develop at the soil surface, which can reduce seed yield and quality. Similarly, many heirloom varieties develop runners that compete with seeds for photosynthates. These energetically costly runners are absent in the more upright (Type II) plant habit. We will begin a second selection program to develop a more upright version of each of these landraces during the summer of 2015. Improving disease resistance and growth habit is expected to increase yields and reduce production costs, making these high-quality heirloom beans more affordable for the public and profitable for producers.

#### ACKNOWLEDGEMENTS

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A.



B.



**Figure 1.** Electrophoresis of phaseolin PCR products per Kami et al. (1995). A: Lanes 1-4 are heirloom varieties, 5-8 are controls. Lanes 5 and 7 are Andean control varieties with "T" type phaseolin. Lanes 6 and 8 are Mesoamerican with "S" type phaseolin. 1, Zuni Gold; 2, Anasazi; 3, Raquel; 4, Orca; 5, Jalo EEP 558; 6, UCD Pink 9634; 7, UC 0801 Cranberry; 8, BAT 93. B: Lanes 1-5 represent heirloom varieties, lanes 6 and 7 are Mesoamerican and Andean controls, respectively. 1, Rattlesnake; 2, Bolita; 3 Tarahumara Purple Star; 4, Rio Zape; 5, Cherokee Trail of Tears; 6, Matterhorn; 7, UC Canario 707; L, ladder.



**Figure 2:** The prostrate growth habit of Zuni Gold, center, during the 2014 field season at UC Davis.

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# DETERMINATION OF IDEAL CONDITIONS TO DO ARTIFICIAL CROSSES IN *Phaseolus lunatus* L.

### Antônia Maria de Cássia Batista de Sousa, José Wellington de Moura Soares, Débora Macêdo Araújo da Silva, Hélio Nelson Brito Monteiro, Marcones Ferreira Costa, Regina Lucia Ferreira Gomes, Angela Celis de Almeida Lopes

Universidade Federal do Piauí, 64049-550 Teresina (PI), Brazil (acalopes@ufpi.edu.br and rlfgomes@ufpi.edu.br)

**INTRODUCTION:** Plant breeding has an important role with regard to increasing food production and improving nutrition for people, especially for poor people. In breeding programs, knowledge of floral biology and ideal environmental conditions is essential for holding crosses in a species. In this sense, this study was performed to determine the ideal conditions to perform artificial crosses, in order to obtain pods by hybridization, allowing the study of inheritance and therefore better use of genetic resources of lima bean.

**MATERIALS AND METHODS:** We conducted the study from January to July 2014, in a greenhouse of the Department of Plant Science at the Federal University of Piauí (UFPI) in the Teresina city, Piauí state. We use four lima bean access from the Germplasm Active Bank (BAG) of UFPI, which contrasted as the growth habit and cycle. The UFPI-628 and 728-UFPI accesses have an upright growth habit and early cycle; already UFPI-666 and -465 are UFPI have climbing habit of growth and late cycle. We did the crosses in the period of 7: 30 am to 10: 00 am; and 4:00 pm to 6:00 pm. In this period were collected moisture data, internal and external temperature in the greenhouse, number of days to flowering, flowering time, the opening hours of flowers, inflorescences with 30 buttons in pre-anthesis were marked and directly observed each time interval during in the morning and afternoon. During the morning, we observed the plants from 8: 00 am to 11: 00 am; and in the afternoon, from 2:00 pm to 6: 30 pm.

**RESULTS:** The accessions differ in the number of days to the start of flowering, flowering duration and the number of days to maturity of the pods (Table 1). The duration of flowering average was 47.75 days, ranging between 28.25 and 62.75 days, and UFPI-465 and UFPI-628 showed the smallest and the largest flowering period, respectively. By evaluating the time of lima beans anthesis, we observed that the period with the highest number of open blooms in the morning is from 9: 00 am to 10: 00 am; and in the afternoon, between 4: 30 pm and 5:30 pm. With respect to the percentage of pods, we found that the highest rate (16%) was observed in the combination of UFPI 628 X UFPI-666; the lowest rate was observed in the combination of UFPI 465 X UFPI 728, with 3.92%.

**Table. 1** - Means of each trait: number of days to the start of flowering (NDIF), flowering duration (DF) and the number of days to maturity of the pods(NDMV), evaluated in four lima bean accesses in Teresina - PI, 2014.

ACESSOS	NDIF	DF	NDMV
UFPI 465	97.00 a	62.75 a	138.25 a
UFPI 666	73.00 b	57.75 b	125.5 b
UFPI 728	40.5 c	42.25 c	79.5 c
UFPI 628	31.00 d	28.25 d	78.25 c
Means	60.38	47.75	105.38

Table 2- Percentages of pods obtained in artificial crosses in lima bean. Teresina-PI, 2014.

Combinations	Percentages of pods
UFPI 628 X UFPI 465	4.34%
UFPI 628 X UFPI 666	16.00%
UFPI 728 X UFPI 465	5.00%
UFPI 728 X UFPI 666	11.11%
UFPI 666 X UFPI 628	11.11%
UFPI 465 X UFPI 628	5.26%
UFPI 465 X UFPI 728	3.92%

**CONCLUSIONS:** Anthesis of lima bean flowers occurs in the morning and afternoon. In June there was more pod formation. The ideal conditions for the artificial crosses in the morning occurred with internal and external average temperature of 29.8°C and 34.2°C, respectively. Already in the afternoon, the ideal conditions of internal and external temperatures were 32,8°C and 30,5°C, respectively. In both periods, the humidity was 64%. The UFPI-628 genotype behaved like great pollen receiver, while the UFPI-465 genotype produced the lowest percentage of pods when it was used as a donor of pollen or receiver.

#### ACKNOWLEDGMENTS

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# GENETIC DIVERGENCE FOR TRAITS RELATED TO GRAIN YIELD IN LIMA BEAN ACCESS (Phaseolus lunatus L.)

### José Wellington de Moura Soares, Antônia Maria de C. Batista de Sousa; Vinícius Santos Freitas, Gabriel de Moraes Cunha Gonçalves, Pablo Alves de Sousa, Ângela Célis de Almeida Lopes, Regina Lucia Ferreira Gomes

#### Universidade Federal do Piauí, 64049-550 Teresina (PI), Brazil (acalopes@ufpi.edu.br and rlfgomes@ufpi.edu.br)

**INTRODUCTION:** The conservation and characterization of plant genetic resources in germplasm banks has been the basis for its use in breeding programs, resulting in the development of new cultivars. The characterization and evaluation can provide the knowledge of the variability, diversity and genetic potential, encouraging the use of available genetic resources. Thus, this study aimed to characterize 24 lima bean accessions from the Germplasm Active Bank (BAG) of the Federal University of Piauí (UFPI).

**MATERIALS AND METHODS:** It was characterized 24 lima bean accesses from the BAG UFPI, from February to September 2014, in greenhouse of the Department of Plant Science, in the Agricultural Sciences Center/UFPI, in the city of Teresina, PI, located 05°05'S, 42°05'W and altitude of 72,7m. We evaluated qualitative characters (background color, pattern color and the second color pattern) and quantitative (number of days to the beginning of flowering, number of pods per plant, pod weight, pod length, pod width, number of grains per pod, 100 seed weight, seed length, seed width and seed thickness), according to the Biodiversity International (IPGRI, 2001). Quantitative data were subjected to analysis of variance and average cluster test of Scott-Knott (P < 0.05%). In multivariate analysis, we use the method of unweighted hierarchical cluster grouping in pairs (UPGMA), with the use of the Mahalanobis distance. The identification of the importance of the characters was based on the Singh's method (1981).

**RESULTS:** The analysis of qualitative characters showed that the predominant background color in the accesses was cream (26.6%), followed by white (23.3%), light brown (13.3%), brickand-black color (10 %), and the colors brown, dark brown, red, pink and purple, which occurred in a lower percentage (3.3%). The pattern color of the seeds was absent in most of the accesses (60.6%). The second color pattern was absent in most of the accesses (63.6%), followed by light brown and dark brown colors (18.18%). The high percentage of white seeds could be related to the loss of variability by the farmers' selection for marketing. Analysis of variance of quantitative traits has shown the existence of variability among accessions. The dendrogram generated by UPGMA enabled the formation of four distinct groups. Group I consists of four accesses (UFPI-780, FAVA MOITA, UFPI-628 and G 25 165), which had the lowest averages for the beginning of flowering, number of pods per plant, pod weight, 100-grain weight. As for seed thickness, obtained the highest average. In this group only UFPI-780 access has not determined the growth size. Group II formed by access (UFPI-787, UFPI-251 and UFPI-537) who obtained the highest average for the number of pods per plant, seed length and width of the seed and the lowest average to average pod length, width the seeds and seed thickness. Group III (UFPI-465, UFPI-666, UFPI-784, UFPI-220, UFPI-622, UFPI-788.2 and UFPI-623) comprised accesses who obtained the highest average for the beginning of flowering and the lowest average for the number of pods per plant. In Group VI accessions were collected (UFPI-788, UFPI-782, UFPI-781. UFPI-779. UFPI-508. UFRRJ-G 20. UFPI-463. UFPI-777. UFPI-599 and G 26 200) that obtained average high for pod weight. The character that contributed most to the divergence

between the approaches was the number of pods per plant. The earliest hits were G-26200, G-25165, UFPI-628 and Fava bush - PB; and the later were UFPI-465, UFPI-788.

Figure 1 Dendrogram of genetic dissimilarity between 24 lima bean accesses (*Phaseolus lunatus* L.) of the Active Germplasm Bank of UFPI obtained by UPGMA method based on quantitative descriptors. Teresina, PI, 2014.



**CONCLUSIONS:** The morphological characterization was efficient in the study of genetic diversity among the lima bean accesses of the Germplasm Active Bank of the Federal University of Piauí. Background colors prevalent in the accessions were cream and white, probably due to the loss of genetic variability. The number of pods per plant showed a greater contribution to discrimination of access in similarity groups. Accesses heterotic groups may been used in the breeding programs to generate genetic variability.

#### ACKNOWLEDGMENTS

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#### NUTRITIONAL QUALITY EVALUATION OF GRAIN AND BIOMASS OF LIMA BEAN (*Phaseolus lunatus*)

# Regis Araujo Pinheiro<sup>1</sup>, Gilberto A Peripolli Bevilaqua<sup>1</sup>, Irajá Ferreira Antunes<sup>1</sup>, Violeta Bachieri Duarte<sup>2</sup>

<sup>1</sup>Embrapa Clima Temperado, BR 392, km 78, CxP 403, Pelotas, RS, Brazil; <sup>2</sup>Cooperativa Bionatur Sementes, Candiota, RS, Brazil. E-mail: <u>gilberto.bevilaqua@embrapa.br</u>

#### **INTRODUCTION**

The family farming systems require the use of plants with multiple purpose that can be used as ground cover, green manure, human and animal nutrition, and exhibit good growth capacity and adaptation to developing in low fertility soils. Furthermore, Altieri (2002) states that these cropping systems had profound changes in recent years, observing the drastic reduction of crops of commercial interest number and of the genetic diversity used for cultivation. The lima beans can replace other land cover crops and green manure, as Mucuna sp. and Crotalaria sp. with advantages such as the use of biomass and grain for feed and food, however requiring accuracy analysis to better recognize the nutritional potential of culture.

According to Vieira (1992) the lima bean is a major legume grown in tropical regions and has the potential to provide vegetable protein to the population, decreasing dependence, almost exclusively, of common beans (*P. vulgaris*).

Embrapa Clima Temperato has a germplasm bank of lima bean composed of 70 cultivars collected throughout the southern region of Brazil and some coming from the tropical region. These genotypes have been evaluated over the past few years and show a large variation between the grains, cycle and size. The aim of this study is to analyze the nutritional composition of grain and biomass of lima bean accesses from germplasm bank of Embrapa Temperate Climate.

#### **MATERIAL AND METHODS**

Genotypes 195A and 198, from the germplasm bank were analyzed, and the control used was cowpea, cultivar Amendoim from the Cooperative of Family Farmers of São José do Norte (Cooafan), RS, Brazil.

The soil for cultivation was Haplaquult, typical of floodplains, presenting poorly drained and low fertility, with the follow physic-chemical characteristics: 1,2% of organic matter, 2 mg kg<sup>-1</sup> de phosphorus (P), 35 mg kg<sup>-1</sup> of potash (K), 20% of clay e pH 5,8. After preliminary analysis of the soil was performed correction with limestone, and adding organic compost, rock phosphate and granodiorite powder, manually entered in dosis of one t ha<sup>-1</sup>. For installation of observation units were sown in november, four lines of each variety, 6m long, spaced 0.50 m apart at a density 2 to 3 plants m<sup>-1</sup>.

Analyses of grain and biomass at flowering were performed in the laboratory of Food Science and Nutrition Animal Embrapa Temperate Climate. The methodologies employed for determination of dry matter, ash, lignin, neutral detergent fiber, acid detergent fiber, ether extract, lignin and crude protein were described according to Silva & Queiroz 2002; National ...(2001).

#### **RESULTS AND DISCUSSION**

Regarding the crude protein content in the grain, the G 195A showed slightly higher than the genotype 198, however similar at check treatment (Table 1). When analyzed the levels of neutral

and acid digestible fiber, the lima bean genotypes were similar, but bellow of cowpea. In relation of mineral matter the results of genotypes were very similar. In relation at ether extract the lima bean genotypes were superior cowpea checking and G 198 had higher value. These values are above those found by Azevedo et al. (2003) which analyzed seven lima beans genotypes in the Piauí State, Brazil, found values between 26.70% - 17.95% for crude protein, 4.10% -3.06% for mineral matter, and from 1.49 to 0.88 % for ether extract.

**Table 1:** Nutritional composition of grain and biomass in flowering stage, of lima bean (*Phaseolus lunatus*) in Pelotas, Brazil.

Genotypes	%DM	%CP	%NDF	%ADF	%MM	%EE	%LIG	
	Grain							
G 195A	87.0	28.4	18.9	4.5	5.0	1.09	-	
G 198	87.2	25.1	22.2	5.7	4.5	1.38	-	
Cowpea (C)	86.6	28.2	32.1	8.7	4.6	0.74	-	
			Biomass					
G195A	94.0	20.4	46.7	33.9	5.9	-	10.6	
Cowpea (C)	90.0	17.1	54.0	35.1	11.7	-	9.1	

DM: dry matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; MM: mineral matter; EE: ether extract; LIG: lignin Cheal: acumea av Amendaim

Check: cowpea cv. Amendoim

Regarding the nutritional composition of lima bean biomass, the results were similar to those presented by cowpea, however, the first presented numerically higher, especially when analyzing the crude protein content (Table 1). With regard to the fiber content, cowpea shown numerically lower results in relation of lima bean, however similar for both types of fibers analyzed. The results can indicate the best nutritional quality of lima bean for feeding animals and humans. For the other hand, in relation to mineral matter the cowpea was superior to lima beans. For the lignin content both genotypes showed similar results however, the same can be considered quite high for use in animal feed.

**CONCLUSION:** Lima bean genotypes from the germplasm bank of Embrapa Clima Temperado can be regarded as an excellent protein source for agricultural families, providing a richer diet, in addition, they present high rusticity, becomes a kind of extreme importance in crop diversification in agroecological systems

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#### **GERMINATION INDUCTORS IN SNAP BEAN SEEDS**

# E.C. Oliveira<sup>1</sup>, L.H.C. Almeida<sup>1</sup>, A. Moritz<sup>1</sup>, L.S.A. Takahashi<sup>1</sup> and E. Miglioranza<sup>1</sup>

<sup>1</sup>Agronomy Department, "Universidade Estadual de Londrina" (Londrina State University) - UEL, Brazil

#### **INTRODUCTION**

Nowadays, the number of companies and seed treatment products has grown due to the supply favoring germination and even the final increase in productivity of crops.

Products, such as fungicides and insecticides, besides the fact of protecting seeds from the attack of diseases and plagues, respectively, they can confer bioactivator effects, helping in the initial growth of the plants (Dan *et al.*, 2012). Besides the utilization of these products, other technologies are being adopted in the treatment of seeds, such as hormones (Yokoya *et al.*, 2010), biostimulants (Khan *et al.*, 2009) and nutrients (Farooq *et al.*, 2012).

Based on the previous statement, the purpose of this paper has been to present preliminary research results on different treatment in the germination of snap beans seeds.

#### **MATERIAL AND METHODS**

The study was conducted in a seed analysis laboratory that belongs to the Londrina State University-UEL, Brazil. UEL-2 seeds for growing snap beans were used in the experiment, constant growth rate, originating from two batches based on the harvest year (2012 and 2014). The experimental design used was entirely randomized by the factorial scheme (2x5) with four repetitions of 50 seeds, remaining on a germination layer at a temperature of  $25^{0}$ C, on a substrate of paper towels, previously moistened by distilled water at a proportion of 2.5 times the mass of the substrate. The treatments and the respective doses used were: 1) untreated (check) sample, only soaked in distilled water; 2) GA<sub>3</sub> (10 µM Kg seeds<sup>-1</sup>); 3) Biostimulant (6.0 ml Kg seeds<sup>-1</sup>); 4) Vegetal bioactivator (3.0 ml Kg seeds<sup>-1</sup>); 5) nutrient complex (2.0 ml Kg seeds<sup>-1</sup>). The evaluations were performed for nine days, through a germination test and expressed in the percentage of normal plantlets. The germination values obtained did not adhere to the normality and homogeneity of variance, for this reason they were transformed by the optimum power of Box-Cox (BOX and COX, 1964), and displayed the original data in percentage values. The germination results for all the treatments were submitted to variance analysis and their averages compared by the Duncan test at a significance rate of 5%.

#### **RESULTS AND DISCUSSION**

The germination results revealed significant effects (p<0.05) among the treatments, displaying differences among the inductors for UEL-2 snap beans (Table 1) only for the year 2014. The interaction between the years and the treatments was significant, demonstrating the effect on the product was capable of improving germinative performance in snap beans seeds and increasing the storage period.

Treatments	Harve	sts
Treatments	2012	2014
1) Untreated check	75.00 aA	67.00 bA
2) GA <sub>3</sub>	80.00 aA	84.00 aA
3) Biostimulant	69.00 aA	85.00 aA
4) Bioactivator	78.00 aA	40.00 bB
5) Nutrient complex	86.00 aA	79.00 aA
C.V.(%)	9.:	56
m.s.d: columns = 12.28; rows 17.47.		

**Table 1.** Percentage of seed germination in snap beans seeds harvested in two distinct years (2012 and 2014) treated with different germination inductors.

Averages followed by the same small letter in the column and capital letter in the row do not differ from each other by the Duncan test (p<0,05).V.C. – variance coefficient; m.s.d. – minimum significant difference.

According to the results, the Bioactivator treatment was verified and displayed more pronounced superior performance in the year 2012 as compared to 2014. These results corroborate with those obtained by Castro et al. (2007), whereas increased germination of soybean seeds using this type of product. The Bioactivator effect is linked to the fact as being responsible for activating various physiological reactions, one of them being the expression of plasmatic membrane proteins, which may favor seeds in a longer harvest period. In contract, Tavares et al. (2007), evaluating the physiological effect in the application of different doses of Bioactivators (0 ml, 5.0 ml, 10.0 ml, 20.0 ml, and 30.0 ml of a commercial product per Kg of seeds), in soybeans, no significant difference was observed among the treatments, for germination. Only partial results have been verified in the research up to now, no positive effect in the germination of the UEL-2 snap beans seeds was observed for the other products.

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# GERMINATION OF SNAP BEANS SEEDS BASED ON THE PRESOAKING TIME IN VEGETAL BIOSTIMULANT

# E.C. Oliveira<sup>1</sup>, A. Moritz<sup>1</sup>, L.H.C. Almeida<sup>1</sup>, L.S.A. Takahashi<sup>1</sup> and E. Miglioranza<sup>1</sup>

<sup>1</sup>Department of Agronomy, "Universidade Estadual de Londrina" (Londrina State University) - UEL, Brazil

#### **INTRODUCTION**

Vegetal hormones influence the germination process in seeds, in the promotion, as well as in gibberellins, regarding the inhibition, and also in abscisic acid (Bertolin *et al.*, 2010). Although, endogenously the seed has some amounts of growth promoting hormones and practices, such as the presoaking of seeds in biostimulants is becoming more and more frequent (Vieira, 2001; Dario *et al.*, 2005).

Even though diverse research studies have proven the effects on the germination of seeds with biostimulants, there are still few studies on the ideal dosages and especially the necessary time for the exposure of seeds to these products, so that the absorption is sufficient and then the guaranteed effects take place.

This manner, due to the usage of biostimulants in the germination of the seeds, the objective of this study was to evaluate the germination response in two snap beans crops based on the presoaking time.

#### **MATERIAL AND METHODS**

The seeds in the (UEL-1 and UEL-2) snap beans two crops used in this study came from "Universidade Estadual de Londrina" (Londrina State University), Brazil. The seeds were harvested in 2014 and stored in favorable conditions until the study began. The seeds were submitted to a presoaking treatment in a vegetal Biostimulant composed of: 0.5 g L<sup>-1</sup> indole butyric acid (auxin), 0.9 g L<sup>-1</sup> kinetin (cytokinin) and 0.5 g L<sup>-1</sup> of gibberellic acid (gibberellins), a dose of 6.0 mL Kg<sup>-1</sup> per seed, for 1, 2, 3, 4, 5, and 6 hours. Four repetitions were used on 25 seeds, placed between germitest-type paper towels, moistened with water in a proportion 2.5 times the weight of the paper, and then this remained in a rolled up position, which was kept in a germination chamber at 25°C. The evaluations were performed for nine days. The applied experimental design was completely randomized, and the germination results of the seeds, as well as the post-germinative characteristics were submitted to the F test, and the averages were adjusted for determining the germination curves of the crops based on the presoaking time.

#### **RESULTS AND DISCUSSION**

The presoaking periods significantly influenced the results (p<0.01) in the germination of UEL-1 and UEL-2 snap beans seeds, (Figure 1). The results from both crops were adjusted to a quadratic polynomial equation. Although the two crops were adjusted to similar models, the values of the estimated maximum point were different by about 0.5 hour, the values of the estimated maximum point was about 0.5 hour.

The UEL-1 crop achieved its maximum estimated point as 86% of germination in about 3 hours of presoaking of its seeds, compared to the results from the UEL-2 crop, its maximum point was reached after 3.5 hours of presoaking as 88% of the seeds germinated (Figure 1).



**Figure 1**. Germination of the UEL-1 and UEL-2 snap beans seeds based on the presoaking time in vegetal Biostimulant.

The germination evolution of the crops behaved similarly, regardless of the presoaking time, except for the sixth day for six hours of presoaking in the UEL-2 crop, whereas there was a drop from 90% to 60% that occurred in germination (Figure 2).



**Figure 2.** This displays the evolution percentage based on days of germination in the snap beans seeds, a) UEL-1 and b) UEL-2 based on the presoaking time in vegetal biostimulant.

The results up to now comply with many research studies on the presoaking time of seeds in biostimulants, as according to diverse authors this time is variable according to the species, size, and even the vigor of the seeds (Vieira, 2001; Dario et al., 2005; Bertolin *et al.*, 2010).

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#### CHARACTERS ASSOCIATED WITH VIVIPARITY IN COMMON BEAN

# Laís A. Pereira<sup>1</sup>, Magno A. P. Ramalho<sup>1\*</sup>, Angela F. B. Abreu<sup>2</sup>, Scheila R. Guilherme<sup>1</sup>

Department of Biology,

<sup>1</sup>Universidade Federal de Lavras, Lavras, MG, Brazil, <sup>2</sup>Embrapa Arroz e Feijão \*Corresponding author: <u>magnoapr@dbi.ufla.br</u>

**INTRODUCTION:** Brazilian farmers spread out the plants of common bean, after harvesting, to the time of threshing in the field. If harvest coincides with a rainy period, the great soil moisture content contributes to the germination of seeds while still in the pods, phenomenon known as viviparity, drastically reducing the production. The main solution is to obtain cultivars tolerant to high moisture content at the harvest time. For the selection of plants / progenies with less viviparity it is necessary to identify characters that can make selection easier. This study was carried out to verify the correlation between the pod wall thickness (PWT) and the percentage of germination of seeds in the pods (PGSP).

**MATERIALS AND METHODS:** The experiments were carried out at Universidade Federal de Lavras, Minas Gerais, Brazil. The lines ESAL 686 and Pérola were used as parents. ESAL 686 belongs to the Andean gene group and has pod walls of greater thickness. The Pérola line is of Mesoamerican origen and thinner pod. From the cross between the lines it was possible to evaluate 93 progenies  $F_{3:4}$  with sown in February 2014.

After the harvest, the pods were removed. Part was used for measuring pod wall thickness (PWT), and the other part was used for evaluation of the percentage of germination of seeds while still in the pods (PGSP). For measurement of PWT, three pods/plant/plot were taken at random. The seeds were removed and a valve of the pods was subjected to measurements of thickness by an external digital micrometer, DIGIMESS brand, code 110.284, 0-25 mm capacity, and precision of  $\pm 0.002$  mm. Measurement was made in the center of one of the valves of each pod.

For evaluation of PGSP, five pods/plot were evaluated leading to six replications. For that purpose, the pods were rolled up, two by two, in sheets of germination paper previously moistened with distilled water. The rolls were kept in germinators at 25°C with 12 hours of light in the Seed Analysis Laboratory. The total number of seeds and the number of germinated seeds were counted on the seventh day so as to obtain the PGSP.

The data were subjected to analysis of variance, according to the procedure presented by Steel et al.(1997). Broad sense heritability ( $h^2$ ) of the progenies was estimated from analysis of variance, estimates of the mean squares of progenies (MSP) and mean square error (MSE) were used, as described by Ramalho et al. (2012). Phenotypic correlation ( $r_{XY}$ ) was estimated between the mean values of the progenies for PWT (X) and PGSP (Y) (Bernardo, 2010). The correlated response RC<sub>Y(X)</sub> in trait Y (PGSP) was estimated by selection of the best 10% of progenies with greatest PWT (X), in a way similar to the model proposed by Falconer & Mackay (1996).

**RESULTS AND DISCUSSION:** The use of progenies was efficient in evaluation of PGSP, with heritability greater than 75%. The gain expected from selection, selecting the 10% with the lowest PGSP was -60.5% (Table 1). In the case of PWT, the heritability was 62%. One of the objectives of this study was to verify which of the two traits evaluated would allow greater efficiency in selection.

The estimate of correlation between PWT and PGSP was negative and different from zero  $(r = -0.5^{**})$ . This fact contributed so that the response correlated by selection in PWT and expected gain in PGSP was less than that directly seen in the trait, though still expressive. Based on this last result, it may be inferred that the greater the pod wall thickness, the lower the germination. Considering that in the two methodologies there is similar difficulty of evaluation, use of the germination in the pod test, in principle, proved to be more promising. Unfortunately, there is difficulty in showing that this trait reflects tolerance to high moisture under field conditions, above all, due to the lower experimental precision in the evaluations under field conditions.

**Table 1**. Estimates of heritability  $(h^2)$  and gain expected from selection (GS) and correlated response (RC<sub>Y(X)</sub>) obtained for the traits of pod wall thickness (PWT) and percentage of germination of seeds while still in the pods (PGSP). Lavras, 2014.

Indiana avaluated	Trait under selection		
indices evaluated	PWT (mm X 100)	PGSP (%)	PWT/PGSP
$h^2\%$	62	77	-
Mean of the progenies (Mo)	16.25	26.19	26.19
Mean selected	20.23	5.61	12.69
GS	2.47(15.2%)	-15.85(-	-
		60.5%)	
$RC_{Y(X)}$	-	-	-10.40 (-39.69%)
* 0 1 1 1 0 1 1	$\cdot 1$ $\cdot$ DUT 1	· · DOOD	

\* Correlated response of selection carried out in PWT and gain in PGSP.

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#### PREDICTION OF THE GENETIC POTENTIAL OF SEGREGATING POPULATIONS IN CARIOCA BEANS BY THE JINKS & POONI METHOD

# Anatércia Ferreira Alves<sup>1\*</sup>, Gilmar Silvério da Rocha<sup>2</sup>, Renato Domiciano Silva Rosado<sup>3</sup>, José Eustáquio de Souza Carneiro<sup>4</sup>, Pedro Crescêncio Souza Carneiro<sup>4</sup> and Ildon Rodrigues do Nascimento<sup>1</sup>

<sup>1</sup>Universidade Federal do Tocantins, Rua Badejós, Lote 7, Chácaras 69/72, Zona Rural. Cx.postal 66. 77402-970, Gurupi, Tocantins State, Brazil; <sup>2</sup> Instituto Federal Catarinense ; <sup>3</sup>Souza Cruz ; <sup>4</sup>Universidade Federal de Viçosa. \*e-mail: anaterciaa@yahoo.com.br

**INTRODUCTION:** In recurrent selection it is necessary to carry out a judicious choice of the lines to be studied when success is desired, for these should possess the favorable phenotypes which one intends to recombine together (Ramalho et al. 1993). In qualitative traits, the decision in the choice of the parents is performed easily through the viewing, however, in concerning with quantitative trait, in the same way as grain yield, the evaluation step is fundamental to conduct the choice of the parents and there are methodologies which can be utilized for this goal. From among the most utilized methodologies in the cropping of bean plant, one has the Jinks & Pooni method (1976), which makes it possible to predict the potential of a particular population in generating lines superior to a given standard in the  $F^{\infty}$  generation, through estimates of means and variance in the early generations, the disposal of non-promising populations at the beginning of the program being possible.

So, the purpose of that work was to predict the genetic potential of 20 segregating populations in the  $F_2$  generation of the carioca type by the Jinks & Pooni method (1976).

**MATERIALS AND METHODS:** The experiments were conducted in the experimental field of the Crop Science of the Universidade Federal de Viçosa (UFV) in Coimbra-MG in the 2010's dry season crop. 20 segregating populations coming from the recurrent selection third cycle of the carioca bean breeding program of the UFV. These populations were evaluated in the  $F_2$  generation along with the controls Pérola, BRSMG Talismã, Ouro Negro, BRSMG Madrepérola and BRSMG Majestoso. The experimental design in 5 x 5 lattice, with three replications. The plots consisted of five rows of 4 m.

The estimates of the genetic and phenotypic parameters were obtained through the data of single plants of each  $F_2$  population. At first, the phenotypic variance ( $\partial P$ ) of each population was estimated by the means of the variance of the plots which were given the same treatments in the different replications. The broad sense heritability  $(h^2)$  was obtained by the ratio among the genetic () and phenotypic () variances. The prediction of the genetic potential of each population was evaluated according to the Jinks & Pooni methodology (1976), which estimates the probability for the population to give rise to lines which excel a particular standard. That probability corresponds to the area on the right of a given value of x on the abscissa of the normal distribution, calculated by using the properties of the standardized normal distribution, estimating the variable Z by the expression Z = (x - m)/s, in which: x = means of the standard line (*L*); in this the mean of cultivar BRSMG Madrepérola (control), added of 20% ( $\Sigma$ ) = 21.80 g/plant) was utilized; m = mean of the lines in the  $F\infty$  generation in which, in a model with no dominance, corresponds to the mean of any segregating generation; in the present study, corresponds to the means of  $F_2$  generation, this is,  $\mathbf{m} = \mathbf{F}_{n1}$  and; s = phenotypic standard deviation among the lines  $(s = \beta \delta_{n1}^2)$ . The genetic variance among the lines  $(\delta_{n1}^2)$  corresponds to as many as two times the additive genetic variance  $(\delta_{n1}^2)$ present in F<sub>2</sub>. For a model with no dominance, the phenotypic variance of F<sub>2</sub> generation ( $\mathcal{F}_2$ ) contains  $\partial_A^2 + \partial_B^2$ . So,  $2\partial_A^2 = 2\partial_B^2 - 2\partial_B^2$ . Considering that the environmental variance of  $F_2$ generation can be estimated by the variance of the lines (controls), one has that  $s = (\delta_1^2 = \sqrt{2\delta_1^2 + \delta_2^2} = (2\delta_1^2 - \delta_2^2)$ . Therefore, for a given "i" population,  $Z_1 = (L - F_2) / (2\delta_1^2 - \delta_2^2)$ . As an environmental variance estimate  $(\delta_1^2)$  the mean of the variances of the cultivars utilized as controls was utilized as controls (67. 70).

**RESULTS AND DISCUSSION:** Cultivar BRSMG Madrepérola was utilized as a standard for having presented the highest grain yield among the controls. Its yield was added of 20% ( $\bar{L} = 21.80$  g/plant), since one wishes to obtain lines which outyields cultivar BRSMG Madrepérola. In terms of grain yield per plant, the greatest means were found in populations 2, 1, 18, 16 e 13, which presented means higher than that of cultivar BRSMG Madrepérola (Table1). Considering the probability of extracting lines which outyield the standard by 20% (PSP), the most promising segregating populations were 2, 1, 6 e 18. As may be verified in these four populations, the Jinks & Pooni procedure seeks to conciliate both elevated genetic means and variances. Similar results on bean plants were also obtained by Carneiro et al. (2002), standing out the potential of the Jinks & Pooni methodology (1976) in the choice of segregating populations.

Populations	Yield (g/planta)	OF.	$\delta^2_{ct}$	h <sup>2</sup> (%)	Zi	PSP (%)
População 2	19.68	99.69	31.99	32.09	0.18	42.86
População 1	18.69	131.96	64.26	48.70	0.22	41.29
População 18	18.46	80.27	12.57	15.66	0.35	36.32
População 16	18.40	73.33	5.63	7.68	0.38	35.20
População 13	18.28	73.24	5.54	7.56	0.40	34.46
População 6	17.84	101.18	33.48	33.09	0.34	36.69
População 12	17.67	90.99	23.29	25.60	0.39	34.83
População 4	17.34	52.47	-15.23	-	0.73	-
População 5	16.97	69.09	1.39	2.01	0.58	28.10
População 8	16.95	73.26	5.56	7.59	0.55	29.12
População 9	16.94	55.46	-12.24	-	0.74	-
População 11	16.85	73.82	6.12	8.29	0.55	29.12
População 17	16.56	70.37	2.67	3.79	0.61	27.09
População 7	16.42	87.05	19.35	22.23	0.52	30.15
População 20	15.81	75.86	8.16	10.76	0.65	25.78
População 14	15.36	73.59	5.89	8.00	0.72	23.58
População 10	15.06	68.42	0.72	1.05	0.81	20.90
População 15	15.02	61.39	-6.31	-	0.91	-
População 3	14.63	76.18	8.48	11.13	0.78	21.77
População 19	14.01	64.89	-2.81	-	0.99	-
Pérola	15.27	55.44				
BRSMG Talismã	16.92	75.61				
Ouro Negro	14.03	64.34				
BRSMG Madrepérola	18.17	75.25				
BRSMG Majestoso	15.93	67.84				

**Table 1.** Means of the grain yield (g/plant) and estimates of phenotypic variance  $(\mathfrak{d}_{F_1})$ , genetic variance  $(\mathfrak{d}_{F_2})$ , heritability (h<sup>2</sup>), value of Z and its respective probabilities of obtaining lines which outyield cultivar BRSMG Madrepérola by 20% (PSP) for each F<sub>2</sub> population.

Populations 4, 9, 15 and 19 presented negative values of genetic variance. That result points out that the environmental variance (67.70), estimated by means of the mean of the phenotypic variances of the controls, can be overestimated. In any way, the means of the grain yields of these populations were poor and therefore, they were not chosen according to the methodology utilized.

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# PHENOTYPIC STABILITY OF THE TRAITS RELATED TO THE GROWTH HABIT IN COMMON BEAN

#### Lucas Rezende Pinheiro, Danuza Araújo de Souza, Monik Evelin Leite, Filipe Couto Alves, João Bosco dos Santos<sup>\*</sup>

<sup>1</sup>Universidade Federal de Lavras (UFLA), CEP 37200-000, Lavras, MG – Brazil E-mail: <u>\*jbsantos@dbi.ufla.br</u>

#### **INTRODUCTION**

Cultivar with broad adaptation is the one that performs well in several environments. One of the limiting characteristics for high yield in bean cultivars is the absence of upright plant under conditions of long days in high humidity and at high temperatures (MELO et al., 2007). Considering the selection of lines based on the growth grade, the character has presented heritability between 60% and 70%. However, the gains are not high mainly due to the high interaction of genotype by environment (CARMO et al., 2007; MELO et al, 2007; GONÇALVES et al., 2009). The identification of cultivars with greater phenotypic stability has been a widely used alternative to mitigate the interaction and obtaining more foreseeable yield. Considering the above, this research was conducted to evaluate cultivars with different growth habits, in the main growing seasons in Southern Minas Gerais, Brazil, to identify the stability of genotypes for the characters components of the bean growth habit.

#### MATHERIAL AND METHODS

The experiments were performed in six seasons during the years of 2012 and 2013 in a randomized complete block design with 3 replications. We evaluated 20 genotypes of four different growth habits, i.e. plant type I, II, III, and IV. Data of days to 50% of plants with the first flower, central main stem length, height of the first pod, number of nodes on the main stem, average length of internodes, number of secondary branches, number of pods per plant, average number of seeds per pod, number of seeds per plant, stem diameter, 100 seed weight, ratio between the length and the width of the central leaflet and scale grade of the growth habit were obtained. The stability of the genotypes was assessed by GGE Biplot method in three-way form of data entry (traits x environments x genotypes). Stability of the 20 genotypes was evaluated in six seasons together using all characters. In GGE Biplot the first two principal components (PC1 and PC2) are derived from the effects of the genotypes + genotype x environment interaction in a single value (YAN and KANG, 2003).

#### **RESULTS AND DISCUSSION**

The axes PC1 and PC2 explained more than 60% of the variance. It is observed that the least stable genotypes belong to the extreme groups of plant type, Flor de Mayo and Small White of type IV and, Radiante and Eriparsa of type I. The phenotypic stability, considering the joint analysis of the 13 characters and six crops is shown in Figure 1.

The most important traits for the instability of type I growth habit genotypes were the weight of 100 grains and the ratio of the length of the central leaflet. For genotypes of plant type IV the average length of internodes, the length of the main stem and the number of nodes on the main stem were the most important traits.





Figure 1. Analysis of stability by GGE Biplot of 20 genotypes evaluated in six crops and considering all traits. Cultivars: Growth habit Type I: Esal 693 (1), Radiante (2), Eriparsa (3); Type II: Valente (4), BRS-Estilo (5), RP-1 (6), BRS-Esplendor (7), BRS-Cometa (8), BRS-Supremo (9), Rio Tibagi (10); Type III: BRSMG-Perola (11), Talismã (12), Jalo-EE558 (13), BRSMG-União (14) Pérola (15), Ouro Negro (16), Carioca comum (17), VR-16 (18) Type IV: Flor de Mayo (19), Small White (20). Traits: Average length of internodes (CMI); Main stem length (CHC); Number of nodes on the main stem (NNC); 50% of plants with at least the first open flower (PPF); Number of seeds per pod (NGV); Number of grains per plant (NGP); Number of secondary branches (NRS); Number of pods per plant (NVP); Stem diameter (DC); Ratio between the length and the width of the ;central leaflet (RCF); One hundred grains weight (MCG); Height of the first pod (IPV).

The improvement should aim broad or specific adaptation to particular region, but always with emphasis on high stability. If the interaction should be avoided or exploited depends on the differences in environments are predictable or not. If the interaction is attributed to unpredictable environmental factors such as the change of climate variables, year by year, the interaction should be avoided by selecting cultivars with stable performances, for a range of conditions. If the interaction is caused by variations of predictable factors such as soil type and cultural practices, the interaction can be exploited by selecting cultivars specifically adapted to a particular environment (ANNICCHIARICO, 1992). Therefore, cultivars with growth habit II or III can be more stable.

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# GRAIN YIELD OF DRY BEANS UNDER RAINFALL CATCHMENT SYSTEMS AND AT FOUR ROWS SOWING

# E.S. Osuna-Ceja<sup>1</sup>, J.S. Padilla-Ramirez<sup>1</sup>, M.A. Martínez-Gamiño and L. Reyes-Muro<sup>1</sup>

<sup>1</sup>Bean Program, INIFAP-Experimental Station Pabellon. Apartado Postal 20, Pabellón de Arteaga, Ags., México, C.P. 20660. \*E mail <u>osuna.salvador@inifap.gob.mx</u>

**INTRODUCTION:** The temperate semiarid Highlands of Mexico is characterized by extreme variation in amount and distribution of rainfall. In this region, dry bean production is affected by drought, since more than 80% of the cultivated area is under rainfed conditions, marginal soils with low content of nutrients and organic matter, where scarce and erratic rainfall affects seriously the productivity of the crop (Aguilar-Benítez *et al.*, 2012). In this context, it is necessary to use varieties having drought adaptation. In addition, it is also needed to include efficient agronomic practices that help to catch, retain and use the rainfall water and that can contribute to increase and stabilize the yields in this region with severe problems of intermittent drought (Osuna *et al.*, 2012).

**MATERIALS AND METHODS:** During summer 2014 a work was established with dry beans under rainfed conditions at Sandovales, Ags., Mexico, at an altitude of 2040 meters above sea level. Rainfall during crop season was 466 mm and average temperature was 16.5°C, soil type is a Planosol, with less than 0.40 m deep, with 2% of organic matter, sandy-clay texture, 2% slope and pH of 6.8. It was evaluated the dry bean sowing in a crop agroecosystem consisted of stripes which integrated the sowing beds of 1.60 m wide with four rows separated 30 cm between them, under "in situ" rainfall water catchment and compared with the traditional farming system, sowing in furrows to 0.76 m to single-layer, without rainfall water catchment. The area cultivated to evaluate both production systems was 0.5 has. Sowing was carried out on June 26 in both production units. A precision seeder was used, which has versatile design to allow sowing in beds with four-row sowing coupled to a water catchment system (roller aqueel printing watershed on the seed bed to catch rainfall water on-site and reduced erosion) which is located on the back of the planter. This equipment was designed by the program of mechanization of Research Station of Pabellon, INIFAP (Rojas et al., 2014). In addition, to the cropping system in stripes with beans to four rows in contour, it was implemented a ridge system (practice that involves lifting soil at 20 cm high on the sides of the bed of planting spaced to store water and reduce erosion) and made an application of foliar fertilization during the grain filling, with urea and phosphoric acid at 2% and 1%, respectively (12 kg of urea and 6 liters of phosphoric acid in 600 liter of water, plus 0.25 liters of adherent). The applied solution to leaves is equivalent to 5.5 kg of N and 4.5 P kg per hectare. Ten plots of 2 m wide by 5 m long within each experimental unit were randomly taken to determine grain yield and its components. Information of quantified characteristics was analyzed determining descriptive basic statistics for each of the systems evaluated. In addition, means comparison was analyzed according to Student's t.

**RESULTS AND DISCUSSION:** Table 1 presents estimates of some parameters describing the variation of grain yield (GY) of dry beans and its components, obtained under two management systems. The average values of GY were statistically different ( $P \le 0.05$ ) for both management systems evaluated. The highest GY was obtained under the cultivation system in strips with bed sowing at four rows and rainwater catchment [SCF (1.43 t ha<sup>-1</sup>)] with respect to the traditional system [ST (0.74 t ha<sup>-1</sup>)]. The SCF/ST relationship (1.43/0.74 = 1.93); indicates that SCF exceeded 93% ST. This difference in GY was associated to catchment rainwater, planting method

and the foliar fertilization. Thus, the change of ST to the SFC, productivity of the dry bean is increased. From the results it can be inferred that the variation of GY, are due to the due to agricultural practices implemented such as modification of the method of planting and schemes of collection of rainwater. The increase in plant density from 50 to 186 thousand plants ha<sup>-1</sup> reduced pod number per plant, however, grain yield was compensated with greater number of pods per unit area at high plant density.

Sowing System	Ν	Minimum	Maximum	Mean	Median	Standard Deviation	C.V %						
Grain yield (t ha <sup>-1</sup> )													
Traditional <sup>1</sup>	10	0.59	0.89	0.74 <b>b</b>	0.76	0.10033	13.62						
$\mathrm{SCF}^{\ddagger}$	10	1.2	1.69	1.43ª	1.44	0.199	13.9						
Straw (t ha <sup>-1</sup> )													
Traditional	10	0.49	0.74	0.60 <b>b</b>	0.6	0.0899	14.93						
SCF	10	0.81	1.13	0.96ª	0.96	0.1173	12.21						
			Pods pe	r plant									
Traditional	10	20	49	33.1ª	33	8.0201	24.23						
SCF	10	22	29	24.7 <b>b</b>	24.7	2.3118	9.35						
			Seeds p	er pod									
Traditional	10	3.44	4.75	4.14 <b>a</b>	4.14	0.4587	11.09						
SCF	10	3.23	6.18	4.18 <b>a</b>	1.18	0.9099	21.75						
			Hundred see	ds weight (g)									
Traditional	10	25.5	49.9	35.9 <b>a</b>	35.9	7.3171	20.37						
SCF	10	30.7	38.7	35.0 <b>a</b>	35	3.2236	9.2						
		ŀ	Plant densities (	(thousand ha <sup>-1</sup>	<sup>1</sup> )								
Traditional	10	33000	85800	50160 <b>b</b>	39600	18976.19	37.83						
SCF	10	170000	202500	186000 <b>a</b>	186000	11498.79	6.18						

Table 1.	Variation	and	comparison	of	mean	grain	yield	(t	ha <sup>-1</sup> )	of	beans	and	yield	in
	relation to	sow	ing system co	m	onents	s. Sand	ovales	s, 2	014.					

<sup>1</sup>Monoculture of beans in 76 cm furrows; ‡ Cultivation system in stripes to the contour with planting in bed at four rows. Values having same letters between factors and levels in the same column are equal to 95% probability according to Student's t.

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# NINE BEAN CULTIVARS COMMON TYPE CARIOCA UNDER FIVE DIFFERENT PLANTING DENSITIES: YIELD, YIELD COMPONENTS AND REACTION TO DISEASES

# Martins, F.A.D.<sup>1,2</sup>; Soares, B. L<sup>1</sup>; Cardilo, B.E.S.<sup>1</sup>; Teixeira, H.<sup>2</sup>; Andrade, M. J. B.<sup>1</sup>

<sup>1</sup>Universidade Federal de Lavras, Minas Gerais State, Brazil. <sup>2</sup>EPAMIG, Minas Gerais State, Brazil. E-mail: fabioaureliod@gmail.com

**INTRODUCTION:** Seeking to maximize the gains obtained by breeding with the release of new cultivars, research in plant science must respond with studies that improve the management enabling the search for higher yields, combined with economic and environmental sustainability. Simple adjustments of management, such as the determination of plant population, allow the cultivation reach higher yields, regardless of the size of the farmer. The aim of this study was to evaluate the behavior of nine modern cultivars of carioca bean when grown under five seeding rates.

**MATERIALS AND METHODS:** Nine field trials were carried out simultaneously in no tillage system, each with a bean cultivar of carioca group: BRS 9435 Cometa, BRS Estilo e BRS Notável, coming from Embrapa Rice and Beans; IAC Alvorada, IAC Formoso e IAC Imperador coming from the Agronomic Institute of Campinas; IPR Tangará from the Agronomic Institute of Paraná; BRSMG Madrepérola e BRSMG Majestoso, obtained in common bean breeding program for Minas Gerais (Epamig, Embrapa, UFLA e UFV). The tests were conducted in Lambari, south of Minas Gerais, Brazil plated on 02/26 and harvesting 09/06/2014. The local climate is humid subtropical or CWa (Köppen classification). The experimental design was a randomized block design with six replications and five treatments populations (83, 116, 150, 183 and 250.000 plants ha<sup>-1</sup>). Seeds were sown with seed drill equipped with distribution of seeds with disk system, density 20 seeds m<sup>-1</sup> and posteriorly (11 days after sowing) performing thinning to obtain the populations desired. The experimental plot had eight lines of 3m, spaced 0.50 m were considered useful the four central lines. Fertilization followed the recommendation of soil analysis and, management had chemical weed control and spraying of insecticides and fungicides recommended for these culture, when necessary. During the crop cycle were three evaluations of disease symptoms (March 21, April 4 and 29). At harvest sampled up ten plants for determination of yield components (pods per plants, grains per pods and weight of 100 grains) and determined the yield of the plot, corrected to standard humidity of 13%. Data were subjected to analysis of variance. In cases of significant effect of sowing densities was performed regression analysis and selected equations with good fit to the relationship between the variables, based on the significance of the model and the value of  $R^2$ . In the case of significance for cultivars, the means were grouped by the Scott-Knott test at 5% probability.

**RESULTS AND DISCUSSION:** The analysis of variance indicated that the cultivars influenced all response variables, populations did not affect the beans number per pod and weight of 100 grains, and the interaction cultivar x populations was not significant in any of the situations. As for yield, the cultivars were grouped into four groups, especially the cultivars BRS Notável and BRSMG Madrepérola with higher yields (Table 1), consistent with the results obtained by MARTINS et al. (2014). The combination of pods per plant and grains per pod impacted more the productivity than the weight of 100 grains, indicating that plants with more pods and more grains are more efficient than those which produce coarse grains. The cultivar BRSMG Majestoso was affected by four pathogens, the IAC Alvorada was affected by pathogens

throughout the crop cycle and BRS Estilo showed excellent disease resistance. The yield performance was linear with increasing population, a result that would certainly be different if the range of populations was greater. The number of grains per plant and pods per plant, showed a quadratic behavior, demonstrating the plasticity inherent in the common bean (JADOSKI et al., 2000).

Table 1. Grain yield of the average values (YIELD), weight of 100 grains (W100), number of								
pods per plant (P/	P) and seed	s per poc	l (P/P),	and diseas	ses identified i	n each evaluat	tion.	
Cultivar	Yield	100GW	G/P	P/P	1 <sup>st</sup> disease	2 <sup>nd</sup> disease	3 <sup>rd</sup> disease	
	(kg/ha)	(g)			evaluation	evaluatiuon	evaluation	

	(kg/ha)	(g)			evaluation	evaluatiuon	evaluation
BRSMG Madrepérola	2.177,87 a	22,76 b	5,62 a	17,04 a	Pythium spp.	Alternaria spp.	-
BRS Notável	2.138,65 a	22,32 b	4,58 c	16,81 a	-	Alternaria spp.;	Uromyces
						Fusarium spp.	appendiculatus
IAC Formoso	2.070,68 b	22,48 b	5,24 b	16,93 a	Pythium spp.	Alternária	-
BRSMG Majestoso	1.980,01 b	20,99 c	5,58 a	16,78 a	Pythium;	Alternaria spp.;	-
					Rizoctonia spp.	Fusarium spp.	
IAC Imperador	1.854,50 c	21,60 c	4,22 d	17,94 a	-	-	Uromyces
							appendiculatus
BRS Estilo	1.840,33 c	22,69 b	5,07 b	14,52 b	-	-	-
BRS Cometa	1.776,40 c	22,07 b	4,54 c	15,47 a	Pythium spp.	Alternaria spp.,	-
						Fusarium spp.	
IPR Tangará	1.510,47 d	22,41 b	5,31 b	12,90 b	Rizoctonia spp.	Alternaria spp;	-
-						P. griseola	
IAC Alvorada	1.494,02 d	25,25 a	4,18 d	12,90 b	Pythium spp.	Alternaria spp.	Uromyces
							appendiculatus

Means followed by the same letter in the column do not differ by the Scott-Knott test at 5% probability.

Figure 1. Effects of plant population on grain yield and pods per plant.



**CONCLUSIONS:** The yield of grain crops increased with the increase in population density, even with reduction in the number of pods per plant. The BRS Notável and BRSMG Madrepérola cultivars are consolidated as highly productive genotypes. The cultivars have different reactions to naturally occurring pathogens.

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MARTINS, F.A.D.; SOARES, B. L.; MATA, D. C.; ANDRADE, M. J. B.; OLIVEIRA, D. P.; FIGUEIREDO, M. A. Carioca bean cultivars under different sowing density. Annual Report of the Bean Improvement Cooperative, v.57, p.301-302, 2014.

# AGRONOMIC PRACTICES ENHANCE PINTO BEAN PRODUCTION UNDER SEMI-ARID CONDITIONS

### H. F. Schwartz, M. A. Brick, J. B. Ogg, K. Otto and M. S. McMillan

Colorado State University, Fort Collins, Colorado

**INTRODUCTION:** A five-year study was conducted at the Colorado State University (CSU) research farm near Fort Collins, CO. The study compared the performance of three pinto bean cultivars with varying growth habits grown under furrow irrigation to determine if double row arrangement on seed beds increased seed yield or altered seed size compared to single row arrangement on seed beds. Upright type II cultivars Croissant (CSU release, 2011) and Stampede (North Dakota State University release, 2010) were compared to the prostrate type III cultivar Montrose (CSU release, 2001).

**MATERIALS AND METHODS:** A 4-bed mechanical planter was used to plant 1 or 2 rows per bed with rows spaced 15 cm apart on the bed), and beds spaced 75-cm apart at 207,500 seed/hectare from 2010 to 2014. Three or four replicates were used each year and experimental units consisted of 4-beds, 8 meters in length. Standard grower practices were applied for fertilizer, weed control, and disease and insect management. Data included yield and seed size. All data were analyzed statistically with PC SAS combined over years.

**RESULTS**: Growing conditions were favorable for plant development with trace infections of common bacterial blight and insect pests each year. Adequate furrow irrigation water (~ every 7 days prior to flowering) and fertilizer supported optimum plant development and pod set. Several significant interactions were noted among factors year, entry, and rows per bed. Multi-year average yield varied from 2141 to 4747 kg/hectare and 100-seed weight varied from 32.2 to 37.6 grams, depending on entry, row arrangement, and/or irrigation interva. Upright growth habit cultivars Croissant and Stampede had 5% higher yield under double row arrangement compared to single row arrangement (Table 1); with no effect on average seed weight. Irrigation interval (modified after flowering) showed an increase of 19, 34 and 51% for Croissant, Montrose and Stampede when irrigated 7 days versus 14 days after flowering during 2013 and 2014 (Table 2). Seed weight was increased with a 7 *vs* 14-day irrigation schedule by 1, 5 and 10% for Croissant, Montrose and Stampede.

	Yield	Rows/Bed	2010	2011	Average			
Entry			Yield (kg/hectare)					
Croissant		1	4780	3879	4330 bc			
		2	5221	4273	4747 a			
Stampede		1	5113	3827	4470 abc			
		2	5275	4002	4638 ab			
Montrose		1	5109	3971	4540 ab			
		2	4528	3795	4163 c			

**Table 1**. Yield and seed weight for three cultivars planted on 1 or 2 rows per 75 cm bed, and furrow irrigated weekly during 2010 and 2011 at Fort Collins, CO.

Means with the same letter are not significantly different according to the LSD ( $P \le 0.05$ ).

Seed Weight	Rows/Bed	2010	2011	Average		
Entry		Seed Weight (grams/100 seed)				
Croissant	1	35.2	38.1	36.7 ab		
	2	34.4	38.0	36.2 b		
Stampede	1	33.7	36.3	35.0 c		
	2	31.2	36.0	33.6 d		
Montrose	1	35.8	38.5	37.2 ab		
	2	35.2	40.0	37.6 b		

# Table 1 (cont.)

Means followed by the same letter are not significantly different according to the LSD ( $P \le 0.05$ ).

**Table 2**. Yield and seed weight for three cultivars planted on 2 rows per 75 cm bed, and furrow irrigated every 7 or 14 days after flowering during 2013 and 2014 at Fort Collins, CO.

	Irrigation	2013	2014	Average
Yield	Interval (days)			
Entry		,	Yield (kg/hectare	2)
Croissant	7	2620	2653	2637 bc
	14	2354	2082	2218 c
Stampede	7	3184	3155	3170 a
	14	2252	2029	2141 c
Montrose	7	3036	2792	2914 ab
	14	2458	2162	2310c

Means followed by the same letter are not significantly different according to the LSD ( $P \le 0.05$ ).

	Irrigation	2013	2014	Average			
Seed Weight	Interval (days)						
Entry		Seed V	Seed Weight (grams/100 seed				
Croissant	7	34.0	31.7	32.9 b			
	14	32.1	32.6	32.4 b			
Stampede	7	34.5	36.3	35.4 a			
	14	31.0	33.4	32.2 b			
Montrose	7	34.4	34.2	34.3 ab			
	14	33.1	32.2	32.7 b			

Means followed by the same letter are not significantly different according to the LSD ( $P \le 0.05$ ).

**DISCUSSION:** These results suggest that pinto bean growers should be able to increase yield and maintain desirable seed size using double row arrangement over traditional single row arrangement per bed combined with other cultural practices that include soil ripping and a reasonable interval between irrigation. Growers should carefully choose cultivars, cultural practices, and integrated pest management strategies that are appropriate for their growing conditions to benefit from these planting arrangements.

# PRODUCTIVITY, ADAPTABILITY AND STABILITY OF PRODUCTION OF BLACK BEAN LINES IN DIFFERENT ENVIRONMENTS OF MINAS GERAIS, BRAZIL

O.G. Brito<sup>1</sup>, A.J. de Carvalho<sup>1\*</sup>, J.E. de S. Carneiro<sup>2</sup>, J.A.A. Moreira<sup>3</sup>, M. Martins<sup>4</sup>, L.C. Melo<sup>5</sup>, L.C. Faria<sup>5</sup>, H.S. Pereira<sup>5</sup>, A.A. de Souza<sup>1</sup>, M.L. Lacerda<sup>1</sup>, V.B. de Souza<sup>1</sup>, T.L.P.O. Souza<sup>5</sup>

<sup>1</sup>Universidade Estadual de Montes Claros, <sup>2</sup>Universidade Federal de Viçosa, <sup>3</sup>Embrapa Milho e Sorgo, <sup>4</sup>Universidade Federal de Uberlândia, <sup>5</sup>Embrapa Arroz e Feijão \*Corresponding author: abjocar@yahoo.com.br

**INTRODUCTION:** The black bean is the second most consumed in Brazil, accounting for 18% of the planted area in the country (CTSBF, 2012), and it is also consumed in other countries. The current breeding programs seek to select lines with high productivity, adaptability and stability, to supply a greater number of growing regions. This work aimed to select common bean lines of black commercial class, with higher productivity, adaptability and stability of production between lines evaluated in Value for Cultivation and Use tests (VCU) of common bean in different environments of Minas Gerais State.

MATERIAL AND METHODS: The experiments were set in Sete Lagoas, Uberlândia, Janaúba and Jaíba, in spring-summer (water), summer-autumn (drought) and autumn-winter (winter) crops, from 2010 to 2013, totaling nine environments. The treatments consisted of 12 precommercial lines and four control cultivars of black common bean, selected by agreement between breeding programs of UFV, UFLA, EPAMIG and EMBRAPA Rice and Beans. We used conventional tillage, with plowing and two disking. Bean plants were sown at a spacing of 0.5 m between rows, distributing about 15 plants per meter. The plots consisted of four rows of 5 m long and the useful area included the two central rows, discarding 0.5 m from each boarder of the rows. The crop was fertilized according to the official recommendation for Minas Gerais and all environments had supplementary irrigation by sprinkler. We evaluated the yield of the lines, considering humidity of 13%. Data were subjected to analysis of variance involving all environments. The effects of the lines, when significant, were compared by Scott-Knott test at 5% significance. Moreover, adaptability and stability analyses of the lines were performed by the method of Annicchiarico (1992) using the GENE program (Cruz, 2013). We adopted confidence level of 75%. The selection of the lines regarding adaptability and stability was defined in terms of Wi, which must be greater than 100%.

**RESULTS AND DISCUSSION:** The CNFP 10793, 10103 CNFP and VP-26 pre-commercial lines and the BRS CAMPEIRO commercial line presented the highest yields. The CNFP 10793 (Wi = 114.18), CNFP 10103 (Wi = 104.08), VP-26 (Wi = 101.88) and BRS CAMPEIRO (Wi = 103.42) lines showed adaptability and satisfactory stability. According to the Wi obtained values, it is possible to say that these lines can produce, with 75% confidence, 14.18, 4.08, 1.88 and 3.44% more than the overall average of the studied lines. In additon the "BRS SPLENDOR", "OURO NEGRO" e "BRS VALENTE" commercial cultivars presented performance below the CNFP 10793, 10103 CNFP and VP-26 pre-commercial lines (Table 1), which reinforces their potential to be released as commercial cultivars suitable for cultivation in the State of Minas Gerais, since they include, besides high productivity, good adaptability and yield stability.

Lines	Yield (kg ha <sup>-1</sup> )	Wi <sup>2</sup>	Classification <sup>3</sup>
CNFP 10793	1942,93 a <sup>1</sup>	114,18	1
BRS CAMPEIRO	1759,22 a	103,42	3
CNFP 10103	1741,04 a	104,08	2
VP-26	1723,74 a	101,88	4
BRS ESPLENDOR	1666,04 b	99,47	5
CNFP 11980	1632,81 b	97,84	6
CNFP 11977	1603,26 b	91,27	9
CNFP 11992	1598,41 b	93,16	7
VP-24	1584,96 b	88,05	11
OURO NEGRO	1576,19 b	84,68	15
VP-27	1556,56 b	92,27	8
BRS VALENTE	1543,04 b	88,04	12
VP-28	1538,41 b	90,02	10
CNFP 11990	1410,26 c	86,22	13
VP-29	1376,96 c	85,41	14
VP-25	1200,07 c	60,66	16

**Table 1:** Grain yield (GY), genotype recommendation index (Wi) and classification of common bean breeding lines of the "Black" commercial class grown in different environments of Minas Gerais State, Brazil.

<sup>1</sup> Means followed by the same letter do not differ by the Scott-Knott test at 5% of significance. <sup>2</sup> Genotype recommendation index by Annicchiarico's method; <sup>3</sup>Classification, 1 as the most stable.

**CONCLUSIONS:** The CNFP 10793, 10103 CNFP, VP-26 and BRS CAMPEIRO lines presented the highest yields, adaptability and yield stability in all evaluated environments.

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#### CHARACTERIZATION OF EARLY GENOTYPES OF COMMON BEAN

# Adriano Stephan Nascente<sup>1</sup> and Leonardo Cunha Melo<sup>1</sup>

<sup>1</sup>Brazilian Agricultural Research Corporation (EMBRAPA), Rice and Beans Research Center, P.O.Box 179, 75375-000, Santo Antônio de Goiás, Goiás, Brazil

**INTRODUCTION:** The use of early cycle cultivars of common bean (*Phaseolus vulgaris* L.) that allow achieving high grain yields in the shortest time thus providing irrigation water and power savings and hence the cost of production is an old desire of farmers. In this sense, Embrapa Rice and Beans developed earlier cycle genotypes to meet this demand. However, these elite lines have to be characterized in more detail in order to develop a management system that allows fully exploit their genetic potential. Growth analysis is a technique which details the allocation of photosynthate partition as a function of the age of the plant. Determination of dry matter (plant and its parts: stems, leaves, pods and seeds) is the most suitable for the growth analysis (Taiz & Zeiger, 2004). The information generated can be used to verify the adaptation of the crop to new environments, interspecific competition and management systems effects (Andrade et al., 2009). The aim of this study was to was to characterize the agronomic performance of three elite genotypes of common bean with early cycle by growth analysis technique.

MATERIAL AND METHODS: The irrigated field experiment was performed on autumn/ winter (May to July) in 2014 at the Capivara farm from Embrapa Rice and Beans in Santo Antônio de Goiás, GO, Brazil. An irrigated field experiment with a randomized block experimental design with eight replications was conducted in Brazil during the 2014 growing seasons. The treatments consisted of common bean genotypes with early maturity, CNFC 15873, CNFC 15874 and CNFC 15875. Sowing of common bean was mechanically held on May 20, 2014, spaced 0.50 m between rows and with 15 viable seeds per meter. Fertilization in sowing furrows in all treatments was 45 kg ha<sup>-1</sup> of N as urea, 50 kg ha<sup>-1</sup> of K as potassium chloride 26 kg ha<sup>-1</sup> of P as triple superphosphate. In the  $V_4$  vegetative stage of the common bean (four trifoliate leaves), a topdressing fertilization of 45 kg ha<sup>-1</sup> of N as urea was performed for all plots. Other cultural practices were performed according to the recommendations of the crops to keep the area free of weeds, disease and insects. It was collected plants weekly in a linear meter in each plot for the realization of the growth analysis. Plants were separated into stems, leaves, pods and seeds. We made the mass accumulation graphs of dry matter of each plant structure and total. At harvest time it was made the evaluation of the yield and yield components of each genotype. Data were subjected to an analysis of variance, and the means were compared by Tukey's test at p < 0.05.

**RESULTS AND DISCUSSION:** The CNFC 15874 genotype showed the highest dry matter mass of seeds (119.32 g m-1) and total (236.96 g m-1) in relation to genotypes CNFC 15873 (89.78 g m-1 and 200.30 g m-1, respectively) and CNFC 15875 (93.29 g m-1 and 178.24 g m-1, respectively) at 72 days after sowing (Figure 1). This further development of CNFC 15874 also allowed the highest grain yield (3615 kg ha-1), which differed significantly from genotypes CNFC 15873 (2660 kg ha-1) and CNFC 15875 (2677 kg ha-1), which did not differ each other (Table 1). The highest accumulation values for seed and total dry matter of CNFC 15874 are related to the genotype potential. It was observed that untill 60 days after sowing, CNFC 15873 genotypes (29.98 g m-1) and CNFC 15875 (32.78 g m-1) accumulated the maximum mass of dry matter in leaves. After this period, leaves dry matter started to decline indicating that there was translocation of their photoassimilates to the seeds. On the other hand, the CNFC 15874 genotype accumulated dry matter in the leaves up to 66 days (46.23 g m-1). This longer period of CNFC 15874 accumulating mass of dry matter in the leaves seems to be crucial to provide the higher yield of

this genotype. According to Wien et al. (1976) in the formation and filling of seeds phases about 45% of photoassimilates in the leaves and stems are translocated into the seeds. From the results it can be seen that the growth analysis technique was effective to explain the higher yield of CNFC 15874 genotype in relation to the others tested genotypes.



**Figure 1.** Growth analysis of three common beans genotypes (CNFC 15873, CNFC 15874 and CNFC 15875) cultivated in Santo Antônio de Goiás, Goiás State, Brazil in the growing season 2014.

**Table 1** – Number of pods per plant (NPP), number of seeds per pods (NSP), mass of 100 seeds (MASS) and yield of early genotypes of common beans. Santo Antônio de Goiás, Brazil, growing season 2014.

NPP	NSP	MASS	YIELD	
unit	unit	grams	Kg ha <sup>-1</sup>	
14b*	5a	22a	2660b	
18a	4a	24a	3615a	
15ab	4a	23a	2677b	
	ANOVA (F	probability)		
< 0.047	0.6325	0.8545	< 0.001	
14.07	11.12	3.43	13.76	
	NPP unit 14b* 18a 15ab <0.047 14.07	NPP         NSP           unit         unit           14b*         5a           18a         4a           15ab         4a           ANOVA (F           <0.047	NPP         NSP         MASS           unit         unit         grams           14b*         5a         22a           18a         4a         24a           15ab         4a         23a           ANOVA (F probability)         <0.047	NPP         NSP         MASS         YIELD           unit         unit         grams         Kg ha <sup>-1</sup> 14b*         5a         22a         2660b           18a         4a         24a         3615a           15ab         4a         23a         2677b           ANOVA (F probability)             <0.047

<sup>\*</sup>means followed by the same letter vertically are not significantly different at p<0.05 according to Tukeys's test.

#### ACKNOWLEDGEMENTS

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Wien, H. C. et al. 1976. <sup>14</sup>C-assimilate distribution in *Phaseolus vulgaris* L. during the reproductive period. Journal of the American Society for Horticultural Science 101: 510-513.
# SELECTION OF BLACK SEEDED BREEDING LINES FOR EARLY MATURITY AND HIGH SEED YIELD IN NORTHERN MEXICO

# Sergio Arellano-Arciniega<sup>1\*</sup>, Rigoberto Rosales-Serna<sup>2</sup>, Esteban Salvador Osuna-Ceja<sup>1</sup> and Mercedes Borja-Bravo<sup>1</sup>

 <sup>1</sup>INIFAP-Aguascalientes. km 32.5 Carretera Aguascalientes-Zacatecas. Pabellón de Arteaga, Aguascalientes, México. C. P. 20660. Tel. +52 (465) 958-01-67.
 \*arellano.sergio@inifap.gob.mx.
 <sup>2</sup>INIFAP-Durango. km 4.5 Carretera Durango - El Mezquital. Durango, México. C. P. 34170.

Tel. +52 (618) 826-04-26, ext. 204.

# INTRODUCTION

Black seeded common bean (*Phaseolus vulgaris*) cultivars are needed in México in order to satisfy grain demand for human consume. Most of black seeded cultivars show late maturity and yield loss due to low temperatures registered in late September and early October. An important annual grain deficit (32,000 MT) is observed for black seeded common beans in central and southern México (Sánchez *et al.*, 2001). One of the most appreciated common bean cultivar in Durango and Zacatecas is Negro San Luis, showing late maturity (110-120 days after planting) and rounded shiny black seeds. High seed yield is observed in Negro San Luis plantings during rainy years (>460 mm) and favorable minimum temperature (>10 °C) observed in October. Several black seeded lines have been developed at INIFAP's breeding program with early maturity and higher seed yield. These breeding lines need to be evaluated under variable climate conditions across the Mexican Highlands. Evaluation across environments permits to select cultivars with wide adaptability and enhanced seed yield. The objective of this study was to select black seeded breeding lines showing early maturity, disease resistance and high seed yield.

#### MATERIALS AND METHODS

In 2014, an experiment was planted in july 4<sup>th</sup> including sixteen (F<sub>8</sub>) black seeded breeding lines in Sandovales, Ags. (21° 54' 08" N and 102° 04' 12" W). The climate [BS<sub>1</sub> kw (w)] is semiarid with low relative humidity and sporadic rainfall (250-500 mm during the year) (Medina *et al.*, 2006). The length of the crop cycle was 110 days (July-October) showing favorable minimum temperature (16.5 °C on average) and total rainfall accumulation (466 mm). The soil type was Planosol, with sandy-loam texture, slightly acid pH (6.4), and low organic matter content (<1 %). Yield trial was sown under randomized complete block design with two replications. Experimental plot consisted in two rows 5 m in length and 0.76 m apart. Plant density was 90,000 plants per hectare, fertilizer was applied using the dose 25-35-00 (N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O) and additional foliar fertilization was applied during pod filling period using 5.5-4.5-00 kg ha<sup>-1</sup>. Weed control included two mechanical labor complemented with a weeding performed by hand. Data were taken for the number of days to flowering and physiological maturity, disease reaction (CIAT, 1987), seed yield and 100 seeds weight. Analysis of variance was obtained under a randomized complete block design with two replications and mean comparisons were performed by LSD (Least Significant Difference) test (P ≤ 0.05).

#### **RESULTS AND DISCUSSION**

Highly significant ( $P \le 0.01$ ) differences were observed among breeding lines for all the evaluated traits (except for disease reaction). Negro San Luis showed early flowering (43 days) and delayed maturity (100 days) compared to the breeding lines (flowering 43 to 49 days and maturity 93 to 96 days). Several breeding lines overpassed the seed yield registered by Negro

San Luis considered as the Check (2,650 kg ha<sup>-1</sup>), although statistical similitude was observed. Outstanding lines for seed yield was NGB10026 (3,097 kg ha<sup>-1</sup>) and NGB10020 (3,002 kg ha<sup>-1</sup>). These lines also showed greater seed size (30 to 31 g) compared to the Check (26 g). Some advances were observed in breeding programs for earliness, seed yield and seed size.

Breeding lines (NGB10026 and NGB10020) were selected in order to improve the seed yield obtained at the Mexican Highlands. Lines also showed disease resistance, intermediate maturity and will be included in evaluation programs for seed yield and adaptability trials grown under farmer's fields conditions. Higher seed yields and improved market quality observed in selected black seeded breeding lines could be used to alleviate grain deficits observed in central and southern México (Sánchez *et al.*, 2001).

Breeding Line	Days to flowering	CBB Reaction*	Days to Physiological Maturity	Seed Yield kg ha <sup>-1</sup>	100 seeds weight (g)
NGB10026	47	5	95	3,097 <sup>a</sup>	31
NGB10020	48	5	95	$3,002^{a}$	30
NGB10032	46	5	93	$2,988^{a}$	29
NGB10031	47	5	95	2,828 <sup>ab</sup>	31
NGB12004	44	5	93	$2,793^{ab}$	23
NGBR12004	43	5	94	$2,761^{ab}$	21
NGOB09005	44	5	94	$2,730^{abc}$	25
NGB10043	46	5	93	2,669 <sup>abc</sup>	31
NGB12002	44	5	93	$2,661^{abc}$	21
Sn. Luis (Check)	43	6	100	$2,650^{abc}$	26
NGOB09002	46	5	93	2,341 <sup>bcd</sup>	22
NGB10048	44	5	93	$2,240^{bcd}$	29
NGB10050	49	5	96	2,234 <sup>bcd</sup>	29
NGB10034	47	5	94	2,122 <sup>cd</sup>	28
NGB10059	44	5	94	1,831 <sup>d</sup>	29
NGB10049	45	5	94	1,727 <sup>d</sup>	26
Average	45		94	2,542	27

Table 1. Average values for different traits evaluated in black seeded breeding lines.

\*CBB= Common Bacterial Blight (*Xanthomonas campestris* = *axonopodis* pv. *phaseoli*); <sup>a-d</sup>Different letters at the same column represents significant differences according to LSD test ( $P \le 0.05$ ).

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# YIELD AND ITS COMPONENTS OF CLIMBING BEAN IN TWO YEARS OF SOWING IN TEMPERATE CLIMATE

# José Alberto Salvador Escalante-Estrada<sup>1</sup>., María Teresa Rodríguez-González.<sup>1</sup> y Yolanda Isabel Escalante-Estrada<sup>2</sup>

<sup>1</sup>Postgrado en Botánica. Campus Montecillo. Colegio de Postgraduados. Montecillo, Mpio. de Texcoco, Edo. de Méx, México.56230. E mail: jasee@colpos.mx., mate@colpos.mx;<sup>2</sup>Instituto de Investigación Científica área de Ciencias Naturales. Universidad Autónoma de Guerrero. Chilpancingo Guerrero México. E-mail: y\_escalante@yahoo.com.mx.

# **INTRODUCTION**

The climbing bean for a major expression of the growth and grain yield, needs of trellises that in general they are of wood and that raise the cost of production, in addition they alter the environment due to the deforestation (Escalante and Kohashi, 1983). In Mexico in general, the climbing bean is sowed with maize, which is used as alive espalier. Nevertheless, the bean yield diminishes due to the competence) with maize (Díaz *et al.*, 2010; Delgado *et al.*, 2014). The study of the genotypic variability in climbing bean would be an alternative to increase the grain yield. The aim of the study was to determine: a) the bean genotypes that present highest grain production under rain conditions in temperate climate; and b) the variability in grain yield between years of sowing.

# MATERIALS AND METHODS

The study realized in Montecillo Texcoco, Méx.Mexico (19 ° 29 ' N, 98 ° 53 'W, to 2250 m of altitude), with temperate climate in a clay soil with pH of 7.8. The treatments consisted of the sowing bean (*Phaseolus vulgaris* L.) of indeterminate climbing habit, to density of 4.16 plants m<sup>2</sup> in rows to 80 cm of separation on: a) June 10, 2010 the genotypes: HAV-14, Japonés, FM X16441, Negro-150 and Michoacán of grain black, white, pink, black and red color, respectively; b) on June 15, 2011 the genotypes: HAV-14, Japonés, Oaxaca (grain coffee color with black spots), Negro-150 and Michoacán. The alive trellis was Azul maize .The experimental design was randomized blocks with four replicates. Was registered the occurrence of phenological stages (Escalante and Kohashi, 1993), the maximum temperature (Tmax), minimum (Tmin) and precipitation (PP, mm) during the growing season. To the physiological maturity (PM, harvest), the pod number (PN), grains number (GN), grain size (g per grain, GS), number of grains per pod (GP) and grain yield (GY). An analysis of variance and Tukey ( $\alpha = 0.05$ ) test were applied.

# **RESULTS AND DISCUSSION**

#### **Climatic conditions and Phenology**

In 2010 during the crop development, the mean of Tmáx was of 26°C, the Tmín of 9 °C and the PP of 326 mm. The emergency (E) of the bean was to the 10 days after sowing (das), the beginning of flowering (BF) to the 58 das for HAV-14; 75 das Japanese; 65 das to FMX16441; 60 das Negro 150 and 56 das Michoacán. The PM was to the 110 das. In 2011, the mean of the Tmáx and Tmín during the development of the crop was of 25 °C and 10 °C, respectively. The PP seasonal was 504 mm. The E was to 12 das; the BF to 58, 62,77 and 75 das, for HAV-14, Japanese, Oaxaca and FMX16441, respectively. The PM was to the 130 das.

#### Yield and its components

In the Table 1, is observed that in both years, bean cultivars under study present differences as for the BF, grain yield and its components. In both years, HAV-14 presented the highest GY with 91 gm<sup>-2</sup> (2010) and 126 g m<sup>-2</sup> (2011), followed of Japonés, Negro-150 and Michoacán. FMX16441 only sowed in 2010 presented 70 gm<sup>-2</sup>; and in 2011 Oaxaca showed a GY of 56 gm<sup>-2</sup>. The changes in GY were related with the GN and PN. The differences in GY between years (31 gm<sup>-2</sup>) were due to changes in rainfall. The bean reached a height of 180 cm approximately.

YEAR	CULTIVAR	PN m <sup>-2</sup>	GN m <sup>-2</sup>	GP	GS g	GY gm <sup>-2</sup>
2010	HAV-14	90 a	251 a	2.8	0.362 a	91 a
	JAPONÉS	48 c	194 b	4.0	0.284 b	55 c
	X16441	53 c	171 b	3.3	0.408 a	70 b
	NEGRO-150	57 c	185 b	3.2	0.297 b	55 c
	MICHOACÁN	76 b	228 a	3.2	0.250 b	57 c
	Mean	65	206	3.3	0.320	66
	Tukey α=0.05	12	30	1.5	0.050	20
2011	HAV-14	116 b	348 a	3.0	0.362 a	126 a
	JAPONÉS	79 c	339 ab	4.3	0.230 d	78 b
	OAXACA	61 c	183 c	3.0	0.304 b	56 c
	NEGRO-150	87 c	261 c	3.0	0.333 a	87 b
	MICHOACÁN	140 a	321 b	2.3	0.280 c	90 b
	Mean	101	307	3.2	0.298	91
	Tukey α=0.05	20	45	1.3	0.55	18

**Table 1.** Yield and its components of cultivars of climbing bean. Montecillo, Texcoco Méx.Mexico. Summer 2010 and 2011.

#### CONCLUSIONS

For the region of study, differences between climbing bean cultivars are observed in the grain yield and its components. HAV-14 stands out for higher production of pods, grain and yield. The differences in grain yield between years of study are due to changes in rainfall.

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# COMMON BEAN AND LOW COST INOCULATION AS COMPONENTS OF SUSTAINABLE AGROFORESTRY SYSTEMS IN DURANGO, MÉXICO

# Rigoberto Rosales-Serna<sup>1\*</sup>, José Ángel Sigala Rodríguez<sup>1</sup>, Marcos Torres Meráz<sup>2</sup>, Homero Sarmiento López<sup>1</sup>

 <sup>1</sup>INIFAP-Durango. km 4.5 Carretera Durango - El Mezquital. Durango, México. C. P. 34170. Tel. +52 (618) 826-04-26, ext. 204. \*rosales.rigoberto@inifap.gob.mx
 <sup>2</sup>Instituto Tecnológico del Salto. Mesa del Tecnológico, s/n. El Salto, Pueblo Nuevo, Durango. C. P. 34950.

**INTRODUCTION:** Agroforestry provide a feasible opportunity to realize multiple products in the same soil portion and an opportunity for landowners to diversify their management systems and sources of economic income (Clason and Robinson, 2000). Common bean (*Phaseolus vulgaris* L.) is considered as an important option to produce food in agroforestry systems used for sustainable agriculture in the state of Durango in North-Central, México. In recent years the area used for fast-growing commercial forest plantations (*Pinus greggii*) has been increased in Durango in order to reduce forest wood extraction and forest degradation. Enhanced productivity is necessary for landowners in order to avoid economic losses caused by the long time period observed during pine timber production. In Durango, the main food and forage crops are common beans (*Phaseolus vulgaris*), maize (*Zea mays*) and oat (*Avena sativa*). Alternatives are also needed in order to reduce chemical fertilizer use and production costs in annual and perennial crops. The objective of this study was to evaluate the effect of inoculation on forage and seed yield in common bean, maize and oat grown under a Christmas-tree plantation used as agroforestry system in Durango, México.

#### **MATERIALS AND METHODS**

In 2014, an agroforestry system was implemented in Durango, México, including a 7 years-old Christmas-tree plantation (*Pinus greggii*) and annual crops, such as common bean (cv. Pinto Saltillo), maize (cv. CAFIME) and oat (cv. Avemex). Annual crops were sown when the rainy season started (july 9<sup>th</sup>) using strips between pine tree lines planted 3 m apart (strips) and 1.5 m among plants. Pine trees showed average values of 2.9 m for plant height, 6.9 cm for stem basal diameter and 1.2 m for canopy diameter. Annual crops were planted in alternate strips using two rows with variable length (20-130 m) and 0.81 m apart. Similar intercropping system was established in an adjacent plot without pine plantings to be used as a Control. In both environments two pyranometers (Kipp & Zonen SP Lite 2) were installed over 80 cm from the soil surface in order to evaluate global solar radiation (watts/m<sup>2</sup>) and the shade effects in annual crops.

For each annual crop, three inoculation treatments were evaluated, including chemical fertilization (F), inoculation (I) and combined use for fertilizer and inoculation (F + I). Fertilizer was applied at the rate of 25-35-00 (N-P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O) for common bean and 30-30-00 for maize and oat. Inoculation included *Azozpirillum brasilense* and *Glomus* spp. applied to seeds prior sowing, according to the product recommendation. Weeds control included mechanical labor (3) complemented with two hand weeding.

Experimental plot consisted in one strip with three replications per treatment. In each crop strip three plant samples were taken during grain filling period in maize and oat in order to obtain dry forage production. Samples consisted in two rows with 5 m in length by 0.81 m in width  $(8.1 \text{ m}^2)$ . At maturity, three plant samples were also taken in each crop strip for seed yield

determinations. Analysis of variance was obtained under randomized complete block design with five replications and mean comparisons were performed by LSD test ( $P \le 0.05$ ).

# **RESULTS AND DISCUSSION**

Highly significant ( $P \le 0.01$ ) differences for dry forage and seed yield (oat and common bean) were observed among intercropping systems (oat) and inoculation treatments (oat and common beans) (Table 1). Highest dry forage and seed yield in oat was observed at traditional intercropping system due to shade effects observed in the agroforestry system. Inoculation treatment showed the lowest dry forage production in both intercropping systems and lower values were also observed for seed yield in the agroforestry system. Oat showed poor adaptation under the agroforestry system which includes Pinus greggii, inoculation and an average shade level reaching 24 %. Maize showed similar results for dry forage and seed yield among agroforestry systems and inoculation treatments. Common beans registered high average for seed yield under traditional intercropping (1,445 kg ha<sup>-1</sup>) and agroforestry system (1,392 kg ha<sup>-1</sup>). Similar results were observed for common beans seed yield among inoculation treatments under the agroforestry system, while the lowest seed yield (1,008 kg ha<sup>-1</sup>) was observed in the inoculation treatment at traditional intercropping system. Incentive programs need to be implemented in Durango in order to encourage adoption of the agroforestry systems, thus, farmers would have food production during tree growth as well as an income derived from tree harvest. Pinto Saltillo, common bean cultivar, represents an important productive option for sustainable agroforestry and traditional intercropping systems. Inoculation was insufficient to satisfy crop nutrition requirements especially in oat and common bean under traditional intercropping system.

		Dry Forage	Yield (kg ha <sup>-1</sup> )	Seed Yi	eld (kg h	a <sup>-1</sup> )
Treatm	nent	Oat	Maize	Oat	Maize	Bean
			Agrofores	stry System		
Chemical (CF)	fertilizer	689 <sup>a</sup>	5,500 <sup>a</sup>	293 <sup>ab</sup>	1,930	1,577 <sup>a</sup>
Inoculation (	I)	$268^{ab}$	$2,800^{ab}$	184 <sup>b</sup>	1,095	1,351 <sup>a</sup>
CF + I		512 <sup>a</sup>	6,100 <sup>a</sup>	317 <sup>a</sup>	1,554	1,249 <sup>a</sup>
	Average	$490^{\mathrm{B}}$	4,800	265 <sup>B</sup>	1,526	1,392
			Traditional Inter	rcropping System		
Chemical (CF)	fertilizer	1,157 <sup>a</sup>	4,500 <sup>a</sup>	415 <sup>a</sup>	808	1,804 <sup>a</sup>
Inoculation (	I)	437 <sup>b</sup>	5,100 <sup>a</sup>	312 <sup>a</sup>	1700	$1,008^{b}$
CF + I		1,065 <sup>a</sup>	5,200 <sup>a</sup>	415 <sup>a</sup>	940	1,523 <sup>a</sup>
	Average	886 <sup>A</sup>	4,900	381 <sup>A</sup>	1,149	1,445

**Table 1.** Dry forage and seed yield registered in annual crops planted under agroforestry and traditional intercropping system in Durango, México (2014).

Letters in each column indicate significant differences according to the Least Significant Difference (P  $\leq$  0.05) between intercropping systems (<sup>A-B</sup>) and inoculation treatments <sup>a-b</sup>.

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# EFFECT OF AGAVE INTERCROPPING SYSTEM AND INOCULATION ON COMMON BEAN, MAIZE AND OAT PRODUCTIVITY IN DURANGO, MÉXICO

# Rigoberto Rosales-Serna<sup>1\*</sup>, José Ángel Sigala Rodríguez<sup>1</sup>, Irma Idelisa Reyes Lara<sup>2</sup>, Homero Sarmiento López<sup>1</sup>

<sup>1</sup>INIFAP-Durango. km 4.5 Carretera Durango - El Mezquital. Durango, México. C. P. 34170. Tel. +52 (618) 826-04-26, ext. 204. \*rosales.rigoberto@inifap.gob.mx.
<sup>2</sup>Instituto Tecnológico del Valle del Guadiana, km 22.5 Carretera Durango-México, Villa Montemorelos, Dgo., México. C. P. 24371.

# **INTRODUCTION**

In Durango, sandy, rocky, superficial and high slope gradient soils causes significant soil losses due to erosion registered under intensive monoculture systems used in the Mexican Northern-Highlands (Flores et al., 2014). Common bean (Phaseolus vulgaris L.) is considered as an important option to produce food under intercropping systems proposed for sustainable agriculture in the state of Durango, México. Large plot areas and the intensive mechanization used for agriculture are some of the factors for soil deterioration in common bean monoculture systems implemented in Durango. Alternatives for intercropping systems and organic plant nutrition are needed in order to perform sustainable agriculture and reducing soil and water losses. The use of chemical fertilizer causes significant cost increments for crop production, thus alternative low cost options are being tested in Durango. Agave species (Agave durangensis, A. salmiana) are perennial crop used in Durango for sustainable agriculture but requiring long time periods since plantings to the plant harvest for industrial use. Agave plant is appreciated by land owners and it is used to obtain forage, aguamiel, agave syrup, pulque and mezcal. Maize (Zea mays) and oat (Avena sativa) are also important crops in Durango and are mainly used for forage and grain production (maize). The objectives of this study were to evaluate the effect of an Agave intercropping system and inoculation on forage and seed yield obtained for common bean, maize and oat in Durango, México.

# **MATERIALS AND METHODS**

In 2014, an intercropping system was implemented in San Francisco de Malpaís, municipality of Nombre de Dios, in the State of Durango, México. Plant species included perennial: (twelve-year old Agave plantation) and annual crops, such as common bean (cv. Pinto Saltillo), maize (cv. CAFIME) and oat (cv. Avemex). Annual crops were sown when the rainy season started (july  $11^{th} - 18^{th}$ ) and row plantings were used in maize and common bean; while seed spreading method was utilized for oat sowings. Inoculation treatments were also evaluated including chemical fertilizer (F), inoculation (I) and the combined use for fertilizer and inoculation (F + I). Fertilizer was applied at the rate of 25-35-00 (N-P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O) for common bean and 40-40-00 in maize and oat. Inoculation treatment included *Azozpirillum brasilense* and *Glomus* spp. applied prior plantings according to product recommendations. Weeds control included mechanical labor (2) in common bean and maize, while chemical control was used in oat (2,4-D amine).

Plant samples (five) were taken during grain filling period in maize and oat in order to evaluate dry forage production. In maize, samples consisted in two rows 5 m in length by 0.81 m in width (8.1 m<sup>2</sup>) and five (0.0625 m<sup>2</sup>) samples were also randomly taken in oat strips using the 25 cm x 25 cm sampling square. At maturity, five plant samples were taken in each crop strip for

yield determinations. Analysis of variance was obtained under completely randomized design with five replications and mean comparisons were performed by Tukey's test (HSD,  $P \le 0.05$ ).

# **RESULTS AND DISCUSSION**

Highly significant ( $P \le 0.01$ ) differences for dry forage and seed yield were observed among inoculation treatments in oat and maize (Table 1). Oat registered higher dry forage yield due to its adaptation and positive response for the effect of chemical fertilization (11.5 t ha<sup>-1</sup>) and the combined effect of chemical fertilization plus inoculation (13.1 t ha<sup>-1</sup>). An overestimation of forage yield was also observed due to the sowing system and sampling method used in this crop. Maize obtained the lowest dry forage yield, compared to oat, even in the inoculated treatment (1.3 t ha<sup>-1</sup>). Significant response was observed for maize and oat dry forage production using fertilizer and fertilizer plus inoculation.

Common bean registered adaptation under Agave intercropping system and showed higher seed yield, compared to maize and oat, but statistical similitude was observed between inoculation treatments. Similar results were observed in previous experiments developed in Durango (Pajarito *et al.*, 2012). Adaptation and natural nitrogen fixation leaded low responses in common beans to fertilizer and combination for chemical fertilizer and inoculation. Common beans represent an important option as a component in Agave intercropping systems used in Durango. Incentive programs need to be implemented in order to encourage the use of intercropping techniques, which include common beans, in order to practice sustainable agriculture in Durango. Results need to be corroborated and suggested that inoculation was insufficient to support plant nutrition mainly in maize and oat.

Table 1	. Dry	forage	and	seed	yield	registered	in	annual	crops	planted	under	an	Agave
intercropping system in Durango, México.													
Inoculat	ion			Oat		Maize			Oat	Maiz	ρ	Re	an

Inoculation	Oat	Maize		Oat	Maize	Bean		
Treatment	Dry Forage	e Yield (t ha <sup>-1</sup> )	Seed Yield (kg ha <sup>-1</sup> )					
Chemical fertilizer (CF)	11.5 <sup>a</sup>	2.9 <sup>a</sup>		345.8 <sup>b</sup>	573.3 <sup>a</sup>	775.0		
Inoculation (I)	4.5 <sup>b</sup>	1.3 <sup>b</sup>		268.5 <sup>b</sup>	$188.0^{b}$	563.0		
CF + I	13.1 <sup>a</sup>	2.6 <sup>a</sup>		631.0 <sup>a</sup>	738.7 <sup>a</sup>	760.0		
Average <sup>1</sup>	9.7	2.3		415.1	500.0	699.3		

<sup>a-b</sup> = Letters in each column indicate significant differences according to Tukey's test ( $P \le 0.05$ ).

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# BIOMASS AND YIELD OF RUNNER BEAN (*Phaseolus coccineus* L.) IN ASSOCIATION WITH MAIZE

# Néstor Jorge Rojas Victoria<sup>1</sup>; José Alberto Salvador Escalante Estrada<sup>1</sup>; María Teresa Rodríguez González<sup>1</sup>

<sup>1</sup>Postgrado en Botánica, Colegio de Postgraduados. Campus Montecillo. Km 36.5 Carretera México-Texcoco, 56230. Teléfono 01(595) 952 02 00 ext. 1330. Montecillo, Texcoco, Estado de México, México.
E-mail: nerovic@colpos.mx, jasee@colpos.mx, mate@colpos.mx

# **INTRODUCTION**

The runner bean (*Phaseolus coccineus* L.) is a legume native from Mexico, that which is grown on small farmland (Castillo *et al.* 2006). The pods, leaves and flowers have a potential for food use. For planting the Ayocote is necessary to use trellises (Escalante and Kohashi, 1993) in order to achieve its distribution in space, to capture solar radiation and consequently to produce higher dry matter. The conventional trellis is wooden poles or metal mesh structures, which significantly raise the cost of production. An alternative is to plant the Ayocote in living trellis of maize that serves as a support. Delgado *et al.* (2014) pointed that in trellis of corn, growth and yield of beans are reduced by competition. The objective of the study was to determine the biomass production and yield of Ayocote according to different cultivars of maize used as living trellises.

# MATERIAL AND METHODS

The study was conducted at the Colegio de Postgraduados Campus Montecillos, Méx, México under conditions of seasonal rainfall during 2013. Planting Ayocote bean (cv Tlaxcala has grain violet), of indeterminate growth habit climber (Type IV), was on May 24, with three cultivars of landrace maize Chalqueño: black (BB), blue (BM) and yellow (YM). The density was 5 plants m<sup>2</sup>. The experimental design was a randomized block with four replications. During the growing season, temperature (°C) and precipitation (PP) were recorded. Also the days to occurrence of phenological stages: emergency (E), vegetative stage (VS), flowering (R6) and physiological maturity (R9) for beans, under the criteria proposed by Escalante and Kohashi (1993). At physiological maturity (PM) or harvest, the grain yield (GY, 10% humidity, g m<sup>-2</sup>) and its components as pod number pod m<sup>-2</sup> (PN); grains per pod (GP); weight of 100 grains (WG); grain number m<sup>-2</sup> (GN), total biomass o dry matter (g m<sup>-2</sup>, TB) were measured. Harvest index (HI) was estimated with the relationship HI= [GY / TB]\*100. An analysis of variance and Tukey test ( $\alpha$ = 0.05) were applied.

#### **RESULTS AND DISCUSSION**

The days to occurrence of phenological stages were similar between treatments. In ayocote, the emergence (E) was at 12 days after sowing (das), the (R6) at 70 das and (R9) 126 das. The seasonal precipitation (PP) was 512 mm, from this 41% occurred in the VS and 59% of the R6 to PM. The highest GY of Ayocote was with BM (352 gm<sup>-2</sup>); followed of Ayocote with association with BB. On the other hand, the association with BM recorded the highest TB (538 gm<sup>-2</sup>), this may be due to increased HI, PN, GN and WG (Table 1). The GY was different between treatments. These differences may be related with the canopy architecture of living trellis.

Cultivar	TB gm <sup>2</sup>	HI%	$PN m^{-2}$	PG	WG g	GN m <sup>-2</sup>	GY gm <sup>-2</sup>
YM-Ayo	486b	72a	182b	4a	48.4b	728a	352a
BB-Ayo	395c	56c	157c	3b	47.7c	471c	224c
BM-Ayo	538a	61b	224a	3b	49.3a	672b	331b
CV %	5.7	10.9	14.0	15.5	4.7	17.6	9.3

**Table 1** Total biomass, harvest index, yield and its components in Ayocote.Montecillo,Méx.México. Summer 2013.

Values with the same letter are statistically equal, Tukey test ( $P \le 0.05$ ). TB: Total biomass; HI: Harvest index; PN: pod number; WG: weight of 100 grains; GN: Grain number m<sup>-2</sup>; GY: grain yield. YM: Yellow maize; BB: Black maize; BM: Blue maize; Ayo: runner bean.

# CONCLUSION

Differences in Ayocote biomass and yield are in function the cultivar of maize used as living trellises. The grain yield of Ayocote is higher when blue maize is used as live trellis.

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# PRUNES IN CLIMBING BEAN AND ITS EFFECT ON BIOMASS, HARVEST INDEX, GRAIN YIELD AND ITS COMPONENTS

# José Alberto Salvador Escalante-Estrada<sup>1</sup>., María Teresa Rodríguez-González.<sup>1</sup> y Yolanda Isabel Escalante-Estrada<sup>2</sup>

<sup>1</sup>Postgrado en Botánica. Campus Montecillo. Colegio de Postgraduados. Montecillo, Mpio. de Texcoco, Edo. de Méx, México.56230. E mail: jasee@colpos.mx., mate@colpos.mx;<sup>2</sup>Instituto de Investigación Científica área de Ciencias Naturales. Universidad Autónoma de Guerrero. Chilpancingo Guerrero México. E-mail: y\_escalante@yahoo.com.mx.

# **INTRODUCTION**

Pruning is an agricultural practice which modifies the phenotype in order to increase yield or yield efficiency. In dry beans, the pruning of stem and branches has increased yield (Escalante *et al.*,1992). The aim of this study was to determine in climbing bean, the effect of removing the apical bud of the main stem on biomass, grain yield and its components.

# **MATERIALS AND METHODS**

The experiment was stablished under glasshouse of June 24 to October 4 in Montecillo Méx. México (19 ° 29 ' N, 98 ° 53 'W, to 2250 m of altitude), of temperate climate. The sowing bean (*Phaseolus vulgaris* L.) HAV-14 of indeterminate climbing habit was in five-liter pots, in June 24. In each plant, the apical bud was removed when stem had 2, 4 and 6 nudes (treatments) to 39, 50 and 65 days after sowing (das), respectively. The test was without removal. At physiological maturity (harvest 130 das) were evaluated by plant, the total biomass based in dry weight (TB), nude number (NN), raceme number (RN), pod number (PN), grain number (GN), grain size (GS), grain per pod (GP) following the criteria indicated in Escalante and Kohashi (1993). The experimental design was randomized completely with five replicates. An analysis of variance and Tukey test were applied.

# **RESULTS AND DISCUSSION**

Pruning in beans increased biomass, harvest index, number of nodes, pods, grains and consequently grain yield (Table 1). The reduction in the interference for light due to the pruning might explain the above mentioned response. Similar trends have also been reported in climbing beans in warm climate for Escalante *et al.* (1992). The grain size and the number of grain per pod no significant changes due to the pruning, indicating that these components are genetically stable. These results indicate that by pruning can increase the production of pods (of importance in string beans) and grains or seeds to increased food availability.

# CONCLUSIONS

Under the conditions of the present study, the pruning in climbing bean HAV-14, increases the production of biomass, the harvest index, the grain yield and its components. The size of grain and the number of grains for pod are not affected by the pruning.

Treatments	TB g	HI %	GY g	GN	GS g	PN	GP	RN	NN
2	46 b	54 a	25 b	69 b	0.362	17 a	4.0	11 ab	26 ab
4	55 a	50 a	28 a	76 a	0.368	19 a	4.0	12 a	29 a
6	42 c	47 ab	20 c	54 c	0.370	13 b	4.1	9 b	25 b
Test	38 d	37 b	15 d	41 d	0.366	13 b	3.1	8 c	18 c
Mean	46	47	22	60	0.366	16	3.8	9	25
Tukey α=0.05	3	10	2	4	0.042	2	1.0	3	3

Table 1. Biomass, grain yield and its components per plant of beans HAV-14 in relation to pruning.

Total biomass based in dry weight (TB), Harvest index (HI), grain yield (GY), grain number (GN), grain size (GS), pod number (PN), grain per pod (GP), raceme number (RN), nude number (NN).

# CONCLUSIONS

Under the conditions of the present study, the pruning in climbing bean HAV-14, increases the production of biomass, the harvest index, the grain yield and its components. The size of grain and the number of grains for pod are not affected by the pruning.

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# APPLICATION OF MIXED MODELS TO EVALUATE GENOTYPE AND ENVIRONMENT INTERACTION IN COMMON BEAN

# R.C. Franzon, M.C. Gonçalves-Vidigal, G.F. Lacanallo, M.P. Caixeta and P.S. Vidigal Filho

Dep. Agronomia, Universidade Estadual de Maringá, Av. Colombo, 5790, Maringá, PR, Brazil.

**INTRODUCTION:** The common bean (*Phaseolus vulgaris* L.) is the most widely produced grain legume used for direct human consumption in many parts of Africa, Latin America and southern Europe (Broughton et al. 2003). In general, in the common bean breeding programs the objective is to identify inbred lines that have wide yield adaptability and stability. For this, the use of mixed models (REML/BLUP) allows a detailed study of genotype × environment interaction ( $G \times E$ ), and reduce the errors from the effects of this interaction (Resende, 2004). In addition, the Harmonic Mean of the Relative Performance of Genotypic Values (HMRPGV) method is recommended for analysis of unbalanced data (Resende, 2007). The preference of Brazilian customers has forced breeding programs to develop new Carioca cultivars. Due to the importance of Carioca common bean group, by the method of Harmonic Mean of the Relative Performance of Genotypic Values 18 genotypes of Genotypic Values (HMRPGV).

**MATERIALS AND METHODS:** The fourteen elite inbred lines and four commercial cultivars of common bean were evaluated in 13 environments in Paraná (PR), Santa Catarina (SC) and Rio Grande do Sul (RS), Brazil, during the years of 2012/2013 and 2013/2014 (Table 1). The experimental design utilized was complete randomized blocks with three replications. Each experimental unit was constituted of four rows of 5.0 m length, spaced with 0.5 m, and the useful area was formed by 4.0 m<sup>2</sup> from the two central rows. The yield adaptability and stability were analyzed using the Model 54 of SELEGEN-REML/BLUP software (Resende, 2002).

State	Environment	Elite inbred lines/Cultivars
	Maringá 2012/13	Pérola, IPR Campos Gerais, IPR Uirapuru,
PR	Maringá 2013/14	CNFP 10104, C4-7-7-2-2, C4-7-8-1-2, CHC 98-42, CHP 01-238, CNFC 10762, CNFP
	Ponta Grossa 2012/13	02-11, LP 09-181, LP 09-40, TB 02-23 e TB 03-13.
	Canoinhas 2012/13	
	Lages 2012/13	
	Ponte Serrada 2012/13	Pérola, IPR Uirapuru, C4-7-8-1-2, CHP 01-
SC	Chapecó 2012/13	238, CHC 98-42, CNFC 10762, CNFP 10794,
	Chapecó 2013	FT 08-47, FT 08-75, LEC 01-11, LEP 02-11,
	Ituporanga 2013	LP 09-181, LP 09-40, TB 02-23 e TB 03-13.
	Águas de Chapecó 2013	
	Urussanga 2013	
RS	Júlio de Castilho 2013	Pérola, C4-7-7-7-2-2, C4-7-8-1-2, CHC 98- 42, CHP 01-238, CNFC 10762, CNFP 10794,
	Maquiné 2013	LP 09-181, LP 09-40, TB 02-23 e TB 03-13.

**Table 1.** Environment and treatments used in the Common Bean Southern Brazilian Network Assays

**RESULTS AND DISCUSSION:** The results obtained for grain yield varied according to each environment (data not shown), with a general mean (GM) of the 13 environments, equivalent to 2,525.5 kg ha<sup>-1</sup>. Through the analyze statistical Relative Performance of Genotypic Values (RPGV), the inbred lines that showed highest adaptability, permitting to be sowed in several environments with the same pattern of interaction  $G \times E$  were: CHC 98-42, CNFP 10794, CHP 01-238, FT 08-75, LP 09-40, CNFC 10762, C 4-7-8-1-2 and LEC 01-11 with values 3% higher than the general mean. Moreover, the four inbred lines that revealed a greater stability as the Harmonic Mean of the Relative Performance of Genotypic Values (HMRPGV) were CHC 98-42, CHP 01-238, FT 08-75 and CNFP 10794 (Table 2).

RPGV \*GM<sup>2</sup>  $HMGV^1$ Lines/cultivars **RPGV HMRPGV** HMRPGV \*GM CHC 98-42 1,129.5 2,852.62 2,344.78 1,118.8 2,825.59 CNFP 10104 1,0695 2,700.94 3,069.90 1,064.4 2,688.18 CNFP 10794 1,0782 2,723.00 2,115.29 1,059.1 2,674.78 CHP 01-238 1,0636 2,686.01 2,188.16 1,058.1 2,672.24 FT 08-75 1,0516 2,655.69 2,183.22 1,048.3 2,647.57 **IPR** Campos Gerais 1,0551 2,664.67 3,021.80 1,046.1 2,642.00 2.631.30 1.031.9 LP 09-40 1.0419 2.136.64 2.606.15 CNFC 10762 1,0328 2,608.25 1,025.3 2,589.34 2,118.80 C 4-7-8-1-2 1,0494 2,650.23 2,113.22 1,025.0 2,588.55 LEC 01-11 1,0068 2,542.69 2,098.05 1,002.0 2,530.48 0.9960 2,495.76 Pérola 2,515.32 2.068.97 0.9882 LP 09-181 0.9922 2,505.87 2,015.09 0.9857 2,489.39 FT 08-47 0.9781 2,470.10 2,026.59 0.9739 2,459.47 LEP 02-11 0.9505 2,400.56 1,976.45 0.9490 2.396.65 2,326.74 2,222.72 2,302.93 IPR Uirapuru 0.9213 0.9119 C 4-7-7-2-2 0.8855 2,236.43 1,664.36 0.8757 2,211.57 TB 02-23 0.8650 2,184.55 1,762.96 0.8358 2,110.78 TB 03-13 0.8285 2,092,42 1,703.08 0.8159 2,060.63

**Table 2**. Adaptability of genotypic values (RPGV and RPGV\*GM), stability and adaptability of genotypic values (HMRPGV and HMRPGV\*GM) for inbred lines and cultivars evaluated in 13 environments from 2012/2013 to 2013/2014 growing season

<sup>1</sup>Harmonic Mean of Genotypic Values, <sup>2</sup>General Mean.

**CONCLUSION:** The REML/BLUP methodology enabled to determine the genotypic stability and adaptability of the 14 elite inbred lines even with unbalanced data and heterogeneity of variances of the errors. The genotypic values were higher in overall environments for CHC 98-42, CNFP 10794, CHP 01-238 and FT 08-75 inbred lines and showed superior productivity when they were selected by the of Harmonic Mean of the Relative Performance of Genotypic Values (HMRPGV).

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# SEED YIELD AND ITS COMPONENTS IN COMMON BEAN (*Phaseolus vulgaris* L.) UNDER RAIN-FED CONDITIONS

# Romero-Félix, C.S.<sup>1</sup>, C. López-Castañeda<sup>2</sup>, J. Kohashi-Shibata<sup>3</sup>, S. Miranda-Colín<sup>2</sup>, V.H. Aguilar-Rincón<sup>2</sup> and C.G. Martínez-Rueda<sup>4</sup>

<sup>1</sup>Posgrado en Fisiología Vegetal, <sup>2</sup>Genética y <sup>3</sup>Botánica, Colegio de Postgraduados, Montecillo, Texcoco, Estado de México, 56230, y <sup>4</sup>Facultad de Ciencias Agrícolas, Universidad Autónoma del Estado de México, El Cerrillo Piedras Blancas, Toluca, Estado de México, 50200. E-mail: jkohashi@colpos.mx

# **INTRODUCTION**

The 'Flor de Mayo' type is one of the most demanded bean types for commercial use in central Mexico; in the spring-summer season 2012, the area sown under rain-fed conditions was 212, 404 ha, with a mean seed yield of 0.33 t ha<sup>-1</sup>, whereas under irrigation the planted area was 26, 231 ha, with a seed yield of 1.77 t ha<sup>-1</sup> (SAGARPA, 2012). The low seed yield in rain-fed areas is due to the incidence of biotic and abiotic stresses; drought and high temperatures frequently cause abiotic stress, so reducing significantly the seed yield of bean crops. The present research work was conducted to study the variability in seed yield and its components in a group of 'Flor de Mayo' type bean and three black bean varieties from southern Veracruz (Mexico) under rain-fed conditions.

# MATERIALS AND METHODS

The field experiment was carried on at the Experiment Station at Colegio de Postgraduados, Montecillo, Texcoco, Mexico (19º 29' N, 98º 54' W), 2250 m altitude; temperate subhumid climate (CWo) and average precipitation and temperature of 645 mm and 15 °C per year, respectively (García, 1973). The soil used for the experiment was a clay type with pH 8.2, organic matter 2.1 % (Walkey-Black) and nitrogen 0.2 % (MicroKjeldhal). Nine cultivars of 'Flor de Mayo' type and three black common bean varieties from the south of Veracruz, Mexico were used for the experiment. Cultivars were assigned to a complete randomized block design with six replicates; plots were four rows wide with 0.8 m between rows and 5 m long. The experiment was sown in April 24, 2013 in a dry soil with the application of NPK at a rate of 40-40-0 and a plant density equivalent to 150, 000 plants/ha. Irrigation was applied after sowing by three dates; and was suspended with the onset of the rainfall season. Thereafter the crop growth depended on the rainfall only. A second application of NPK at rate of 40-40-0 was made at 50days. An additional application of a foliar fertilizer (Nutriplant Plus®) was performed at the beginning of flowering as the earliest formed leaves looked yellow after a period of wet overcast days. Weeds and pests were controlled with conventional methods. At maturity, aerial biomass, seed yield and yield components (pods m<sup>-2</sup>, seeds m<sup>-2</sup>, seeds/pod and 200-seed weight) were determined. Data were analyzed by using the SAS program, version 9.1 (SAS Institute, 2008). A least significant difference (Lsd.  $P \le 0.05$ ) was calculated for the comparison of means.

# **RESULTS AND CONCLUSIONS**

Seed yield, aerial biomass and yield components showed significant differences among genotypes (P $\leq$ 0.05); values of seed yield, aerial biomass, pods m<sup>-2</sup>, seeds m<sup>-2</sup>, seeds/pod and 200-seed weight ranged from 366 to 522 g m<sup>-2</sup>, 578 to 901 g m<sup>-2</sup>, 334 to 441 pods m<sup>-2</sup>, 1975 to 2881 seeds m<sup>-2</sup>, 5.6 to 6.7 seeds/pod and 34 to 66 g, respectively (Table 1). FM Sol, FM RMC, FM 2000, FM M38 and Michoacán 128 had greater seed yield and aerial biomass than the other genotypes; FM Sol, FM RMC, Michoacán 128, Negro Veracruz, Negro Cotaxtla and Criollo San

Andrés produced more pods m-2, seeds m-2 and seeds/pod than the other cultivars, and FM 2000 had higher 200-seed weight than all the other bean varieties (Table 1). The genotypes with higher seed yield also produced greater aerial biomass; which may be related to a higher availability of assimilate for seed-filling; FM RMC, FM M38 and Michoacán 128 had high seed yield and aerial biomass, and they also had high pods m-2 and seeds/pod.

Cultivar	SY	BM	Pods	Seeds	Seeds/pod	200-seed
	$(g m^{-2})$	$(g m^{-2})$	$m^{-2}$	m <sup>-2</sup>		weight (g)
FM Sol	522	819	357	2348	6.6	55.0
FM RMC	515	841	379	2447	6.5	55.0
FM 2000	513	901	370	2296	6.2	66.0
FM M38	461	832	380	2262	6.0	51.0
Michoacán 128	448	785	403	2402	6.0	46.0
FM Corregidora	410	731	334	1986	6.0	52.0
FM Anita	407	656	361	2056	5.7	53.0
FM Noura	403	713	343	1975	5.8	54.0
Negro Veracruz	390	692	416	2745	6.6	39.0
Negro Cotaxtla	390	642	441	2720	6.2	37.0
Criollo San Andrés	378	718	432	2881	6.7	34.0
FM Bajío	366	578	359	2017	5.6	49.0
General mean	434	742	381	2345	6.1	49.3
Lsd (P≤0.05)	83	135	65	402	0.2	5.5

 Table 1. Seed yield (SY), aerial biomass (BM) and yield components of common bean (*Phaseolus vulgaris* L.) under rain-fed conditions.

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# EFFICIENT USE OF SOIL AND WATER TO PRODUCE DRY BEAN UNDER RAINFED CONDITIONS IN THE NORTH CENTRAL REGION OF MEXICO

# M.A. Martinez-Gamiño<sup>1\*</sup>, E.S. Osuna Ceja<sup>2</sup>, L. Reyes-Muro<sup>2</sup>, M. Borja-Bravo<sup>2</sup>, S. Arellano-Arciniegas<sup>2</sup>, C. Rojas-Santillan<sup>2</sup>, A. Corrales-Suastegui<sup>2</sup>, Serna-Perez<sup>3</sup>, and F. Chavarria-Chaires<sup>3</sup>

<sup>1</sup>Bean program, INIFAP, Experimental Station San Luis; <sup>2</sup>Bean program, INIFAP, Experimental Station Pabellon; <sup>3</sup>Bean program, INIFAP, Experimental Station Zacatecas \* E-mail: martinez.miguelangel@inifap.gob.mx

**INTRODUCTION:** Traditional practices to produce dry bean in the North Central region of Mexico are: soil tillage with plow and disk, furrows are not level, sowing landraces of bean with a growing season of 110 days, pest and weeds are not controlled, chemical or organic fertilizers are not used, plant density of 75,000 plants/ha, and manual harvest. Besides, rainfall distribution does not fit the need of water during flowering, pod development and grain formation stages, causing drought stress to bean crop, which results in low harvest of dry bean (200-300 kg/ha), and usually, cost production overcome income. Despite this, two millions of hectares are planted each year with bean. Farmers are skeptical trying others crops with less risk to drought effect, such as grass and forage crops, because dry bean is a traditional crop, which has been sowed for more than 50 years and because it is part of their diet. The objective of this study was to assess the effect of soil and water conservation practices to produce dry bean under rainfed conditions in the North Central region of Mexico.

**MATERIALS AND METHODS:** In 2014, a study was conducted in La Providencia, Villa de Arriaga, San Luis Potosi, Mexico, where soil texture is sandy loam, climate is temperate dry, annual average temperature is 18.0 °C, the frost free period is from April to early October, and annual average rainfall is 350.0 mm. Two treatments were evaluated: i) soil and water conservation practices (SWCP) and ii) traditional practices (T). The SWCP compounds were reduce tillage with a root cutter or multiarado, furrow level, rainwater-harvesting practices, Pinto Saltillo bean variety, and plant density of 160,000 plants/ha. The T practices were traditional soil tillage with plow and disk, furrows without leveling and rainwater-harvesting practices, Pinto Saltillo bean variety, and plant density of 75,000 plants/ha. In the SWCP treatment, four lines on a 1.60 m wide bed were sowed, where each line was 0.20 m from each other and plants were separated 0.1 m. In the T treatment, sowing was on single lines with furrows separated 0.8 m. The use of Aqueel at sowing time and forming furrow pond reservoirs at 30 days after sowing were the rainwater-harvesting practices employed. Ten random samples were taken in each treatment to calculate dry bean yield and its compounds.

**RESULTS AND DISCUSSION:** From June to November, rainfall was 382.4 mm, but before sowing date, 233.2 mm were registered. Most of this amount of water mas lost by runoff in the T treatment, while more water was storage in soil profile in the SWCP because of leveling furrows. From sowing to flowering, rainfall was 23.4 mm, such amount did not covered the water need of crop, but because of water storage in the SWCP treatment before sowing time, plants were not affected by drought effects as the T treatment was. From flowering to harvest, rainfall was 59.6 mm, this amount did not covered the water need for this important stage of filling grain so that dry bean yield was affected in both treatments.

Statistical analysis showed significant differences between treatments for dry bean yield, where SWCP treatment obtained 828 kg/ha and T treatment 297 kg ha (Table 1). This result

indicates that soil and water were more efficiently used when soil and water conservation practices were combined with sowing four lines on a 1.60 m wide bed in the SWCP treatment. First at all, more rainwater infiltrated in the soil because furrows were leveled and second, the higher plant density (156,000 plants/ha) allowed using most of the soil moisture instead of losing by evaporation. In the T treatment, because furrows were not level, rainwater lost by runoff, so that the soil moisture was lower than that in the SWCP treatment. The low plant population (79,000 plants/ha) did not use all the soil moisture because the small plant architecture (Table 1). It confirms that the new genotypes of bean developed by INIFAP requires a different topological arrangement than the one use with landraces or older varieties than Pinto Saltillo. Pinto Saltillo has a more compact and erected plant architecture than bean landraces, allowing seeding three or four lines of plant, instead of two as farmers traditionally do. Osuna-Ceja et al 2013 reported a bean yield increase of 35 %, by sowing double rows instead of one with Pinto Saltillo in the same area of this study in the North Central region of Mexico.

In table 1, yield compounds are shown. The number of pods/plant were higher in the SWCP than that in the T treatment because of the higher soil moisture. However, the number of grains/pods were lower in the SWCP than that registered in T treatment because the amount of rainfall registered during the grain fill stage was lower than the crop need. As a result, less grain per pod were filled in the SWCP and because the higher demand of soil moisture from a high number of pods/plant. This effect was reflected in the weight of 100 seeds, where T treatment obtained the higher value. Then, the answer to the question, how SWCP got higher dry bean yield that that of T treatment? It is in the number of plants in each treatment. In the SWCP, plant density was 156,000 plants/ha, while in T treatment it was 79,000 plants/ha. The higher plant density and pods/plant allowed obtaining 285 grains of dry bean harvested per square meter in T treatment.

**Table 1.** Dry bean yield, pods per plan, grains per pod, weight of 100 seeds, plant density, and grain yield per square meter with soil water conservation practices (SWCP) and traditional (T) in Villa de Arriaga, San Luis Potosi, Mexico. 2014.

Treatment	Dry bean	Pods/plant	Grains/pod	Weight of	Plant	Grains
	yield			100 seeds	density	yield/m <sup>2</sup>
	Kg/ha				Plants/ha	
SWCP	828 a	7.6 a	2.4 b	29 b	156,000 a	285 a
Т	297 b	2.7 b	4.3 a	35 a	79,000 b	85 b

Means followed by the same letter are not significantly different at 0.05 probability according to Turkey test.

**CONCLUSION:** Soil and water conservation practices combined with sowing four lines on a 1.60 m wide bed in the SWCP treatment allowed to use soil and rainwater more efficiently and to get high dry bean yield than that in T treatment.

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# PRODUCTIVE RESPONSE OF NEGRO COMAPA, AN IMPROVED CULTIVAR TRANSFERRED TO FARMERS OF VERACRUZ, MEXICO

#### Francisco Javier Ibarra-Perez, Oscar Hugo Tosquy-Valle, Ernesto López-Salinas

INIFAP, Campo Experimental Cotaxtla. Km 34.5 carr. Federal Veracruz-Córdoba, Medellín de Bravo, Ver., México <u>Ibarra.francisco@inifap.gob.mx</u>

**INTRODUCTION:** In Veracruz, Mexico most farmers grow common bean (*Phaseolus vulgaris* L.) tropical landraces, which produce very low seed yields. Grain is commonly used for sowing bean cultivars of unknown origin which are very susceptible to rust and angular leaf spot fungal diseases, and to virus particularly bean golden yellow mosaic virus (BGYMV). This situation occurs to a great extent because farmers are not familiar to or do not know the newly improved common bean cultivars developed and released by research institutions. The bean Program for southern Mexico of INIFAP (National Research Institute of Forestry, Agriculture and Livestock) developed and recently released Negro Comapa, a high seed yield cultivar widely adapted to tropical conditions, it carries resistance genes for the main diseases attacking beans in tropical southern Mexico (López *et al.*, 2012). The objectives of this study were to validate yield performance of Negro Comapa in farmer's fields to determine the production response in semi commercial plots compare to regional cultivars, and to transfer a technological component of the bean crop to farmers and farm advisors of Veracruz, Mexico.

**MATERIALS AND METHODS:** Eleven validation plots were conducted during 2011 to 2013 across the State of Vercaruz, Mexico (two in the north, three in central region and six in the south). Negro Comapa was compared to a traditional well-known bean cultivar Jamapa and T 39 as well, used as checks which were grown under rainfed and residual moisture conditions. All three cultivars were sown each in 800 m<sup>-2</sup> validation plots with a plant density of 250,000 plants ha<sup>-1</sup>. Field crop management was conducted following technical recommendations of Cotaxtla Field Station of INIFAP. Angular leaf spot was observed attacking bean plants during the vegetative phase of the crop in three environments (Orizaba summer 2011, Acayucan Fall-winter 2011 and Cosoleacaque Winter-spring 2013), therefore the incidence reaction of bean genotypes was recorded and the scale 1 to 9 was used (CIAT, 1987). As a means to transferring Negro Comapa to farmers, field days were undertaken each in every of five validation plots located in central and southern areas of Veracruz. As bean plants reached maturity, four plant samples 3 m<sup>-2</sup> each were harvested to estimate seed yield and compare the productive response of Negro Comapa with cultivar checks using the Student t test.

**RESULTS AND DISCUSSION:** Reaction of bean cultivars to angular leaf spot was recorded in three environments and Negro Comapa showed an average tolerance reaction (score 3-4), Jamapa had an intermediate reaction (score 5-6) while T 39 was slightly susceptible (score 6-7). Based on plant samples taken from all validation plots carried on either under rainfed or residual moisture conditions, Negro Comapa produced (1261 kg ha<sup>-1</sup>) an average of 41.2 and 69.6% higher seed yield than Jamapa and T 39, respectively (Table 1). Under rainfed conditions the increase of Negro Comapa (1562 kg ha<sup>-1</sup>) compared to check cultivars was even higher (76 to 82%) than under residual moisture environments (Table 1). This results indicate that by replacing Jamapa by the newly released cultivar, it would be feasible to significantly increase yield production under farmers' field conditions. Negro Comapa was transferred as a newly released improved bean cultivar to 133 farmers and 6 farm advisors of central and south bean production regions in Veracruz, Mexico. Farmers attended field day activities undertaken in validation plots

to get acquainted with plant characteristics and field adaptation to farmers fields in different dry bean production regions in Veracruz, Mexico (Table 2).

Location	Region	Season/vear	Condition	Negro	Negro	Т 39
Location	Region	Sedson/year	Condition	Comapa	Jamapa	1 57
Orizaba	Central	S 2011	Rainfed	939	925	366
Orizaba	Central	S 2012	Rainfed	1424	654	567
Cosoleacaque	South	S 2012	Rainfed	2322	1080	1642
Average				1562	886	858
Increase compare		76	82			
José Azueta	South	W-P 2011	RM	1294	1014	644
Acayucan	South	F-W 2011	RM	1567	789	989
San A. Tuxtla	South	F-W 2011	RM	1058	1068	460
San A. Tuxtla	South	F-W 2011	RM	958	1003	815
Tlapacoyan	North	F-W 2011	RM	785	785	524
Tlapacoyan	North	W-S 2012	RM	652	496	399
Orizaba	Central	F-W 2012	RM	1272	942	746
Cosoleacaque	South	W-S 2013	RM	1600	1066	1025
Average				1148	895	700
Increase compare	ed to check	as (%)			28	64
General mean				1261 893 *		743 **
Increase compare	ed to check	as (%)			41	67

**Table 1.** Negro Comapa seed yield (kg ha<sup>-1</sup>) obtained from validation plots conducted in 11 farmer's fields of three productions regions in Veracruz, Mexico.

W-S=Winter-spring, F-W=Fall-winter and S=Summer, RM=Residual moisture.

\*, \*\* Significant and highly significant differences based on t Student test.

Table 2.	Negro	Comapa	transferred	to	farmers	and	farm	advisors	by	means	of	field	day
validation	plots co	onducted i	in farmers' f	ield	ls in Vera	acruz	, Mexi	ico.					

Location		Season/year	Farmers	Advisors	Total
José Azueta	1	W-S 2011	28	2	30
Acayucan		F-W 2011	29	1	30
Orizaba		S 2012	28	1	29
Cosoleacaq	ue	S 2012	31	0	31
Cosoleacaq	ue	W-S 2013	17	2	19
Total			133	6	139

W-S=Winter-spring, F-W=Fall-winter and S=Summer.

**CONCLUSIONS:** Negro Comapa produced an average seed yield (1,261 kg ha<sup>-1</sup>) higher than cultivar checks, Jamapa and T 39. Field performance, agronomic characteristics and field adaptation of Negro Comapa was transferred to farmers and farm advisors of central and south production regions in Veracruz, Mexico.

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# NEGRO COMAPA, A NEW BLACK BEAN CULTIVAR ADAPTED TO SOUTHEAST MEXICO

# Ernesto López-Salinas, Oscar Hugo Tosquy-Valle, Francisco Javier Ibarra-Pérez, Bernardo Villar-Sánchez and Jorge Alberto Acosta-Gallegos

INIFAP, Campo Experimental Cotaxtla. Km 34.5 carr. Federal Veracruz-Córdoba, Medellín de Bravo, Ver., México salinaser@hotmail.com

**INTRODUCTION:** In southeastern Mexico, the black dry bean seed type has the largest commercial demand among consumers, and a large market in central Mexico. In the southeastern region, dry bean production is affected by biotic (pests and diseases) and abiotic factors mainly drought and poor soils (Acosta et al., 1999). The National Research Institute of Forestry, Agriculture and Livestock (INIFAP) Dry Bean Program released for commercial use a cultivar named Negro Comapa, which has shown high productivity, and tolerance to the main fungal and viral diseases. It has wide adaptation to different production areas where beans are planted in the humid tropics of southeastern Mexico (López et al., 2010). The objective of this report is to announce the release of Negro Comapa, its origin, main agronomic characteristics and production performance.

Pedigree and breeding history: Negro Comapa originated from the triple cross: (VAX-4/A-801)//DOR-500, made in 1998 at the International Center for Tropical Agriculture (CIAT) in Cali, Colombia. Negro Comapa was developed through individual selection in F<sub>2</sub> made in Popayan, Colombia, mass selection in  $F_3$  followed by bulk selection in  $F_4$  to  $F_6$  in Darien, Colombia. The breeding line was introduced to Mexico in 2002 as the MN13337-9 breeding line, with a code identification as follows: (VAX-4/A-801)F1// DOR-500- (M) O-6P- (M) D-MC-MC-MC. The VAX-4 elite line was used as a source of resistance to common bacterial blight (Xanthomonas campestris pv. phaseoli); A-801 was used as a source of resistance genes to angular leaf spot (Phaeoisariopsis griseola) and DOR-500 to golden bean yellow mosaic virus (BGYMV) and rust (Uromyces appendiculatus var. appendiculatus). The breeding line released as Negro Comapa was evaluated and validated in Mexico with the code CIAT-103-21. Negro Comapa was first evaluated in adaptation nurseries in 2002-03 followed by preliminary yield trials conducted in 2004-05. Later, CIAT-103-21 was included in a regional yield trial evaluated in eight environments (2007-09) of Veracruz and Chiapas, Mexico, followed by validation plots conducted in farmers' fields in two years (2010-11) and grown under residual moisture, rainfed and irrigated conditions.

**Agronomic features:** Negro Comapa exhibits the type-II upright intermediate vine (indeterminate) growth habit (Singh, 1982). Negro Comapa reaches the flowering period between 43 and 45 d after planting when grown under rainfed and residual moisture conditions, with a range of 70 and 75 d to attain physiological maturity. The canopy is about 0.44 m in height and the seed of Negro Comapa is opaque, black and small in size (< 19.3 g 100 seed<sup>-1</sup> weight). Among other important traits, Negro Comapa is resistant to bean common mosaic virus (BCMV) tolerant to angular leaf spot and to BGYMV, diseases that commonly occur in the bean producing areas of southeastern Mexico.

**RESULTS:** Data obtained from the adaptation nurseries indicated that Negro Comapa yielded 1,222 kg ha<sup>-1</sup>, which represented 68.6% higher seed yield than the check cultivar Negro INIFAP. In preliminary trials conducted in 10 environments of Veracruz and Chiapas, Mexico, Negro Comapa exceeded the seed yield of Tacaná, Negro INIFAP and Jamapa by 25.1, 37.4 and 59.8%,

respectively. Information of regional yield trials conducted under rainfed conditions indicated that Negro Comapa exceeded seed yield of cultivar checks Papaloapan and a group of local bean varieties including Jamapa and T39 by 32.9 and 68.7%, respectively. Under soil residual moisture conditions the increase ranged from 8.5 to 32.5 % (Table 1). Among the diseases that naturally occurred in locations of Veracruz and Chiapas, only BCMV, angular leaf spot and BGYMV significantly affected crop performance, and Negro Comapa showed resistance to first two pathogens and tolerance to BGYMV, while T39 was highly susceptible to such diseases. In validation plots, Negro Comapa also outperformed Jamapa and T39 either in rainfed, soil residual moisture or irrigation conditions. The average seed yield across environments of Negro Comapa was 1,427 kg ha<sup>-1</sup>, 40% higher than the average obtained by cultivar checks. Data obtained either from yield trials and/or validation plots confirmed the high yield potential and better adaptation of Negro Comapa in comparison to dry bean varieties commonly grown by farmers.

Location/State	Season/year <sup>&amp;</sup>	Moisture regime <sup>§</sup>	Negro Comapa	Negro Papaloapan	Check <sup>†</sup>
Comapa, Ver.	Summer 2007	Rainfed	1,987	1,317	1,077
Córdoba, Ver.	Summer 2007	Rainfed	2,104	1,363	1,727
Orizaba, Ver.	Summer 2009	Rainfed	1,970	1,879	789
Average			2,020	1,520	1,198
Medellín de Bravo, Ver.	F-W 2009-10	RM	686	572	751
Martínez de la Torre, Ver.	F-W 2009-10	RM	1,279	993	773
San Andrés Tuxtla, Ver.	F-W 2009-10	RM	2,656	2,625	2,391
Rodríguez Clara, Ver.	F-W 2009-10	RM	667	538	242
Ocozocoautla, Chis.	F-W 2009-10	RM	454	566	175
Average			1,148	1,059	866
Overall average			1,475	1,232	991

**Table 1.** Negro Comapa seed yield (kg ha<sup>-1</sup>) compared to Papaloapan and other cultivars used as checks when grown in seven field locations of Veracruz and one of Chiapas, Mexico.

<sup>†</sup> Comapa and Córdoba, check cultivar was Jamapa, while T39 was used as check in the rest of the locations.  ${}^{\&}F-W = Fall-winter$ .  ${}^{\$}RM = Soil residual moisture.$ 

**CONCLUSIONS:** Negro Comapa showed high field performance and wide adaptation to tropical areas of southeastern Mexico. This new bean cultivar has a tolerance reaction to BGYMV and resistance to BCMV and angular leaf spot. It is registered in MX as new cultivar (FRI-066-100910).

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# NAMING AND RELEASE OF VERDIN, OPAQUE DRY BEAN CULTIVAR TOLERANT TO TERMINAL DROUGHT

# Oscar Hugo Tosquy-Valle, Ernesto López-Salinas, Francisco Javier Ibarra-Pérez, Bernardo Villar-Sánchez, Néstor Francisco-Nicolás, José Raúl Rodríguez-Rodríguez, Jorge Alberto Acosta-Gallegos

INIFAP, Campo Experimental Cotaxtla. Km 34.5 carr. Federal Veracruz-Córdoba, Medellín de Bravo, Ver., México tosquy.oscar@inifap.gob.mx

**INTRODUCTION:** This is to announce the release of Verdín, an early maturing, opaque black bean cultivar (*Phaseolus vulgaris* L.). In Veracruz and Chiapas, Mexico, almost all dry bean fields are planted with opaque black bean cultivars, since this is the commercial grain class most demanded in southern Mexico. In both federal states, the main factor limiting yield production of dry beans under the residual moisture or rainfed cropping systems is terminal drought, which can severely reduce grain yield (20-100%). INIFAP's (National Research Institute of Forestry Agriculture and Livestock) Bean Breeding Program for southern Mexico has developed an improved common bean cultivar named Verdín, which is tolerant to terminal drought with high yield potential and wide adaptation to southern Mexico. The objective is to report the origin, main agronomic characteristics and field production performance of Verdín across environments in Veracruz and Chiapas, Mexico.

Pedigree and breeding history. Verdin was derived from the triple cross SXB 114/DOR605//SXB 123 made in 2003. Both parents, SXB 114 and SXB 123, were selected to possess drought tolerance and the BGYMV resistance of DOR 605. The purpose of the cross was to introduce the BGYMV resistance into high-vielding erect drought tolerant black bean breeding lines. Verdín was developed through pedigree selection combined with five seasons of mass selection in different locations of Colombia, S.A. The F<sub>1</sub> was obtained by gametic selection and mass selection in the F<sub>2</sub> was conducted under drought stress at CIAT, Cali, Colombia. Individual selections were performed in  $F_{2-3}$  (anthracnose) and  $F_{5-6}$  (plant type and seed color), combined with mass selections for angular leaf spot in F<sub>4</sub> and for seed yield under drought stress in F<sub>5</sub>, F<sub>7</sub> and F<sub>8</sub> generations. The F<sub>6-8</sub> derived line was introduced to Mexico in 2009 as SEN-70; this breeding line was field tested from 2011 to 2013 in 11 locations across Chiapas and Veracruz, Mexico as part of the regional yield trial series conducted under both, residual moisture and rainfed conditions. In spring season of 2013, SEN-70 and other 22 breeding lines were evaluated and compared to both checks, Negro Jamapa and Negro Tacaná (López et al. 1997), as part of a yield trial carried on under both, irrigated and terminal drought, conditions. SEN 70 was selected based on yield performance, drought susceptibility index proposed by Fisher and Maurer (1978) and the productivity efficiency index (Graham, 1984).

**Agronomic features.** Vedín exhibits the type-II upright short vine (indeterminate) growth habit. Plants average 59 cm in height, are more upright than Jamapa, and exhibit an overall upright appearance similar to Negro Tacaná. Verdín produces purple blossoms, matured pods are yellowcream in color and seeds are small (24.7 g/100 seeds) opaque black. One of the traits that has a high agronomic acceptance is that Verdín is an early maturing cultivar with 68 d after planting with a range of 67 to 70 d, depending on season and location of the tropical and subtropical conditions of southern Mexico, compared with a range of 73 to 75 and mean of 74 d for Jamapa. The earliness allows Verdín reduce the risks of yield losses due to the occurrence of terminal drought. Verdín is resistant to anthracnose and to BGYMV. It possesses the single dominant hypersensitive *I* gene which conditions resistance to seedborne Bean Common Mosaic Virus (BCMV), but is sensitive to the temperature-insensitive-necrosis inducing strains of BCMNV like NL 3, diseases that are present in bean farmer's fields of Veracruz and Chiapas.

**RESULTS:** Verdín was tested for four years (2011-14) over 17 locations in Veracruz and Chiapas, Mexico. Over 11 locations, Verdín yielded 1446 kg ha<sup>-1</sup> and significantly out-yielded the commercial check varieties: Negro Papaloapan (12.8%) and Negro Comapa (10.2%). Verdín was field tested under irrigation and terminal drought and selected based on drought tolerance (DSI = 0.8) and productivity efficiency (RYEI = 1.45) indices. Estimated soil water balance in field yield trials carried on in 2013-14 indicated that terminal drought occurred in four out of six locations in Veracruz and Chiapas, Mexico (data not shown); in such locations, Verdín was the most productive cultivar under both, terminal drought (1121 kg ha<sup>-1</sup>) and non-stress conditions (1568 kg ha<sup>-1</sup>). The yield increase of Verdín compared to commercial check cultivars was more important under terminal drought than without drought stress; in average seed yield of Verdín was 34 and 41% higher than the commercial check varieties, Negro Tacaná and Negro Jamapa (Table 1).

ideations in verderuz and two in emapas, wexico.					
Location/State	Season /year	Verdín	Negro Tacaná	Negro Jamapa	
La Candelaria, Medellín, Veracruz	FW 2013	1253	747	854	
Ocozocoautla, Chiapas	FW 2013	687	468	549	
La Candelaria, Medellín, Veracruz	WS 2014	1140	713	1024	
Cintalapa, Chiapas	WS 2014	1407	1065	738	
Average with terminal drought		1121	748	791	
Increase compared to checks (%)			49	41	
Martínez de la Torre, Veracruz	FW 2013	1427	1200	1318	
Martínez de la Torre, Veracruz	WS 2014	1709	1471	924	
Average without drought		1568	1335	1121	
Increase compared to checks (%)			17	40	
Average		1270	944	901	
Increase compared to checks (%)			34	41	

**Table 1.** Seed yield (kg ha<sup>-1</sup>) of Vedin compared to regional check cultivars evaluated over four locations in Veracruz and two in Chiapas, Mexico.

FW = Fall-Winter. WS = Winter-Spring.

**CONCLUSIONS:** Verdín, a new improved common bean with a type-II upright medium vine growth habit, early maturing cultivar, drought tolerant and high yield efficiency under irrigation and terminal drought. Verdín is small opaque black-seeded cultivar that complies with the commercial grain characteristics most farmers and consumers demand in southern Mexico.

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#### **2015 MEMBERSHIP LIST**

George Abawi Dept. of Plant Pathology NYSAES Cornell University 113 Barton Laboratory, Geneva, NY 14456 gsa1@cornell.edu

Anatercia Ferreira Alves Rua Presidente Castelo Branco 2565, Ap 15, Centro Gurupi - Tocantins 77405-090 BRAZIL anaterciaa@yahoo.com.br

Steve Antonius 600 N. 15th St. Rochelle, IL 61068 Steve.antonius@delmonte.com

H.M. Ariyarathne Horticultural Crop Res. & Dev. Inst., PO Box 11, SRI LANKA herathma@yahoo.com

Parthiba M. Balasubramanian Agriculture & Agri- Food Canada Lethbridge Research Centre 5403 – 1 Ave., S. PO Box 3000 Lethbridge, Alberta T1J 4B1 CANADA Parthiba.Balasubramanian@AG R.GC.CA

Messias Jose Bastos de Andrade Departmento de Agricultra Universidade Federal de Lavras Cx. P. 3037, CEP 37200-000 Lavras-MG BRAZILmandrade@ufla.br Jorge A. Acosta Gallegos Cerro del aire 127 Col. Colinas del Climatario Queretaro, Queretaro 76090 MEXICO jamk@prodigy.net.mx

Manuel Amane Coordinacao, Programa Feijao I.N.I.A. C.P. 3658 Mavalane, Maputo MOZAMBIQUE manuel\_amane@hotmail.com

Iraja Ferreira Antunes Embrapa Clima Temperado - C. Postal 403 Pelotas, Rio Grande do Sul 96001-970 BRAZIL iraja.antunes@embrapa.br

Demerson Arruda Sanglard ICA - UFMG Avenida Universitaria 1.000 Bairro Universitario Montes Claros - MG- CEP 39.404-457 BRAZIL demerson.ufmg@gmial.com

Jim Ballerstein NYSAES Dept. of Hort. Sci. Cornell University 630 W. North St., Geneva, NY 14456-0462 jwb2@cornell.edu M.W. Adams 5607 Colby Rd. Crystal, MI 48818

American Pulse Association 2780 W. Pullman Road, Moscow, ID 83843-4034

Sergio Arellano-Arciniega km 32.5 Carretera. Ags.-Zac. C.P. 20660, A.P. 20, Pabellon de Arteaga, Ags. MEXICO arellano.sergio@inifas.gob.mx

Fabio Aurelio dias Martins Santa Rita 153 Jardin Gloria Lavras - MG 37200-000 BRAZIL fabioaureliod@gmail.com

Priscila Zaczuk Bassinello Rua S-05, no 499, apto 703 Sector Bela Vista Goiania - Goiás, 74823-460 BRAZIL priscila.bassinello@embrapa.br

Bean Coordinator EIAR Melkassa Research Center P. O. Box 436, Nazreth ETHIOPIA Bean Research Group Awassa Research Center P. O. Box 6 Awassa ETHIOPIA James S. Beaver Dept. of Crop and Agro-Environmental Sci Univ. of Puerto Rico, Mayaquez Call Box 9000, Mayaguez, PR 00681-9000 PUERTO RICO j\_beaver@hotmail.com; james.beaver@upr.edu

Tchabana Bere Agronome / Phytopatologiste Institute Togolais de Research Agronomique ITRA / CRASS, PB 129, Kara TOGO tchabanab@yahoo.fr

Fred A. Bliss 214 Inca Pl. Davis, CA 95616 Fbliss@dcn.org

Mark A. Brick Dept. of Soil & Crop Sciences Colorado State University Fort Collins, CO 80524 mbrick@colostate.edu

Robin Buruchara CIAT r.buruchara@cgiar.org KENYA

Ana Campa Negrillo Ctra Oviedo s/n (Serida) SPAIN acampa@serida.org Steve Beebe CIAT 7343 NW 79th Terrace Medley, FL 33166-2211 s.beebe@cgiar.org

Kirstin Bett Dept. of Plant Sciences University of Saskatchewan 51 Campus Dr. Saskatoon, SK S7N 5A8 CANADA k.bett@usask.ca

Jeffrey Boersma 93 Wungong Rd Wungong, WA 6112 AUSTRALIA jeff97boersma@yahoo.com.au

Brotherton Seed Company, INC. Box 1136 Moses Lake, WA 98837 john@brothertonseed.com heidi@brothertonseed.com

Louis Butare Chief, Programme Legumineuses ISAR, Rubona B.P. 138, Butare RWANDA

Bruno Campion CRA - Unità di Ricerca per l'Orticoltura 26836 Montanaso Lombardo Lodi ITALY bruno.campion@entecra.it Beijing Book Co., Inc. Periodicals Dept. Sub. No. 660B0011#2015 701 East Linden Ave, Linden, NJ 07036-2495 journals@cnpbbci.com

Gilberto Bevilaqua RUA Almirante Barroso 1056 Centro Pelotas - RS 96010-280 BRAZIL gilberto.bevilaqua@cpact.embrapa .br

Joao Bosco dos Santos Departmento de Biologia UFLA, C.P. 3037 CEP 37200-000 Lavras-MG BRAZIL jbsantos@dbi.ufla.br

Juliana Buratto IAPAR Rod. Celso Garcia Cid (PR-445), Km 375 Londrina - Paraná BRAZIL jsburatto@iapar.br

Donald Caine 2330 Easlan Dr Plover, WI 54467 dljed@charter.net

Steve Cannon 1017 Crop Genome Informatics Lab Wallace RD Iowa State University, Ames, IA 50010 steven.cannon@ars.usda.gov Cliff Chan Harris Moran Seed Company Molecular Genetics 9241 Mace Blvd., Davis, CA 95618 c.chan@hmclause.com

Chief Programme Legumineuses FOFIFA B.P. 1444 Ambatobe, Antananarivo 101 MADAGASCAR

Karen Cichy USDA-ARS 434 Plant & Soil Sciences Bldg. Michigan State University, East Lansing, MI 48824-1325 karen.cichy@ars.usda.gov

Vicky Crone USDA National Agric. Library Current Serial Records, Room 002 10301 Baltimore Ave., Beltsville, MD 20705 vicky.crone@ars.usda.gov

Emmalea Ernest University of Delaware Carvel Research & Education Center 16483 County Seat Hwy, Georgetown, DE 19947 emmalea@udel.edu

Deidre Fourie ARC-Grain Crops Institute Private Bag X1251 Potchefstroom 2520 SOUTH AFRICA FourieD@arc.agric.za

Valérie Geffroy Institut de Biologie des Plantes Université Paris Sud Bat 630 FRANCE valerie.geffroy@u-psud.fr Syama Chatterton Lethbridge Research Center AAFC 5403 1 Ave South POB 3000 Lethbridge, AB T1J 4B1 CANADA Syama.Chatterton@agr.gc.ca

Rowland Chirwa Coordinator, SABRN Chitedze Res. Stat. P. O. Box 158, Lilongwe MALAWI r.chirwa@cgiar.org

Steve Cloyd University of Wisconsin Plant Pathology Library, 584 Russell Lab 1630 Linden Drive, Madison, WI 53706 scloyd@library.wisc.edu

Felipe Aranha de Andrade Rua Jose Rogue Salton 250 Apart. 706, Torre 4 Terra Bonita Londrina - PR 86047-622 BRAZIL felipearanhaa@hotmail.com

J. Alberto Escalante Estrada Colegio de Postgraduados Campus Montecillo km 36.5 Carretera, Montecillo, Mex 56230 MEXICO jasee@colpos.mx

Giovanni Galli Rua 5 de julho, 1379 Centro Palotina - Parana 85950-000 BRAZIL

Robert J. Gehin Harris Moran Seed Co. 1677 Muller Rd. Sun Prairie, WI 53590 r.gehin@hmclause.com Chief Programme Haricot ISABU BP 795, Bujumbura BURUNDI

CIAT Regional Bean Programme PO Box 2704 Arusha TANZANIA

Cornell University Library 110 Olin Library Ithaca, NY 14853

Trazilbo Jose de Paula, Jr. EPAMIG Vila Gianetti 47 Vicosa, MG 36570-000 BRAZIL trazilbo@gmail.com

Kathryne Everts 27664 Nanticoke Rd. Salisbury, MD 21801 keverts@umd.edu

Kadiatou Gamby Toure Entomologiste Chef De Programme Fruits Et Legumes Institut D'Economie Rurale CRRA, BP 262, Sotuba

Dimitar Genchev Dobroudja Agricultural Institute 9520 General Tochevo BULGARIA genchev@dai-gt.org Paul Gepts Dept. of Plant Sciences/MSI 1 Shields Avenue University of California, Davis, CA 95616 plgepts@ucdavis.edu

Regina Lucia Ferreira Gomes Rua Manoel Felicio de Carvalho 1864, Ininga Teresina - PL 64-49-690 BRAZIL r.lfgomes@hotmail.com

Maria Teresa Rodriguez Gonzalez Colegio de Postgraduados Campus Montecillo km 36.5 Carretera, Montecillo MPIO. De Texcoco 56230 MEXICO mate@colpos.mx

Phillip Griffiths NYSAES 314 Hedrick Hall 630 W. North St., Geneva, NY 14456-0462 pdg8@cornell.edu

Janice Harte 114 G.M. Trout Food Science Building Michigan State University East Lansing, MI 48824 harteja@msu.edu

Gerrit Hoogenboom AgWeatherNet Washington State University 24106 N. Bunn Rd, Prosser, WA 99350-9687 gerrit.hoogenboom@wsu.edu Chris Gillard Ridgetown College 120 Main St., E. University of Guelph Ridgetown, ON N0P 2C0 CANADA cgillard@ridgetownc.uoguelph.ca

Anderson Goncalves da Silva Conjunto Cidade Nova I, Rua 2, no.91 Bairro Coqueiro 67130-770 Ananindeua, PA BRAZIL agroanderson.silva@yahoo.com.br

Kenneth F. Grafton NDSU Dept. 7500 314 Morrill Hall, 7500 P.O. Box 6050, Fargo, ND 58105-6050 k.grafton@ndsu.edu

Michael Grusak USDA-ARS Children's Nutrition Research Center 1100 Bates St Houston, TX 77030 mike.grusak@ars.usda.gov

Jerry Haynes Jack's Bean Company LLC 402 N. Interocean, Holyoke, CO 80734-1000 office@jacksbean.com

George L. Hosfield 208 Artists Alley Blowing Rock, NC 28605-9615 georgehosfield@bellsouth.net Graciela Godoy-Lutz 406 Plant Science Department of Plant Pathology University of Nebraska, Lincoln, NE 68583-0722 ggodoy@unlnotes.unl.edu

Maria Celeste Goncalves Vidigal Av. Colombo 5790-cep:87020-900 Univ. Estadual de Maringa Maringa,Parana,87020-900 BRAZIL mcgvidigal@uem.br

Tom Grebb P.O. Box 215 Quincy, WA 98848 tom@centralbean.com

John Hart USDA-ARS TARS 2200 P.A. Campos Ave., Suite 201 Mayaguez, PR 00681 John.Hart@ARS.USDA.GOV

Adam Heuberger Proteomics and Metabolomics Facility Colorado State University 2021 Campus Delivery, Fort Collins, CO 80523 adam.heuberger@colostate.edu

Khwaja G Hossain SB 108 330 3rd Street, NE Mayville State University, Mayville, ND 58257 k.hossain@mayvillestate.edu Anfu Hou Unit 100-101 Route 1Y5 Morden, Manitoba R6M 1Y5 CANADA houa@agr.gc.ca

Francisco Ibarra-Perez CE Cotaxtla, INIFAP Carretera Veracruz- Cordoba km 34.5 Medellin de Bravo, Verecruz 94270 MEXICO fcojip@hotmail.com

Lodi Lama Jean Paul Institut National Pour Etude Et La Rachereche Agronomiquest Bean Program P.O. Box 2037, M'Vuazi Research Center, Kinshasa Dr. CONGO WESTERN DRC

Venugopal Kalavacharla 205 Baker Annex Delaware State University 1200 N DuPont Hwy, Dover, DE 19901-2277 vkalavacharla@desu.edu

Joseph Kasukurthi 2110 University Ave, APT# 210, Madison, WI 53726 josephrajvikas@gmail.com

Paul Kimani Dept of Crop Science-Kabete University of Nairobi P. O. Box 30197, Nairobi KENYA kimanipm@nbnet.co.ke Benjamin Hughey P.O. Box 746 Warden, WA 98857 ben.hughey@purelineseed.com

Carmen Jacinto-Hernandez Tepetlaoxtoc Mna-5, L-2. Fracc. Lomas de Cristo, , Texcoco, Estado de México. CP 56253 MEXICO carmenjh9@yahoo.com

Rachana Jhala Dept. of Plant Pathology 406 PSH University of Nebraska, Lincoln, NE 68583-0722

Kris Kappenman ADM-Seedwest 4666 Faries Parkway, Decatur, IL 62526 kappenman@adm.com

Chris Kelley Kelley Bean Company 1520 Ave "B" Scottsbluff, NE 69361 ckelley@kelleybean.com

Ted Kisha Curator, Phaseolus Collection WRPIS 59 Johnson Hall, Pullman, WA 99164-6402 Theodore.kisha@ars.usda.gov Jessie Hunter American Pulse Association 2780 W. Pullman Road, Moscow, ID 83843 hunter@americanpulsecrops.org

Antony Jarvie PANNAR Research Services Pty (LTD) Box 19 Greytown 3250 SOUTH AFRICA antony.jarvie@pannar.co.za

Abner José de Carvalho Rua Marcelino Nunes da Silva, 284 Bairro Jardim Imperial. Janaúba - Minas Gerais 39440-000 BRAZIL abjocar@yahoo.com.br

Alexander Karasev University of Idaho Dept of PSES, AgSci Rm. 242 875 Perimeter Dr. - 2339, Moscow, ID 83844-2339 akarasev@uidaho.edu

James D. Kelly 1066 Bogue St Michigan State University East Lansing, MI 48824 kellyj@msu.edu

Ken Kmiecik 714 Seneca Pl. Madison, WI 53711 kakmiecik@sbcglobal.net Joseph Kasukurthi 2110 University Ave, APT# 210, Madison, WI 53726 josephrajvikas@gmail.com

Paul Kimani Dept of Crop Science-Kabete University of Nairobi P. O. Box 30197, Nairobi KENYA kimanipm@nbnet.co.ke

Josue Kohashi-Shibata Centro de Botanica. Col. De Postgrad Montecillo, Edo. De Mexico C.P. 56230 MEXICO jkohashi@colpos.mx

Arlindo Leal Boica Junior Via de Acesso Prof. Paulo Donato Castellane,s/n 14884-900 Jaboticabal, SP BRAZIL

Jonathan Lynch 102 Tyson Bldg Penn State University University Park, PA JPL4@psu.edu

Mr. Godwill Makunde Bean Coordinator, Agron. Inst. Dept. of Research & Spec. Serv. PO Box CY-550, Causeway, Harare ZIMBABWE Chris Kelley Kelley Bean Company 1520 Ave "B" Scottsbluff, NE 69361 ckelley@kelleybean.com

Ted Kisha Curator, Phaseolus Collection WRPIS 59 Johnson Hall, Pullman, WA 99164-6402 Theodore.kisha@ars.usda.gov

Enéas Ricardo Konzen Rua Santos Dumont 119-Vila Independencia Piraciacaba, SP CEP13418-120 BRAZIL eneas\_florestal@yahoo.com.br

Allan Lobato Universidade Federal Rural da Amazônia Caixa Postal 411 CEP: 68625-971 Paragominas -Pará Brazilallan.lobato@ufra.edu.br ; allanllobato@yahoo.com.br

Maria Aparecida Milagres Machado Rua Geraldo Alves Pereira 460 Planalto Montes Claros - MG 39404-036 BRAZIL cidammachado@yahoo.com.br

Mirella Marconato Di Bello Alameda Angelo Curtareli 110 Nova Aparecido Jaboticabal, Sao Paulo 14883-324 BRAZIL mirellamarconato@hotmail.com James D. Kelly 1066 Bogue St Michigan State University East Lansing, MI 48824 kellyj@msu.edu

Ken Kmiecik 714 Seneca Pl. Madison, WI 53711 kakmiecik@sbcglobal.net

Paul Kusolwa Sokoine Univeristy of Agriculture Department of Crop Science Tiba Road, P.O. Box 3005, Morogoro Tanzania kusolwap@gmail.com

Richard Lowe Pure Line Seeds, Inc. 1004 Peach Tree Dr Moscow, ID 83843 dick.lowe@purelineseed.com

Domenico Magnifico Tera Seeds SRL Cons. Via della Rotaia 4/5 47035 Gambettola (FC) ITALY dmagnifico@teraseeds.com

Samuel Markell NDSU Dept. 7660 PO Box 6050 N.D. State University, Fargo, ND 58108-6050 samuel.markell@ndsu.edu Frédéric Marsolais Southern Crop Protection & Food Res Centre AAFC 1391 Sandford St. Lonodon, ON N5V 4T3 CANADA Frederic.Marsolais@agr.gc.ca

Phil McClean Department of Plant Sciences, NDSU Dept # 7670 PO Box 6050, 270B Loftsgard North Dakota State University, Fargo, ND 58108-6050 phil.mcclean@gmail.com

Phil Miklas USDA-ARS-IAREC 24106 No. Bunn Road Prosser, WA 99350-9687 phil.miklas@ars.usda.gov 509-786-9258

Wezi Mkwaila Dept of Horticulture LUANR P.O. Box 219, Lilongwe MALAWI wezimkwaila@gmail.com

Kennedy Muimui Misamfu Regional Research Cntr. PO Box 410055 Kasama ZAMBIA

Dr. Savi Natarajan Soybean Genomics and Improvement, PSI-ARS-USDA Bld. 006, BARC-West 10300 Baltimore Ave., Beltsville, MD 20705 savi.natarajan@ars.usda.gov Mark Massoudi AG BIOTECH INC. PO Box 1325, San Juan Bautista, CA 95045 info@agbiotech.net

Maeli Melotto University of Texas, 501 S. Nedderman Dr. Biology Department Box 19498, Arlington, TX 76019 melotto@uta.edu

Amos Miningour Institute de L'Environnement Et de Recherches Agricoles INERA, 01 BP 476, Ouagadougou 01 BURKINA FASO

Vania Moda-Cirino IAPAR Rod. Celso Garcia Cid (PR-445), Km 375 Londrina - Paraná BRAZILvamoci@iapar.br

James R. Myers Dept. of Horticulture, ALS 4017 Oregon State University Corvallis, OR 97331 myersja@hort.oregonstate.edu

National Bean Programme Coordinator Southern DRCongo INERA Kipopo P.O. Box 224, Lubumbashi DR CONGO liungameschac@yahoo.fr/ilunga.m eschac@gmial.com Netzahualcoyotl Mayek-Perez Centro de Biotecnologia Genomica-IPN Blvd. Del Maestro esq. Elias Pina Col. Narcisco Mendoza, 88710 Reynosa, Tamaulipa MEXICO nmayek@ipn.mx

Thomas Michaels Dept. of Horticultural Sci. 1970 Folwell Ave. University of Minnesota, St. Paul, MN 55108 michaels@umn.edu

Telesphore Mirindi Cirhuza Chief D' Antenne PNL/INERA MULUNGU (D.R. Congo) BP 327, Cyangugu RWANDA

Bertrand Monsimier Vilmorin Route Du Manoir 49250 La Menitre FRANCE bertrand.monsimier@vilmorin.com

Masoud Naderpour Seed and Plant Cert. Res. Instit. (SPCRI) Nebovvat Blvd, Sohrevardi P.O. Box 31535-1516, Karaj IRAN mnaderpour\_spii@yahoo.com

Rosa Navarrete-Maya Sur 121 MZ 17 L 14 Col. Juventino Rosas Iztacalco, Mexico, DF 08700 MEXICO rosa navarrete@hotmail.com Susan Nchimbi-Msolla Dept. of Crop Science and Production Sokoine University of Agriculture P.O. Box 3005, Chuo Kikuu Moragoro TANZANIA nchimbi@suanet.ac.tz; smsolla@yahoo.com

Luciano Nogueira Avenida Jaime Ribeiro 888 Bloco 3, Apartamento 14 Vila Industrial Jabotical - SP 14883-125 BRAZIL lucianonogueiraagro@gmail.com

Dâmiany Pádua Oliveira Rua Lasmar 116 Vista Alegre Perdoes - Minas Gerais,37260-000 BRAZIL damy\_agro84@hotmail.com; damiany.padua.oliveira@gmail.com

Arie Oppelaar Monsanto Holland BV Wageningse Afweg 31 NETHERLAND Sarie.oppelaar@monsanto.com

Esteban S. Osuna Ceja km 32.5 Carretera. Ags.-Zac. C.P. 20660, A.P. 20, Pabellon de Arteaga, Ags. MEXICO osuna.salvador@inifap.gob.mx

Julie S. Pasche NDSU Walster Hall 323 Dept 7660, PO Box 6050, Fargo, ND 58108-6050 Julie.Pasche@ndsu.edu James Nienhuis Dept. of Hort, 1575 Linden Drive University of Wisconsin Madison, WI 53706nienhuis@wisc.edu

Laurant Nounamo Systems Agronomist,Dorrespondant National IRAD Institut De Recherche Agricole Pour Le Developpment/ Irad, P.O. Box 2067, Yaounde CAMEROUN

Eli Carlos Oliveira Rua Luiz Lerco, 399 Ap # 705 Torre # 01 Londrina – Paraná 86047 – 610 BRAZIL elioliveira.agro@gmail.com

Pedro F. Ortega Murrieta Martires de Cananea 475 Col. Ley 57 Hermosillo, Sonora 93100 MEXICO ortega.pedro@inifap.gob.mx

PABRA Coordinator Kwanda Agric. Research. Inst. P. O. Box 6247 Kampala, UGANDA

Talo Pastor-Corrales USDA-ARS, Soybean Genomics and Improvement Laboratory Bldg.006 Rm. 118 BARC-West 10300 Baltimore Ave., Beltsville, MD 20783 talo.pastor-corrales@ars.usda.gov Steve Noffsinger Seneca Foods Corp. 301 Seneca Way P.O. Box 105, Dayton, WA 99328 snoffsinger@senecafoods.com

Barry Ogg Dept. of Soil & Crop Sciences Colorado State University Fort Collins, CO 80523-1170 Barry.Ogg@colostate.edu

Renato Franco Oliveira de Moraes Avenida Jaime Ribeiro 888, Bl. 3, Ap. 33 Vila Industrial 14883-125 Jaboticabal, SP BRAZIL renatomoraes2@hotmail.com

Juan M. Osorno Dept. of Plant Science NDSU Dept. 7670, P.O. Box 6050 North Dakota State University, Fargo, ND 58108-6050 juan.osorno@ndsu.edu

James Palmer Michigan Crop Improvement Assoc. P.O. Box 21008 Lansing, MI 48909 palmerj@michcrop.com

Peter Pauls 44 James St W Guelph, Ontario N1G 1E4 CANADA ppauls@uoguelph.ca Calvin H. Pearson Colorado State University Western Colorado Research Center 1910 L Road, Fruita, CO 81521 calvin.pearson@colostate.edu

Yakende Rodrigue Prosper Institut Centrafrican de Rechereche Agriconomique ICRA BP 1762 , Bangui CENTRAL AFRICA REPUBLIC

Eleazar Reyes Barraza Col. Florida Monterrey, Nuevo Leon 64810 MEXICO elreyes@itesm.mx

Charlene Robast Vilmorin Route Du Manoir 49250 La Menitre FRANCE charlene.robast@vilmorin.com

Gonzalo Rojas-Cifuentes Dept. of Plant Science NDSU Dept. 7076 266A Loftsgard Hall, P.O. Box 6050, Fargo, ND 58108-6050 Gonzalo.Rojas@ndsu.edu

Rigoberto Rosales Serna Encinos 158 Residential Los Pinos Durango, Dgo. Mex. 34162 MEXICO rigoberto serna@yahoo.com Alexis Plouy Monsanto 21120 Hwy 30, Filer, ID 83328 alexis.plouy@monsanto.com

Magno Antonio Patto Ramalho Dept. de Biologia - UFLA Cx. Pos. 3037 37200-000 Lavras, M.G BRAZIL magnoapr@ufla.br

Zulene Antonio Ribeiro Via de Acesso Prof. Paulo Donato Castellane,s/n FCAV/UNESP, Dep. De Fitossanidade 14884-900 Jaboticabal, SP BRAZIL zribeiro@fcav.unesp.br

Fernando da Silva Rocha Institute de Ciencias Agrarias -ICA UFMG Campus Regional de Montes Claros Avenida Universitaria 1.00 Montes Claros - MG 39404-547 BRAZIL rochafsplant@yahoo.com.br

Carlos Rojas-Santillan km 32.5 Carretera. Ags.-Zac. C.P. 20660 , A.P. 20, Pabellon de Arteaga, Ags. MEXICO rojas.carlos@inifas.gob.mx

Juan Carlos Rosas EAP/ZAMORANO Calle Pastizales, Bloque E, Casa No. 5 Residencial La Hacienda, P.O. Box 93, Tegucigalpa, HONDURAS jcrosas@zamorano.edu Tim Porch USDA ARS SAA TARS 2200 P.A. Campos Ave., Suite 201 Mayaguez, PR 00680 PUERTO RICO timothy.porch@ars.usda.gov

Anna Regina Tiago Carneiro Rua Geraldo Alves Pereira 460 Planalto Montes Claros - MG 39404-036 BRAZIL anna-regina@hotmail.com

Ron Riley Basin Seed Co. 10766 Lake Shore Dr. Nampa, ID 83686 ron.riley@basinseed.com

A. Paula Rodino Miguez Dept of Plant Breeding Carballeira 8-Salcedo SPAIN aprodino@mbg.csic.es

Mariuci Romero Lopes Via de acesso Prof. Paulo Donato Castelane FCAV/UNESP Jaboticabal, Sao Paulo 14884-900 BRAZI Lmariuci\_lopes@hotmail.com

Janice M.W. Rueda ADM 4666 Faries Parkway Decatur, IL 62526 Janice.Rueda@adm.com Ivan A. Russkikh Belarus State University Nezavisimosti Prospect, 4 220030 Minsk BELARUS russkikh@bsu.by

Helton Santos Pereira Rodovia GO-462 (Goiânia -Nova Veneza), km 12 (Embrapa Arroz e Feijão) Santo Antônio de Goiás Goiás,75375-000 BRAZIL helton@cnpaf.embrapa.br

Lesole Sefume Department of Agricultural Research Research - Lethoso P.O. Box 829, Maseru LETHOSO

Matt Shellenberger Pro Vita PO Box 628 Kuna, ID 83634 Matt@Provita-Inc.com

Shree P. Singh Kimberly Research and Extension 3793 N. 3600 East University of Idaho, Kimberly, ID 83341 singh@kimberly.uidaho.edu

Jodi Souter 6-809 Kristjanson Rd. Saskatoon, SK S7S 1M8 CANADA jrs293@mail.usask.ca Jeff Safe Crites Seed Inc. 16500 Rd. 5 NW P.O. Box 8, Quincy, WA 98848 jeff@critesseed.com

Jim Schild Scotts Bluff County Extension 4502 Avenue I Scottsbluff, NE 69361 Jschild1@unl.edu

Serials ACQ Dept. Iowa State University 204 Parks Library Ames, IA 50011-2142

Ron Shellenberger Pro Vita PO Box 628 Kuna, ID 83634 Rons@Provita-Inc.com

Thomas H. Smith Crop Science Building University of Guelph 50 Stone Rd. E. Guelph, ON, N1G 2W1 CANADA thsmith@uoguelph.ca

Thiago Souza Embrapa Rice and Beans GO-462, km 12, Zona Rural Santo Antonio de Goiás, GO CEP: 75375-000 BRAZIL thiago.souza@embrapa.br Marta Santalla Ferradas Pablo Iglesias 28 5A 36210 VIGO SPAIN msantalla@mbg.cesga.es; msantalla@mbg.csic.es

Howard F. Schwartz C205 Plant Sciences Dept. of Bioagr. Sci. & Pest Mgmt. Colorado State University, Fort Collins, CO 80523-1177 howard.schwartz@colostate.edu

Serials Dept Penn State University 126 Paterno Library University Park, PA 16802-1808

Norman Simelane Agricultural Research Division Lowveld Experiment Station P.O Box 11, Matata Swaziland

Svetla Sofkova Maritza Vegetable Crop Res. Inst. 32 Brezovsko Shosse Strb, 4003 Plovdiv BULGARIA Svetlas\_76@yahoo.com

Starke Ayres Seed (Pty) Ctd. Plot 17, Farm Hartebeesfontein 9th Road, Bredell, 1623 Gauteng SOUTH AFRICA casper@starkeayres.co.za James R. Steadman Dept. of Plant Pathology 406 PSH University of Nebraska, Lincoln, NE 68583-0722 jsteadman1@unl.edu

Swets Information Services 160 Ninth Ave Unit B Runnemede, NJ 08078 napub@us.swets.com

John Theuws Kempenlaan 7 B-3600 Genk BELGIUM johntheuws@telenet.be

Joseph Michel Tohme C I A T 7343 NW 79th Terrace Medley, FL 33166-2211 j.tohme@cgiar.org

Jennifer Trapp SENECA Dayton, WA molassesface@hotmail.com

Dr. Michael Ugen NARO-NACRRI P. O. Box 7084 Kampala UGANDA Andrzej Stera ul.Grzyminska 25a/15 POLAND andrzej.stera@tlen.pl

Rogério Teixeira Duarte Rua Francisco Travaini 167 Santa Luzia Jaboticabal, Sao Paulo 14884-900 BRAZIL rogerio.tduarte@yahoo.com.br

Henry J. Thompson Colorado State University Cancer Prevention Lab 1173 Campus Delivery, Fort Collins, CO 80523-1173 henry.thompson@colostate.edu

Juarez Tomaz IAPAR Rod. Celso Garcia Cid (PR-445), Km 375 Londrina - Paraná BRAZIL tomaz@iapar.br

Shing Jy Tsao 1184 Fynes Ct. San Jose, CA 95131 jocelyn@ntu.edu.tw

University of California Library Bioscience & Natural Res. 2101 VLSB #6500 Berkeley, CA 94720-0001 Kathy Stewart-Williams Idaho Crop Improvement Association 2283 Wright Ave, Suite C Twin Falls, ID 83303 kathysw@idahocrop.com

Julie S. Thayer 59 Johnson Hall Washington State University Pullman, WA 99164 jthayer@wsu.edu

Alyson Thornton Harris Moran 1677 Muller Rd. Sun Prairie, WI 53590 A.Thornton@hmclause.com

Oscar H. Tosquy-Valle CE Cotaxtla, INIFAP Carretera Veracruz- Cordoba km 34.5 Medellin de Bravo, Verecruz 94270 MEXICO tosquy.oscar@inifap.gob.mx

Mark A. Uebersax 2846 West Braden Road Perry, MI 48872 uebersax@tds.net

University of Queensland Library Level 1, Dughig Building St Lucia Campus St Lucia QLD 4072 AUSTRALIA
Carlos Urrea Panhandle Research & Extension Center 4502 Avenue I University of Nebraska, Scottsbluff, NE 69361 Currea2@unl.edu

Bert Vandenberg Dept. of Plant Sciences 51 Campus Drive, Univ of Saskatchewan Saskatoon, SK S7N 5A8 CANADA bert.vandenberg@usask.ca

Maryam Vazin University of Guelph Dept. of Plant Agriculture 21-78 College Ave West Guelph, ON, N1G 4S7 CANADA vazinm@uoguelph.ca

Oswaldo Voysest 1225 Bushnell St Beloit, WI 53511-6430 ovoysestv@aol.com

Lyle Wallace 3405 NW Orchard Ave Apt 252 Corvallis, OR 97330 LW2671@gmail.com

Molly Welsh P.O. Box 6 Colton, WA 99113 wandh@pullman.com

Andi Woolf Idaho Bean Commission 821 W. State St. Boise, ID 83702 andi.woolf@bean.idaho.gov Arlene Valmadrid East-West Seed Co., Inc. Km 54 Cagayan Valley Road Sampaloc San Rafael, Bulacan 3008 Philippines arlene.dionglay@eastwestseed.com

Greg Varner MI Dry Bean Res. Board 8437 N. Blair Road Breckenridge, MI 48615-9726 varnerbean@hotmail.com

Elise Vendeuvre Vilmorin Route Du Manoir 49250 La Menitre FRANCE elise.vendeuvre@vilmorin.com

Dan Wahlquist Syngenta Seeds, Inc. 6338 HWY 20 - 26 Nampa, ID 83687 dan.wahlquist@syngenta.com

Robyn Walton 21120 HWY 30 Filer, ID 83328 robyn.c.walton@monsanto.com

Jeffrey White ALARC, USDA-ARS 21881 North Cardon Lane Maricopa, AZ 85138 jeffrey.white@ars.usda.gov

Mildred Zapata Serrano UPR – Mayaguez (RUM) PO Box 9000, Mayaguez, PR 00681-9000 mildred.zapataserrano@upr.edu Gerthon van de Bunt Pop Vriend Seeds B.V. P. O. Box 5 NETHERLANDS gvandebunt@popvriendseeds.nl

Jose E. Vasquez 602 12th St. N. Moorhead, MN 56560 jose.e.vasquez@ndsu.edu

Rogerio Faria Vieira Grain Legume Researcher EPAMIG - Vila Gianetti 47 Vicosa, MG 36571-000 BRAZIL rfvieira@epamig.br

J. G. Waines Botany and Plant Sciences University of California Riverside, CA 92521-0124 giles.waines@ucr.edu

Ivo Eduardo Wellington Thereza Cristina de Jesus Julião, nº740; Jardim Nova Aparecida Jaboticabal, SP CEP: 14883-296 BRAZIL wellington ie@hotmail.com

Dale Williams Plant Sciences #7670 P.O. Box 6050 North Dakota State University, Fargo, ND 58108-6050 Dale.Williams@ndsu.edu

## 2014 FINANCIAL STATEMENT BEAN IMPROVEMENT COOPERATIVE

## **BALANCE AS OF January 1, 2014 INCOME**

\$ 13,709.00

		2014
	2014 Dues	\$ 5070.00
	Extra Articles for 2013 Report	\$ 25.00
	2015 Dues prepaid	\$ 80.00
	Excess from Portland BIC meeting	\$ 1,603.79
	Back Issues	\$ 80.00
	Bank Interest	\$ 91.17
	TOTAL INCOME	\$ 6,949.96
EXPI	ENSE	
	Labor Charges	\$ 732.16
	Postage, Copy Charges and Office Supplies	\$ 2,591.60
	Printing and shipment – Volume 56	\$ 2,878.00
	PayPal Fees	\$ 142.56
	TOTAL EXPENSE	\$ 6 344 32

## **BALANCE AS OF December 31, 2014**

\$ 13,684.64