ANNUAL REPORT OF THE

BEAN IMPROVEMENT COOPERATIVE

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

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Report of The

BEAN IMPROVEMENT COOPERATIVE

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Coordinating Committee

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SITE REGULARLY UPDATED

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

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Cover: Dry bean harvest in North Dakota courtesy of Juan Osorno

THE 62nd ANNUAL REPORT OF THE BEAN IMPROVEMENT COOPERATIVE

The Bean Improvement Cooperative (BIC) invites all members and other interested parties to join us at the 30th Biennial Meeting from November 3 through November 6, 2019, in Fargo, North Dakota. The local BIC meeting organizers include: Juan Osorno Juan.Osorno@ndsu.edu, Phil McClean phillip.mcclean@ndsu.edu , Julie Pasche Julie.Pasche@ndsu.edu , and Mike Grusak Mike.Grusak@ARS.USDA.GOV . In addition, the associated meetings with our colleagues in the North American Pulse Improvement Association will be from November 6 to 9, 2019 [NAPIA host contact: BunyaminTar'an bunyamin.taran@usask.ca]. The Phaseolus Crop Germplasm, BIC Genetics and the Regional W-3150 Committees are scheduled for November 6. A field trip is also planned. Please refer to the information provided by the local organizing committee in the current report, look for additional information and updates on the website for the conference and on the BIC web site http://www.bic.uprm.edu/.

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 June 30, 2019. We will continue to recognize our founding members through the **Frazier-Zaumeyer Distinguished Lectureship**. The Lectureship will be awarded at the meeting in Fargo 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 was moved this year to http://www.bic.uprm.edu/ and is curated by Dr. Tim Porch (USDA-ARS, Mayaguez, PR) in collaboration with the University of Puerto Rico. Please feel free to contact us with any new ideas, contributions, or updates for the BIC website. A recent suggestion was to develop a specific links page for all the new genomic resources available for Phaseolus. I would like to personally thank Dr. James Kelly for maintaining the BIC website during my tenure as BIC President for the past ten years.

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

Dr. Phillip Miklas, BIC President

BIC COMMITTEE MEMBERSHIP - 1957 to 2019

Coordinating Committee (approximate year of appointment):

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

Awards Committee:

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

Genetics Committee

2004 **Bassett** (Chair), Beaver, Blair, Gepts, McClean, Miklas, Welsh (ex officio)
2005 **Beaver** (Acting Chair), Blair, Gepts, McClean, Miklas, Porch, Welsh (ex officio)
2007 Beaver, Blair, Gepts, McClean, Miklas, **Porch** (Chair), Welsh (ex officio)
2008 Bett, Blair, Gepts, McClean, Miklas, **Porch** (Chair), Urrea, Welsh (ex officio)
2014 **Bett** (Chair), Ferreira, Gepts, Goncalves-Vidigal, Kalavacharla, Kelly, Kisha (ex officio), McClean, Osorno, Porch, Urrea
2018 Bett**,** Ferreira, Gepts, Goncalves-Vidigal, Kalavacharla, **Kelly** (Chair), Kisha (ex officio), McClean, Osorno, Porch, Urrea

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

2017 David M. Kramer, Hannah Distinguished Professor, in Photosynthesis and Bioenergetics at Michigan State University [Frazier - Zaumeyer Distinguished Lectureship]
Maria Celeste Gonçalves-Vidigal, Maringá State University, Brazil, Plant Breeder,
Gregory V. Varner, Michigan Dry Edible Bean Advisory Board, Research Director
Irvin E. Widders, Michigan State University, Director of FtF Legume Innovation Lab
Deidre Fourie, ARC GCI, Potchefstroom, S. Africa, Plant Pathologist [Achievement Award]
Clare Mukankusi, CIAT-Uganda, Plant Breeder [Achievement Award]
Rian Lee, North Dakota State University, Research Technician [Technical Merit]
Evan M. Wright, Michigan State University, Research Technician [Technical Merit]

Please consider nominating your colleagues for the 2019 BIC Awards. Details on nominating colleagues are provided below

2019 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 60-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 thirtieth Anniversary of the BIC Biennial Meeting in Fargo, ND, on the 5th of November 2019.

BIC AWARD NOMINATION

Return by June 30, 2019 to:

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

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

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]

FIRST ANNOUNCEMENT FOR THE BIENNIAL BIC/NAPIA 2019 MEETING IN FARGO, NORTH DAKOTA

BIC = Nov 3 – NOV 6, 2019 NAPIA = NOV 6-8, 2019

The meeting will be held at the Radisson Hotel, downtown Fargo. A block of rooms has been set aside, and reservations can be made by contacting the hotel directly.

Hotel: Radisson Downtown Fargo: https://www.radisson.com/fargo-hotel-nd-58102/fargo
Address: 201 Fifth Street North
Fargo, ND 58102
Phone: (701)-232-7363

Details on Registration will be posted on BIC website: http://www.bic.uprm.edu/

LOCAL HOSTS:

Juan M. Osorno (juan.osorno@ndsu.edu) Phil McClean (phillip.mcclean@ndsu.edu)
Julie Pasche julie.pasche@ndsu.edu Mike Grusak (Mike.Grusak@ARS.USDA.GOV)

BIC business contact: Phil Miklas Phil.Miklas@ARS.USDA.GOV
NAPIA business contact: Bunyamin Taran (bunyamin.taran@usask.ca)

TENTATIVE SCHEDULE FOR BIC/NAPIA Meeting - 2019

Day	*Date*	*Activity*	*Time*
Sunday	Nov 3	Welcome mixer BIC	6-9P
Registration BIC			4-6P
Monday	Nov 4	BIC talks and posters	8A-7P
Dinner on your own		evening	
Tuesday	Nov 5	BIC talks and poster	8A-6P
BIC banquet			6:30-8:30P
Wednesday	Nov 6	W3150/PCGC/ BIC Genetics Committee Meetings	8A-12P
Tour: TBD?			1:30 -5:30P
NAPIA mixer			7-9P
Thursday	Nov 7	NAPIA	8A-5P
		NAPIA Awards Luncheon	12 noon
Friday	Nov 8	NAPIA	8A-5P

IN MEMORY OF COLIN LEAKEY

Professor Colin Louis Avern Leakey passed away on January 29, 2018 in Lincoln, England. Colin was born on December 13, 1933 to Louis and Frida (Avern) Leakey in Cambridge. Beginning in the 1930s, his parents conducted pioneering paleoanthropological research on human origins in East Africa. Colin's upbringing was mainly in East Anglia where he attended Gresham's School in Holt, Norfolk. After school, Colin served a year on the staff of Lord Mountbatten who was then Commander in Chief of the Mediterranean Fleet. He then obtained a B.S. degree from Cambridge University in Natural Sciences. He later trained in tropical agriculture and plant pathology at Exeter University and the University of the West Indies, Trinidad. In 1972, having already taught a heavy course load at Makerere University, Uganda, in plant pathology, crop protection, plant breeding, and other courses like crop ecology and statistics, he was awarded a PhD by Cambridge University.

Dr. Leakey was supported by the Commonwealth Mycological Institute to Uganda's Kawanda Research Station as pathology specialist in improvement of *Phaseolus* beans. In addition to beans and other legumes, he worked on coffee, cocoa, cotton and vanilla diseases. In the early 1970s, political turmoil in Uganda led to the end of British funding and Colin's return to the UK, where he became active in Cambridge University's bean program. With the late Alice Evans they produced IBPGR's crop descriptors booklet for *P. vulgaris* which forms the basis for European registrations.

In the 1980s he set up Peas and Beans Ltd. with colleague Anthony Brown. Their aim was to develop new cultivars from bean cuisines around the world that could be adapted to the British climate. Projects included Manteca beans from Chile and a new type of red-seeded bean with improved processing characteristics. He then set up a small private research station in Girton, Cambridge where he continued breeding work and conducted field trials. Later, he re-located to Lincoln where he remained till the end of his life.

Colin developed several notable cultivars. 'Horsehead', a dark-red mottled bean, originated from a cross of a Colombian red-mottled with a disease resistant, green-podded "French bean" (Diacol Nima/Cofinel). 'Stop' is a dark red kidney with non-leaching color, slightly smaller seed size with thinner skins, and adapted to shorter seasons. Its parentage is 'Brazil 2'/'Royal Red' kidney. 'Prim' is perhaps Colin's most notable bean. It was bred from different germplasm ('Swedish Brown'/'Opal') but was intended to emulate Manteca beans. Coscorron and Manteca beans were discovered by Colin in his consulting work in Chile, and he was intrigued by their reputation as "beans for the rich man's table" because they were low flatulence inducing. In Uganda, Colin had been asked by nutritionists to increase digestibility of beans for use as weaning food. These threads came together to inspire his development of a low flatulence bean, 'Prim'. He thought that Manteca and Coscorron beans had reduced tannin levels which increased digestibility. Recent work has shown that Manteca beans have less dietary fiber, less indigestible protein and starch, but with similar concentrations of oligosaccharides, and are free of proanthocyanins and condensed tannins that reduce protein digestibility and iron absorption. Colin often worked on the boundaries of market classes as well as with rather more obscure market classes. Examples would include 'Dordogne', a small-sized white kidney bean, 'Gogo' an oval shaped green-seeded (persistent color) bean, and Victor', a determinate early maturing 'Coscorron' bean for shell-outs. The BIC recognized his accomplishments with the Meritorious Service Award in 1989.

Dr. Leakey's publications reflect a wide range of interests. Books contributed to include Crop Improvement in East Africa, Agriculture in Uganda, and Food Crops of the Lowland Tropics. There is a series of major reports on topics such as Breadfruit Reconnaissance Study in the Caribbean, Pulse crops in Chile and a feasibility study on the Establishment of a date palm plantation in Oman. There are also many papers published in peer refereed journals.

He is survived by his wife Susan, daughters Emma, Tess and Tamsin, and 7 grandchildren.

IN MEMORY OF ALI NAVABI

The Plant agriculture community and members of the Ontario Agricultural College at the University of Guelph sadly inform the Bean Improvement Cooperative membership of the death of Dr. Alireza (Ali) Navabi on March 10, 2019. Ali became an active participant in the BIC after his appointment in 2008 to Agriculture Agri-Food Canada as the breeder in the joint Ag Canada - University of Guelph bean breeding program, located at the University of Guelph. In 2014 Ali took a position with the University of Guelph as the Grain Farmers of Ontario (GFO) Professor in Wheat Breeding.

Ali was an innovator in bean breeding and genetics. He was particularly interested in nitrogen fixation and believed that this trait needed to be improved to reduce the environmental impact of growing beans. He co-authored over 20 publications on beans, including 10 bean variety descriptions. He actively promoted people working with him and (successfully) nominated his technician for the first Technical Merit Award of the BIC. Ali was a strong advocate of student participation in international conferences, including the BIC meetings, and was generous with his financial and technical support to encourage students to attend and excel in their oral and poster presentations.

Ali has had significant success as a wheat breeder as well. The program expanded enormously to include variety development of spring and winter types and lines released by the program were supported for variety registration early this year.

Ali's CV includes 65 papers published in peer-reviewed journals, 30 disclosures of advanced breeding lines to the University and over 100 presentations at national and international conferences. Ali was a leader in scientific organizations, including as: associate editor of the Canadian Journal of Plant Science for 4 years, editor-in-chief of the Canadian Journal of Plant Science for 3 years, President of the Canadian Society of Agronomy from 2016-2017, and as executive board member for Plant Canada from 2015-2017. His contributions were recognized by his induction as a Fellow of the Canadian Society of Agronomy in 2016 and his recognition by the Ontario Seed Growers' Association as a Seed Champion in 2018.

Ali's death seems sudden even though he shared the news of his pancreatic cancer diagnosis with friends and colleagues a year ago. He openly discussed how he had arranged his chemo treatment regime for weekends so that he could continue teaching undergraduate courses, advise grad students and manage a bourgeoning wheat breeding program, during the week. From his continuing presence in the department, it seemed as if he might prevail, through quiet determination. But sadly, it was not to be.

Students will remember Ali's passionate dedication to teaching and mentoring. He was a strong advocate for students at all levels and a believer in their abilities. He was a true colleague in the Plant Ag Department and worked in many ways, including as chair of the social committee, to make it a positive and supportive community.

IN MEMORY OF SOON JAI PARK

Dr. Soon Park passed away peacefully on December 23, 2018, in his 81st year. He is survived by his wife Ki Ok, two grown daughters, one granddaughter and several siblings living in Canada and South Korea. Dr. Park was born January 22, 1937 in South Korea. He received three post-secondary degrees from Seoul National University, the University of Hawaii and North Dakota State University. Following graduation, he accepted rice breeding positions in South Korea and the Philippines (IRRI), before coming to Canada to develop soybean cultivars for King Grain in Chatham Ontario. Most of his colleagues remember him as a dry bean breeder for Agriculture and Agri-Food Canada in Harrow Ontario. Dr. Park retired in 2008 after 27 years of service. During his tenure, Dr. Park was affectionately known as 'Whirlwind Soon' and 'The Tornado'.

Dr. Park developed non-nod, super-nod and ineffective nodulation mutants for biological nitrogen fixation genetics and breeding studies in common bean. He actively bred lines for several market classes of dry bean with resistance to anthracnose, bean common mosaic (BCMV), common bacterial blight (CBB), the complex of root rot species and for white mold, among other characteristics. Soon Park made extensive use of exotic germplasm to broaden the genetic base of common bean cultivars for Canadian bean growing environments, including interspecific crosses with *P. coccineus* and *P. acutifolius* to introduce resistance to common bacterial blight, root rot and white mold. Dr. Park released several germplasm lines, more than 25 dry bean cultivars in navy, kidney, black and pinto market classes, two soybean cultivars as well as cultivars in specialty classes such as otebo, azuki (*Vigna angularis*) and mung beans (*Vigna radiata*). His navy bean cultivar Nautica is still popular in Ontario today, more than 15 years after its release. Pintoba, a pinto bean cultivar developed in Dr. Park's program, was widely grown in Manitoba for many years. Although his research effort was devoted to dry bean breeding, he had a lifelong interest in rice and soybean, as well as other alternative pulses like pigeon pea (*Cajanus cajan*). Dr. Park wrote one book chapter and published 65 refereed and 50 non-refereed research articles. Near the end of his career, he focused on molecular marker assisted techniques to improve the efficiency on conventional bean breeding approaches, with a number of accomplishments.

Dr. Soon Park served as associate editor of the Canadian Journal of Plant Science, was an honorary member of the Canadian Seed Growers' Association since 1998, received the Meritorious Service award from the Bean Improvement Cooperative (BIC) in November 2001, and received recognition by the Korean-Canadian Science and Engineering Association.

IN MEMORY OF GONZALO ROJAS-CIFUENTES

Dr. Gonzalo Rojas-Cifuentes, assistant director of the North Dakota Foundation Seedstocks Program, passed away at his home surrounded by his family and friends on October 22, 2018 in Fargo, ND. He had worked at North Dakota State University for more than 15 years. Gonzalo was diagnosed with pancreatic cancer four months before his departure.

Gonzalo was born September 1, 1962 in Santiago, Chile to Osvaldo Rojas-Cuadras and Marta Cifuentes-Briceño. He graduated from the University of Chile in 1992 with a degree in Agronomy. Soon after his marriage to Viviana Rivera, they moved to Fargo, ND in 1994 to work on his M.S. degree in crop production at North Dakota State University (NDSU) with Dr. Al Schneiter. His research focused on the effect of cadmium on different crop species. In 2001, Gonzalo earned his Ph.D. degree in plant breeding/genetics at NDSU, advised by former NDSU potato breeder Dr. Richard Novy.

Between 2001 and 2009 he worked as a postdoctoral research fellow with the NDSU dry bean breeding program, first with Dr. Ken Grafton until 2006, then with Dr. Juan Osorno until 2009. During this period, Dr. Grafton took on some administrative responsibilities while remaining the program leader and Gonzalo worked as assistant breeder overseeing and executing the everyday activities of the breeding program. Among other responsibilities, Gonzalo was also in charge of the disease screening of all the breeding lines from the program. Gonzalo's efforts contributed to the development and release of important cultivars in North Dakota such as Eclipse black bean, and Lariat, Stampede, ND-307 pinto beans, among others. In addition, he did some studies documenting the environmental effects on seed darkening on pinto bean varieties, as well as the effects of different row spacings on seed yield and seed losses of newer upright cultivars.

In 2009, he was hired as the assistant director of the NDSU Foundation Seedstocks Program. Part of his job was to inspect and ensure the quality of seed stock of all varieties produced by NDSU plant breeding programs, including dry bean varieties. Gonzalo was instrumental in the coordination and management of the seed distribution programs. He would spend hours roguing seed production fields in order to ensure genetic purity of the varieties. In addition, Gonzalo coordinated the production and distribution of seed of dry bean varieties recently released by the University of Nebraska. He also taught a course in the Department of Plant Sciences on seed production and technology.

Gonzalo and Viviana, as natives of Chile, initially had not planned to stay in the U.S. for more than three years. However, after living in the U.S. 19 years, they were proud to earn their U.S. citizenship in 2012. "I am proud to be Chilean by birth and American by choice," said Gonzalo at the time.

In May 2018, Gonzalo was awarded the prestigious President's Volunteer Service Award for his volunteer work in Guinea, West Africa in September 2017. The award recognizes Americans who engage in volunteer service and inspire others to do so. Gonzalo provided training for faculty and students at the Institute Superior of Agronomy and Veterinary of Faranah, Guinea's only agricultural university. Traveling was one of Gonzalo's passions.

With his easy smile and friendly manner, he made friends wherever he went. He will be missed by many.

COMMON BEANS AND LIMA BEANS IN THE NORTHERN ANDES: EVOLUTIONARY RIDDLES AND POTENTIAL UTILITY

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Introduction: Genetic variability of the *Phaseolus* genus in the northern Andes is complex and intriguing. Colombia was the crossroads of Mesoamerican (e.g. maize, common bean), Amazonian (e.g. cassava) and Andean (e.g. potato, common bean) crops. While cultivated common bean has two major gene pools, and the wild ancestor presents much wider variability (Chacon et al. 2005), unique germplasm in the northern Andes is reported: wild populations in Ecuador and N Peru with 'I' phaseolin (Rendón-Anaya et al., 2017b); and wild and cultivated germplasm from Colombia. The region has seen the precursor of the species coming from Central America, then the migration of its wild form to give birth to the Mesoamerican and Central-Southern Andean genepools (Chacon et al. 2007; Rendón-Anaya et al. 2017a). Landraces intercrossed with each other and with their sympatric wild relatives. These facts make the northern Andes a scenario of migration, extinction, local evolution, introduction and genetic interchange. This review reflects on the evolutionary role of the region, and possible implications for genetic improvement.

The distribution of wild species: A consideration of wild *Phaseolus* and its evolution demands assuring that we are dealing with wild plants and not escapes from cultivation that would be derived from human intervention. In this regard, explosively dehiscent pods observed on living and herbarium specimens and findings in original forest habitats as reported by collectors are critical information. In contrast to escaped Lima bean (there is one accession G25246B from Ashanti, Ghana, in CIAT genebank), escapes from cultivation of *P. vulgaris* do not survive well in natural vegetation.

The geography of the western hemisphere is a determining factor in the evolution of *Phaseolus,* and its multiple wild species, and is the context for interpreting genetic evidence from various sources. The geographic setting of NW South America was established about 80 million years ago (Graham 2011), and the Isthmus of Panama could have closed the gap between the northern and southern continents some 13-15 million years ago (Montes et al. 2015). The genus *Phaseolus* is about 8-10 million years old (Delgado-Salinas et al. 2006), and has diversified in Mesoamerica, where most species are distributed today (Freytag & Debouck 2002). The age of *P. vulgaris* and *P. lunatus* as independent species and their separation from related species are very difficult to define but have been estimated at 2 and 1 mi years, respectively (Gepts et al. 2000, separation from *P. dumosus*; and Serrano-Serrano et al. 2010, separation from *P. augusti – P. pachyrrhizoides*). While speciation is an ongoing process, the var. *mexicanus* and the var. *aborigineus* of *P. vulgaris* are living examples of it (Delgado-Salinas et al. 1988).

The presence of wild *Phaseolus vulgaris* was initially reported independently for Argentina (Burkart 1941) and Guatemala (McBryde 1947); and of wild *P. lunatus* for Mexico to Panama, Colombia, Venezuela, Brazil and Peru (Piper 1926) (but in contrast with the former two, without any specific location). Germplasm collections carried out in 1960-2019 in the American tropics

and subtropics have since notably expanded our knowledge and shown that wild common bean is present in mid-to-high altitude forests from Chihuahua, Mexico (Nabhan 1985) down to Córdoba, Argentina (Drewes 2006). The range of wild Lima beans extends from northern Mexico (Sonora, Tamaulipas) to northern Argentina (Chaco, Formosa) (Debouck 2018). Note that because of the altitude requirements of wild common bean, its distribution is discontinuous, less so for wild Lima bean, and thus the range of the latter is wider. Molecular markers applied to seed storage proteins (Gepts et al. 1986) and later to mitochondrial (Khairallah et al. 1992) and nuclear DNA (Kwak & Gepts 2009; Tohme et al. 1996) have shown higher diversity in the wild forms as compared to the cultivated ones. These works have demonstrated that the two gene pools existing in the cultivated common and Lima beans, traditionally observed on seed traits (Evans 1976; Kaplan 1971), pre-date domestication (estimated at about 7-8,000 years before present: Chacón-Sánchez & Martínez-Castillo 2017; Mamidi et al. 2011). The same organization into gene pools found in the associated pathogens (anthracnose, angular leaf spot: Pastor-Corrales 1991; Guzmán et al. 1995, respectively) also supports that hypothesis.

Migration: The presence of wild common and Lima beans in natural vegetation of both North and South America raises the question on how this situation has come about. Keeping in mind that humans came into the Americas through Beringia some 20,000 years ago (Wells 2003), this means that wild common and Lima beans were already present in the American tropics and subtropics of both continents. So, returning to the question of how to explain the presence of wild common bean in South America, given the number and diversity of species in Mesoamerica, one logical scenario would be that of an early migration of *P. vulgaris* into the Andes from Mesoamerica through the Isthmus of Panama, and some analyses would support that hypothesis (Bitocchi et al. 2012; Blair et al. 2012; Mamidi et al. 2013; Schmutz et al. 2014).

However, a collection (DGD-1956) of August 1986 in San Pablo, Cajamarca, Peru, changed the picture dramatically, and a group of wild common beans was disclosed in SW Ecuador and NW Peru (Debouck et al. 1993). Further analysis with different markers constantly showed the uniqueness of this group as compared to the other wild gene pools (beebmcb et al. 2017; Bitocchi et al. 2013; Blair et al. 2012; Chacón-Sánchez et al. 2007; Freyre et al. 1996; Kami et al. 1995; Khairallah et al. 1992; Koenig et al. 1990; Kwak & Gepts 2009; McClean et al. 2004; Mina-Vargas et al. 2016; Tohme et al. 1996). Assumed to have strayed from its home in Mesoamerica in the distant past, it was found to be ancient (Kami et al. 1995), with a separation from the rest of wild *P. vulgaris* about 0.6 mi years ago (Chacón-Sánchez et al. 2007) or 0.9 mi years ago (cpDNA) (Rendón-Anaya et al. 2017a), and eventually interpreted as a sister species of common bean (Rendón-Anaya et al. 2017a, b). Later this ancestral stock of *P. vulgaris* underwent another speciation event: the formation of the two major gene pools (Ariani et al. 2017; Chacón-Sánchez et al. 2007; Rendón-Anaya et al. 2017a). A few DNA polymorphisms shared between Mexico and the Southern Andes (Chacón-Sánchez et al. 2007; Khairallah et al. 1992; Tohme et al. 1996) may account for that early split, evidencing a migration of *P. vulgaris* both north- and southwards from its primordial cradle in the northern Andes. A more recent southward migration from Mexico accounts for much of the genetic diversity seen in Central America (Ariani et al. 2017; Chacón-Sánchez et al. 2007) and ending up in Colombia (with the 'B' and 'CH' phaseolins). As expected, the wild common beans in the central and southern Andes show less genetic diversity as compared to Central America because they could not expand in longitude (Ariani et al. 2017; Bitocchi et al. 2013; Schmutz et al. 2014). Also, one should note that during the two migrations southwards through the Isthmus *P. vulgaris* migrated alone without other species of the *Phaseoli* that could have enriched its gene pool (Lioi &

Hammer 1989; Rendón-Anaya et al. 2017a). In this regard, the migration of *P. dumosus* into Andean South America, namely the humid interandean valleys and eastern slope where it is feral (Schmit & Debouck 1991), would have been too recent to enrich its gene pool with the exception of a few natural hybrids.

A similar scenario exists for Lima bean. From an ancestral stock in Mesoamerica the tertiary gene pool of Lima bean would evolve and after a first migration into the Andes this stock had time to diversify into two closely related species: *P. augusti* and *P. pachyrrhizoides* (Caicedo et al. 1999), and an insular off-type: *P. mollis*. A group of wild Lima beans was found in SW Ecuador and NW Peru (Debouck et al. 1987), and analysis with different markers showed it to be the ancestral wild form of large-seeded cultivated Lima bean (Gutiérrez-Salgado et al. 1995; Motta-Aldana et al. 2010; Fofana et al. 1997). Thus, speciation resulted in the formation of the Andean gene pool of Lima bean, and 0.5 mi years ago the formation of the so-called Mesoamerican gene pool (it has colonized the tropical lowlands of both Mesoamerica and South America however) quickly splitting into two branches MI and MII (Serrano-Serrano et al. 2010). In addition, a slightly different group of wild Lima beans related to the Andean pool seems present in Boyacá, Colombia (Chacón-Sánchez & Martínez-Castillo 2017).

While migratory birds have been called upon to explain the trans-isthmic migrations of wild beans (Ariani et al. 2017; Rendón-Anaya et al. 2017a), an alternate scenario is the one of beans moving through natural seed dispersal and reproduction in favorable conditions under climatic variations since the late Tertiary period. Because wild beans have toxic seeds (Sotelo et al. 1995), particularly true for wild *P. lunatus* (Seigler et al. 1989), it is not fully clear why migratory birds or long-range moving mammals would look for them as food, drop them in the right habitats thousands of miles from the original ones, assuming that the seeds would survive after passing through the digestion track. The diversity displayed by the markers again favor the slow accumulation of mutations through time because of migration and drift. Local extinction of many populations because of unsuitable growing conditions also played a prominent role in the two complex patterns observed today.

It might be relevant here to recall the breeding systems of the six species related to *P. vulgaris,* which are genetically autocompatible outbreeders with active nectaries on the floral disk. The seed set is significantly increased if the flowers are visited by bees and bumblebees (Darwin 1858). The terminal stigma is variously shaped, from extrorse in *P. coccineus* (Webster et al. 1980) to capitate in *P. albescens* (Ramírez-Delgadillo & Delgado-Salinas 1999) to introrse in *P. debouckii* (Rendón-Anaya et al. 2017b). As a consequence, allogamy can be high in wild forms, explaining the genetic diversity within populations (Rodriguez et al. 2016). In wild Lima beans where the flowers are much smaller, nectaries on bracteoles may play a role, and outcrossing rate as high as 47% has been reported (Baudoin et al. 2004).

Domestication: Amerindians confronted a distribution of the wild forms of both species not dramatically different from the one known today since we are still in the same interglacial period (Clark et al. 2009). The primitive hunter-gatherers at some point stopped observing and making periodical harvests, and started planting wild forms in what is today Mexico and in the Central Andes. The exact location of the domestication process has been much investigated, under the afore-mentioned assumption, showing two independent foci for the common bean (Bitocchi et al. 2013; Chacón-Sánchez et al. 2005; Kwak et al. 2009) and the Lima bean (Chacón-Sánchez et al. 2012; Chacón-Sánchez & Martínez-Castillo 2017). At the beginning this activity was limited to

planting and harvesting wild forms (Debouck 2016). Through this process Amerindians selected for bigger seeds and pods, although the transition to fully modern sizes has not appeared yet in the archaeological record (Kaplan & Lynch 1999). But they did not alter the breeding system, and thus crosses between the wild forms and the quasi-domesticated forms continued to occur. As a result, intermediate or weedy forms continued to appear in the contact zone, in some regions of Colombia, Peru, Bolivia, Argentina and Mexico until today (Beebe et al. 1997; Freyre et al. 1996; Hoc et al. 2006; Zizumbo-Villareal et al. 2005, respectively), and similarly in Lima bean (Félix et al. 2014). So, admixtures continue to cause headaches to scholars trying to count the number of migrations or to locate the domestication spots!

Weedy types in Colombia perhaps give a window on this past activity and likely human intervention. These weedy types have a vigorous climbing habit to compete in a thicket environment or in coffee groves. While attributed to spontaneous hybridizations within a breeding complex, evidence also suggested human intervention in selection and dissemination. One type known as 'Vagamundo'or 'Vagabundo' with 'CH' phaseolin has pink seed with red stripes. It is widely distributed in Colombia in the Cauca river valley and in valleys leading to the eastern plains. This grain color would not occur readily in crosses with wild bean, nor would a wild-weedy complex alone result in wide distribution. We suggest that some weedy types might be remnants of an incipient agriculture (beans were used 8,600 years before present in the Middle Cauca Valley: (Dickau et al. 2015)), whereby weedy types were spread by early cultivators in thicket environments that permitted production and collection at seed maturity, with no additional crop management.

This scenario invites reflection on what we understand by "domestication" and what assumptions are implicit in that understanding. One view is comparable to a pedigree breeding system whereby the parentage of a domesticate can be traced linearly to some unique original ancestor that was selected for valuable spontaneous mutations by plant domesticators (primitive plant breeders). Over years additional mutations would have been selected within this parental's progenies and maintained in linear pedigree fashion. An alternative view suggested here is more similar to a recurrent selection system, whereby occasional outcrossing led to introgression of genes and new genetic constitutions and phenotypes. This latter model must be included in our conceptualization of domestication to explain how the genetic composition of wild *Phaseolus* and indeed, that of our modern germplasm came to be.

Potential utility of northern Andean Phaseolus: While specific classification of native Colombian germplasm remains ambiguous and depends on the method and the germplasm under study, a recognition of the role of the northern Andes in the evolution of wild species of *Phaseolus* raises questions about the possible utility of this germplasm including those domesticated accessions that are unique to this region. Early work on the genetic structure of the species using phaseolin seed protein led to the suggestion that domestication had occurred in Colombia based on type 'B' phaseolin in local wild and cultivated populations (Gepts and Bliss 1986) (although we had difficulty in distinguishing type 'B' consistently from type 'S'). However, phaseolin types 'CH' and 'L' were more distinctive and were found in both local wilds and cultivars, further contributing to the hypothesis of local domestication activity (Toro et al. 1990; Tohme et al. 1996; unpublished data, CIAT). The designation of 'L' phaseolin was derived from the landrace 'Labrancero', a local climbing bean. Bush cultivar G4691 with type 'CH' is unique in presenting flowers with no wing petals. A study of CIAT's bean core collection suggested a North Andean cultivated gene pool, but this again was heavily influenced by

variation in seed proteins: phaseolins, lectins, and α-amylase inhibitors (Islam et al. 2001). This should now be viewed as too narrow an information base to posit a unique gene pool, however, other evidence on the unusual nature of this germplasm emerged from pathological studies. While presenting a Mesoamerican morphological phenotype, accessions that classed in this group presented disease reactions similar to Andean beans, suggesting a long-term evolution in situ (Islam et al. 2002).

The potential of this germplasm for breeding is largely unexplored, and few examples exist that are indicative of its value. One striking example resulted from the use of a Colombian wild bean in a breeding scheme that had been suggested to obtain introgression of unique genes from wild ancestors (Tanksley et al. 1996). Breeding line 115M was derived from the backcross of a Colombian wild accession to cultivar 'Negro Tacaná' (DOR 390 in CIAT's coding system) and presented excellent yield (Wright and Kelly 2011).

Following the analysis of wild accessions of common bean for phaseolin type, purified phasolin protein was extracted from seed and subjected to in vitro hydrolysis, imitating the digestive process in the gut. Phaseolin of one such Colombian accession with type 'L' presented 93% hydrolysis versus 58% in 'S' type and 71% in 'T' type, suggesting the potential to employ this phaseolin for the nutritional improvement of common bean (Montoya et al. 2008). Crosses and selections have been advanced, although a test of such phaseolins with potential for high digestibility has not been carried out in mammals and is lacking.

A cultivated accession with Mesoamerican phenotype from Nariño department in Colombia was identified for excellent adaptation to low soil phosphorus, and exhibits unusually good photosynthate remobilization under other types of stress as well. A QTL study highlighted segments contributing as much as 100 kg/ha to yield (Diaz et al. 2018).

G19833 or Chaucha Chuga was collected by one of the current writers in northern Peru, and subsequently was recognized to be unusually tolerant to low soil phosphorus (Yan et al. 1995a and b). So unusual was G19833 that it was selected to develop a reference genome of the species. Later sequencing revealed that in fact it has significant introgression from a Mesoamerican genome –an observation that highlights the dynamism of the evolution of local cultivated germplasm (Lobaton et al. 2018).

Other accessions from this region (G23818B, G23823E, 23834E) have presented high concentration of iron in grain and have served as sources of this trait in breeding for nutritional value of common bean. These appear to be intergene pool crosses that have occurred naturally in farmer's fields where Andean and Mesoamerican types have been cultivated side by side or in mixtures.

Unfinished work and conclusions: Regarding collections, Colombia lies squarely on the north-south pre-colonial routes of trade that moved germplasm in both directions. Thus, local common bean germplasm is heavily influenced by that of both Mesoamerica and the southern Andes. To have a clearer picture of wild common bean in NW South America, it might be easier to tackle first those similar questions on the Lima bean model. With the increase of rural transportation in the 20th century, seed movement of Lima bean could have been less important than with common bean. With less long-distance transportation of Lima bean landraces and cultivars, there could be less blurring due to gene flow between cultivated forms and the wild (Beebe et al. 1997; Papa &

Gepts 2003). In this regard, areas well outside the main stream of seed movements (e.g. the eastern extreme of the Andes in Venezuela) might be worth visiting, as wild beans are known to be present there (Aymard 1999). Also, within Colombia true wild beans are reported by local residents in Antioquia for a possible transition with Panama and Costa Rica, and could exist in Nariño department for a transition with the sibling species (Rendón-Anaya et al. 2017b). In order to take full benefit of the advances in genomics to solve the puzzle, a much better sampling of the wild forms in South America is urgently needed. These collections remain to be accomplished. Finally, there is little knowledge of *Phaseolus* germplasm in the western extreme of the Guyana shield, which has an environment that should be amenable to bean cultivation, and which has been largely isolated. Common bean is reported here (Aymard 1999), but little or nothing is known about cultivars there.

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DOCUMENTING A FIFTH STAGE FOR CONTINUED GROWTH AND DEVELOPMENT IN DRY BEANS

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A Fifth Stage for Growth and Development of Dry Beans - is Defined.

Understanding the importance of Stage Five for Dry Beans comes from seventeen trials with eight Pinto (PT) and eight Great Northern (GN) Varieties from 2009-2017. Data from the Ag Lab weather station [Scottsbluff 6NW, Period of Record 1996-07-11 to-Present (AKA Mitchell Farms) Period of Record 1912 to 1996-07-11. Latitude 41.95N°. Longitude -103.70E0°. Elevation 1254m. Client UNL Extension – Panhandle] dating back to 1912 was used in calculating Growing Degree Days (GDD) using the maximum temperature (T_{max}) and minimum temperature (T_{min}) for each day in the following formula:

Table 1 – Five Stages of Growth and Development for Dry Beans

Vegetative Development

1. 21 days for Emergence and Stand Establishment (June 7* – June 28)**
2. 21 days for Rapid Vegetative Growth (June 29 – July 19)**

Reproductive Development

3. 21 days for Flowering and Pod Development (July 20 – August 9)**
4. 21 days for Pod Fill (August 10 – August 30)**

Physiological Maturity and Seed Maturation

5. 21 days for Physiological Maturity and Seed Maturation to reach 14% moisture (August 31 – September 20)**

* June 7 is an average date of planting for Dry Beans in Western Nebraska.

** These dates vary from one year to the next depending upon changes in weather.

($GDD = (T_{max} + T_{min}/2) - T_{base\ of}\ 50$)

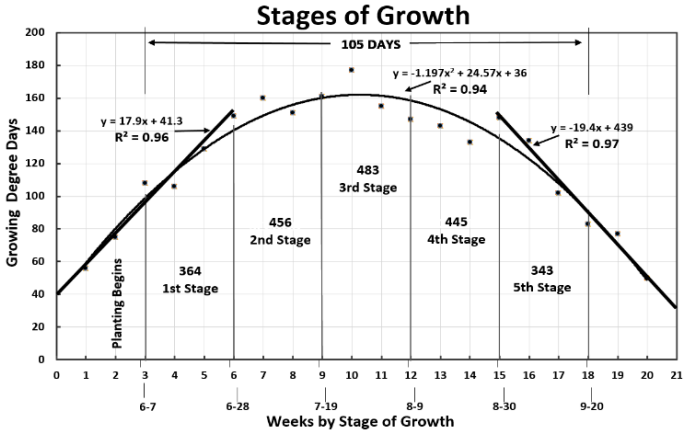
Solar energy at any given location can be converted to GDD.

The typical four stages of growth and development are well documented and are used with either Green Beans (GB) or Dry Beans (DB). Both warm-season crops depend upon solar energy in moving from one stage to another. Green Beans are harvested for their fleshy green pods and immature seed prior to stage four; in contrast, DB are harvested after stage four for their mature edible seed at 14% moisture. Maturity is recorded for DB when eight out of ten pods turn buckskin in color. The moisture of the seed in the pod is about 30% at this time and about 30% of the leaves remain on the plant. Dry down of seed from 30% to the desired 14% moisture for harvest is longest if the plants are left standing or can be hastened by undercutting a furrow irrigated crop or by using a desiccant on a crop to be direct harvested.

A Fifth Stage for Growth and Development of Dry Beans - is a Continuation of the First Four Stages.

In the Northern hemisphere, daily temperature increases as days lengthen in the spring and decreases as days shorten in the fall. Average weekly GDDs from May 18 to October 11 averaged over 2009-2017 were used in a quadratic equation that results in Figure 1 which accounts for 94% of the variation in GDD during these 21 weeks.

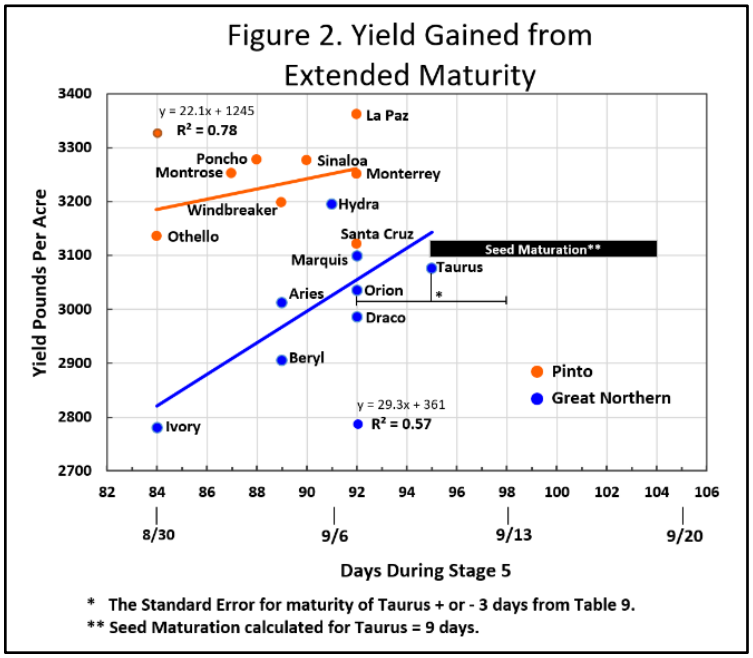
Figure 1: Growing Degree Days Within Five Stages of Growth



The first thing that catches the eye is that all five stages for growth and development of the DB fit neatly under the arched curve beginning with the average planting date of June 7 and terminating 105 days later, on September 20. Secondly, weekly GDD from May 18 to June 28 is linear and accounts for 96% of the variation in GDD during this period while gaining 18 GDD per week. Stage one fits neatly within **June** starting with the June 7 planting date. Then, weekly GDD for eight weeks in the fall from August 23 to October 11 is also linear and accounts for 97% of the variation in GDD while losing 19 GDD per week. Stage five fits neatly within the first three weeks of **September.** Finally, stages two, three and four fit neatly within **July** and **August** during the heat of summer when the sun's rays are directly overhead.

A Fifth Stage for Growth and Development of Dry Beans - is Justified as it is here that Yield is Gained and Seed Maturation Occurs.

All 21 days of stage five are included in Figure 2 and all but one of the 16 varieties in these trials matured within the first eight days of stage five. Maturity is followed by seed maturation where moisture of the seed in the pod decreases from 30% to 14% in readiness for harvest as shown with Taurus.



The white space to the right of a variety's average maturity is where seed maturation occurs during stage five. For example: Taurus matured in 95 days with a standard error of the mean of plus or minus three days as shown in Figure 2. This is the *first* variable encountered as seed maturation begins or ends for a given variety during stage five. The *second* variable deals with the system of management used in production of the crop. If Taurus is grown in 22" furrow irrigated rows it could be undercut at maturity as seed maturation begins then and lasting for about six days; or it could be left standing like a direct harvested crop to go through seed maturation which takes longer if a desiccant is used to burn off the remaining leaves to hasten dry down. A *third* variable is the weatherwhich can alter seed maturation for either the undercut or desiccated plants in hastening or delaying seed maturation. A *fourth* variable controlled by the grower is variety selection based upon maturity ranging from 84 to 92 days. For example: the early-season PT Othello and GN Ivory if undercut at maturity (84-days) will have completed seed maturation and harvest prior to maturity of the six full-season (92-day) varieties. In contrast, if the late-season Taurus is undercut at maturity (95-days) it will have completed seed maturation and harvest on the twentieth day of stage five as shown in Figure 2.

An Important Point: All 16 varieties during all nine years of these trials had not completed seed maturation within stage five on September 20. Nevertheless, during the longest growing season of 2017; all varieties in all trials did complete seed maturation and were harvested nine day after stage five on September 29, which was 114 days after planting. The first 28-degree frost during these nine years occurred on October 4, 2009.

In summary: A fifth stage for growth and development is essential in maximizing yield and ensuring seed maturation prior to harvesting Dry Edible Bean.

NEW RACES OF ANGULAR LEAF SPOT AND ANTHRACNOSE IN EASTERN AFRICA AND IDENTIFICATION OF SOURCES OF RESISTANCE

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INTRODUCTION: Angular leafspot (*Pseudocercospora griseola*) and anthracnose (*Colletotrichum lindemuthianum*) are among the most important biotic constraints to bean productivity in east, central, southern and West Africa, and worldwide (Teran et al, 2013). Other major bean diseases in eastern Africa include root rots (caused by *Pythium* and *Fusarium spp.*), bean common mosaic and common bacterial blight (Wortmann et al, 1998). Under favourable conditions, yield losses of 50 to 100 percent have been reported in susceptible cultivars. Breeding varieties with resistance to one or more these diseases is considered the most effective and sustainable strategy of reducing losses due to these diseases. Consequently, breeding for resistance to major diseases has been a major goal of bean improvement programs in eastern Africa for the last three decades (Kimani et al, 2005). Several resistant varieties have been developed and released for production in nearly all bean growing countries in East, Central, West and Southern Africa (Buruchara et al, 2011). However, several varieties have succumbed to diseases they were resistant to at the time of release. This has led to the hypothesis that new strains of these pathogens may have arisen. However, no systematic studies have been conducted to provide supporting evidence. Knowledge of pathogen variability is essential for effective screening to identify useful sources of resistance and development of resistant varieties. The objectives of this study were to: i) Determine if there is new pathogenic variation in major bean growing regions of Kenya, ii) Determine reaction of commercial varieties to recently collected isolates of five major diseases, and iii) Identify better sources of resistance for future breeding.

MATERIALS AND METHODS: Sixty-two isolates of angular leaf spot, 51 of anthracnose, nine of root rots, 101 of common bacterial blight and 32 of bean common mosaic virus were made from bean leaves, pods and seeds collected from 54 bean growing districts in western, central, Rift Valley, eastern and coastal regions of Kenya during the 2010 short rain (Oct-December) and 2011 long rain season (April-June). The survey covered 12 agro-ecological zones (AEZ). Standard laboratory and greenhouse procedures were followed during isolation, inoculation and multiplication of inoculum for root rots (Abawi and Pastor Corrales, 1990), angular leafspot and anthracnose (Pastor-Corrales et al 1994; 1998; Wagara et al, 2005); common bacterial blight (Makandawire et al 2004; Vandermark et al, 2009) and bean common mosaic virus (Spence and Walkey, 1994). A 1-9 disease scale, where 1-3 is resistant, 4-6 intermediate, and 7-9 is susceptible (van Schoonhoven and Pastor-Corrales, 1987) was used for scoring diseases. The pathogen variability of 57 isolates of angular leaf spot from 35 districts, and 31 of anthracnose was studied using their respective current 12 differentials. The isolates also were used to screen for resistance in 13 large seeded commercial varieties, 8 small and medium seeded varieties, 4 climbing bean varieties and 7 new sources of resistance to angular leafspot, anthracnose, bean common mosaic virus, common bacterial blight and root rots.

RESULTS AND DISCUSSION: Twenty-three races of *P. griseola* were identified, 12 of which were represented by only one isolate. Only 11 races were found in two or more districts. Race 63-63 was the most virulent and caused leaf spots on all 12 bean differentials, whereas race 63-55 was the most widely distributed among the surveyed regions (10 of 57 isolates). Races 63-55, 63-63, 63-54 and 63-35 were found to be the most dominant races in areas studied. Two new races, 31-31 and 63-31 were reported for the first time in Kenya. Forty-five isolates were of the Mesoamerican group, with only 12 isolates of Andean group, suggesting co-evolution of the pathogen with *P. vulgaris* in this host-pathogen interaction. Twelve physiological races of *Colletotrichum lindemuthianum* were identified. Of the 12 races, seven (1, 2, 17, 23, 38, 55 and 485) had been previously identified, while five (65, 73, 81, 87 and 89) are new. Races 65 (8 of 31 isolates) and race 73 (4 of 31 isolates) were the most frequent in surveyed regions. G

2333 was highly resistant to all the isolates. Five sources of resistance (G10909, MEX54, AND 1062, RWR719 and VAX6) and commercial cultivars showed high compatibility with most of the races (Table 1). Differential varieties AB 136 and G 2333 were resistant to all races and therefore can be used in breeding programs in this region. Future bean breeding efforts should consider the new racial diversity of *P. griseola* and *C. lindemuthianum* because most of the commercial varieties used in this study were highly susceptible.

Table 1. Reaction of commercial bean varieties and new sources of resistance to infection by isolates of angular leaf spot, anthracnose, bean common mosaic virus, common bacterial blight and root rots from 54 districts in Kenya.

Variety	Market class	Growth habit	Genepool	Angular leaf spot	Anthracnose	BCMV	Common Bacterial blight	Root rots
GLP2	Red mottled	I	Andean	7.4	6.2	5	4.3	7
GLP 24	Red Kidney	II	Andean	1	2.5	4.6	5.9	5
KAT69	Red mottled	II	Andean	7.4	8.8	6.8	2.6	7
GLP 585	Small red	II	Mesoamerican	7	4.3	3.7	5.3	5
KAT 56	Medium red	I	Mesoamerican	8	6	3.7	6.1	6
KAT B9	Medium red	II	Mesoamerican	8	9	6.8	2.6	9
KAT B1	Yellow	I	Mesoamerican	8	9	6.5	4.1	9
GLP x 92	Pinto	II	Mesoamerican	6.6	2.5	5	5.7	6
Mex 142	Navy	II	Mesoamerican	7	8	9	8	6
Sources of Resistance								
G10909	Small red	IV	Mesoamerican	1	1	7.9	7.4	6
Mex 54	Cream/beige	III	Mesoamerican	1	1	6.3	5.7	4
G2333	Small red	IV	Mesoamerican	1	1	5.4	5	7
VAX 6	Small red	I	Mesoamerican	8	7	2.8	1.4	5
RWR 719	Small red	II	Mesoamerican	7	1	4.8	4	1
BRB 191	Red mottled	II	Andean	4	9	3	2	1
AND 1062	Red kidney	I	Andean	7	8.1	4.2	4	1

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AGGRESSIVENESS OF *Pseudocercospora griseola* STRAINS COLLECTED IN MINAS GERAIS STATE, BRAZIL

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INTRODUCTION

The angular leaf spot (ALS), caused by the fungus *Pseudocercospora griseola*, is one of the main diseases of common bean. This disease occurs in many common bean-growing areas of Brazil (Abadio et al., 2012). However, lines with durable resistance is a challenge because of the high variability of pathogen (Silva et al., 2007). The continuous monitoring and characterization of predominant strains in different infested areas are important to disease control. Therefore, the aim of this work was to collect, isolate and characterize the aggressiveness of *P. griseola* strains from two common bean-growing areas of Minas Gerais state, Brazil.

MATERIALS AND METHODS

Leaves and pods with angular spot lesions were collected in common bean fields at Lavras-MG (LV) and Lambari-MG (LB). From the lesions, strains of *P. griseola* were obtained from monosporic cultures. For aggressiveness test an experiment was carried out to each strain of *P. griseola* in a completely randomized design, with three replicates. Twenty strains from each enviroment were inoculated in four common bean lines (Madrepérola, Pérola, MA-III and Ouro Negro). The inoculum was obtained by, colony mycelium discs of each *P. griseola* strain in test tubes containing PDA (potato-dextrose agar) medium and maintained at 24 °C in BOD, for 12 days. Seeds of each line were sown in polystyrene trays. Eight days after sown, the plants (V2 stage) were inoculated with a conidial suspension (concentration of $2.0x10^4$ conidia/mL). Disease severity was assessed 18 days after inoculation using diagrammatic scale (1 to 9 scores) proposed by Librelon et al. (2015). The plants that exhibited scores 1 to 3 were considered resistant, and those with scores 3.1 or above were considered susceptible. An analysis of variance was carried out considering the environments, strains and lines. Means were compared by Scott-Knott test ($p = 0.05$), using R software.

RESULTS AND DISCUSSION

In joint variance analysis interaction lines x strains was significative ($p < 0.05$) showing that reaction of common bean lines inoculated with strains from different environments was not coincident (Figure 1). Line Ouro Negro was resistant to Lambari strains but was susceptible several strains from Lavras showing that there is specific resistance (Table 1 and Figure 1). Although *P. griseola* resistance is quantitative, the interaction lines x strains exemplifies a type of interaction that characterizes vertical resistance (Figure 1), also observed by Pereira (2015). In general the aggressiveness of the Lavras strains was higher than those from Lambari. According to Table 1, the most aggressive strains were LB6, LB19, LV20 and LV18.

Table 1: Aggressiveness of *P. griseola* strains from Lambari (LB) and Lavras (LV) when innoculated on common bean lines.

	Lines							
Strains	Pérola		Madrepérola		MA-III		Ouro Negro	
LB1	1.1	**C***	4.6	**C***	1.2	**D***	1.0	**E***
LB2	1.8	**C**	3.8	**D**	1.2	**D**	1.2	**E**
LB3	1.3	**C**	3.2	**E**	1.5	**D**	1.3	**E**
LB4	1.1	**C**	3.7	**D**	1.2	**D**	1.3	**E**
LB5	2.5	**A**	4.1	**D**	1.9	**C**	1.8	**E**
LB6	2.9	**A**	5.2	**B**	2.0	**C**	2.2	**D**
LB7	2.7	**A**	4.5	**C**	1.9	**C**	1.6	**E**
LB8	1.9	**B**	3.7	**D**	1.5	**D**	1.1	**E**
LB9	1.4	**C**	3.4	**D**	1.2	**D**	1.2	**E**
LB10	1.3	**C**	2.8	**F**	1.1	**D**	1.0	**E**
LB11	2.0	**B**	3.9	**D**	1.2	**D**	1.4	**E**
LB12	1.1	**C**	2.2	**F**	1.5	**D**	1.1	**E**
LB13	3.1	**A**	4.8	**C**	1.4	**D**	2.3	**D**
LB14	2.2	**B**	3.3	**E**	2.7	**B**	1.6	**E**
LB15	1.4	**C**	4.0	**D**	1.4	**D**	1.1	**E**
LB16	1.9	**B**	6.6	**A**	1.6	**D**	1.2	**E**
LB17	2.3	**B**	5.8	**B**	1.4	**D**	1.2	**E**
LB18	1.4	**C**	2.8	**F**	1.2	**D**	1.3	**E**
LB19	2.4	**A**	5.6	**B**	2.3	**B**	1.5	**E**
LB20	2.3	**B**	4.6	**C**	1.4	**D**	1.2	**E**
LV1	1.3	**C**	3.8	**D**	2.7	**B**	1.7	**E**
LV2	1.0	**C**	3.1	**E**	2.0	**C**	1.1	**E**
LV3	1.1	**C**	3.5	**D**	1.9	**C**	1.1	**E**
LV4	1.3	**C**	5.9	**B**	3.5	**A**	1.4	**E**
LV5	1.4	**C**	3.3	**E**	2.4	**B**	1.1	**E**
LV6	1.1	**C**	3.1	**E**	1.6	**D**	1.1	**E**
LV7	1.0	**C**	2.4	**F**	1.4	**D**	1.0	**E**
LV8	1.4	**C**	4.3	**C**	1.6	**D**	2.6	**D**
LV9	1.5	**C**	3.1	**E**	1.3	**D**	2.9	**D**
LV10	1.9	**B**	2.5	**F**	2.1	**B**	2.0	**D**
LV11	1.9	**B**	4.5	**C**	1.7	**C**	3.9	**B**
LV12	1.9	**B**	4.7	**C**	1.8	**C**	4.1	**B**
LV13	1.3	**C**	3.6	**D**	2.4	**B**	3.7	**B**
LV14	2.1	**B**	5.5	**B**	1.9	**C**	5.1	**A**
LV15	2.2	**B**	4.0	**D**	1.9	**C**	3.8	**B**
LV16	1.4	**C**	4.3	**C**	2.7	**B**	5.6	**A**
LV17	1.8	**C**	3.3	**E**	1.9	**C**	3.1	**C**
LV18	2.7	**A**	4.9	**C**	2.3	**B**	4.8	**A**
LV19	1.9	**B**	4.9	**C**	1.9	**C**	5.5	**A**
LV20	2.6	**A**	5.7	**B**	2.9	**A**	5.1	**A**

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

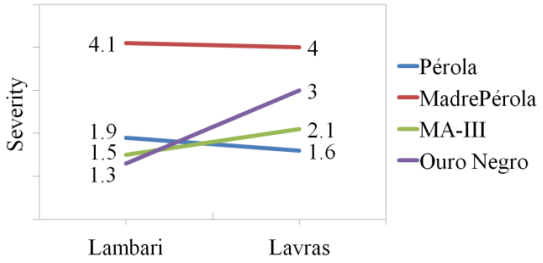

Figure 1: ALS severity mean scores of common bean lines inoculated with *P. griseola* strains from Lavras and Lambari.

Figure 2: Common bean lines inoculated with *P. griseola* strains from Lavras: LV14 (left) and LV10 (right).

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POPULATION STRUCTURE OF RACES OF *Colletotrichum lindemuthianum*

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INTRODUCTION

Anthracnose is a disease caused by the fungus *Colletotrichum lindemuthianum* (Sacc and Magnus) Briosi and Cavara. Anthracnose can lead to a yield reduction of up to 100% in common bean (*Phaseolus vulgaris* L.), when the conditions are favorable for the pathogen (Sileshi et al., 2014). *C. lindemuthianum* is characterized by a wide pathogenic variability with approximately 256 races identified worldwide (Nunes et al., 2013; Padder et al., 2017). There are different strategies to estimate this variability, such as amplification of the ribosomal DNA (rDNA) ITS (Internal Transcribed Spacer) regions via PCR (Polymerase Chain Reaction). ITS markers are used to amplify the ITS1 and ITS2 regions, which are repeated several times in the genome (Fungaro, 2000). For that reason, sequencing of these regions is an efficient way to estimate genetic variability in fungi. This study had as objective to evaluate the occurrence of the genetic differentiation of races of *C. lindemuthianum* from Brazil through the sequencing of the ITS regions.

MATERIALS AND METHODS

We evaluated 40 isolates of *C. lindemuthianum* from Mato Grosso, Paraná and Santa Catarina States, Brazil. These isolates belong to the mycotheca from common bean breeding of Núcleo de Pesquisa Aplicada à Agricultura (Nupagri). Out of the 40 isolates, 32 were already characterized as distinct physiological races. DNA extraction was carried out according to methodology described by Raeder and Broda (1985). DNA samples were quantified with Quant-iT^{TM} fluorimeter. For ITS region amplification reaction we used ITS1F (Gardes and Bruns 1993) and ITS1 (White et al. 1990) as forward primers, while ITS4 (White et al., 1990) was the reverse one. Amplicon's purification was conducted with PureLink PCR Purification Kit (Invitrogen), following the manufacturer's recommendations. Amplified fragments were sequenced at Centro de Estudos do Genoma Humano e Células-Tronco at the Universidade de São Paulo (USP). We carried out clustering analyses of isolates with Structure 2.3.4 software (Pritchard et al., 2000) based on the Bayesian model. To determine the optimal number of clusters, we used 10 independent runs of K=2-10. Each run had a burn-in of 10,000 interactions followed by 100,000 data-collecting interactions using Markov chain Monte Carlo (MCMC) method. Structure Harvester program defined the optimal values of K using ΔK method (Earl and von Holdt, 2012).

RESULTS AND DISCUSSION

Analysis of the genetic structure, based on sequence distribution, revealed the presence of three clusters (Figure 1). Cluster I was composed of races 1, 3, 7, 8, 10, 17, 55, 67, 72, 75, 79, 81, 87, 91, 105, 114, 283, 351, and 2047. Cluster II was formed by consisted of races 0, 2, 9, 23, 27, 31, 65, 67, 72, 73, 75, 83, 89, 101, 121 and 581. Sequences from Genbank of races 23, 31, 89, and

MAFF 305390 isolate were also included in this cluster. Cluster III was composed of races 13, 73, 346, and race 2 from GenBank. Andean races 1, 3, 7, 17, and 55 were allocated as admixture in the Cluster I, whereas Mesoamerican races were allocated in all clusters. In this work, we observed the presence of admixture between Andean and Mesoamerican populations. Besides that, Andean races were allocated in Cluster I and II, while Mesoamerican races were allocated in all groups. Another study evaluated Andean and Mesoamerican isolates of *C. lindemuthianum* through repetitive DNA sequence patterns (Mahuku and Riascos, 2004). The authors did not find genetic differences between Andean and Mesoamerican *C. lindemuthianum* isolates. The present study concludes that there is high variability among *C. lindemuthianum* races when analyzed at the molecular level. These results suggest that sequence analysis of ITS rDNA regions of anthracnose's pathogen may be a valuable tool to identify this pathogen through design of specific primers.

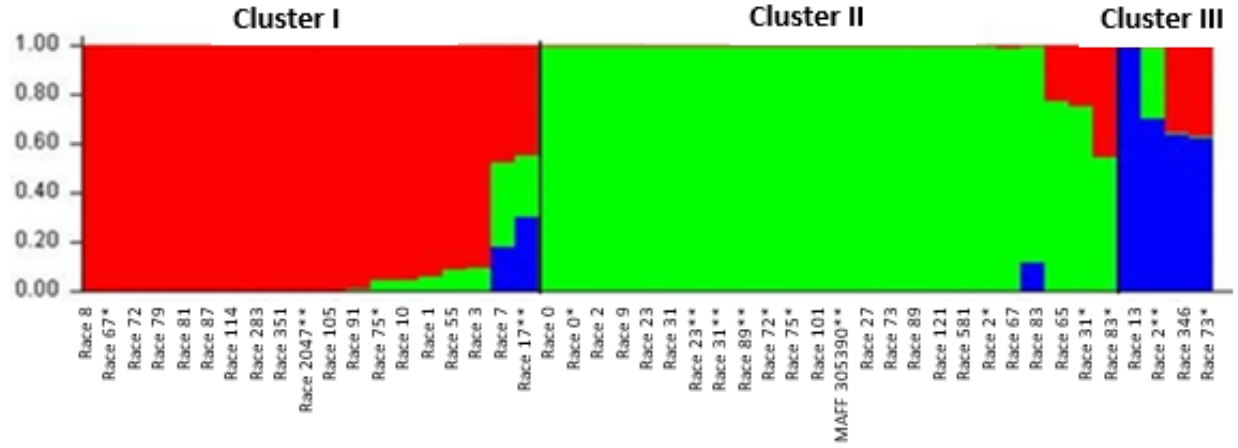

Figure 1- Population genetic structure of 40 isolates of *C. lindemuthianum* and 7 sequences of GenBank through the sequencing of the ITS regions. Each individual accession is represented by a colored bar, partitioned to reflect an individual's relative proportion of genetic membership in a given cluster *K*.

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EFFECTS OF ANTHRACNOSE (*Colletotrichum lindemuthianum*) ON GERMINATION AND VIGOR OF COMMON BEAN SEEDS FROM MATO GROSSO STATE, BRAZIL

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INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is a crop of great economic and social importance for several countries, especially Brazil, but the average Brazilian productivity is low due to several factors, especially the occurrence of diseases, among them anthracnose, caused by the fungus *Colletotrichum lindemuthianum*, is considered one of the most severe, especially if infected seeds are used in planting, the main way of disseminating the pathogen over long distances (LeClair et al., 2015). Infected seeds can cause damages in germination and vigor, furthermore, deterioration, booth reduction in the field, introduce pathogens into new areas and distribute initial foci of infection (Mancini et al., 2016). More than 10 different races of *C. lindemuthianum* have been characterized in the state of Mato Grosso, which evidences the importance of the disease in the region (Felipin-Azevedo et al., 2014). The objective of this research was to evaluate the effects of anthracnose infectation on seed germination and vigor of commercial common bean (*Phaseolus vulgaris* L.) cultivars from the state of Mato Grosso, Brazil, in which healthy and infected seeds with *C. lindemuthianum* were compared. There was a significant difference, where seeds infected with anthracnose had lower rates of germination and vigor when compared to healthy seeds.

MATERIAL AND METHODS

Five commercial common bean cultivars were collected in the state of Mato Grosso (Pérola, Branquinho, Carioca Pitoco, Dama and Esplendor (black)), separated in lots of contaminated and healthy seeds. Infected seeds were considered as having characteristic symptoms, such as dark lesions on the cotyledons. The experiment was carried out in the Laboratório de Sementes do Núcleo de Pesquisa e Biotecnologia (Nupagri), at Universidade Estadual de Maringá. For the germination and vigor test, 200 seeds of each sample were used, with four replicates with 50 seeds of the cultivars in the 2 conditions (infected or not with anthracnose) in a germinator regulated at a constant temperature of 25º C. The seeds were arranged in boxes of the gerbox type, previously sterilized. As substrate, paper sheets of filter paper were used moistened with distilled water. The counts of % germination and vigor were performed on the 5th and 9th days after installation (respectively). The averages obtained were compared by the Tukey test, at 5% probability.

RESULTS AND DISCUSSION

Healthy seeds had germination rates higher than those observed for anthracnose-infected seeds, by the Tukey test at 5% probability. This result is in agreement with the results of Corrêa et al. (2008), who also detected higher germination rates in treatments with lower inoculation rates of *C. lindemuthianum* fungus, as well as lower germination rates as indexes increased of fungus inoculation. One of the main seed pathogens affecting *P. vulgaris* L. cultivars in the world is *C. lindemuthianum*, which under mild temperature conditions and high humidity can cause losses of up to 100% in production (Padder et al., 2017).

For the vigor test, there was a significant difference between the healthy and infected seeds, and the healthy ones had a higher vigor rate, by the Tukey test at 5% probability. In order to study *C. lindemuthianum* infection in common bean seeds, Migliorini et al. (2017) found that as *C. lindemuthianum* infection rates increased, seed vigor reduced, corroborating the results found in this research.

The presence of the pathogen in the seeds acts as a source of primary inoculum dissemination, which can cause epidemics in the initial phase of the crop, resulting in seeds with lower germination rate and vigor, resulting in lower plant populations and consequently production decrease (Padder et al., 2017). In this sense, this study contributes to a better understanding of the producers regarding the use of healthy seeds to increase common bean production.

Table 1 - Rate of germination of healthy and infected anthracnose seeds of common bean evaluated at day 5

Cultivars	Seeds conditions	
	Healthy	Infected
Pérola	94.00 Aa	9.50Bb
Carioca Pitoco	86.75 Aab	13.00Bb
BRS Esplendor	85.00 Abc	13.50Bb
Dama	80.50 Abc	15.00Bb
Branquinho	78.00 Ac	26.75 Ba

Means followed by distinct uppercase letters in the row and lowercase in columns differ from each other by the Tukey test ($P \leq 0.05$).

Table 2 - Rate of vigor (normal seedlings) of healthy and infected anthracnose seeds of common bean evaluated at day 9

Cultivars	Seeds conditions	
	Healthy	Infected
Carioca Pitoco	85.75 Aa	10.00 Ba
Pérola	79.00 Aab	5.50 Ba
BRS Esplendor	78.00 Aab	12.25 Ba
Dama	76.75 Aab	9.00 Ba
Branquinho	69.50 Ab	14.00 Ba

Means followed by distinct uppercase letters in the row and lowercase in columns differ from each other by the Tukey test ($P \leq 0.05$).

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CHARACTERIZATION OF *Colletotrichum truncatum* ISOLATES TRANSMITTED FOR LIMA BEAN PLANT THROUGH SEEDS

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INTRODUCTION

In the Northeast of Brazil, lima bean (*Phaseolus lunatus* L.) is an important source of food and income for the population of this region. However, the occurrence of pests and diseases especially anthracnose (Carvalho et al., 2015) and the use of low quality seeds has contributed to the reduction of the crop productivity. The objective of this study was to evaluate the transmissibility of the pathogen via the seed and to characterize the isolates in relation to mycelial growth (mean colony diameter and mycelial growth rate index) and sporulation (colony conidia cm-2).

MATERIALS AND METHODS

Lima Bean seeds of the Boca de Moça variety (access UFPI-1215), with symptoms of fungal infection (S/W) and no apparent symptoms (S/N) were sown in pots containing a mixture of vegetable soil and manure (2:1) totaling 18 replicates of each treatment (18 pots with S/W and 18 S/N). After germination and for approximately 25 days, daily observations of the plants were performed to detect in the unifoliolate lesion of the basal leaf compatible with infectious symptom caused by fungus for subsequent isolation and culture in bean-dextrose-agar (FDA) culture medium and incubation at 28 ± 1 ° C, with 12-hour photoperiod (Cavalcante et al., 2012). Among the isolates obtained, two were characterized after pathogenicity evaluation: EMSC 06 (plants whose seeds showed symptoms of S/W) and EMSC 07 (plants whose seeds did not present S/N symptoms) in relation to the diameter of the colony (Carvalho, 2009) index of mycelial growth rate (Oliveira, 1991) and conidia production (Carvalho, 2009).

RESULTS AND DISCUSSION

During the evaluation period, all S/W plants (100%) generated from symptomatic seeds presented unifoliolate basal leaves with symptoms of lesions caused by fungi whereas in seedlings generated from seeds with no apparent symptoms (S/N plants) in only six plants (33%) appeared the symptoms. The cultural characteristics: colony diameter and conidia cm^{-2} production varied according to the isolate. The isolate EMSC 06 had a higher colony diameter and a higher number of conidia cm^{-2} than the EMSC 07 isolate when grown in FDA medium, temperature 28º ± 1ºC and absence of light. In relation to the index of mycelial growth rate, the isolates did not differ from each other (Table 1). The high occurrence of lesions on unifoliolate leaves indicates that the seeds represent the form of transmission of the anthracnose pathogen in lima bean and the high potential of damage to the crop, since the severity of the disease is related to the moment of the attack of the pathogen (Mota et al., 2017, Costa et al., 1994). In the evaluation of the pathogenicity of the isolates, we observed reddened lesions, predominantly located in the veins. Later, as the disease progressed, there was a fall in leaves or one or more leaflet, as described by Carvalho (2009), confirming the pathogenicity of the isolates and the transmissibility of the fungus to the plant via seed. However, transmission occurs to a lesser extent from seeds with no apparent symptoms. Thus, the incidence of anthracnose in lima bean can be reduced by using good quality seeds.

Table 1. Cultural characteristics of* Colletotrichum truncatum *isolates when cultivated in Beans-Dextrose-Agar medium, at a temperature of 28º ± 1ºC and absence of light. Teresina - Piauí, Brazil.

Isolates characteristics	EMSC 06	EMSC 07
Diameter of the colony (cm)	5.17 a	4.81 b
CV (%)	0.86	4.53
Index of mycelial growth rate	1.48 a	1.44 a
CV (%)	1.94	3.41
Conidia cm^{-2} ($x10^5$)	24.67 a	3.15 b
CV (%)	45.26	63.45

Means followed by the same letter in the line of each cultural characteristic, do not differ among themselves by the Tukey test at 5% probability.

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MOLECULAR CHARACTERIZATION AND MAPPING OF THE ANTHRACNOSE RESISTANCE GENE IN THE ANDEAN COMMON BEAN CULTIVAR PERLA

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INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) stands out as a culture of great importance in Brazil and worldwide. Among the main factors that affect common bean productivity, we can point out incidence of diseases (Singh and Schwartz, 2010). In this sense, anthracnose, caused by the fungus *Colletotrichum lindemuthianum* deserves special attention, as it can cause production losses up to 100% in susceptible cultivars, and in regions of subtropical climate, with moderate temperatures and high humidity. It is most likely to be transmitted through contaminated seeds (Padder et al., 2017). Previous inheritance studies about anthracnose resistance, using races 65 and 73, revealed the presence of a single dominant resistance gene in Perla cultivar (Taboada et al., 2016). This cultivar is also resistant to race 2047, which shows to be an important source of anthracnose resistance. Therefore, this study had as objective to characterize and to map the anthracnose resistance gene of the Perla cultivar of common bean.

MATERIALS AND METHODS

This research was carried out under greenhouse conditions at the Common Bean Breeding and Molecular Biology Laboratory of Núcleo de Pesquisa Aplicada à Agricultura (Nupagri). The inheritance test was conducted in F_2 generation from the cross Perla × Cornell 49-242, inoculated with the races 65 and 73 (Taboada et al., 2016). Complementary allelism tests were conducted in F_2 populations derived from crosses between Perla and the following cultivars: Cornell 49-242, TO, G2333, Jalo Vermelho, Jalo de Listras Pretas, Corinthiano, Crioulo 159 and BGF-20. Genomic DNA was extracted from 100 F_2 individuals derived from the cross Perla and Cornell 49-242 as previously described (Afanador et al., 1993). Bulked segregant analysis was performed to identify markers linked to the *Co-Perla* allele in the F_2 population (cross Perla × Cornell 49-242). Therefore, two contrasting bulk DNA samples of equal amounts of DNA (Michelmore et al., 1991) were generated five resistance dominant plants from F_2 generation (bulk resistant) and five F_2 recessive plants (bulk susceptible). Among the 30 molecular markers tested, only the STS markers CV542014 and $g683_{500}$ were polymorphic in the parental plants, as well as in the resistant and susceptible bulks. Linkage analyzes were performed using Mapmaker/EXP 3.0 software (Lincoln and Lander, 1993) and the map was constructed with MapChart (Voorrips, 2002).

RESULTS AND DISCUSSION

The inheritance study confirmed the presence of one dominant gene in Perla cultivar conferring resistance to *C. lindemuthianum* races 65 and 73. To verify the independence of the gene identified in Perla, allelism tests were performed (R × R) with 65 and 2047 in F_2 populations derived from crosses of Perla and the cultivars: Cornell 49-242, TO, G2333, Jalo Vermelho, Jalo Listras Pretas, Corinthiano, Crioulo 159 and BGF-20. All F_2 populations showed segregation that fit a ratio of 15R:1S, indicating the action of two dominant genes, one of them present in Perla and the other in each respective tested cultivar. These results indicate that the Andean cultivar Perla possesses an unreported gene. The molecular analyzes revealed that the markers $CV542014_{390}$ and $g683_{500}$, previously mapped on LG Pv01 (McConnell et al., 2010) are linked in coupling phase to *Co-Perla* resistance gene at 10.8 cM and 7.6 cM, respectively (Figure 1). These molecular markers were tested in BAT 93/Jalo EEP558 (BJ) RI population. Results revealed a segregation ratio of 37 (+): 34 (-) ($\chi^2 = 0.13$; $p = 0.72$) for a good fit to a 1:1 ratio. It seems that Perla is a promising anthracnose resistance source for common breeding programs as they currently lack the use of effective Andean resistance genes against this disease. Through marker-assisted introgression of this gene into commercial cultivars, common bean programs will be able to enhance resistance against anthracnose.

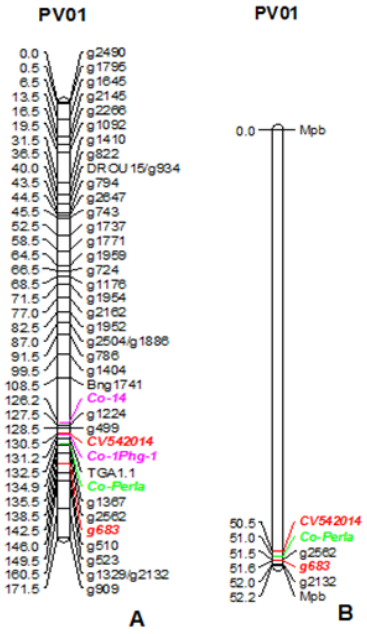

Figure 1– Genetic distance and location of the *Co-Perla* gene. Approximate distances between the markers are expressed in centimorgans (cM). Green color represents *Co-Perla* gene, while the red represent polymorphic markers (A). Illustrative physical map depicted in approximately millions of base pairs (Mpb) of the common bean Pv01 linking group based on version 1 of Phytozome (B). The map was constructed with MapChart (Voorrips, 2002).

ACKNOWLEDGEMENTS

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PRODUCTION *in vitro* AND *in vivo* OF SEXUAL STRUCTURES OF *Glomerella* spp. STRAINS FROM COMMON BEAN

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INTRODUCTION

The occurrence of fungal diseases is one of the factors that affect common bean yield. Common bean anthracnose and scab are fungal diseases that present similar symptoms in the stem, but distinct in pods. These diseases are caused by species of the genus *Colletotrichum*, which the fungus *C. lindemuthianum* is the causal agent of anthracnose. However, in the case of scab, there are still doubts about the etiological agent of the disease. Isolates from anthracnose and scab lesions have been investigated, and *Glomerella* spp. (teleomorphic phase) and *Colletotrichum* spp. (anamorphic phase) have been obtained and carried out the morphological, cytological and molecular characterization (Barcelos et al., 2014). Sexual reproduction is one of the main mechanisms of increasing genetic variability in fungi (Leslie, 1993), so it is important to study the sexual structures of the pathogen. This work aimed to evaluate the perithecia formation of four *Glomerella* spp. strains *in vitro* and *in vivo* when inoculated in common bean cultivars. This information contributes to understanding the sexual recombination in these species and can aid in the common bean breeding in order to obtain cultivars with durable resistance.

MATERIALS AND METHODS

Three strains of *Glomerella* spp. from scab lesions (13-2A, 13-1A1 and 51-2A) and one from anthracnose (38-3A) were evaluated. The analysis *in vitro* was conducted in DIC with plot subdivided in time and three replicates, the plot consisting of a Petri dish. Strains were grown in Petri dishes with M3S medium and maintained in BOD for 18 days at 22°C in the darkness. After 11 and 18 days the colonies were evaluated for number and diameter of perithecia produced per plate, using the Stemi 2000-C stereomicroscope from Zeiss. For the *in vivo* analysis, two experiments were performed in DIC in 4x2 factorial scheme in plot subdivided in time with three replicates. The strains were inoculated, with a syringe containing conidia and ascospores, in two common bean cultivars: Pérola and Majestoso. In the first experiment the plot consisted of a pot containing three plants. Pots were kept at 25ºC and 75% humidity until the last reproductive stage in greenhouse. The presence/absence of perithecia in the lesions of leaves, stems and pods were evaluated. In the second experiment, detached leaves and hypocotyls of common bean plant (stage V3) were inoculated in the laboratory, kept in Petri dishes and incubated in BOD at 23°C. The plot consisted of a Petri dish. For both experiments, after 11 and 18 days of inoculation, the same traits described in evaluation *in vitro* were assessed.

RESULTS AND DISCUSSION

In the analysis *in vitro*, significant increase was observed in the number of perithecia on evaluated period, and the 38-3A strain produced a large amount of perithecia (Table 1). All strains produced perithecia at 18 days on detached leaves of both cultivars in analysis *in vivo*. Only strain 13-1A1 produced perithecia when inoculated on plants, of cultivar Majestoso at 18 days, with 372 perithecia and an average diameter of 72.6 µm, in greenhouse. However, this

strain produced larger perithecia (90.9 µm) in the detached leaf evaluation of this cultivar (Table 1). Diameter of peritecia was larger in analysis *in vitro* than analysis *in vivo*. Perithecia in the pods were not observed and the strains inoculated in the cultivar Pérola caused symptoms of scab. Scab and anthracnose lesions were observed in the cultivar Majestoso.

Table 1 Number and diameter (µm) of perithecia *in vitro* and *in vivo* of *Glomerella* spp strains at 11 and 18 days after inoculation on cultivars Pérola and Majestoso.

Strains	Number of perithecia					
	In vitro		*In vivo*			
	11º day	18º day	11ºday		18º day	
			Pérola	Majestoso	Pérola	Majestoso
51-2A	199.3 B*	592.7 B	148.7 A	98.3 A	274.7 B	127.0 A
13-2A	4.3 C	9.0 D	25.0 B	3.0 C	331.3 A	9.7 B
13-1A1	231.0 B	359.0 C	3.3 C	9.0 C	87.7 C	130.0 A
38-3A	1024.0 A	1419.3 A	-	27.6 B	205.7 B	115.0 A
Diameter of perithecia						
51-2A	389.4 B	217.4 A	107.1 A	97.7 A	104.9 A	85.4 A
13-2A	600.6 A	274.3 A	104.6 A	61.8 A	96.3 A	81.6 A
13-1A1	294.6 C	218.3 A	79.9 B	74.3 A	76.3 B	90.9 A
38-3A	107.9 C	189.6 A	-	81.6 A	103.7 A	100.6 A

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

After the evaluation period was observed a large amount of perithecia in the senescent leaves and petioles that had already fell from the plants in both cultivars. In the leaves that were wet, perithecia in abundance were observed (Figure 1). This observation suggests that natural formation of perithecia in the common bean plants in the last reproductive stage may be occurring in the field that can explain the isolation of many *Glomerella* strains. This is the first report of the production of *Glomerella* spp. perithecia *in vivo* in common bean plants.

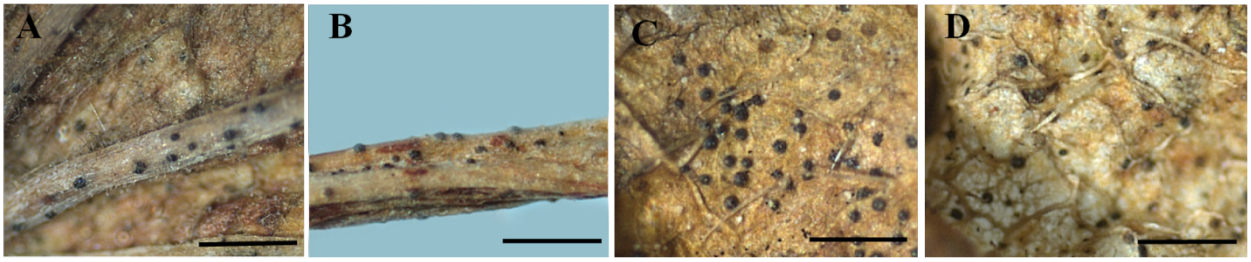

Figure 1 Perithecia of *Glomerella* spp. in senescent leaves and petioles of the cultivars Pérola (A, C) and Majestoso (B, D) when inoculated with the strains 51-2A, 13-2A, 13-1A1 and 38-3A respectively. Scale: 1mm.

ACKNOWLEDGEMENTS: CNPQ, CAPES and FAPEMIG for financial support.

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EFFECT OF MULTIPLE INOCULATIONS OF AN AGGRESSIVE *MACROPHOMINA PHASEOLINA* ISOLATE FOR SCREENING COMMON BEAN GENOTYPES UNDER HIGH TEMPERATURES

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INTRODUCTION: Ashy stem blight (ASB) caused by the fungus *Macrophomina phaseolina* (Tassi) Goidanich (*Mp*) is an important disease in common bean (*Phaseolus vulgaris* L.) in hot environments worldwide (Kaur et al., 2012). Partial resistance has been identified in Andean common bean and *P. acutifolius* A. Gray genotypes by the cut-stem method in the greenhouse (Viteri and Linares, 2017; 2019). To optimize the screening process and avoid the loss of valuable genotypes, it is important to determine the number of *Mp* inoculations necessary to identify ASB resistance. Thus, our objective was to assess the response of common bean genotypes from diverse origins to one, two, and three inoculations of an aggressive *Mp* isolate.

MATERIALS AND METHODS: Twenty-four genotypes were planted in a randomized complete block design with four replications in the greenhouse at the Lajas Research Substation, University of Puerto Rico in September, 2018. Plants were inoculated one, two, and three times at the fourth internode of the main stem and lateral branches close to the base of the plant with the aggressive PRI18 *Mp* isolate, according to the methodology described by Viteri and Linares (2017). Inoculated plants were exposed to high temperatures (mean day $\geq$ 29 °C) and moisture ranged from 50-70% after the inoculation. Ashy stem blight severity was noted for each plant 50 days after the inoculation using a 1-9 scale where 1= no sign of *Mp* infection and 9= the fungus infection passed the third node and may cause plant death (Viteri and Linares, 2017). Also, the area under disease progress curve (AUDPC) was calculated from 7 to 50 days.

RESULTS AND DISCUSSION: Significant differences were observed between replications, genotypes, number of inoculations, and the interaction genotype x number of inoculations for ASB severity and the AUDPC (Table 1). However, there were not significant differences between the mean severity scores (8.2 and 8.1 LSD= 0.4; $P \leq 0.05$) for two vs. three inoculations. In fact, all the genotypes tested had susceptible scores (> 6.0) when two or three inoculations were conducted (Table 2). Thus, when plants are exposed at higher temperatures, it is not recommended to use more than one inoculation with an aggressive *Mp* isolate to select genotypes with resistance or partial resistance to ASB. With respect to the common bean genotypes response, 'Othello' and BAT 477 were susceptible as expected, while Andean A 195 and 'PC 50' had partial resistance to a single inoculation of PRI18 *Mp* (Table 2). Similar results were reported in previous studies (Viteri and Linares, 2017; 2019). Andean genotypes PRA 154 and PRA 155 also showed intermediate resistance to one inoculation of PRI18 *Mp*, whereas breeding lines PRA 152 and PRP 153 had susceptible scores (Table 2). These four breeding lines possess pyramided resistance to white mold disease (Singh et al., 2016; 2017). However, differences in the disease response might be related to the presence of more Andean parents in the pedigree of PRA 154 and PRA 155 that might have genes/QTL for higher levels of ASB resistance. Genotypes with heat and drought

tolerance (e.g., 'Matterhorn' and SER breeding lines) (Urrea et al., 2009) generally were susceptible to ASB with the exception of SEA 5 which had an intermediate score (5.4) to one inoculation of PRI18 *Mp* isolate. This may reflect that SEA 5's pedigree includes 'San Cristobal 83' and BAT 477, two genotypes that showed resistance to ASB in previous studies (Pastor-Corrales and Abawi, 1988), as parents (Singh et al., 2001).

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Table 1. Portion of analysis of variance of 24 common bean genotypes, and multiple inoculations of PRI18 *Macrophomina phaseolina* at the University of Puerto Rico, Lajas in 2018.

Source	df	Mean squares	
		Severity	AUDPC
Replication	3	24.6*	52525.8*
Genotype (G)	23	18.8*	36929.2*
Number of inoculations (N)	2	37.0*	53152.3*
G x N	46	5.4*	6425.9*

*$P \leq 0.05$

Table 2. Mean severity scores and area under disease progress curve (AUDPC) of 12 common bean genotypes inoculated one, two, and three times with the PRI18 *Macrophomina phaseolina* and evaluated at 50 days in the greenhouse at the University of Puerto Rico, Lajas in 2018.

Genotype	One inoculation		Two inoculations		Three inoculations	
	Scores†	AUDPC	Scores	AUDPC	Scores	AUDPC
A 195	6.0	180.3	7.8	192.9	7.0	171.1
BAT 477	7.8	241.9	7.9	245.9	9.0	297.5
Matterhorn	8.6	263.8	9.0	300.1	8.8	286.1
Othello	9.0	284.4	9.0	300.6	9.0	309.8
PC 50	4.9	161.0	6.9	210.9	8.3	237.1
PRA 152	7.9	209.1	7.8	215.7	6.8	179.4
PRP 153	9.0	277.4	8.5	250.3	8.8	274.8
PRA 154	4.8	128.2	6.6	156.2	6.1	181.1
PRA 155	4.6	130.4	8.3	233.6	7.3	204.3
SEA 5	5.4	154.4	9.0	295.8	8.9	285.7
SER 5	7.6	232.3	8.9	276.9	9.0	252.0
SER 22	7.5	231.9	9.0	299.7	8.6	222.3
Mean	6.9	207.9	8.2	248.2	8.1	241.8
LSD ($P \leq 0.05$)	1.9	62.9	1.6	47.1	1.6	55.8

†A 1-9 scale was used, where 1= no sign of *Mp* infection, 5=*Mp* invasion past the first node and invaded 50% of the second internode, and 9= the fungus infection passed the third node with or without plant death.

MECHANISM OF THE RESISTANCE CONFERRED BY THE *bc-1* and *bc-2* ALLELES TO *Bean common mosaic virus* IN COMMON BEAN

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INTRODUCTION: Recessive resistance to *Bean common mosaic virus* (BCMV) in common bean (*Phaseolus vulgaris* L.) is conferred by six alleles: *bc-u*, *bc-1*, *bc-1*^{2}, *bc-2*, *bc-2*^{2}, and *bc-3* (1). The *bc-3* allele functions as mutated eIF4E translation initiation factor providing resistance through interfering with the virus replication process, while the nature of other recessive resistance alleles remains unclear (6). Here, a role of *bc-1* and *bc-2* was investigated through assessment of replication and cell-to-cell movement, separately from systemic spread, for eight BCMV isolates representing pathogroups I, IV, VI, VII, and VIII, in a set of bean differentials expressing different combinations of six recessive resistance alleles. The data showed the replication and cell-to-cell movement of most of the studied BCMV isolates in common beans bearing *bc-1* and *bc-2* alleles was not affected, but their ability to move systemically was impaired. This suggested *bc-1* and *bc-2* alleles may affect long distance movement and systemic spread of BCMV in common bean. Also, *bc-1* and *bc-2* alleles were found to provide better resistance to BCMV when combined with each other than that of expressed singly.

MATERIALS AND METHODS: The reference BCMV isolates RU1-P, RU1-OR, and 1755a were described previously (2, 3, 4). BCMV isolate 3915 was found in a field sample 91-3915 collected in Willamette Valley, OR, in 2012. BCMV isolates 3PF, RU1-CA, and PG1 were collected in 2015 near Davis, CA, by Dr. P. Guzman. Isolate 313615 was found in 2013, in a common bean germplasm collection of the USDA-ARS Plant Germplasm Unit, Pullman, WA. BCMV isolate Viva2 was collected from a common bean at the VIVA farm near La Conner, WA in 2016. All virus isolates were propagated in the bean cultivar 'Dubbele Witte' under greenhouse conditions. The biological typing, whole-genome sequencing, and sequence analysis were conducted as described (2). Additional experiment was conducted to assess the resistance efficiency to BCMV in seven differentials bearing recessive resistance alleles and their effect on virus replication, cell-to-cell and long-distance movement in the plant (5).

RESULTS: Based on the pathogenicity profiles exhibited on bean indicators, BCMV isolates 3915, RU1-CA were assigned to PG IV and VI respectively, and the rest of studied isolates, 3PF, Viva2, 313615, and PG1 were classified as belonging to PG I. However, when screened on the seven bean differentials lacking the *I* gene, virus replication and cell-to-cell movement in inoculated leaves of all studied BCMV isolates were not affected in *bc-1* and *bc-2* carrying cultivars such as 'Redlands Greenleaf C' (*bc-u*, *bc-1*) , 'Redlands Greenleaf B' (*bc-u*, *bc-1*^{2}) , 'Sanilac' (*bc-u*, *bc-2*) and 'UI 35' (*bc-u*, *bc-1*^{2}, *bc-2*^{2}). The proportion of infected leaves among all the upper, non-inoculated leaves was dependent on BCMV isolate and bean cultivar: for 'RGLC' and 'RGLB', the average infection rate ranged from 0 to 46%, while for 'Sanilac', the rate ranged from 1 to 67%, and infection rate of upper non-inoculated leaves varied from 0 to 88% in 'UI 35'. The whole genomes of all studied BCMV isolates were cloned and sequenced, using the approach described previously (2). Upon sequence assembly, 3PF, Viva2, 313615, PG1 and 3915 were all

found to be 10,053-nt long, excluding the poly (A). Based on conceptual translation, all five isolates encoded a single polyprotein of the same size (3,222 aa). Upon sequence assembly, RU1-CA was found to be 10,001-nt long, excluding the poly (A). Based on conceptual translation, RU1-CA genome encoded a single polyprotein of 3,202 aa.

CONCLUSIONS: The whole genomes of five novel BCMV isolates 3PF, Viva2, 313615, PG1 and 3915 were sequenced and analyzed. As can be seen from Figure 1, two genome areas in the P1/HC-Pro and NIa/NIb cistrons appeared to reveal more sequence diversities. Based on the sequence analysis, pathogenicity determinants potentially affecting BCMV isolates' biological response in a *bc-2* genotype (cv. 'Sanilac'), was located in the 5'-terminal region spanning P1, HC-Pro and P3 cistrons, nucleotides 1 to 3,829. The data obtained in this research suggest that *bc-1* and *bc-2* alleles provide variable level of resistance to many BCMV strains through interfering with the systemic spread of the virus in common beans, and the efficiency of the resistance conferred by these two alleles could be improved when combined with each other and with other resistance genes.

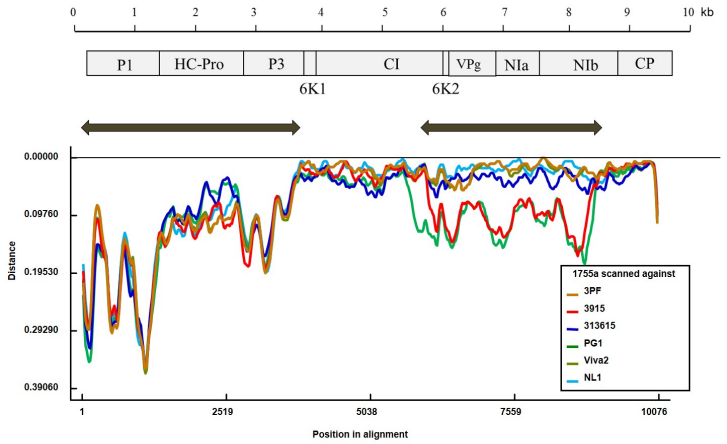

Fig. 1. Recombination analysis of the 5 studied *Bean common mosaic virus* (BCMV) isolates, 3915, 3PF, Viva2, 313615, and PG1, in comparison to the control BCMV isolates 1755a and NL1 (from Feng et al. 2018). Manual distance plot based on the aligned full-length nucleotide sequences of BCMV isolates 3915, 3PF, Viva2, 313615, PG1, 1755a, and NL1. Sequence of isolate 1755a (PG-VIII; accession number KT175570) was used as the reference. X axis represents nucleotide position in the alignment, Y axis represents relative distance from the reference sequence which is calculated using Kimura model (Kimura, 1980).

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BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF A LIMA BEAN STRAIN OF *Bean common mosaic virus* IN COMMON BEANS

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INTRODUCTION: *Bean common mosaic virus* (BCMV) is a destructive pathogen infecting many cultivated legume vegetables. In 2017, two lima bean (*Phaseolus lunatus* L.) plants in a public garden in Honolulu, HI, exhibited mosaic, vein banding, and growth retardation, and were identified as BCMV-infected. After whole genome sequencing, the genomes of both samples were found nearly identical and exhibiting the closest similarities (93%) with peanut stripe virus strain of BCMV. A single name BCMV-A1 was retained. Through biological typing, A1 was assigned to PG-I, with limited asymptomatic systemic spread in differential cultivars expressing single *bc-1* or *bc-2* alleles. Severe systemic necrosis in cultivar 'Dubbele Witte' and necrotic or chlorotic reaction in inoculated leaves of five other bean differentials were also observed during biological characterization. Meanwhile, partial genome sequences of two common bean isolates of BCMV collected in Idaho were found sharing 99% identities with the A1 sequence. The data obtained suggest that BCMV-A1 represented a new, lima bean strain of BCMV and might pose a potential threat for common bean production.

MATERIALS AND METHODS: In May 2017, BCMV isolates A1 and A2 were collected from two symptomatic *P. lunatus* plants at a community garden in Honolulu, HI, where BCMV was reported earlier (4). Potyvirus infection in these two plants was determined using a lateral flow assay (Agdia, Elkhart, IN) and leaf samples were transferred to the University of Idaho. BCMV isolates F17:0298A and F17:0298D were collected in Boise, ID, from two *P. vulgaris* plants exhibiting seed-borne infection, and provided by Elizabeth Vavricka (Idaho State Department of Agriculture) in August 2017. The origin of reference BCMV isolates RU1-P, RU1-OR, and 1755a was described previously (1, 2). All virus isolates were propagated in the bean cultivar 'Dubbele Witte', except for A1 and A2 (inducing systemic necrosis and plant death in 'Dubblele Witte') propagated in *Nicotiana benthamiana*, using mechanical inoculation and maintained under greenhouse conditions as described previously (1). The biological typing, genome cloning strategy, sequencing, and sequence analysis for BCMV isolate A1 was conducted as described previously (1, 3).

RESULTS: When tested on 11 differential lines of common bean, both A1 and A2 isolates were identified as belonging to pathogroup I (PG-I), since based on symptom observation, A1 and A2 established systemic infection only in 'Dubbele Witte' and 'Stringless Green Refugee'. In 'Dubbele Witte', severe systemic vein necrotic was developed and the plant wilted and died at around 3 wpi as the result of the virus infection, which was never seen upon infection with other BCMV isolates. Necrotic or chlorotic symptoms were induced in inoculated leaves, and variable level of asymptomatic systemic infection was confirmed by TAS-ELISA in four other inoculated cultivars, 'Redlands Greenleaf C', 'Redlands Greenleaf B', 'Sanilac', and 'UI-35'. This suggested

BCMV-A1 was able to replicate and move cell-to-cell in inoculated leaves, but its systemic spread was partially or completed blocked by *bc-1* or *bc-2* alleles. The whole-genomes of A1 and A2 were sequenced directly from overlapping RT-PCR fragments using a set of primers developed for this study. Upon sequence assembly and comparison, both sequences were found nearly identical and to be 9,995-nt long, excluding the poly (A). The genome encoded a single polyprotein of 3,202 aa. The partial, 699-nt long genome fragments of BCMV isolates F17:0298A and F17:0298D were sequenced and found to have only 3 nucleotides difference between each other (BCMV-ID), and both sharing the 99% identity with isolate BCMV-A1.

CONCLUSIONS: To investigate the genetic diversity distribution along the entire genome, the whole genomes for BCMV-A1 and four other representative BCMV strains were aligned and subjected to recombination analysis. Fig. 1 shows the comparison of the five BCMV sequences using the manual distance plot analysis, with the full-length A1 sequence used as reference. As can be seen from Fig. 1, most of the genomic diversity between these representative BCMV isolates was concentrated in the 5' terminal area spanning 5'-UTR and P1 cistron, nucleotides 1 to 935. The fact that BCMV-A1 can partially overcome single *bc-1* or *bc-2* alleles expressed in some common bean cultivars suggested that this lima bean isolate could potentially threaten common bean production. Indeed, the high genomic identities (99%) between BCMV-ID and BCMV-A1provided a direct evidence of wider distribution and threat of A1 in the *P. vulgaris* field.

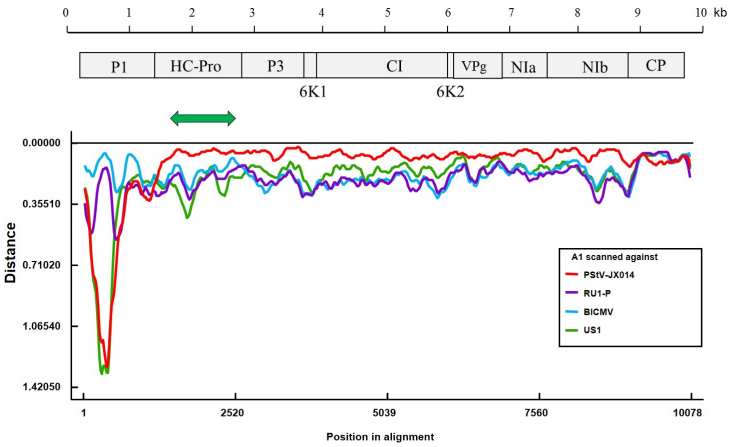

Fig. 1. Distribution of the genetic diversity along the whole genome of the *Bean common mosaic virus* (BCMV) isolate A1. Manual distance plot based on the aligned full-length nucleotide sequences of BCMV isolates from the main BCMV lineages; BCMV-A1 sequence was used as a reference. The green double-arrow indicates the position of the BCMV-ID genome fragment. X axis represents nucleotide position in the alignment, Y axis represents relative distance from the reference sequence which is calculated using Kimura model (Kimura, 1980).

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EPISTATIC INTERACTION BETWEEN RUST RESISTANCE LOCI *Ur-3* AND *Ur-5*

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INTRODUCTION

Gene pyramiding involves the amalgamation of several desirable genes from various parents into a single genotype for a specific trait such as disease resistance. Because gene pyramiding broadens the genetic base of cultivars, it is a very important strategy for managing pathogens with high virulence diversity such as those that cause the rust, anthracnose, and angular leaf spot diseases of common bean. Most of the reported disease resistance in common bean are conferred by single and dominant genes. Combining these genes into a single cultivar is often challenging specially when using phenotypic markers to confirm the presence of these genes in the pyramiding process. One of the complications is epistasis or the interaction between genes in which one gene (epistatic) masks the presence of another gene (hypostatic). Epistasis is widespread in common bean and particularly among rust resistance genes. When using specific races of the rust pathogen to combine two genes or more genes, often certain genes hide the presence of other genes. For example, the *Ur-3* and *Ur-6* rust resistance genes are epistatic to the *Ur-11* gene. The resistant reactions of the *Ur-3* and *Ur-6* genes are visualized as necrotic spots known as the hypersensitive reaction (HR). Conversely, the resistant reaction of *Ur-11* is tiny rust pustule. When plants combining the *Ur-3* and *Ur-11* genes are inoculated with race 53 (31-1), to which both genes are resistant, these plants display only the HR (grade 2) type of reaction of *Ur-3* and not the reaction of *Ur-11*. These results indicate that *Ur-3* is epistatic to *Ur-11* (Stavely 2000, Pastor-Corrales and Stavely 2002). Similarly, the combination of *Ur-4* and *Ur-5* loci results in epistasis in which *Ur-5* masks the presence of *Ur-4* (Valentini et al., 2015). Thus, epistatic interactions make difficult the pyramiding of genes using specific races of the rust pathogen. Successful pyramiding of these with races of the rust pathogen requires labor-intensive phenotyping with two or more races of the rust pathogen. On the other hand, the pyramiding of these genes is easily accomplished using highly accurate DNA markers that are tightly linked to the genes of interest. In the present study, we report the epistatic interaction between the *Ur-3* and *Ur-5* rust resistance genes using two recently developed KASP markers closely linked to these genes.

RESEARCH METHODS

A total of 92 F_2 plants from the Aurora (*Ur-3*) x Mexico 309 (*Ur-5*) cross were phenotyped with races 41 (15-1) and 53 (31-1) of *U. appendiculatus*. Both parents were resistant to both races; Aurora displayed a hypersensitive (HR) reaction to races 41 and 53 while the resistant reaction of Mexico 309 to both races was expressed as tiny pustules (TP). Race 53 was used as a phenotypic marker that accurately identifies the presence of *Ur-3* alone or in combination with other rust resistant genes. We also included eight plants each of the check cultivars Pinto 114, Early Gallatin (*Ur-4*), Golden Gate Wax (*Ur-6*), and PI 181996 (*Ur-11*). Two KASP markers developed in our laboratory were also used; KASP SS68, closely linked to *Ur-3* (Hurtado-Gonzales et al., 2017) and KASP marker SS183, a newly developed marker tightly linked to *Ur-5* (Hurtado-Gonzales et al., 2019). Published methodologies were used for rust phenotyping (Stavely 1984) and KASP genotyping (Hurtado-Gonzales et al., 2017).

RESULTS AND DISCUSSION

The expected segregation ratio of combining *Ur-3* and *Ur-5* rust resistance genes would be 9:3:3:1 if these genes were independent from each other. The inoculation of the 92 F_2 plants with races 41 and 53 revealed a ratio different from 9:3:3:1, that suggested that these two genes interacted with each other in epistasis. The inoculation of the F_2 plants from the Aurora (*Ur-3*) x Mexico 309 (*Ur-5*) revealed that the hypersensitive reaction (HR) of *Ur-3* was epistatic to tiny pustule (TP) type of reaction of *Ur-5* when these two genes were combined. We observed a segregation ratio of 12HR:3TP:1S. This type of epistasis is known as "Dominant epistasis 1" where the dominant allele of one gene (*Ur-3*) hides the effects of both alleles of another gene (*Ur-5*). Table 1 depicts all the different allelic stages and rust phenotypes observed on a subset of F_2 plants. This epistatic interaction was confirmed with KASP markers SS68 and SS183 (Table 1).

Table 1. The parents and F_2 plants from the Aurora and Mexico 309 cross phenotyped with rust races 41 and 53 of the bean rust pathogen and genotyped with KASP markers SS68 and SS183. Alleles of KASP SS68 are T:T for *Ur-3* and A:A for *ur-3*. Alleles of KASP SS183 are C:C for dominant *Ur-5* and T:T for *ur-5*. Rust phenotypes are denoted as hypersensitive response (HR) or tiny pustules (TP).

Common bean cultivar or F_2 plant	KASP SNP allele		Genotype allele		*Uromyces appendiculatus*		*Ur*-gene
	SS68	SS183	SS68 *Ur-3*	SS183 *Ur-5*	41	53	
Aurora	T:T	T:T	BB	AA	HR	HR	*Ur-3*
Mexico309	A:A	C:C	AA	BB	TP	TP	*Ur-5*
2-4183-40	A:A	T:T	AA	AA	S	S	*ur-3, ur-5*
2-4183-25	T:T	T:T	BB	AA	HR	HR	*Ur-3*
2-4183-9	A:T	T:T	AB	AA	HR	HR	*Ur-3*
2-4183-6	T:T	C:C	BB	BB	HR	HR	*Ur-3, Ur-5*
2-4183-7	A:T	C:T	AB	AB	HR	HR	*Ur-3, Ur-5*
2-4183-8	A:A	C:C	AA	BB	TP	TP	*Ur-5*
2-4183-1	A:A	C:T	AA	AB	TP	TP	*Ur-5*

The results of this study demonstrate that the SS68 and SS183 markers will facilitate the development of common bean cultivars combining the *Ur-3* and *Ur-5* with other rust resistance genes.

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MAPPING AND MARKER DEVELOPMENT FOR THE *Ur-5* RUST RESISTANCE LOCUS

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INTRODUCTION

Several molecular markers have been developed over the past 25 years tagging the *Ur-3*, *Ur-4*, *Ur-5*, *Ur-6*, *Ur-7*, *Ur-11*, *Ur-13*, and *Ur-14* rust resistance genes. The BIC website includes a comprehensive list of rust resistance DNA markers together with the primer sequences and PCR conditions. The accuracy of some of these markers is limited due to the recombination between the markers and the resistance loci. Thus, there is a need to develop more accurate and tightly linked markers that can be used in a high-throughput approach avoiding the gel-based labor intensive methods. The Mesoamerican *Ur-5* rust resistance locus provides resistance to more than 60 out of 88 races of *Uromyces appendiculatus,* maintained at ARS-Beltsville. Pyramiding the *Ur-5* locus with other rust resistance loci can be difficult due to epistatic interactions. Currently, the most reliable method of detection for the presence of *Ur-5* locus is using multiple races of the rust pathogen. The objective of this study was to develop and validate an *Ur-5* SNP-based marker that can reliable be used in breeding programs to develop common bean cultivars with *Ur-5*.

MATERIALS AND METHODS

This study expands the initial work reported by Valentini et al (Valentini et al., 2015). A total of 378 F_2 plants from the Early Gallatin (*Ur-4*) x Mexico 309 (*Ur-5*) cross were phenotyped with races 41 (15-1), 53 (31-1), 73 (6-15), and 108 (22-52) of *U. appendiculatus*. A bulk segregant analysis (BSA) was performed using only plants susceptible to races 41 and 53. Susceptible bulks were genotyped using the BARCBEAN6K_3 SNP chip and SSRs were designed to construct a linkage map around the *Ur-5* locus. A total of 33 common bean cultivars including 18 previously sequenced by Song et al (2015) and 15 cultivars from this study were whole genome sequenced for additional SNP discovery, haplotype analysis, and marker development around the *Ur-5* region. KASP markers were designed around the *Ur-5* locus and used to screen $F_{2:3}$ recombinant families derived from the Early Gallatin x Mexico 309 F_2 cross. SSR and KASP markers were tested as described by Hurtado-Gonzales et al., (2017). Marker validation was done using 265 common bean cultivars from several market classes, including (A) all common bean lines from the Mesoamerican Diversity Panel that were susceptible to races 67 and 108. The MDP cultivars were further phenotyped with races 49 and 53; (B) all Mesoamerican common bean cultivars from the Cooperative Dry Bean Nursery that were evaluated as resistant under field conditions at Beltsville. These cultivars were inoculated with a mixture of races 38 (5-0), 39 (21-0), 40 (4-1), 41 (15-1), and 43 (13-2), from 2010 to 2016; and (C) approximately 73 common bean cultivars from the BeanCap panel. In addition, a selected subset of cultivars was also phenotyped in the greenhouse with races 38, 41, and 43 for further dissection and confirmation of the presence of the *Ur-5* locus.

RESULTS

The 378 F_2 plants from the Early Gallatin (*Ur-4*) x Mexico 309 (*Ur-5*) cross were phenotyped with races 41, 53, 73, and 108. Mexico 309 was resistant and Early Gallatin was susceptible to races 41 and 53, respectively. Conversely, Early Gallatin was resistant and Mexico 309 was susceptible to

races 73 and 108, respectively. Based on the reaction of the 378 F_2 plants to races 41 and 53, a total of 291 plants were resistant and 87 were susceptible (χ^2= 0.794, *p* value=0.373). This segregation fits 3 resistant to 1 susceptible ratio, confirming that the resistance in Mexico 309 was conferred by a single dominant locus. Initial linkage analysis positioned the *Ur-5* gene in the short arm of Pv04. Screening of 378 F_2 lines with SSR marker BARCPVSSR04569 and KASP marker SS64 only uncovered six recombinant genotypes. Available $F_{2:3}$ recombinant families were further genotyped with eight KASP and two SSRs markers across the flanked region by markers BARCPVSSR04569 and SS64. This region was approximately 1.02 Mb in size. Little to no recombination was observed among the different tested markers. KASP marker SS183, one of eight KASP markers, was selected for validation across a panel of 265 bean lines. Common bean lines with a rust resistance phenotype to race 53 (tiny pustules) and susceptible to races 49, 67, and 108 were considered as having the *Ur-5* resistance locus and further tested with KASP marker SS183. The KASP marker SS183 is positioned at 576,802 bp on Pv04 (version 1 of Pv genome).

DISCUSSION

The *Ur-5* rust resistance locus has been previously described as a complex locus comprised of multiple closely linked individual loci conferring resistance to various races of *U. appendiculatus* (Stavely 1984). In this study, we report that *Ur-5* is a single rust resistance gene positioned in a "cold-spot" of recombination, due to the extremely low number of observed recombinant plants from a wide cross (Andean line x Mesoamerican line). The low level of recombination in this specific genomic region of Pv04 has been also observed in other crosses such as the Ouro Negro x Ruda cross and in RIL populations for which BARCBEAN6K_3 SNP genotype data has been obtained (Valentini, et al., 2017, Miklas, personal communication). We speculate that this low level of recombination could be the reason for the segregation observed by Stavely (1984), suggesting that the resistance in Mexico 309 was conferred by a large block of genes. KASP marker SS183, identified while screening recombinant $F_{2:3}$ families, was positioned at the end of a large block without recombination. This marker was validated on a panel of 265 phenotyped common bean lines with 100% accuracy. And additional validation is in progress. Its application in common bean breeding will accelerated the incorporation of the *Ur-5* in a wider group of common bean cultivars. The results reported in this study serve as an excellent foundation for understanding the complex organization of the short of chromosome arm Pv04 which has also been reported to carry the *Ur-14*, *Ur*-PI 310762 and other disease resistance genes.

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NEW SOURCES OF WHITE MOLD RESISTANCE DERIVED FROM WIDE CROSSES IN COMMON BEAN AND EVALUATED IN THE GREENHOUSE AND FIELD USING MULTI-SITE SCREENING NURSERIES

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Our research goal is to identify putative sources of resistance to white mold in adapted backgrounds at multiple sites located in major bean-production areas of the northern United States. In 2018, breeders sent 9 lines for field evaluations and 11 lines for greenhouse straw test assays. Trials were conducted using these lines, plus three controls G122 (partial resistance), Beryl (susceptible), and Bunsi (field avoidance). One test line was eliminated from the field test due to poor germination.

Field trial data from six sites showed a significant difference in lines (**Table 1**; ANOVA $p = 0.005$). Two lines, N14229 and VCP-13, had lower disease ratings than Bunsi, although no less disease than G122. Greenhouse data from four sites did not identify any lines that had straw test ratings with better performance than G122 (**Table 2**; ANOVA $p = 0.085$). Collectively, these preliminary results suggest at least two lines showing promise for disease resistance in the field.

Table 1. Results of 2018 WMMN field tests*

Line	WI	WA	OR	MI	ND	NE	Mean	Grouping
Beryl	9.0	7.7	7.8	8.7	3.3	5.7	7.0	A
PT11-13-B	7.3	6.8	8.0	5.3	5.9	4.8	6.4	A B
PRP-153†	6.3	6.3	6.5	6.0	4.9	5.5	5.9	A B C
SR16-5	9.0	4.0	7.2	6.0	3.1	3.7	5.5	A B C D
NDZ-14083	8.0	4.2	6.3	6.0	3.6	4.7	5.5	A B C D
B16504	8.0	5.5	5.2	5.7	3.2	3.7	5.2	A B C D
ND121630-GN	7.7	3.5	4.8	7.0	4.7	3.2	5.2	A B C D
Bunsi (Ex Rico)	7.7	5.3	3.7	7.0	2.8	3.3	5.0	B C D
VCP-13‡	4.7	5.8	5.0	2.0	3.0	5.8	4.4	C D E
N14229	7.0	2.7	3.0	3.0	4.6	3.0	3.9	D E
G122	4.7	2.2	2.0	3.7	2.8	1.0	2.7	E

*CIAT scale- 1-9; 1= no disease, 9 = dead plant; †Line was called NE5-16-98 in 2017 testing; ‡Line was called NE5-16-101 in 2017 testing

Table 2. Results of 2018 WMMN greenhouse tests**

Line	WA	OR	MI	NE	Mean	Grouping
ND121448	7.2	6.9	9.0	7.5	7.6	A
N14229	7.2	6.0	8.1	8.2	7.4	A B
Beryl	6.7	6.9	8.2	7.3	7.3	A B
B16504	7.2	6.0	8.5	6.4	7.0	A B C
NDF120287	6.7	5.4	7.8	7.0	6.7	A B C D
NDZ-14083	5.2	6.4	8.8	6.0	6.6	A B C D E
SR16-5	5.8	5.4	8.8	6.3	6.6	A B C D E
PRP-153†	6.3	6.0	8.8	4.9	6.5	A B C D E
PT11-13-B	2.8	7.2	8.4	5.9	6.1	A B C D E
Bunsi (Ex Rico)	6.2	6.9	4.8	5.4	5.8	A B C D E
ND122386	5.1	6.0	6.6	4.6	5.6	B C D E
ND121630	3.3	5.7	6.5	5.0	5.1	C D E
VCP-13‡	2.3	7.2	6.1	4.4	5.0	D E
G122	3.3	3.9	6.6	5.0	4.7	E

**Petzoldt & Dickson scale:
1-3 = resistant,
4-6 = intermediate,
7-9 = susceptible

†PRP 153= NE5-16-98 in 2017 testing;
‡VCP 13= NE5-16-101 in 2017 testing

Table 3. Combined WMMN greenhouse test data for years 2016, 2017 and 2018

Line	NE	OR	WA	WI	CO	MI	Mean	Grouping
Beryl_2018	7.3	6.9	6.7	NA	NA	8.2	7.28	A B C D E F G
NDF140433_2016	8.8	6.3	6.3	9.0	5.7	7.5	7.28	B C D E F G
B16504_2018	6.4	6.0	7.2	NA	NA	8.5	7.03	B C D E F G H
N14229_2017	7.3	7.9	6.3	5.5	7.8	NA	6.96	B C D E F G H
B15430_2017	7.6	7.0	7.8	6.1	6.2	NA	6.94	B C D E F G H
WM91212-4-3_2016	7.3	5.9	6.5	8.3	7.4	6.2	6.92	B C D E F G H
PT9-5-6_2017	7.5	6.9	6.1	6.3	7.5	NA	6.86	B C D E F G H I
NDF140461_2016	8.3	6.6	6.9	6.6	4.2	8.0	6.74	B C D E F G H I
NDF120287_2018	7.0	5.4	6.7	NA	NA	7.8	6.74	B C D E F G H I
ASR 1865_2016	7.1	4.8	5.2	7.8	6.1	8.9	6.64	B C D E F G H I
NDF140423_2016	8.3	6.0	6.7	6.8	4.8	7.2	6.62	B C D E F G H I
NDZ-14083_2018	6.0	6.4	5.2	NA	NA	8.8	6.61	B C D E F G H I J
SR16-5_2018	6.3	5.4	5.8	NA	NA	8.8	6.59	B C D E F G H I J
NDF140405_2016	7.3	6.1	6.6	7.6	5.2	6.7	6.57	C D E F G H I J
Beryl_2017	7.2	5.6	7.9	4.3	7.7	NA	6.54	C D E F G H I J
PRP-153†_2018	4.9	6.0	6.3	NA	NA	8.8	6.49	C D E F G H I J K
SR16-5_2017	7.1	7.6	5.8	4.7	7.2	NA	6.45	C D E F G H I J K
NDF141308_2016	7.6	4.2	5.0	8.0	7.2	6.7	6.44	D E F G H I J K
R13752_2016	6.9	4.4	6.3	8.3	6.2	6.3	6.39	D E F G H I J K
NDF140427_2016	7.1	5.4	5.7	8.3	4.8	7.0	6.37	D E F G H I J K
Beryl_2016	7.7	5.1	5.8	5.8	5.8	8.0	6.35	D E F G H I J K
NDF140409_2016	7.5	6.1	6.2	8.2	4.9	5.0	6.33	D E F G H I J K
NDF140415_2016	7.5	6.1	5.4	7.3	5.4	6.0	6.28	E F G H I J K
NDF140408_2016	6.8	6.0	6.0	7.4	5.2	6.1	6.24	F G H I J K
NDF140406_2016	8.2	5.0	6.3	4.9	5.1	7.9	6.22	F G H I J K
NDZ14083_2017	7.2	6.6	6.1	4.8	6.4	NA	6.21	F G H I J K
PT11-13-B_2018	5.9	7.2	2.8	NA	NA	8.4	6.07	F G H I J K L
NDF140460_2016	7.4	6.0	4.7	7.3	3.5	7.5	6.07	F G H I J K L
Bunsi_2018	5.4	6.9	6.2	NA	NA	4.8	5.83	G H I J K L
PS08-039A-5_2016	5.9	5.0	5.3	7.2	5.1	5.9	5.74	H I J K L
Cayenne§_2017	4.7	5.2	5.9	5.5	6.9	NA	5.65	H I J K L
Bunsi_2017	6.6	5.1	6.3	4.2	5.8	NA	5.62	H I J K L
Ex Rico (Bunsi)_2016	7.8	4.9	5.8	5.6	4.3	5.1	5.60	I J K L
R12844_2016§	5.5	5.2	5.7	5.8	4.6	6.8	5.60	I J K L
ND122386_2018	4.6	6.0	5.1	NA	NA	6.6	5.57	I J K L M
ND121630_2017	5.8	3.2	6.1	4.0	6.8	NA	5.17	J K L M N
ND121630_2018	5.0	5.7	3.3	NA	NA	6.5	5.12	J K L M N
VCP-13‡_2018	4.4	7.2	2.3	NA	NA	6.1	5.01	K L M N
G122_2016	5.2	4.2	4.1	4.7	4.4	6.9	4.91	L M N O
G122_2018	5.0	3.9	3.3	NA	NA	6.6	4.70	L M N O
ND122386_2017	4.4	5.3	3.8	3.5	4.0	NA	4.21	M N O
G122_2017	5.2	2.8	3.8	4.0	4.6	NA	4.05	N O
USPT-WM-12_2016	4.2	3.7	3.8	4.3	3.3	5.0	4.03	N O
031-A-11_2016	4.2	3.7	4.4	3.0	3.3	5.2	3.96	N O
NE5-16-101‡_2017	3.5	2.7	2.4	3.3	5.1	NA	3.40	O
NE5-16-98†_2017	4.0	3.1	2.8	3.3	3.0	NA	3.25	O

†PRP 153= NE5-16-98; ‡VCP 13= NE5-16-101; §R12844 = Cayenne

Two sites (CO and WI) did not report greenhouse data in 2018, and one (MI) in 2017. Over the past three years, we evaluated a total of 31 bean lines in the greenhouse and/or multi-site fields **(Table 3)** for identification of new sources of resistance to white mold. Eleven line-year entries with ratings above Beryl were eliminated from **Table 3** for lack of space (data available on request).

FUNGICIDE SENSITIVITY OF 207 *Sclerotinia sclerotiorum* ISOLATES FROM DRY BEAN AND SOYBEAN

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INTRODUCTION: Dry beans are valued at $ 1.01 billion and soybean at $41.0 billion per annum. Disease caused by *Sclerotinia sclerotiorum* produces up to 100% yield loss when environmental conditions are favorable. Since there are neither dry bean nor soybean varieties completely resistant, fungicide applications are integral to reduce yield loss from *S. sclerotiorum*. This fungus is thought to have intermediate risk of fungicide resistance, and resistance has been reported in Brazil from soybean[1] and in China from canola[3]. In the US, potential for resistance to thiophanate methyl was found among isolates from soybean[2], yet no studies have been conducted on dry bean. Continued use of fungicides may lead to resistance or low sensitivity to the most commonly used fungicides. This study aims provide a contemporary look of fungicide sensitivity from dry bean and soybean in the US.

MATERIALS AND METHODS: In 2015, 2016, and 2017, we conducted a survey of *S. sclerotiorum* from soybean in NE, IA, WI, and MI, resulting in a collection of 1,362 isolates, which included farmer fields and fungicide field trials. In 2003 to 2012, 366 isolates were collected and characterized previously from white mold screening nurseries or producer fields throughout dry bean and snap bean production areas across the US, with others collected in prior years. From these, we randomly selected 207 isolates: 93 from dry beans (18 ND, 16 MI, 16 WA, 15 CO, 11 NE, 2 DE, 2 MN, 2 WI, 1 OR, 6 from Brazil and, 4 from Mexico) and 114 from soybeans (48 NE, 25 MI, 24 IA, and 17 WI). Isolates were from farmer fields (FF), fungicide field trials (FFT), and fields without fungicide applications, the latter were called baseline. Fungicide sensitivity was determined for four technical products representing different modes of action: thiophanate methyl (TM) in group 1, tetraconazole in group 3, boscalid in group 7, and picoxystrobin in group 11. First, we determined fungicide sensitivity of 21 baseline isolates plus 21 additional isolates from FF and FFT. We used serial dilution with 6 concentrations for TM (0.75, 1.00, 1.50, 2.00, 2.50, 10.00 ppm), for tetraconazole (0.5, 1.0, 2.0, 3.0, 5.0 ppm), for boscalid (0.025, 0.050, 0.100, 0.200, 0.800 ppm), and picoxystrobin (0.01, 0.02, 0.04, 0.06, 0.10 ppm), with 4 repetitions per isolate and 2 experimental replications. Plates were incubated in darkness at 23 ± 2 °C for 42 h and diameter measured with digital calipers. A dose-response curve was fit to estimate the EC_{50} for each fungicide. Second, we identified the concentration with the best prediction of EC_{50} for each fungicide, known as discriminatory concentration (DC), by linear regression of % mycelial growth vs. log(EC_{50}). The concentration yielding the highest coefficient of determination (r^2) was selected as the DC for each fungicide. For the remaining isolates among the 207, growth on the DC was used to estimate EC_{50}, which is termed EC_{50}(D).

RESULTS: The DCs were: 2.0 ppm for tetraconazole, 0.2 ppm for boscalid, and 0.01 ppm for picoxystrobin. It was not possible to reliably estimate the EC_{50} of TM, instead, we used the highest concentration as DC (10 ppm) and classified isolates as either sensitive or resistant. Among all 207 isolates, the average EC_{50}(D) were 1.038 ppm to tetraconazole, 0.093 ppm to boscalid, and 0.013 ppm to picoxystrobin, and all isolates were sensitive to TM. Comparisons were made with isolates grouped by source (FF, FFT and baseline Fig. 1A-C) and also by host (drybean and soybean; Fig. 1D-F). Significant differences were found between sources for sensitivity to boscalid (Kruskal-

Wallis $p < 0.001$) and to picoxystrobin ($p = 0.038$). In both cases, there was a significant difference in baseline isolates versus farmer fields (boscalid Bonferroni-adjusted $p = 0.014$; Fig. 1A and picoxystrobin $p = 0.032$; Fig. 1B). Sensitivity to boscalid was the only fungicide that showed a difference between farmer fields and fungicide field trials ($p = 0.001$; Fig. 1A). No significant differences were observed for tetraconazole. There was a significant difference in sensitivity to boscalid for isolates from dry beans and soybeans (Kruskal-Wallis $p < 0.001$; Fig. 1C). No significant differences were observed for picoxystrobin or tetraconazole. Further investigation of fields with the least sensitive isolates is needed in order to know whether or not these isolates are indicative of reduce sensitivity in these fields.

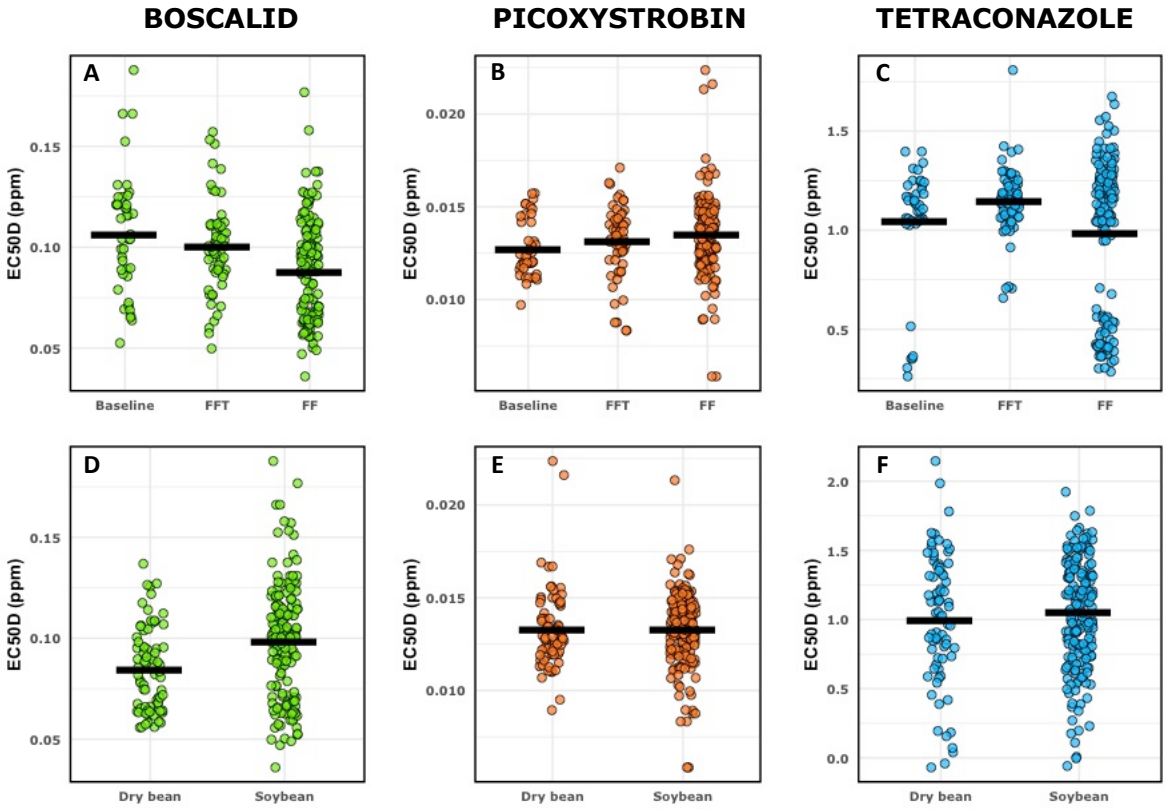

Figure 1. Fungicide sensitivity (EC50(D)) of 207 *Sclerotinia sclerotiorum* isolates to boscalid (**A** and **D**), picoxystrobin (**B** and **E**) and tetraconazole (**C** and **F**). Isolates were either grouped according to the type of fungicide field treatment (**A**–**C**) or host crop (**D**–**F**). FFT= fungicide field trials; FF= farmer fields

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SCREENING FOR PARTIAL RESISTANCE TO WHITE MOLD ON COMMON BEAN ELITE LINES OF CARIOCA MARKET CLASS

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INTRODUCTION

White mold (WM), caused by the fungus *Sclerotinia sclerotiorum*, is a devastating disease in irrigated areas of common bean in Brazil. Genetic resistance is a key component of the WM management. Approximately 70% of the cultivars of common bean used in Brazil belong to the *carioca* market class (beige with brown stripes). Common bean elite lines developed by Federal University of Lavras (UFLA), Federal University of Viçosa (UFV), and Embrapa Rice and Beans have been evaluated each year at several locations in the State of Minas Gerais through the "Value for Cultivation and Use" (VCU) trials. In general, the VCU trials are not conducted in areas with history of WM. The objective of this study was to screen in field elite lines of *carioca* with partial resistance to WM to be further included in the advanced trials where the most promising genotypes with partial resistance to WM are evaluated in greenhouse (physiological resistance) and in field trials.

MATERIAL AND METHODS

Two sprinkler irrigated trials (Coimbra and Oratórios) were conducted during the fall-winter season of 2017 in areas with a history of WM. Twenty-one genotypes from UFV, UFLA and Embrapa belonging to the *carioca* market class were evaluated. A randomized block trial with three replicates was used. Plots were two 4 m-long rows, spaced 0.50 m apart with 15 seeds per meter. As plants matured, lodging was scored using a 1-to-9 scale, in which 1 = no lodging and 9 = > 90% lodged. WM was scored visually using a 1-to-9 scale, in which 1 indicates no diseased plants and 9 indicates 80-100% diseased plants and/or 60-100% infected tissues (Miklas et al. 2001). Dry plants from 4 m^2 of each plot were harvested for seed (adjusted to 13% moisture content) yield determination. Individual and combined ANOVA were performed.

RESULTS AND DISCUSSION

WM pressure was moderate/high in Oratórios and high in Coimbra. Site x genotype interaction was significant for yield and lodging, but nonsignificant for WM score. Correlation between WM score and yield was significant in Coimbra (r = -0.51, $p < 0.001$) and Oratórios (r = -0.62, $p < 0.001$). Correlation between lodging and WM score was significant in Coimbra (r = 0.76, $p < 0.001$) and Oratórios (r = 0.54, $p < 0.001$). The lines VC 35, VC 37, CNFCMG 246D, RCPVIII-1 and the cultivars Pérola and BRSMG Uai were the genotypes located in the groups of lower WM score and higher yields in both trials. The lines VC 36 and VC 39, which stood out in the VCUs conducted in areas free of WM, had bad performance under WM pressure. The lines VC 35, VC 37, CNFCMG 246D, and RCPVIII-1 were selected for the advanced trials.

Table. White mold scores (1 to 9) and seed yield of genotypes of common bean (*carioca* market class) in two districts of the State of Minas Gerais, Brazil, in 2017.

Genotype[1]	White mold score[2]	Yield (kg ha^{-1})	
		Coimbra	Oratórios
VC 35	5.5 B[3]	2378 A	2333 A
VC 37	5.3 B	2013 A	2696 A
CNFCMG 246D	4.8 B	2093 A	2379 A
CXI-1	5.8 A	1818 B	2446 A
RCPVIII-1	5.7 B	1992 A	2192 A
CNFCMG 198D	4.6 B	2362 A	1788 B
Pérola	5.7 B	2130 A	2004 A
BRSMG Uai	5.2 B	2073 A	2054 A
CXI-26	6.2 A	2177 A	1854 B
CNFCMG 134M	5.5 B	1793 B	2183 A
BRS Estilo	5.8 A	2171 A	1767 B
CNFPMG 126M	5.3 B	1633 B	2117 A
VC 34	6.4 A	1946 A	1721 B
CNFP 10762	6.3 A	2165 A	1488 B
VC 38	6.0 A	1656 B	1992 A
CXII-15	5.6 B	1590 B	1900 B
CXII-13	5.7 B	1347 B	2038 A
VC 36	6.3 A	1646 B	1704 B
CXII-16	6.3 A	1829 B	1508 B
CNFP 11948	6.7 A	1395 B	1550 B
VC 39	6.6 A	1402 B	1279 B
Mean	5.8	1886	1952
CV (%)	13	17	16

[1]Cultivars in bold were included for comparison.
[2] Mean white mold scores for the two trials.
[3] In each column, means followed by the same letters belong to the same group (Scott-Knott test, $p = 0.05$).

ACKNOWLEDGMENTS
CNPq, FAPEMAT and FAPEMIG for financial support.

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EFFECT OF MULCHING WITH GRASSES AND CHEMICAL TREATMENT ON WHITE MOLD (*Sclerotinia sclerotiorum*) IN COMMON BEAN (*Phaseolus vulgaris* L.)

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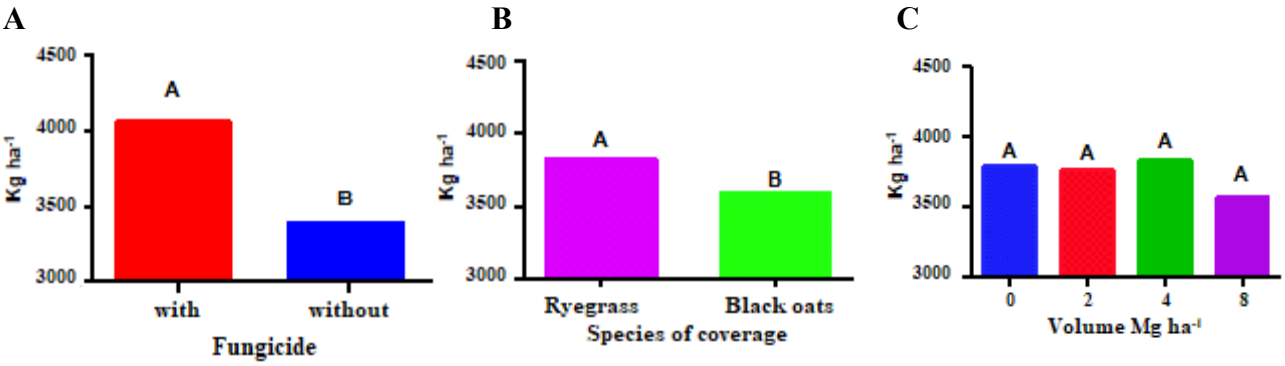
INTRODUCTION: White mold of common bean, caused by the soilborne pathogen *Sclerotinia sclerotiorum*, is one of the worst yield-limiting diseases in brazilian plantations. The employment of straw mulch on soil surface on the reduction of pathogen sclerotia's germination and white mold severity were reported previously (Civardi et al. 2019). However, most cover crops traditionally used for this purpose have high decomposition rates in tropical climates, not allowing sufficient soil cover and consequently protection of *S. sclerotiorum* hosts. The common bean crop in Brazil is the second largest in the world, exceeding 3.0 million tons on around 2,7 million ha annually. Although brazilian total production is half of India's, the south american country employs only 18% of the area used by India (FAOSTAT 2019). Hence, there is a strong demand for eco-friendly methods that provide efficient soil protection to manage white mold and improve bean production systems. Cultural practices recommended for the management of soilborne pathogens such as *S. sclerotiorum* must be associated with no-tillage cropping (NT), considered essential for sustainability of annual crops, once NT systems benefit the physical, chemical and biological soil components and therefore the dynamics of plant diseases (Silva et al. 2018). Despite the limited management options of white mold in common bean, cultural control with straw mulch for the no-tillage system and conscious chemical control with specific fungicides are efficient practices that can help the management of the disease. The aim of this work was to verify the effects of mulching with different vegetal species, volumes of straw mulch and chemical treatment with fungicide on *Sclerotinia sclerotiorum* by verifying grain yield of common bean.

MATERIALS AND METHODS: The field experiment was set during the planting season of 2017/2018 in a commercial farm named 'Fazenda Ubiratã' in the municipality of Londrina, Paraná-Brazil (23°20'23"S, 51°12'32"W and altitude of 532 m). The soil was classified as en eutrophic Red Latosol, with smooth undulating relief. Climate, according to the Köppen classification, is of Cfb type, mesothermal, humid subtropical (IAPAR, 1994; OLMOS-ITURRI et al, 1984). The field had been previously cropped with forage turnip (*Brassica rapa* L.) followed by infestation of the area with sclerotia collected from neighboring areas, in order to guarantee a high amount of initial inoculum. Sowing of common bean cv. IAPAR 81 was performed on 21th November 2017 in a no-tillage system, in a density of 12 seeds m^{-1} with 0,40 m spaced rows. The experiment was conducted in randomized plots of 5 x 5.6 m, with 28 m^2 area. The treatments were: a) two types of dead vegetation cover, black oats (*Avena strigosa* Schreb.) and ryegrass (*Lolium perenne* L.); (b) four volumes of straw per hectare (0, 2, 4 and 8 Mg) arranged on parcels soil manually; and c) two applications of Fluazinam (Frownicde® fungicide) at a dose of 1 L ha^{-1}, which were sprayed at growth stage R4 (50% of pods at maximum length - mid pod set) and R5 (one pod with fully developed seeds). The culture was irrigated during its whole cycle in order to increase sclerotia germination. All data was statistically analyzed in a completely randomized design with four replicates, in a 2x4x2 factorial scheme - two types of straw (black oat and ryegrass), four volumes of straw (O, 2, 4 and 8 Mg ha-1) and the use or not of the fungicide. Yield (kg ha^{-1}) was determined by the production from 11.2 m^2 of each plot, followed by comparison of means by Tukey's test at 5% probability level.

RESULTS AND DISCUSSION: Production indexes determined through grain yield showed statistical differences for vegetal cover species and the treatment with fungicide. Sequential treatment with fluazinam at R4 and R5 growth stages ensured a yield of 4074 kg ha^{-1}, meanwhile control plots obtained 3434 kg ha^{-1}, a difference of approximately 16% (figure 1 - A). Similar results were obtained by Silva et al. 2018. Ryegrass cover on soil achieved 3827 kg ha^{-1} and black oats cover 3591 kg ha^{-1} in grain yield (figure 1 - B), with a difference of approximately 6% between vegetal cover species. Different straw volumes didn't have significant responses in this parameter (figure 1 – C). As related by Vieira et al. (2010), increase in grain yield by the use of fungicide is closely related to high incidence and severity of white mold in plots without spraying fluazinam, once the product obtained efficient control of the disease. Despite the fact that different straw volumes didn't affect the disease, ryegrass cover on soil enabled higher grain yield than black oats cover. This gain might be related to differences in vegetal composition, as the higher carbon-nitrogen ratio presented in ryegrass species, vegetal allopathic effects and favorable environmental conditions to common bean development.

Figure 1: Yield (kg ha^{-1}) with and without fungicide treatment (A), with black oats and ryegrass vegetal cover (B) and in addition of four different volumes of vegetal straw (C).



*Different letters differ by Tukey's test ($p<0,05$).

CONCLUSION: Fungicide treatment statistically increased common bean grain yield. Also, ryegrass cover obtained higher production than black oats cover and different straw volumes didn't affect final production.

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GENETIC IMPROVEMENT OF DRY BEAN FOR RESISTANCE TO WHITE MOLD USING A MAGIC POPULATION

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INTRODUCTION: White mold (*Sclerotinia scleriotorum* Lib. de Bary) is considered one of the most important diseases for dry bean in the U.S. with seed yield losses up to 100% (Singh and Schwartz, 2010). North Dakota, the leading state for dry bean production, harvested approximately 275,000 ha in 2017 representing more than $300 million. The use of resistant varieties is an effective strategy for combatting diseases. However, for white mold, resistance has been difficult to incorporate because of low heritability, cumbersome screening methods, few sources of resistance, and inadequate breeding methods (Carneiro et al., 2011). To overcome these limitations, we used a Multi-parent Advanced Generation Inter-Crosses (MAGIC) population to generate higher recombination and increased genetic diversity to enable fine mapping QTL (Osorno et al., 2017), and to facilitate development of improved partially resistant lines with good agronomic performance.

MATERIALS AND METHODS: The development of the WM-MAGIC population used in this research followed the method from Bandillo et al. (2013). It had two purposes: gene mapping and production of inbred lines with combined resistance to white mold and good agronomic performance. By design, most lines from this WM-MAGIC population are in the pinto market class and the founders are shown in Table 1. A random subset of approximately 500 lines out of 1070 were selected and screened using the seedling straw test method proposed by Arkwazee and Myers (2017). Strain 1980 of the pathogen was used to inoculate lines. Disease was visually scored using a 1-9 scale (Arkwazee and Myers, 2017). Two resistant (PC-50 and USPT-WM-12), and two susceptible (Othello and Beryl) checks were included in the greenhouse evaluations. Experimental design was an augmented block with four replications in time.

RESULTS AND DISCUSSION: Disease ratings obtained thus far show a normal distribution as expected for a quantitative trait (Figure 1). One line exhibited a higher level of resistance than the most resistant check PC-50, and 19 lines (14 pinto and 5 great northern) had equal performance to PC-50. Currently, Genome-Wide Association Studies (GWAS) are being conducted with the subset of 500 lines to identify genomic regions associated with resistance.

Table 1 MAGIC Population founders background information

Genotype	Origin	Market Class	Level of resistance in Straw Test	Level of resistance in Field Test
USPT-WM-12	USDA-ARS	Pinto	Good	Good
PT 7-2	USDA-ARS	Pinto	Susceptible	Susceptible
El Dorado	MSU	Pinto	Intermediate	Very Good
CO16079	CSU	Pinto	Good	No Data
ID14-4	Univ. of ID	Pinto	Good	No Data
La Paz	Provita	Pinto	Susceptible	Good Avoidance
Lariat	NDSU	Pinto	Susceptible	Some Avoidance
Powderhorn	MSU	Great Northern	No Data	Good Avoidance

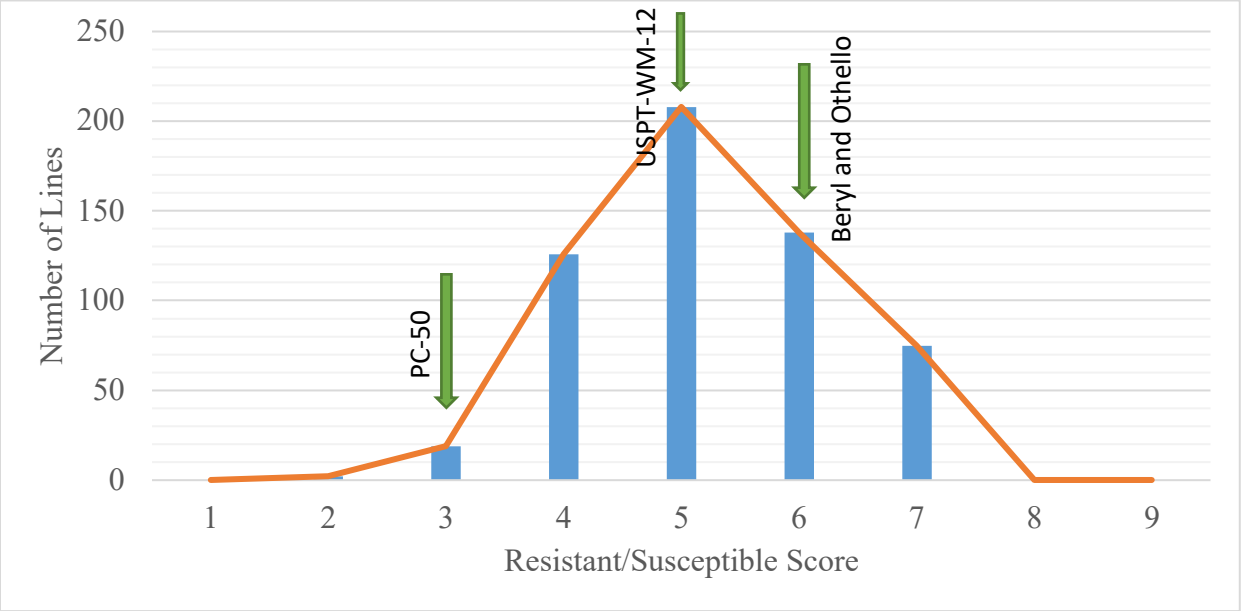

Figure 1. Distribution of 500 lines from WM-MAGIC Population and 4 checks screened using the seedling straw test.

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REACTION TO WHITE MOLD OF EARLY MATURITY CARIOCA SEEDED COMMON BEAN ELITE LINES IN MULTI-ENVIRONMENT TRIALS

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INTRODUCTION

White mold, caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, is one of the most destructive diseases for the common bean (*Phaseolus vulgaris* L.) crop, especially in rainy and irrigated growing seasons in Central Brazil. The development and use of resistant cultivars has been an aim of common bean breeding programs and of the productive sector because this strategy represents a viable alternative easily adopted by growers and is an important tool for integrated management of the disease (Schwartz & Singh, 2013). Promising efforts have evaluated both physiological resistance and resistance associated with disease avoidance mechanisms in the field. New superior genotypes developed by breeding programs should preferentially have both these resistance mechanisms, which are complementary (Miklas et al., 2013). The main goal of this study was to evaluate the reaction to white mold of early maturity (< 85 days) carioca seeded common bean elite lines and control cultivars in field nurseries in Brazil and in a controlled environment assay to select resistance sources to the disease.

MATERIAL AND METHODS

The elite lines and control cultivars tested include genotypes evaluated in the VCU trials (Value for Cultivation and Use trials) of the 2016/2017 cycle conducted by the Embrapa Common Bean Breeding Program in a national network of final field trials. The controlled environment assay was carried out at Embrapa Arroz e Feijão, using the modified straw test reported by Ferreira et al. (2018). The white mold filed nurseries were carried out in Oratorios–MG (lat 20°24'S, long 42°24'W, alt 400 m) and Goianira–GO (lat 16°26'S, long 49°24'W, alt 734 m). In Oratorios–MG, three replicates were used and each plot was composed of two 3.0-m long rows, with 0.5 m between rows and 15 seed m^{-1}, in the fall/winter growing season of 2018. In Goianira–GO, the trial was set up with three replicates and each plot was composed of three 4.0-m long rows, with 0.5 m between rows and 12 seed m^{-1}, in the winter growing season of 2017. For the disease severity evaluation, a visual scale was used with scores from 1 (immune plant or plots without disease symptoms) to 9 (dead plant or plots with 80%-100% of plants exhibiting generalized necrosis). Statistical analyses were carried out using mixed models via REML/BLUP.

RESULTS AND DISCUSSION

Considering the modified straw test, the lines CNFC 16832, CNFC 16729, CNFC 16820, CNFC 16846 and CNFC 16871 shown the best performance for physiological resistance to white mold, with mean genotypic values (eBLUP mean) for disease severity less than that presented by the control cultivar BRS Notavel (4.27). In the field nursery carried out in Goianira–GO, among the nine genotypes with eBLUP mean lower than 4.0 are the lines CNFC 16729 and CNFC 16871,

which also shown physiological resistance by the straw test. In Oratorios–GO, the lines with best performance were CNFC 15502 and CNFC 16242, with eBLUP mean of 3.33 and 3.81, respectively (Table 1). The lines CNFC 16820 and CNFC 15875 are examples of potential resistance sources selected based on their general performance both in the field and in the controlled environment evaluations.

Table 1. Genotypic values (eBLUP) related to white mold severity of early maturity (< 85 days) carioca seeded common bean elite lines and control cultivars evaluated in field nurseries in Brazil (Goianira–GO and Oratorios–MG) and in a controlled environment assay.

Genotype	Modified straw test		Goianira–GO		Oratorios–MG	
	$eBLUP_{(\mu+gi)}$[a]	$eBLUP_{(gi)}$[b]	$eBLUP_{(\mu+gi)}$	$eBLUP_{(gi)}$	$eBLUP_{(\mu+gi)}$	$eBLUP_{(gi)}$
CNFC 16832	3.49	-2.0931	7.70	3.2423	4.54	-0.5566
CNFC 16729	3.55	-2.0321	3.80	-0.6586	5.27	0.1708
CNFC 16820	3.58	-2.0040	4.07	-0.3800	4.91	-0.1929
CNFC 16846	3.64	-1.9424	6.16	1.7098	5.03	-0.0716
CNFC 16871	3.95	-1.6292	3.80	-0.6586	6.72	1.6256
BRS Notavel	4.27	-1.3146	4.21	-0.2406	4.30	-0.7990
CNFC 15875	4.33	-1.2536	3.52	-0.9372	4.54	-0.5566
CNFC 16188	4.45	-1.1353	4.07	-0.3800	5.51	0.4133
CNFC 15873	4.70	-0.8852	7.42	2.9637	4.91	-0.1929
BRS FC104	4.81	-0.7765	4.21	-0.2406	4.06	-1.0415
CNFC 15856	4.88	-0.7006	5.05	0.5953	4.78	-0.3141
TAA Gol	5.19	-0.3891	4.07	-0.3800	5.75	0.6558
BRS Cometa	5.70	0.1203	5.33	0.8739	4.91	-0.1929
CNFC 16831	6.33	0.7481	4.63	0.1773	4.91	-0.1929
IPR Andorinha	7.05	1.4655	3.80	-0.6586	6.24	1.1407
CNFC 16242	7.21	1.6321	3.52	-0.9372	3.81	-1.2840
CNFC 15502	7.31	1.7272	3.24	-1.2159	3.33	-1.7689
IPR Colibri	7.49	1.9118	4.63	0.1773	6.48	1.3832
CNFC 16066	7.49	1.9118	3.66	-0.7979	5.27	0.1708
IAC Imperador	7.50	1.9139	4.07	-0.3800	6.24	1.1407
CNFC 15723	7.85	2.2727	3.24	-1.2159	4.78	-0.3141
CNFC 15708	8.03	2.4520	3.80	-0.6586	5.88	0.7770
Mean	5.58		4.45		5.10	
CV_{gi}%	36.06		29.95		20.00	
CV_{ei}%	32.01		22.98		21.21	
CV_r	1.13		1.30		0.94	

[a]eBLUP mean of the genotype *i*; [b]genotypic value (eBLUP) of the genotype *i*.

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FIELD EVALUATION OF COMMON BEAN GENOTYPES SCREENED FOR PARTIAL RESISTANCE TO WHITE MOLD IN 2018

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INTRODUCTION

Since 2008 we have screened common bean genotypes with different reactions to white mold (WM) caused by *Sclerotinia sclerotiorum* from the "Value for Cultivation and Use" (VCU) trials (Lima et al., 2016). In the VCU trials, elite lines are compared to cultivars for resistance to WM and yield. The VCU trials are required before a cultivar is released in Brazil. From 2011 on, we began to evaluate these screened genotypes in the advanced trials, in which we used as a WM-resistant control the line A195. From 2015 on, the WM-resistant control lines G122 and Cornell 605 were also included in the advanced trials. Here, we present the results of two advanced trials conducted in 2018.

MATERIAL AND METHODS

Two field trials were conducted with 20 common bean genotypes during the fall-winter season of 2018 in areas with a history of WM in Brazil. Trials were conducted in Oratórios, State of Minas Gerais, and in Sorriso, State of Mato Grosso. In Oratórios, trial was set up in an area under no-till management and using a conventional sprinkler irrigation system. In Sorriso, trial was set up under a center pivot where a conventional-till management has been used since 2010. Ten lines (types II and III) and the cultivars Ouro Branco (type I) and Vereda (type III) were selected in the VCUs trials for partial resistance to WM; the cultivars Pérola (type III) and Estilo (type II) for moderate resistance; and the cultivars Madrepérola, Majestoso and Ouro Vermelho (types III) for susceptibility to WM. The following international genotypes with partial resistance to WM were included for comparison: A195, G122 and Cornell 605. These three lines have Andean origin and determinate type I growth habit. A randomized complete block design was used, with four replications in Oratórios and three replications in Sorriso. Plots were two 3 m-long rows, spaced 0.50 m apart. At harvest, plants were rated from 0 to 4, in which 0 = no symptoms, and 4 = 76 to 100% of the plant with symptoms of WM (Hall and Phillips, 1996). WM severity index (WMSI) was calculated for each plot on a percentage basis: Σ (scores of all plants) / [4 × (total number of plants)] × 100.

RESULTS AND DISCUSSION

WM pressure was low/moderate in Oratórios (Table) and low in Sorriso (WMSI varied from 0 to 10%). In Oratórios, correlations between WMI and yield (r = -0.10, p = 0.18) and between WMSI and yield (r = -0.13, p = 0.114) were nonsignificant. In these conditions of relatively low WM pressure, VC 35 was in the group of the most yielded beans in both trials. In the average of the trials, VC 35 yielded 15% more than the most productive cultivar Madrepérola. In Sorriso, in addition, all lines of black beans were in the group of the most productive lines. The lines VC 17 and CNFC 10432, which had good performance in previous trials conducted under different WM

pressure, were in the group of the less productive genotypes, with yields similar to those obtained by the cultivars Majestoso, Estilo and Pérola. The three international genotypes had relatively low yields in both trials. G122 and Cornell 605 were also in the group of the most susceptible genotypes to WM in Oratórios. We concluded that even under relatively low WM pressure some genotypes screened for their partial resistance to WM had higher yield relative to cultivars registered in Brazil.

Table. White mold incidence (WMI) and white mold severity index (WMSI) in Oratórios, and yield in Oratórios and Sorriso, of 20 genotypes screened for different reactions to white mold in advanced trials of 2018.

Genotype[1] (seed market class)	Oratórios-MG			Sorriso-MT	Mean yield (kg ha^{-1})
	WMI (%)	WMSI (%)	Yield (kg ha^{-1})	Yield (kg ha^{-1})	
VC 35 (C)	33 A[2]	18 A	3231 A	3715 A	3473
CNFP 11990 (B)	35 A	18 A	2834 B	3809 A	3322
VP 34 (B)	8 B	4 B	2513 B	3851 A	3182
CNFP 10798 (B)	16 B	8 B	2674 B	3688 A	3181
VC 26 (C)	29 B	12 B	3288 A	2857 B	3073
RCPVIII-1 (C)	33 A	20 A	2793 B	3322 A	3058
VC 37 (C)	27 B	12 B	2933 A	3143 B	3038
Madrepérola (C)	55 A	27 A	3084 A	2933 B	3009
CNFC MG 246-D (C)	2 B	1 B	2793 B	3204 B	2999
CNFC MG 11-08 (C)	38 A	23 A	2977 A	2868 B	2923
CNFC 10432 (C)	30 B	17 A	2854 B	2987 B	2921
Majestoso (C)	17 B	8 B	2743 B	2937 B	2840
VC 17 (C)	28 B	12 B	2741 B	2902 B	2822
Estilo (C)	19 B	10 B	2789 B	2796 B	2797
CNFC 10720 (C)	12 B	5 B	2654 B	2667 B	2661
Pérola (C)	28 B	14 B	2434 B	2562 B	2498
Ouro Vermelho (R)	55 A	33 A	2718 B	2226 B	2472
A195 (A)	23 B	12 B	2223 B	2663 B	2443
G122 (A)	43 A	22 A	1201 C	2884 B	2043
Cornell 605 (A)	46 A	28 A	1571 C	2352 B	1962
Mean	29	15	2652	3018	2835
CV (%)	59	62	11	11	-

[1] Cultivars in italic + bold are moderately resistant to white mold in the field; cultivars in bold are susceptible to white mold in the field. Between parentheses: C = carioca, B = black, A = Andean, R = red. [2] Scott-Knott test ($p < 0.05$).

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MARKER ASSISTED GAMETE SELECTION FOR MULTIPLE DISEASE RESISTANCE AND AGRONOMIC TRAITS IN INTER-RACIAL BEAN POPULATIONS

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INTRODUCTION: Anthracnose (*Colletotrichum lindemuthianum*), angular leafspot (*Pseudocercospora griseola*), root rots (*Pythium and Fusarium* spp), common bacterial blight *(Xanthomonas axonopodis* pv.*phaseoli and Xanthomonas axonopodis pv phaseoli* var. *fuscans)* and bean common mosaic virus are important biotic constraints to bean productivity in east, central, southern and West Africa, and worldwide (Teran et al, 2013; Wortmann et al, 1998). Yield losses of 50 to 100 percent have been reported in susceptible cultivars. Breeding for resistance to major diseases, a major goal of bean improvement programs in eastern Africa, has followed conventional methods (Kimani et al, 2005). Gamete selection and marker assisted selection methods are being adopted to improve efficiency and precision in breeding programs, and to reduce duration for variety development (Miklas et al, 2006; Teran et al, 2013; Singh, 1994). In 2009, a marker assisted gamete selection program was started at the University of Nairobi to pyramid genes for resistance to angular leafspot, anthracnose, root rots, common bacterial blight (CBB) and bean common mosaic viruses (BCMV) into susceptible commercial varieties, and to determine the effectiveness of this procedure. This report highlights progress made in this program.

MATERIALS AND METHODS: Thirty-two multiparent populations were developed from single, three-way and double cross male gametes among seven sources of resistance, which were finally crossed to four large seeded, and four small seeded commercial varieties susceptible to the five diseases. In these crosses, G10909 and Mex 54 were the sources of resistance to angular leafspot, G2333 to anthracnose, BRB 191 to bean common mosaic virus, VAX 6 to common bacterial blight, RWR719 and AND1062 to root rots. Artificial inoculation with local isolates was used to validate reaction to infection. Twenty-two markers were tested for polymorphism. Polymorphic markers were used to identify resistance genes in each plant before plant-to-plant pairwise hybridization; SH13 for angular leafspot, SAB 3 for anthracnose, SW13 for BCMV, SU91 for CBB, and PYAA19 for *Pythium* root rot. The eight double-cross male gametes were finally crossed to a commercial parent as female. Single plant selection for disease resistance among $F_1$and $F_{1.2}$ was conducted at Kabete (1800m) and Tigoni (1900masl) during the 2012 long and short rain seasons. Evaluation and selection for agronomic traits $F_{1.3}$, $F_{1.4}$ and $F_{1.5}$ progeny rows and families was conducted at Kabete in 2013, 2014 and 2015. Preliminary yield tests of 229 $F_{1.6}$ lines were conducted at Mwea in 2016. Advanced yield trials of 92 $F_{6.7}$ lines were conducted in 2017 at Mwea (1150m), Kabete (1820m) and Tigoni (2130m). Disease resistance in 26 elite lines was validated in 2018 by artificial inoculation in a greenhouse at Kabete Field Station. A 1-9 disease scale, where 1-3 is resistant, 4-6 intermediate, and 7-9 is susceptible (van Schoonhoven and Pastor-Corrales, 1987) was used for scoring diseases. Genstat version 15 was used for data analysis.

RESULTS AND DISCUSSION: There were significant differences for disease resistance and agronomic traits among segregating populations, progeny rows, families and advanced lines. Populations KMA1 to KMA16 produced mostly large seeded progenies, while populations KMA

17 to KMA 32 produced medium and small seeded progenies. This was attributed to the contribution of the final female parent. Segregation for grain type continued up to $F_{1.6}$ and $F_{1.7}$ generations. More than 96% of the tested elite lines (25 of the 26) showed combined resistance to at least two pathogens (Table1). Five lines had multiple resistance to five pathogens. The 26 elite lines varied in growth habit, seed size, seed colour and grain yield, and adaptation to low medium and high altitudes. Seven had growth habit IV, 13 type III, 5 type II and one type I. Six had pinto grain type, 5 red kidney, 4 red mottled, 4 small red and six were tan red, brown or black. Yield of elite lines in multilocation trials varied from 1869 to 3860 kg ha-[1] compared with a mean of 1884 kg ha-[1] for the check varieties. This study confirmed the effectiveness of marker-assisted gamete selection to concurrently improve the resistance of common bean resistance to major diseases and agronomic traits.

Table 1. Reaction of elite bean lines inoculated with different pathogens in greenhouse at Kabete, 2018.

Genotypes	[#]Pathogens							[§]Resistances	Number
	ALS	BCMV	CBB	ANT	*Fusarium*	*Rhizoctonia*	*Pythium*		
KMA13-17-17	R	I	R	R	I	R	I	A, C, AN, R	4
KMA13-17-25	R	I	I	R	S	R	I	A, AN, R	3
KMA13-21-10	I	I	I	R	I	R	I	AN, R	2
KMA13-21-11	I	I	I	R	S	R	R	AN, R, P	3
KMA13-21-20	R	I	S	R	I	I	I	A, AN	2
KMA13-22-21	I	R	I	R	I	I	R	B, AN, P	3
KMA13-22-30	I	I	I	R	I	I	I	ANT	1
KMA13-23-13	I	R	S	R	I	R	I	B, AN, R	3
KMA13-23-14	R	I	I	R	I	R	R	A, R,AN, P	4
KMA13-23-22	I	R	I	I	I	R	I	B, R	2
KMA13-24-7	R	I	I	R	I	R	I	A, AN, R	3
KMA13-25-9	R	R	I	R	I	R	R	A, B,AN, R, P	5
KMA13-26-32	R	I	R	R	I	R	I	A, C, AN, R	4
KMA13-27-12	R	R	I	R	I	I	I	A, AN, B	3
KMA13-27-27	R	R	I	R	I	R	I	A, B, AN, R	4
KMA13-27-31	R	I	R	R	R	R	I	A, C, AN, F, R	5
KMA13-28-13	R	R	I	R	S	R	I	A, B, AN, R	4
KMA13-28-2	R	I	R	R	I	R	I	A, C, AN, R	4
KMA13-28-21	R	R	R	R	I	I	R	A, B, C, AN, P	5
KMA13-28-5	R	R	I	R	I	R	R	A, B, AN, R, P	5
KMA13-29-21	R	R	I	R	I	R	I	A, B, AN, R	4
KMA13-29-24	I	R	I	R	I	R	I	B, AN, R	3
KMA13-30-14	I	R	R	R	I	R	R	B, C, AN, R, P	5
KMA13-30-22	R	I	I	R	I	R	I	A, AN, R	3
KMA13-31-62	R	R	I	R	I	I	I	A, B, AN	3
KMA13-32-28	R	I	I	R	I	R	R	A, AN, R, P	4

[#]: R=resistant; I=intermediate; S=susceptible;[§]: A=ALS; B=BCMV; C=CBB; AN=anthracnose; F=*Fusarium*; R=*Rhizoctonia* and P=*Pythium*

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REACTION OF COMMON BEAN GENOTYPES TO FUSARIUM WILT

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INTRODUCTION

Common bean is widely cultivated by small, medium and large farmers in the State of Minas Gerais, Brazil. Significant yield losses have occurred in the State due to the occurrence of fusarium wilt, caused by *Fusarium oxysporum* f. sp. *phaseoli* (*Fop*). The aim of this study was to evaluate the reaction of nine common bean genotypes to two representative *Fop* isolates - FOP UFV 01 (largely used in the common bean breeding programs in Minas Gerais) and FOP UFVJM 01 (isolated from the most important common bean area of the State).

MATERIAL AND METHODS

Three experiments were carried out at Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM), Campus Unaí-MG. In the first experiment, seven common bean genotypes of the commercial groups Black (Meia Noite) and Carioca (Dama, Pérola, BRSMG Madrepérola, VC25, BRS Estilo and BRSMG Uai) were inoculated with the *Fop* isolate FOP UFV 01. In the second and third experiments, five common bean cultivars from the commercial group Carioca (BRS Estilo, Agronorte, BRSMG Uai, Dama and Star) were inoculated with the *Fop* isolates FOP UFV 01 and FOP UFVJM 01, respectively. The experimental design was completely randomized with three replicates (a replicate was a pot with three plants). The inoculation was done by root immersion in a conidia suspension (1 x 10^6 conidia/mL). Disease severity was evaluated at 21 days after inoculation using a 1 to 9 scale (Pastor Corrales and Abawi, 1987). Genotypes with scores from 1.0 to 3.0 were considered resistant, 3.1 to 6.0 intermediate, and 6.1 to 9.0 susceptible.

RESULTS AND DISCUSSION

The reaction of common bean genotypes to fusarium wilt is presented in the Table 1. Important commercial cultivars were susceptible to both *Fop* isolates FOP UFV 01 and FOP UFVJM 01. The genotype VC 25 was resistant to the isolate FOP UFV 01 and the cultivar Dama was resistant to both *Fop* isolates in the three experiments. Cultivars tested by Cruz et al. (2018) were susceptible to the isolate FOP UFV 01, and the Manteigão Fosco 11 presented score 6,0, although it is a cultivar known as resistant to most *Fop* isolates. Resistant genotypes screened in our experiments, including to the aggressive isolate FOP UFV 01, could be recommended for the areas of the State of Minas Gerais infested with the pathogen.

Table 1. Reaction of common bean to *Fusarium oxysporum* f. sp. *phaseoli* (*Fop*) isolates FOP UFV 01 and FOP UFVJM 01.

Genotypes	Fusarium wilt severity (1 to 9 scale)[1]	Reaction[1]
First experiment – *Fop* isolate FOP UFV 01		
BRS Estilo	9.0	S
Meia Noite	7.1	S
BRSMG Uai	6.2	S
BRSMG Madrepérola	4.6	I
Pérola	4.3	I
VC25	3.0	R
Dama	1.2	R
Second experiment – *Fop* isolate FOP UFV 01		
Agronorte	8.7	S
BRS Estilo	7.2	S
BRSMG Uai	5.3	I
Star	5.0	I
Dama	2.4	R
Third experiment – *Fop* isolate FOP UFVJM 01		
BRS Estilo	8.1	S
Agronorte	8.0	S
BRSMG Uai	7.1	S
Star	7.5	S
Dama	2.7	R

[1]Genotypes with scores from 1.0 to 3.0 were considered resistant (R), 3.1 to 6.0 intermediate (I), and 6.1 to 9.0 susceptible (S).

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MYCELIAL GROWTH RATE AND SPORULATION OF ISOLATES OF *Fusarium oxysporum* f. sp. *phaseoli*

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INTRODUCTION

Fusarium wilt is associated to significant yield losses on common beans in Brazil. Our objective was to evaluate the variability of the causal agent, the fungus *Fusarium oxysporum* f. sp. *phaseoli* (*Fop*), by mycelial growth rate and sporulation of isolates collected from different common bean growing areas in Brazil.

MATERIALS AND METHODS

Mycelial plugs (5 mm diameter) of 45 *Fop* isolates were placed in the center of Petri dishes (9 cm diameter) containing 15 mL of PDA and incubated at 25 ^{o}C. Colony diameter was assessed at 72, 144 and 216 hours after incubation. Average mycelial growth rate (mm h^{-1}) was estimated (diameter after 144 hours - diameter after 72 hours + diameter after 216 hours - diameter after 144 hours)/144. After assessing the mycelial growth rate, 10 mL of sterile distilled water was used in each dish to scrape the mycelium. Sporulation of isolates was estimated using a Neubauer chamber. Three dishes were used for each isolate and the experiment was performed twice.

RESULTS AND DISCUSSION

The average mycelial growth rate varied from 0.16 to 0.40 mm h^{-1} in the first experiment and from 0.24 to 0.42 mm h^{-1} in the second experiment. The sporulation varied from 3.3 x 10^5 to 3.1 x 10^7 conidia/mL in the first experiment and from 2.5 x 10^5 to 3.2 x 10^7 spores/mL in the second experiment. Mycelial growth rate did not correlate with sporulation of isolates. The results showed high variability among *Fop* isolates, which was accomplished by a further study (Cruz et al. 2018).

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Table: Mycelial growth rate (mm h^{-1}) and sporulation (conidia mL^{-1}) of isolates of *Fusarium oxysporum* f. sp. *phaseoli* (*Fop*)

Isolates	Mycelial growth rate (mm h^{-1})		Sporulation (conidia mL^{-1})	
	1º Experiment	2º Experiment	1º Experiment	2º Experiment
*Fop*LAPS 157	0.40 a*	0.41 a*	3.3×10^5 f*	2.5×10^5 h*
Fop 03	0.35 b	0.39 a	1.3×10^7 c	4.0×10^6 g
*Fop*LAPS 166	0.35 b	0.38 a	1.2×10^7 d	8.0×10^6 e
*Fop*LAPS 506	0.34 b	0.37 a	9.3×10^6 d	6.7×10^6 f
Fop 07	0.34 b	0.40 a	6.4×10^6 e	3.0×10^5 h
Fop 15	0.32 c	0.33 b	6.4×10^6 e	5.6×10^6 f
*Fop*LAPS 507	0.31 c	0.33 b	5.8×10^6 e	5.3×10^6 f
*Fop*LAPS 168	0.31 c	0.40 a	6.6×10^6 e	7.2×10^5 h
*Fop*LAPS 505	0.30 c	0.36 a	3.1×10^7 a	3.2×10^7 a
*Fop*14645	0.30 c	0.38 a	7.4×10^6 e	4.0×10^6 g
*Fop*11178	0.30 c	0.35 a	1.7×10^6 f	2.5×10^5 h
*Fop*LAPS 164	0.30 d	0.29 c	1.4×10^7 c	1.8×10^6 h
*Fop*UFV08	0.29 d	0.42 a	5.9×10^6 e	1.5×10^6 h
*Fop*14629	0.29 d	0.29 c	1.0×10^7 d	7.5×10^6 f
*Fop*LAPS 160	0.29 d	0.38 a	7.2×10^6 e	4.3×10^5 h
*Fop*UFV02	0.29 d	0.35 a	7.2×10^6 e	1.1×10^7 e
*Fop*LAPS 502	0.29 d	0.33 b	4.3×10^5 f	3.2×10^5 h
*Fop*UFV03	0.28 d	0.30 b	1.1×10^7 d	4.3×10^6 g
*Fop*UFV11	0.28 d	0.34 b	5.6×10^6 e	1.1×10^7 e
*Fop*11257	0.28 d	0.31 b	1.1×10^7 d	8.3×10^6 e
*Fop*UFV14	0.28 d	0.36 a	6.7×10^6 e	5.9×10^6 f
*Fop*LAPS 156	0.28 d	0.33 b	2.8×10^7 a	4.0×10^6 g
*Fop*UFV17	0.27 d	0.40 a	7.2×10^6 e	5.9×10^6 f
Fop 56	0.27 d	0.32 b	8.5×10^6 e	4.3×10^6 g
*Fop*LAPS 161	0.27 d	0.32 b	5.5×10^5 f	8.5×10^5 h
*Fop*LAPS 163	0.27 d	0.41 a	6.1×10^6 e	5.1×10^6 f
*Fop*UFV10	0.27 d	0.32 b	1.3×10^7 c	9.3×10^6 e
*Fop*UFV01	0.27 d	0.26 c	8.0×10^6 e	2.4×10^7 b
*Fop*14435	0.27 d	0.33 b	3.1×10^7 a	2.0×10^7 c
*Fop*LAPS 509	0.26 d	0.29 c	1.5×10^7 c	4.0×10^6 g
*Fop*UFV05	0.26 d	0.27 c	1.6×10^7 c	1.5×10^7 d
*Fop*UFV18	0.26 d	0.29 c	9.3×10^6 d	1.6×10^6 h
*Fop*UFV15	0.26 d	0.32 b	8.5×10^6 e	6.7×10^6 f
*Fop*UFV13	0.26 d	0.34 b	1.1×10^7 d	5.9×10^6 f
*Fop*UFV07	0.26 d	0.35 a	2.5×10^7 b	1.5×10^7 d
Fop 101	0.26 d	0.36 a	7.2×10^6 e	2.5×10^5 h
*Fop*UFV19	0.24 e	0.35 a	8.3×10^6 e	5.9×10^6 f
*Fop*UFV04	0.24 e	0.33 b	9.9×10^6 d	9.8×10^5 h
*Fop*UFV06	0.24 e	0.31 b	4.0×10^6 f	8.3×10^6 e
*Fop*UFV16	0.24 e	0.29 c	1.2×10^7 c	1.2×10^7 h
*Fop*LAPS 510	0.23 e	0.30 c	4.0×10^6 f	9.3×10^6 e
*Fop*14353	0.23 e	0.24 c	7.2×10^6 e	4.0×10^6 g
*Fop*LAPS 503	0.23 e	0.30 c	1.6×10^7 c	9.3×10^6 e
*Fop*UFV12	0.23 e	0.34 b	7.2×10^6 e	4.8×10^6 f
*Fop*UFV09	0.16 f	0.25 c	4.0×10^6 f	1.3×10^7 d
Control	0.00 g	0.00 d	0.00 g	0.00 i

***** Means were grouped by Scott-Knott test ($p < 0.05$).

THE EFFECT OF DIFFERENT STINKBUG *Euschistus heros* DENSITIES ON COMMON BEAN PRODUCTION

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INTRODUCTION

The brown stinkbug [*Euschistus heros* (Fabricius, 1798)] is one of the most important common bean's (*Phaseolus vulgaris* L.) pests and has a great potential to reduce the quantity and commercial quality of the seeds (Andrade et al., 1999; Obando Flor, 2004). The damage is most visible in seeds with a clear tegument, resulting in stains and the reduction in its commercial quality, due to the stinkbug's feeding. The research on stinkbug damage is important towards elaborating strategies for integrated pest management. Thus, the goal of this study was to determine the potential yield reduction of the IPR Celeiro cultivar, which was inoculated with the *Bean Golden Mosaic Virus* (BGMV) and different densities of *E. heros*.

MATERIALS AND METHODS

The experiment was performed in field conditions during the dry season (November, 2017 to February, 2018) in the Instituto Agronômico do Paraná (IAPAR), Londrina - Paraná, Brazil. According to Köppen's classification the climate is humid subtropical (Cfa) (Caviglione et al., 2000). The soil classified as Hapludox. The common bean seeds are from the IPR Celeiro cultivar, they were treaded with fungicide and insecticides and were sown using a conventional tillage system in an area of 0,5 ha. The sow spacing used was of 0,5 m between rows and 0,1 m between plants. When the plants were at V2 phenology, they were infested with *Bemisia tabaci* biotype B, which were contaminated with BGMV. Before the plant's blooming, cages made of polyester (2 x 2 mm mesh) were installed, inside holding 20 or 40 common bean plants. The treatments correspond to 8 population densities, including 0; 0.25; 0.50; 1; 2; 3; 4 and 5 fresh *E. heros* adults, these were released during the plants full blossom, and kept until the plant's physiological maturity. The cages were checked twice a week, replacing any dead stinkbugs. The plants were harvested when dry and recorded the following variables: number of pods; number of grains; total grain weight and commercial grain weight. The commercial classification follows MAPA's n°12 Normative Instruction (Knabben, 2012), which utilizes sieves to classify the grains in accordance to its size. The data was submitted to a variance analysis with means compared using the Scott-Knott test (α=5%).

RESULTS AND DISCUSSION

The seed's weight and commercial grain percentage declined with the increase in stinkbug density (Figure 1). This can be explained by the stinkbug's feeding, resulting in seeds that are smaller, deformed, stained, with loss in germinating vigor and even dead seeds (Obando Flor, 2004). These types of damage are a result of the toxic saliva being injected in the cotyledon or embryo, reducing considerably the productivity. Furthermore, while feeding, the stinkbug may transmit a yeast infection (*Nematosporacoryllio sp*.) which stains and diminishes the products commercial

classification (Quintela, 2002). The 5 stinkbug.m^{-1} density resulted in the smallest seed yield (31,1 grams), and from this total, 80% were classified as "non-commercial" (Figure 1). The control treatment (0 stinkbugs.m^{-1}) had the highest seed yield (53,2 grams). The high proportion of non-commercial seeds was caused due to the BGMV presence and probable interaction between the stinkbug's feeding and the BGMV. Since the cultivar IPR Celeiro (tolerant to BGMV) demonstrated a few plants with the disease's symptoms. Between the different treatments, no statistical difference was found on median number of pods and number of grains. Thus, the presence of stinkbugs after the plant's blossoming did not significantly affect the pod and seed production.

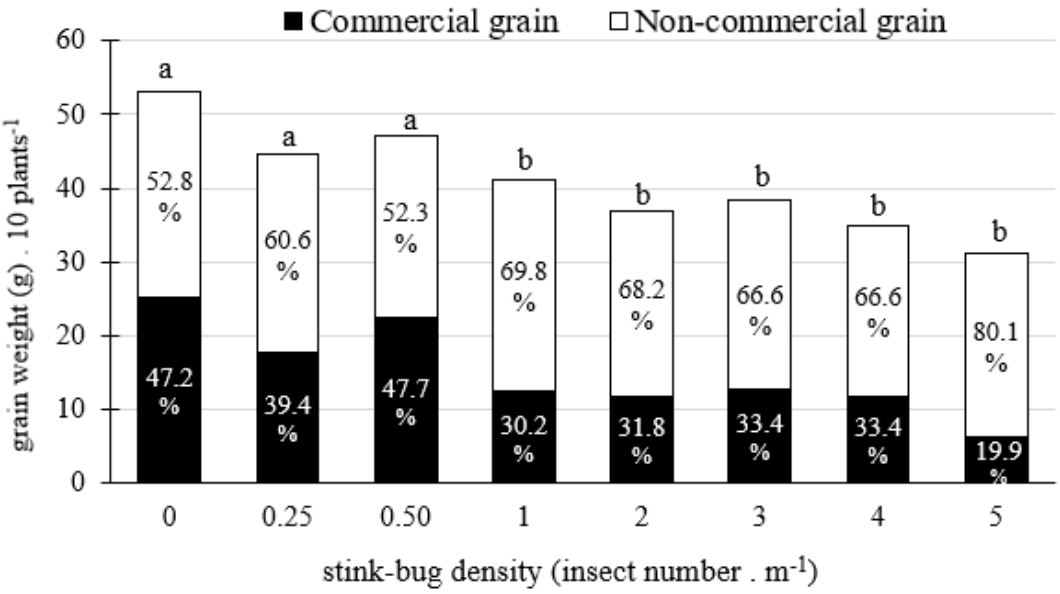

Figure 1. Median seed weight (g) and commercial and non-commercial grain percentage of the common bean (*Phaseolus vulgaris* L.) submitted to different brown stinkbug (*Euschistus heros*) densities. Columns followed by the same letter do not differ according to Scott-Knott, with a 5% significance. Dry season, 2018. Londrina, PR, Brazil.

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RESISTANCE OF *Phaseolus vulgaris* L. GENOTYPES IN DIFFERENT PHENOLOGICAL STAGES TO FALL ARMYWORM

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INTRODUCTION

Host plant resistance is one of the main control methods to be adopted in integrated pest management programs. However, the resistance expression can be affected by several factors, which may be related to the environment, insect and/or plant, such as the crop phenological stage. According to the crop phenology there may be modifications in morphological, physical and/or chemical characteristics which can lead to changes mainly in the plant resistance levels (SMITH, 2005; BOIÇA JÚNIOR et al., 2015). Then, the aim of this work was to evaluate the resistance expression of bean genotypes in different phenological stages to *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae).

MATERIALS AND METHODS

The experiment was carried out under laboratory conditions of 26 ± 2 ° C, 60 ± 10% relative humidity and a photoperiod of 12:12 (L:D) hours. A completely randomized design was adopted in a 3 x 2 factorial scheme, corresponding to the main effect of three bean genotypes and two phenological stages, with 10 replications. The BRS Pérola and IAC Harmonia genotypes were used as susceptible and resistant patterns, respectively, in addition to BRS Supremo, which were evaluated in phenological stages of the third expanded trifoliate leaf (V4) and pod formation (R7). Plants were kept in 5-liters plastic pots placed in a greenhouse and for the experiment the second trifoliate leaf was used from the plant apex.

Leaves were washed with hypochlorite solution (0.5%) and cutted into 5-cm-diameter leaf discs, which were weighed on a precision analytical scale and then individually placed in 9-cm-diameter Petri dishes lined with moistened filter paper. Recently molted third-instar larvae of *S. frugiperda* were starved for 3 h to void their guts and weighed in a precision analytical scale. One larva was released per plate, where they remained feeding until approximately 80% of the leaf disc of one replication was consumed. Leaf discs originated from central leaflets of the trifoliate of each treatment and 30 caterpillars were used as dry weight aliquot. At the end of the experiment the larvae and the leaf discs remaining of each treatment were dried in an oven at 60ºC for 48 hours and weighed on an analytical scale. Through the difference between the initial and final dry weight, the dry mass consumed and the dry weight gain by the larvae in each treatment were determined.

Data were submitted to normality and homogeneity analysis by the Kolmogorov Smirnov and Levene tests, respectively. Then they were submitted to analysis of variance (ANOVA) and when significant the means of the treatments were compared by the Tukey test ($P < 0.05$).

RESULTS AND DISCUSSION

There was lower consumption by *S. frugiperda* larvae in IAC Harmonia compared to BRS Pérola and BRS Supremo (Fig 1A). In addition, the dry matter consumption was about 26.66% lower in leaf discs from plants at R7 stage regarding to the V4 stage (Fig. 1B).

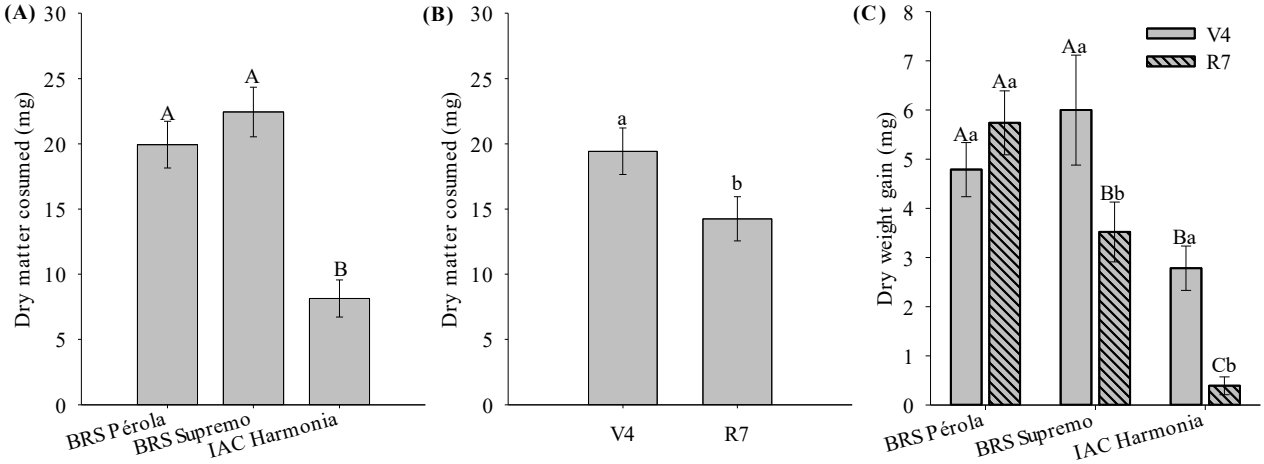

Figure 1. Dry matter consumed and dry weight gain by *Spodoptera frugiperda* larvae in different bean genotypes and phenological stages. Bars topped with the same uppercase letters are not significantly different among genotypes; bars topped with the same lowercase letters are not significantly different among phenological stages by Tukey test ($P > 0.05$). **(A) (B)** Genotypes (G): $F_{2, 54} = 21.76$, $P < 0.0001$; Phenological stage (P): $F_{1, 54} = 7.49$, $P = 0.0084$; G x P: $F_{2, 54} = 0.42$, $P = 0.6577$. **(C)** G: $F_{2, 54} = 39.47$, $P < 0.0001$; P: $F_{1, 54} = 15.93$, $P = 0.0002$; G x P: $F_{2, 54} = 10.33$, $P = 0.002$.

Larvae that fed on BRS Supremo and IAC Harmonia in the V4 stage had greater weight gain compared to those fed on plants in R7, the same was not observed for BRS Pérola. Futhermore when the larvae were fed by plants in the V4 stage, there was no difference in weight gain for BRS Perrola and BRS Supremo larvae, which was not verified when the same genotypes were offered in R7 (Fig. 1C).

The differences in the consumption and larvae weight gain observed for the two phenological stages are probably due to changes in plant characteristics, mainly morphological, as well as the increase of lignin contents, which leads to reduction of consumption and substrate digestibility, thus interfering in the larvae weight gain (SMITH, 2005). Thus, it is concluded that bean phenological stage may, depending on the genotype, interfere in characteristics related to the resistance expression and, consequently, in the resistance levels of bean to *S. frugiperda*.

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ANTIXENOSIS OF *Phaseolus vulgaris* L. GENOTYPES TO *Helicoverpa armigera* (HÜBNER, 1805) (LEPIDOPTERA: NOCTUIDAE)

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INTRODUCTION

Due to its polyphagia *Helicoverpa armigera* (Hübner, 1805) (Lepidoptera: Noctuidae) has been causing damage to several crops in Brazil, such as common bean, since the first record in the country (LIU et al., 2004; CZEPAK et al., 2013). Bean is one of the main annual crops in Brazil and therefore it is necessary to adopt pest insect control methods, such as host-plant resistance, which in view of its advantages and efficiency should always be part of integrated pest management programs (BOIÇA JÚNIOR et al., 2017). Thus, the aim of this work was to evaluate the resistance in the antixenosis category of bean genotypes to *H. armigera* larvae.

MATERIALS AND METHODS

Bean genotypes BRS Pérola, IAC Sintonia, IAC Dama, BRS FC 402 and ANFc-9 were evaluated for antixenosis to *H. armigera* larvae by free-choice and no-choice tests performed under laboratory conditions of 26 ± 2 ° C, 60 ± 10% relative humidity and a photoperiod of 12:12 (L:D) h. The genotypes were sown in 5-liters plastic pots and placed in greenhouse until the phenological stage V4 (emission of second trifoliate leaf). For the experiments, the second trifoliate leaf of each plant was used, which was washed with hypochlorite solution (0.5%), then leaf discs (3.0 cm of diameter) were prepared using a cork borer.

The free-choice test was conducted under a randomized block design, while no-choice test under a completely randomized design, both with 10 replicates. In free-choice test, each replicate was composed of a 14-cm-diameter Petri dish lined with moistened filter paper on which the leaf discs of the evaluated genotypes were arranged circular and equidistantly from the center, where a third instar larva was released by genotype. In no-choice test, each replicate was composed of a 9-cm-diameter Petri dish lined with moistened filter paper and with an individual leaf disc of each evaluated genotype and a third instar *H. armigera* larva was released.

The number of larvae attracted to each leaf disc was evaluated at each evaluation interval (30, 60, 180, 360, 720, 1440 and 1800 minutes) after the larvae release. At the end was calculated the average number of larvae attracted to each treatment, in addition, the leaf area consumed (cm^2) by the larvae was determined by the difference between the initial and final leaf area, measured by ImageJ software. The frequency data of *H. armigera* larvae in each treatment were submitted to Chi-square test ($P < 0.05$). Leaf area consumed data were submitted to the normality and homogeneity analysis by Kolmogorov Smirnov and Levene tests, respectively. Then they were submitted to analysis of variance (ANOVA) and when significant the treatments means were compared by Tukey test ($P < 0.05$).

RESULTS AND DISCUSSION

The mean percentage of larvae attracted to BRS Pérola, IAC Dama and ANFc-9 genotypes were lower in comparison to IAC Sintonia in free-choice test. However, in no-choice test there was no significant difference in the percentage of larvae attracted to each genotype. (Table 1).

Table 1. Percentage of larvae and leaf area consumed (cm^2) by *Helicoverpa armigera* on each bean genotypes in free-choice and no-choice tests.

Genotypes	Larvae (%)		Leaf area consumed (cm^2)	
	Free-choice[1]	No-choice[1]	Free-choice[2]	No-choice[2]
BRS Pérola	14.53 ± 3.05 b	14.97 ± 2.76 a	0.34 ± 0.12 b	1.02 ± 0.23 a
IAC Sintonia	36.75 ± 4.03 a	22.45 ± 5.77 a	1.49 ± 0.26 a	0.94 ± 0.26 a
IAC Dama	13.68 ± 2.48 b	23.13 ± 2.33 a	0.48 ± 0.32 b	0.96 ± 0.21 a
BRS FC 402	23.08 ± 2.99 ab	23.81 ± 2.33 a	1.66 ± 0.49 a	0.80 ± 0.20 a
ANFc-9	11.97 ± 1.53 b	15.65 ± 3.27 a	0.44 ± 0.19 b	0.70 ± 0.17 a
F	-	-	6.23	0.36
χ^2	10.81	2.88	-	-
P	0.0288	0.5789	0.0008	0.8327
DF	4	4	4. 32	4. 43

[1] Mean ± standard error values followed by the same letter in the column are not significantly different by Chi-square test ($P < 0.05$); [2] Mean ± standard error values followed by the same letter in the column are not significantly different by Tukey test ($P < 0.05$).

Leaf area consumed by *H. armigera* larvae in BRS Pérola, IAC Dama and ANFc-9 genotypes were approximately four times lower than in IAC Sintonia and BRS FC 402, in free-choice test (Table 1). In no-choice test there was no difference in leaf area consumed by larvae among genotypes. Thus, probably BRS Pérola, IAC Dama and ANFc-9 present chemical, physical and / or morphological characteristics which are repellent to *H. armigera* larvae and consequently makes these genotypes resistant in antixenosis category to *H. armigera* larvae.

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ESSENTIAL OILS REPELLENT ACTIVITIES ON *Zabrotes subfasciatus* (Bohemann, 1833) (Coleoptera, Bruchinae)

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INTRODUCTION

In Brazil, beans productivity (*Phaseolus vulgaris* L.) is considered low due to some factors. One of them is the damage caused by pest insects, among which *Zabrotes subfasciatus* (Boh., 1833) stands out as the main stored bean plague (CARVALHO et al.2014).

The concern over the abusive use of agrochemicals in agriculture has aroused the interest of researches about alternative pest control tactics, especially those with essential oils (ANDRADE et al.2013). The aim of this paper is to evaluate the essential oils repellent effect of the fruits *Xylopia aromatica* (Lam.) Mart (Annonaceae) and *Schinus molle* L. (Anacardiaceae) on *Z.subfasciatus*.

MATERIAL AND METHODS

The experiment was conducted at the Entomology Laboratory of Instituto Federal Goiano - Campus Iporá, Goiás, Brazil. We employed the randomized blocks design with three treatments and seven repetitions constituted by arenas with four circular plastic containers with approximately 141.83 cm^3 each. The arena was composed by a central container interconnected by plastic tubes, and three containers equidistant arranged lined with filter paper containing 15g of beans. The essential oils were applied to the filter paper so that the insect did not adhere to the grain and the oil acted by fumigation.

The treatments were control (T1), 5µL of *X. aromatica'*s fruit essential oil (T2) and 5µL of *S. molle*'s fruit essential oil (T3). In the central container, 21 couples of *Z. subfasciatus* were released. Evaluations were made 03, 06, 12, 24, 48 and 72 hours after the insects' release counting the number of specimens in the treatments.

The data obtained in the *Z. subfasciatus* repellency test were transformed in $\sqrt{x + 0{,}5}$, the analysis of variance by Tukey's test was applied and the Preference Index (PI) was determined by using the formula cited by Procópio *et al.* (2003).

RESULTS AND DISCUSSION

Based on the analysis of variance it can be observed that there was a significant difference between the treatments. The *X. aromatica'*s fruit essential oil was different from the other treatments presenting the lowest average percentages of attractiveness in all evaluation times, ranging from 1.36 to 5.44% at 03 and 72 hours respectively (Table 1).

The studies performed by Silva et al. (2015) demonstrate the high importance of species of Xylopia's genus as a potential for the development of bio insecticides, which may explain the results obtained by the Preference Index.

The values obtained in the Preference Index ranked the 5 µL dose of *X. aromatica*'s essential oil as a repellent in all the evaluation periods, ranging from -0.88 and -0.762 at 03 and 72 hours respectively (Table 2).

These results compare with those of Campos et al. (2014), which evaluated the insect repellent and insecticidal activity of *Baccharis Articulata's* essential oil on the bean weevil (*Acanthoscelides obtectus*), and obtained preference indexes of -0.50, -0.51, -0.66, -0.62 and -0.62 at the doses of 10, 20, 30, 50 and 100, respectively.

Table 1. Average attractiveness (%) of *Zabrotes subfasciatus* adults in beans treated with different dosages of essential oils of *Xylopia aromatica* and *Schinus molle*'s fruits at different evaluation times. Iporá-GO,2017.

Dose	Time (h) [1]					
	03	06	12	24	48	72
Control	21.42 b	22.44 b	25.51 b	36.39 b	41.49 b	40.47 b
X. aromática	1.36 a	1.36 a	1.36 a	2.38 a	4.08 a	5.44 a
S. molle	21.08 b	24.48 b	26.87 b	39.45 b	44.89 b	47.61 b
P	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
F	34.64	43.74	63.60	74.61	80.15	90.48
C.V. (%)	22.63	20.81	17.78	16.62	14.99	12.37

Averages followed by the same letter in the column do not differ by the Tukey test. ** = significant at 1%. [1]Data transformed to $\sqrt{x + 0{,}5}$. [1]Calculated using the formula cited by Procópio *et al.* (2003).

Table 2. Attractiveness classification according to preference index (P.I) of *Zabrotes subfasciatus* adults at different evaluation times.

Time (hours)	Preference Index± Standard Error	
	Xylopia aromatica	*Schinus molle*
03	-0.880 ± 0,02	-0.008 ± 0,05
06	-0.885 ± 0,02	0.043 ± 0,01
12	-0.898 ± 0,01	0.025 ± 0,01
24	-0.877 ± 0,01	0.040 ± 0,02
48	-0.820 ± 0,02	0.039 ± 0,01
72	-0.762 ± 0,02	0.081 ± 0,01

CONCLUSION

The dose of 5 µL of *X. aromatica*'s fruit essential oil shows potential for repellency on *Z. subfasciatus* under laboratory conditions.

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EVALUATION OF THE EFFECT OF BEAN GENOTYPES-POD IN THE OVIPOSITION OF *Zabrotes subfasciatus* (COLEOPTER: CHRYSOMELIDAE)

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INTRODUCTION

The bean-pod (*Phaseolus vulgaris* L.) belongs to leguminous family, being the same botanical species of beans for dry grains (FILGUEIRA, 2008), and their productivity is considered low due to several factors, including the attack by many pests (YOKOYAMA, 2006).

In storing, the crisomolidae *Zabrotes subfasciatus* (Boh.), originating from the New World, is one of the main pests. The larvae feed only of seeds, causing considerable damage when attacking the cotyledons, building galleries, which may destroy them completely (GALLO et al., 2002).

This pest control may be carried out with insecticides. However, the studies of resistance of plants have been widely conducted in Brazil as an alternative to the control of bruchids. The advantages of this approach are: the decrease in the use of insecticides, low cost, ease of use and, mainly, the compatibility with other control methods. Thus, this study aimed to evaluate the oviposition behavior of *Z. subfasciatus* in different genotypes of bean-pod (BOTTEGA et al., 2013).

MATERIALS AND METHODS

The experiment was conducted on Instituto Federal Goiano - Campus Iporá, Goiás, Brazil. The insects used in the experiments were from the creation, in plastic bottles of 1 liter, closed with castings and coated lids with nylon net. Every 30 days, the material was sieved and the adults were separated to start the infestation on new bottles.

For this test, a randomized-complet design with ten treatments and four replications was used. To carry out essays, the following genotypes were used: Slenderwash, Hab 46, Gold Wax, contend, Yellow Japanese, Tendergreen Improved, Commodore Improved, Provider, White Japanese and Tenderette.

The test was performed without choice, and seven couples of *Z. subfasciatus*, newly emerged were released, in glass containers of 3.9 cm in height and 3.8 cm in diameter containing 10 grams of snap bean of the corresponding treatment. The adults remained in treatments for seven days. After this period, the counting of eggs placed by weevils was carried out. The biological parameters evaluated were: viable, unviable and total eggs.

The results obtained were analyzed by F test of variance (ANOVA), and the averages were compared by the T1ukey test.

RESULTS AND DISCUSSION

The number of viable eggs was not observed significant differences between the evaluated genotypes. When it comes to the number of viable eggs and total, a significant difference was observed between the genotypes. The Commodore Improved genotype was the least preferred for oviposition by insect, and the Tenderette was the one that presented the highest number of viable eggs, unviable and total (Table 1).

Bottega et al. (2013), assessing genotypes of bean-pod, noted that the UEG05 genotype was the least preferred for oviposition of *Z. subfasciatus.* The oviposition variation may occur by the fact that the females of *Z. subfasciatus* are able to use visual and chemical stimulus and in the search and choice of the host, as has been demonstrated for other species, for Messina (1990). According to Lara (1991), the way in which *Z. subfasciatus* set their eggs in grains, not letting them loose, it may be an indication that these insects, select the host.

Table 1. Average number of viable, unviable and total eggs of *Zabrotes subfasciatus* in seeds of common bean genotypes-pod, in no-choice tests. Iporá/GO, 2015.

Genotypes	**No-Choice**		
	No. of eggs[1]		
	Total	Viable	Unviable
Slenderwash	95.50 ab	82.50 a	13.00 b
HAB46	88.00 ab	72.75 a	15.25 ab
Gold Wax	113.7 ab	94.00 a	19.75 ab
Contender	99.50 ab	75.75 a	23.75 ab
A. Japonês	80.00 ab	55.75 a	24.25 ab
T. Improved	114.0 ab	91.00 a	23.00 ab
C. Improved	48.75 b	30.00 a	18.75 ab
Provider	163.0 ab	116.0 a	47.00 ab
B. Japonês	132.5 ab	81.75 a	50.75 ab
Tenderette	195.5 a	132.5 a	60.00 a
F (G)	2.13*	1.81ns	3.04*
C.V. (%)	24.85	51.39	30.20

Medium followed by the same letter in column, did not differ among themselves by Tukey test at 5% probability. For analysis, the data were transformed into (x + 0.5)1/2.

CONCLUSION

It was concluded that there was a lower tendency of preference for oviposition in genotype Commodore Improved, in without choice test.

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BREEDING COMMON BEANS FOR HIGH IRON AND ZINC CONTENT IN GHANA

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INTRODUCTION

Over 3 billion people worldwide suffer from nutrition related disorders resulting from iron and zinc deficiencies (Haas et al., 2016; Blair, 2013; Bouis and Welch, 2010). In Ghana, sixty-six percent (66%) of children aged 6-59 months and forty-two (42%) of women are iron deficient anaemic (Ghana Statistical Service, 2015). Iron deficiency increases the risk of expectant women dying during delivery or in the post-delivery period. Zinc fortification can help reduce childhood infections, especially diarrhoea and pneumonia. Biofortification is considered as the most sustainable, efficient and cost-effective approach to reduce micronutrient deficiencies (Society, 2014). The improvement of common beans, a major protein source in Ghana through biofortification that increase Fe and Zn will reduce acute micronutrient deficiencies and improve the nutrition and health status of women and children. Hence, the objective of this study was to develop high iron and zinc bean genotypes through introgression of genes from four common bean lines.

MATERIALS AND METHODS

Four parents were planted in the field at CSIR-Crops Research Institute Station in Fumesua with routine agronomic practices until harvesting. Three single crosses were made using the four parents; cross 1 (CAL 96 x RWR 2154), cross 2 (MCR-ISD-672 x RWR 2154), and cross 3 (RWR 2154 x NUA 99). The F_1 seeds were used to generate F_2 populations through simultaneous selfing, and backcross (BC_1P_1 and BC_1P_2) with the respective parents, P_1 and P_2. Six treatments (P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2) for each population were evaluated using RCB design with three replications from May to August, 2018. The seeds were harvested and shipped to Rwanda Agriculture Board (RAB) Research Station in Rubona to quantify the iron and zinc contents using X-ray fluorescence (XRF) spectrometry. Generated data were statistically analyzed with the GenStat software package, version 12.

RESULTS AND DISCUSSION

Results showed significant ($p < 0.001$) variation among the generations for iron and zinc contents, which respectively ranged from 58.4 to 106.5 ppm (Fig 1) and 25.0 to 36.7 ppm (Fig 2). All progenies recorded higher iron content (Fe > 75 ppm) than the parents, and two progenies for higher zinc content (Zn > 35 ppm) were consider as a genetic gain. Promising lines with relatively very high iron content (Fe > 90 ppm) were selected and advanced during the second growing season from October to December, 2018.

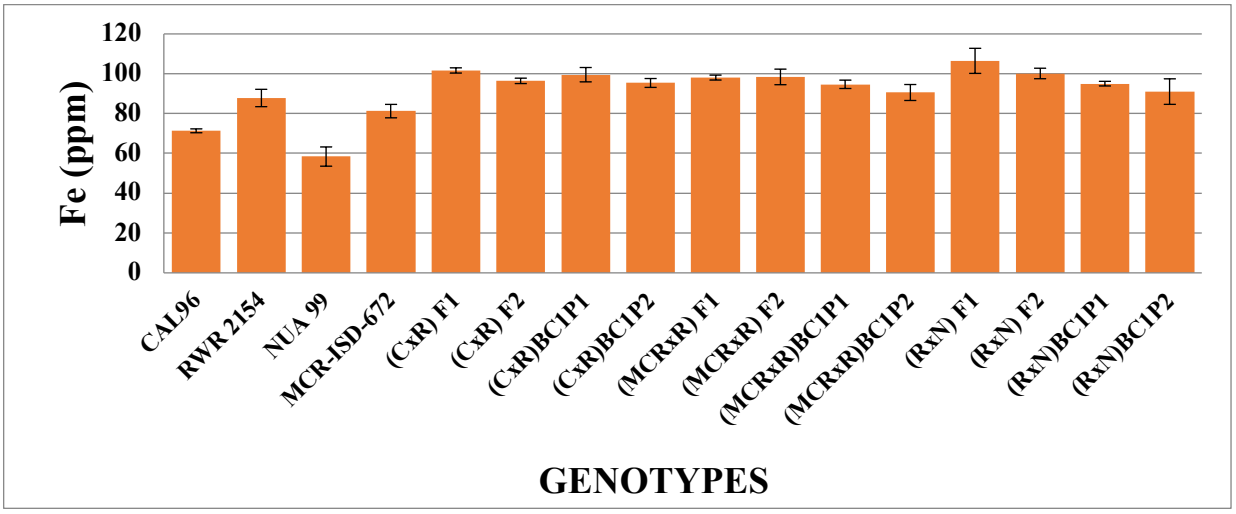

Figure 1. Fe content of six basic generations from three crosses with CAL 96, RWR 2154, NUA 99 and MCR-ISD-672 as parents

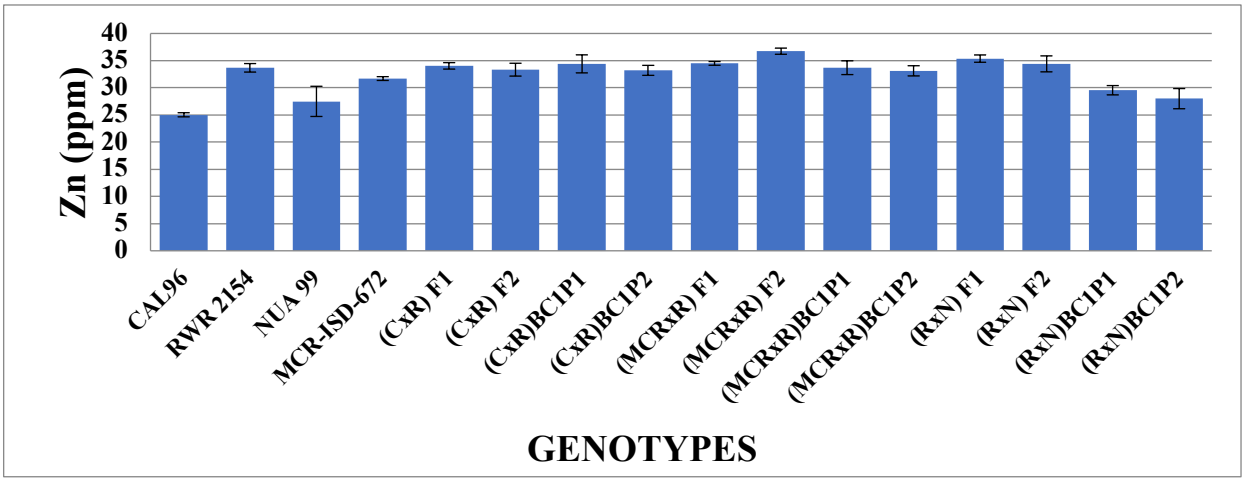

Figure 2. Zn content of six basic generations from three crosses with CAL 96, RWR 2154, NUA 99 and MCR-ISD-672 as parents

Segregating populations would be advanced and evaluated at multiple locations for their adaptability and yield potential.

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IN VIVO (*Gallus gallus*) ASSESSMENT REVEALS THE IRON BENEFITS OF CONSUMING THE FAST COOKING MANTECA YELLOW BEAN (*Phaseolus vulgaris*)

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OBJECTIVES: The common dry bean (*Phaseolus vulgaris* L.) is a globally produced pulse crop and an important source of protein and micronutrients for millions of people across Latin America and Africa. In these regions, energy for cooking is expensive or scarce and long cooking times deter consumers from purchasing beans. In addition, many of the preferred black and red seed types have phytate and polyphenols that limit the absorption of trace minerals. Yellow beans are unique because their seed coats are rich in kaempferol 3-glucoside, a recently discovered promoter of iron absorption. Several market classes of yellow beans are sold throughout Latin America and Africa, where they are marketed at premium prices for their fast cooking tendencies. Exploring the yellow bean's unique heritage to develop new fast cooking varieties that deliver more absorbable iron would be useful for regions where inhabitants have limited access to fuelwood for cooking. This study compared the iron bioavailability of three fast cooking yellow beans from Africa with contrasting seed coat colors (Manteca, Amarillo, Njano) to slower cooking white and red kidney commercial varieties from North America (**Table 1**).

METHODS: Cooked beans were formulated into diets with the complementary food crops of potato, rice and cabbage. Iron bioavailability was measured as the ability to maintain total body iron hemoglobin (Hb-Fe) during a 6 week in vivo (*Gallus gallus*) feeding trial.
Results: Animals fed yellow bean diets had faster growth rates, accumulated more dietary iron and had higher Hb-Fe than animals fed either kidney bean diet (**Figure 1**). In contrast to yellow beans, the kidney beans had almost no kaempferol 3-glucoside (**Table 2**). When compared to the other four bean based diets, the fast cooking Manteca yellow bean diet delivered the largest increase in Hb-Fe in vivo (**Figure 1**).

CONCLUSIONS: Through the added benefit of fast preparation times and improved iron quality after cooking, this study provides evidence that the Manteca market class is worthy of germplasm enhancement as a new convenience food to help alleviate trace mineral deficiencies in regions where beans are widely accepted as a dietary staple.

Funding: USDA-NIFA

Table 1. Description, Sources, Cultivation Status and Cooking Times of the Five Genotypes Used to Evaluate the Iron Bioavailability of the African Yellow Bean.[1]

Name	Seed Type (*Market Class*)	Source	Cultivation	Cooking Time (min)[2]
Ervilha	Yellow (*Manteca*)	IIA; Huambo, Angola	Landrace	15.3 ± 0.22 ^{e}
Uyole 98	Yellow (*Amarillo*)	Tanzania Breeding	Variety	22.3 ± 0.37 ^{d}
PI527538	Yellow (*Njano*)	Burundi; US GRIN	Landrace	26.0 ± 0.63 ^{c}
Snowdon	White Kidney	Michigan State Unv.	Variety	29.4 ± 0.37 ^{b}
Red Hawk	Dark Red Kidney	Michigan State Unv.	Variety	36.8 ± 0.92 ^{a}

[1]This panel consists of medium to large Andean beans ranging from 58 - 81 g/100 seed. IIA, Instituto de Investigação Agronómica; US GRIN, U.S. Germplasm Resources Information Network. [2]Raw seed were soaked in distilled water for 12 hours prior to determining the number of minutes to reach 80% cooking time with an automated Mattson pin-drop device. Values are means ± SEM of four field replicates, each measured in duplicate (n = 8). Means sharing the same letter are not significantly different at $p \leq 0.05$.

Table 2. Iron, Phytate and Kaempferol 3-glucoside Analysis of Bean Based Diets.

Genotype	Ervilha	Uyole 98	PI527538	Snowdon	Red Hawk
					
Diet Analysis[1]					
Iron concentration (μg/g)	53.7 ± 1.5^{a}	46.5 ± 0.36^{b}	54.5 ± 0.91^{a}	47.4 ± 0.37^{b}	52.4 ± 1.1^{a}
Phytate : iron molar ratio	12.1 ± 0.69^{bc}	13.9 ± 0.16^{a}	11.3 ± 0.49^{c}	12.9 ± 0.92^{ab}	12.6 ± 0.86^{bc}
Kaempferol 3-glucoside (nmol/g)	153 ± 5.1^{c}	327 ± 18^{a}	234 ± 5.9^{b}	0.6 ± 0.1^{e}	1.9 ± 0.1^{d}

[1]Food ingredients were cooked, drained and lyophilized prior to milling into a course powder for chemical analysis. Values are means ± SEM of five replicates for each of the bean-based diets. Means sharing the same letter in each row are not significantly different at $p \leq 0.05$.

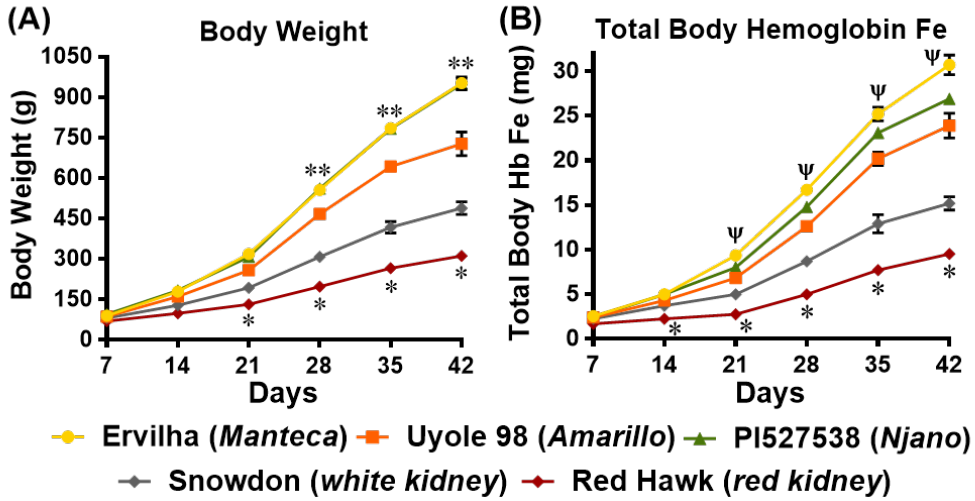

Figure 1. Body weight (A) and total body hemoglobin Fe (B) during the 6 weeks of consuming bean based diets formulated from yellow and kidney beans. Values are means ± SEM (n = 10 - 13 animals per treatment group). * Significantly ($p < 0.05$) lower values measured in the group receiving the Red Hawk diet. ** Significantly ($p < 0.05$) higher body weights measured in the groups receiving the Ervilha and PI527538 diets. Ψ Significantly ($p < 0.05$) higher total body hemoglobin Fe measured in the group receiving the Ervilha diet.

GENOME-WIDE IDENTIFICATION OF THE *VACUOLAR IRON TRANSPORTER (VIT)* GENES IN *Phaseolus vulgaris* AND *Glycine max*

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INTRODUCTION: Iron (Fe) deficiency is a major human nutritional problem (Brear et al., 2013). One of the solutions is the biofortification, that is the process of engineering plants to accumulate nutrients in their edible parts, at levels that can positively impact human nutrition (Sperotto et al., 2012). *VACUOLAR IRON TRANSPORTER* (*VIT*) genes are involved in the storage of Fe in seeds (Kim et al., 2006). Given the essential roles of VIT proteins in iron translocation, the aim of this study was broadening the knowledge of VIT beyond the eudicot models of Arabidopsis (*Arabidopsis thaliana)*. For this purpose, extensive analyses were conducted focused in find putative *VIT* genes in common bean (*Phaseolus vulgaris* L.) and soybean (*Glycine max*).

MATERIALS AND METHODS: In the literature is one annotated gene as *VIT* in the *A. thaliana* genome (AtVIT - AT2G01770), the first step was to BLAST the gene sequence against *A. thaliana* genome using *Phytozome* database. Only the genes with a similarity greater than or equal to 30% of the characterized sequence advanced in this study. Subsequently, the AtVIT gene sequence were used as a query to performer BLASTp and TBLASTx in Phytozome and Ensembl Gramene database to find sequences in *P. vulgaris* and *G. max* genomes. The sequences that correspond to each VIT or putative VIT were loaded from the databases for further analysis. The alignment of nucleotide and protein sequences were generated using ClustalW in Molecular Evolutionary Genetics Analysis - MEGA version 7.0 (Kumar et al., 2018).

RESULTS AND DISCUSSION: The Blast against *A. thaliana* genome using as input sequence the gene AtVIT, enabled to found other four genes. However, none of these genes showed an identity greater than 30% with the reference sequence (Figure 1A). Consequently, to perform the other analyzes, only the AtVIT gene were used. Looking at the bean genome nine genes were found; although, only three revealed high identity compared to AtVIT (Figure 1B). In the soybean, the blast demonstrated eight gene sequences similar to AtVIT, but of these only two genes have the acceptable identity to continue in the analyzes (Figure 1C). The Figure 1D shows that all the sequences selected have a high identity to AtVIT, supporting that the content of these sequences may have a similar biological function.

Different from Arabidopsis, it was found three and two putative *VIT* genes in common bean and soybean, respectively. Moreover, two of the genes found in common bean (Phvul.002G322900 and Phvul.002G322800) are tandem in the genome. These results suggest that a duplication may have occurred in these species. In order to have a greater reliability of the data, not only the amino acid sequences were analyzed but also the nucleotide sequences (Figure 2). In the Figure 2A each line corresponds to a similarity in the exon sequence between the common bean and soybean genes related to the Arabidopsis gene. Almost all the exons of common bean and soybean have a line with the *Arabidopsis* exons. This result demonstrates a great similarity among the nucleotide sequence of these genes, reinforcing the idea that they can be orthologs sequence and probably have the same biological function.

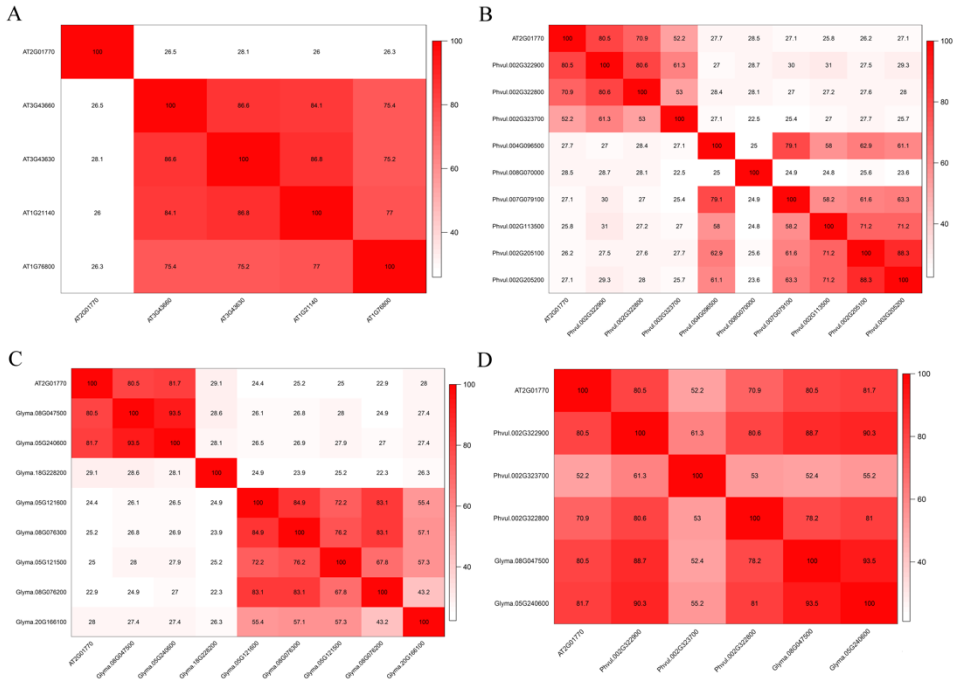

Figure 1. Heatmap board displaying pairwise sequence identity among protein sequences. A white-to-red gradient is used to represent low-to-high sequence identity. A) Sequences of genes from *A. thaliana*. B) Sequences of *A. thaliana* and *P. vulgaris*. C) Sequences of genes from *A. thaliana* and *G. max*. D) Sequences of genes from *A. thaliana*, *P. vulgaris* and *G. max*.

Only one exon from the common bean gene Phvul.002G323700 (Phv6) do not have similarity with the corresponding *Arabidopsis* exon. In order to confirm this result, a comparison among all the genes against the Phv6 gene was done (Figure 2B). It is possible to observe that not only Arabidopsis, but all the other sequences do not have similarity with the last exon of Phv6, suggesting a differentiation of the gene in relation to the characterized VIT gene. A late duplication can be occurred, that made this gene accumulate more mutations over time, with the last exon undergoing a greater number of changes.

In this study, it was possible identify three orthologs in common bean and two in soybean that may belong to the VIT family. These findings indicate that the *in-silico* approach used here successfully identified in a genome-wide context VIT gene family. Beside this, these candidate genes can help for the selection of lines for the genetic breeding and the deepening of these genes for biofortification of the common bean and soybean.

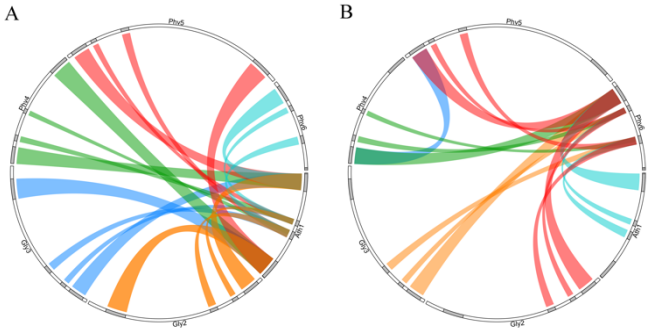

Figure 2. Circle graph demonstrating similarity between the putative VIT genes. In grey is the exon of each gene, in white is the intron. Each line is the sequence with similarity between different sequence. A) Comparison with the *A. thaliana* sequence. B) Comparison with the *P. vulgaris* sequence. Ath1 (AT2G01770), Gly2 (Glyma.08G047500), Gly3 (Glyma.05G240600), Phy4 (Phvul.002G322800), Phy5 (Phvul.002G322900) and Phy6 (Phvul.002G323700).

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EVOLUTION OF THE *VACUOLAR IRON TRANSPORTER (VIT)* GENES IN *Phaseolus vulgaris* L. AND *Glycine max*

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INTRODUCTION: Among the essential micronutrient for most organisms, Iron (Fe) is one of the most important. The vacuolar compartment emerged as the major iron source in seeds, and members of the VIT family are involved in the absorption of Fe into the vacuole for storage (Brear et al., 2013). Due the relevant roles of VIT proteins in the translocation of iron to the seeds, a study was conducted to identify orthologous genes. Motif discovery and gene structure proved to be highly effective for gaining deeper insights into the evolutionary relationships among the *VIT* gene family. Therefore, a phylogenetic analysis in the *VIT* gene family were conducted among three and two putative *VIT* genes of common bean (*Phaseolus vulgaris*) and soybean (*Glycine max*), respectively, found in previous studies.

MATERIALS AND METHODS: In the literature is one annotated gene as *VIT* in the *A. thaliana* genome (AtVIT - AT2G01770). Thus, in order to identify *VIT* genes and their homologs in *P. vulgaris* and *G. max*, a BLAST was done using Phytozome database. Three genes for *P. vulgaris* (Phvul.002G322900, Phvul.002G322800 and Phvul.002G323700) and two for *G. max* (Glyma.08G047500 and Glyma.05G240600) were found. The alignment of nucleotide and protein sequences were generated using ClustalW in Molecular Evolutionary Genetics Analysis - MEGA version 7.0 (Kumar et al. 2018). All conserved residue sites were used to reconstructed evolutionary relationships using Construct Maximum Likelihood Tree. For the determination of the gene structure of the sequences the web server GSDraw (Gene Structure Draw Server) in Plant Intron Exon Comparison and Evolution database (PIECE) was used. The conserved motifs were predicted via scanning peptide sequences through Multiple Em for Motif Elicitation (MEME) suite 4.11.2 web server. The protein structure was predicted using PROTTER. The sequence logo was constructed with WebLogo.

RESULTS AND DISCUSSION: Phylogenetic analysis demonstrated that the putative *VIT* genes were classified into three major clades (Figure 1A). The first clade is compost by the Arabidopsis gene and the two genes of *G. max*. This suggests that these two species have a similar sequence and that the two genes of *G. max* may be due to a recent duplication. The second clade is compost of only one gene (Phvul.002G322800), and the last clade has the other two genes of *P. vulgaris*. The genes Phvul.002G322800 and Phvul.002G322900 are in tandem in the genome. However, they are not present in the same clade. This may suggest that an accumulation of modifications can be occurred, making these two sequences not so similar.

The analyses of gene structure could help understand the gene functions, regulations, and evolution (Feng, Zhang, and Ebright 2016). According to the results shown in Figure 1B, the genes do not have differences in the number of exons and introns, as well as in the size of these elements, with exception of the last exon of Phvul.002G323700 that have a smaller size. All the sequences have four exons and all the phases of the introns are similar in the sequences. This analysis revealed a high degree of conservation among species regarding their gene structure which is also confirmed

by the frequency analysis of amino acids (Figure 2B), showing a high level of similarity the sequences are, except for the last exon.

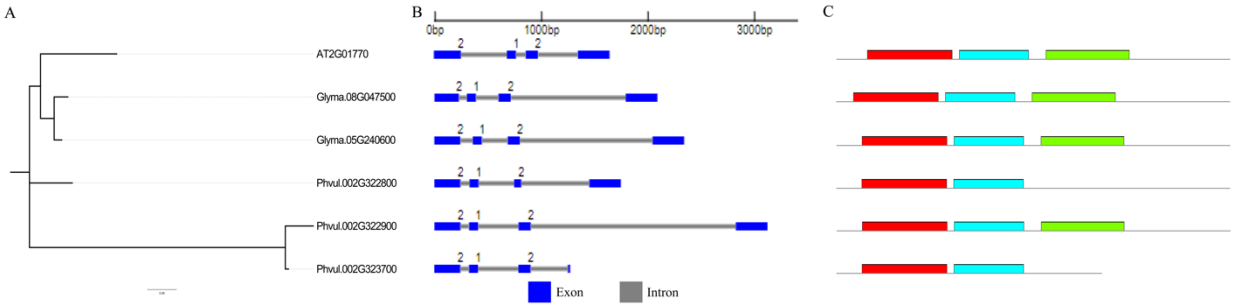

Figure 1. Gene structure schematic diagram for *VACUOLAR IRON TRANSPORTER (VIT)* genes in Arabidopsis, *Phaseolus vulgaris* and *Glycine max* genomes. (A) Compact phylogenetic tree showing the relationships between putative VIT protein sequences. (B) Exon-intron structure of putative *VIT* genes. (C) Motif analysis of VIT protein sequences. MEME analysis explored conserved motifs of the VIT proteins. The different color background indicated different conserved motifs.

Using the MEME program, three conserved motifs in VIT amino acid sequences were identified (Figure 1C). The lengths of the motifs varied from 41 to 50 amino acids. The genes from *Arabidopsis*, *G. max* and one gene of *P. vulgaris* (Phvul.002G322900) have all the three motifs while the other two genes of *P. vulgaris* have only two motifs. The last exon of Phvul.002G323700 is smaller, not being possible the presence of the motif in this sequence, whereas the Phvul.002G322800 gene may have some modifications that made it difficult to identify the motifs in the sequence. The absence of the last motif and/or last exon shows a possible change in the efficiency of the biological function or even a differentiation of the function, as we can see in Figure 2 that this region is potential transmembrane domain.

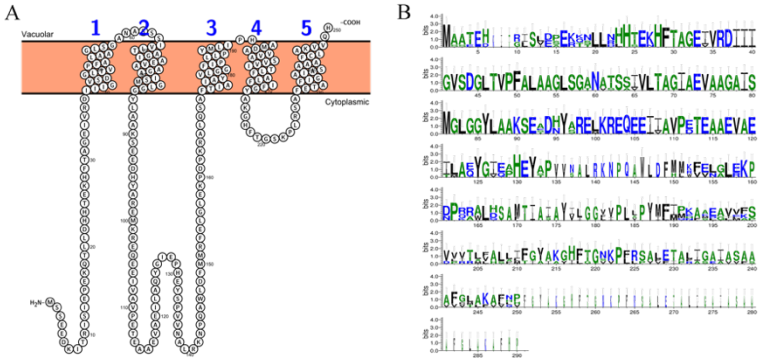

Figure 2. Protein structure of the VIT sequences. A) Predictions of the protein structure of AtVIT sequence, generated by PROTTER tool. B) Amino acid sequence logo of all putative VIT sequences. The logo was generated from WebLogo.

In this study, several associations became apparent when the six putative VIT protein sequences identified from Arabidopsis, *G. max* and *P. vulgaris* were used to construct a phylogenetic tree. The phylogenetic classification provided a basis for identify conserved motifs, which can be useful in the demarcation of class specificity. *P. vulgaris* have 3 putative *VIT* genes, but only one gene has all the three motifs, similar to the characterized Arabidopsis gene. This may suggest that the other genes may have a different biological functions or isoforms with different efficiency. These results contribute to the selection of marker genes for genetic breeding and especially in population studies of common bean and soybean genomes, since alterations in the sequences of these genes can lead to different degrees of efficiency in the transport of iron to the grain.

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EFFECT OF CO-INOCULATION OF *Azospirillum brasiliense* AND *Rhizobium* sp. ON LEVELS OF NITROGEN AND PHOSPHORUS AND PRODUCTION COMPONENTS IN COMMON BEAN (*Phaseolus vulgaris* L.)

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INTRODUCTION: Common bean presents the ability to fix atmospheric nitrogen when in symbiosis with nitrogen-fixing bacteria, which can contribute to reduce the consumption of nitrogen fertilizers. Studies have selected bacteria strains with greater specificity for the bean culture adapted to tropical soils, as is the case of the species *Rhizobium tropici*. In addition to *Rhizobium* species, there are other microorganisms that can bring benefits to the culture. One of the most promising groups is represented by the genus *Azospirillum*, classified as plant growth-promoting bacteria that act through several processes, such as the production of growth hormones and performing nitrogen biological fixation, solubilization phosphates present in soil, among others. Co-inoculation consists in using combinations of different microorganisms, which produce a synergistic effect, obtaining results that overcome those acquired from isolated species. The aim of this study was to evaluate the influence of Biological Nitrogen Fixation (BNF) on bacteria of the genus *Rhizobium* and *Azospirillum*, inoculated separately and in combination.

MATERIALS AND METHODS: The experiment was carried out on the experimental field of State University of Londrina, located in the municipality of Londrina, Paraná-Brazil (23°08'47"S, 50°52'23"W and altitude of 508 meters). Average annual temperature and precipitation stands around 20 °C and 1588 mm, respectively. The common bean genotype IPR TANGARÁ was used. The experimental design set up was a randomized block with 4 seed treatments, represented by 1 - Witness treatment, without inoculation; 2 - Inoculated with *Rhizobium tropici* alone; 3 - Inoculated with *Azospirillum* alone; and 4 - Co-inoculation with *Rhizobium tropicium* and *Azospirillum*, with 5 repetitions, thus 5 blocks. A total of twenty experimental plots were formed, each consisting of 8 lines of 5 meters in length, spaced at 0.45 m, with 10 plants per linear meter. The working area was formed by the 4 central lines, eliminating 1 m from each line's end. Sowing was done using the amount of seeds sufficient to obtain a density of 10 to 12 plants per linear meter, on March 20, 2018. In the full bloom stage (R6), five consecutive plants were collected, packed in a bag paper, taken to the laboratory, separated in root and aerial part and then submitted to forced air circulation drying at an average temperature of 65 ºC until reaching a constant mass. Subsequently the levels of N and P were determined, according to the methodology of Malavolta et al. (1989). Harvesting of the useful area was performed 100 days after sowing (DAS). Components of the production, represented by the number of pods per plant, number of grains per pod and mass of 100 grains were evaluated from 5 plants per block, standardizing grain humidity at 13%. The data were evaluated through analysis of variance and the means were compared by Fisher's test at 5% of significance.

RESULTS AND DISCUSSION: There was no significant difference in nitrogen (N) and phosphorus (P) contents and accumulation in the aerial part expressed in Kg^{-1} (TABLE 1). Similar results were obtained by Hungria et al. (2013), whose work did not verify differences among treatments - a) non-inoculated, b) nitrogen fertilization, c) inoculation without nitrogen fertilization, d) inoculation with *A. brasilense*, e) inoculation with *R. tropici* and f) co-inoculation - in common bean cultivated in the rainy season of 2009/10 in Londrina for the content of nitrogen

from the aerial part. According to Sá and Israel (1991), phosphorus has a direct influence on the initial formation, growth and nodules' functioning. Israel (1987) emphasized that high phosphorus requirements are necessary for the biological N fixation, so that an increase in phosphorus supply promotes an increase in the activity and accumulation of dry phytomass of the nodule. In the present work, the number of grains per pod, pods per plant, mass of 100 grains and dry mass per plant did not have significant difference among the four seed treatments (TABLE 1). This result can be explained by the high incidence of anthracnose disease, caused by the pathogen *Colletotrichum lindemuthianum*, at the reproductive stage. Nevertheless, Peres (2014) detected significant differences in dry mass of bean shoots inoculated with *Azospirillum brasiliense*. Although the absence of significant statistical difference, the number of pods per plant was higher in the co-inoculation with *Rhizobium*, *Azospirillum* and co-inoculated treatment than untreated control. As a result, different inoculation treatments on common bean did not affect the culture's yield.

Table 1 - Number of grains per pod, pods per plant, mass of 100 grains and dry mass per plant related to common bean seed inoculation with *Rhizobium tropici* and *Azospirillum brasiliense* alone and co-inoculated compared with untreated seeds.

Treatments	Nitrogen	Phosphor	Grains per Pod	Pods per Plant	Weight of 100 grains	Dry mass
	--- g kg^{-1} ---	--- g kg^{-1} ---			---- g ----	---- g $plant^{-1}$ ----
1-Untreated control	47,04	3,43	4,93	10,85	25,77	8,64
2-*Rhizobium tropici*	46,90	3,21	5,47	12,17	24,28	9,72
3-*Azospirillum brasiliense*	48,35	3,44	5,66	12,07	27,06	9,45
4-*Rhizobium* + *Azospirillum*	45,06	3,38	5,19	12,70	25,07	9,36
Average	46,84	3,36	5,31	11,95	25,55	9,29
C.V. (%)	8,92	7,36	10,64	11,26	8,41	17,31
Fc	0,526	0,526	1,267	1,986	1,506	0,499
Pr>Fc	0,6727	0,6727	0,3296	0,1699	0,2632	0,6899

Treatments means don't differ from each other according to Fisher's Test (f<0,05).

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UREIDES SAP CONTENT IN COMMON BEAN GENOTYPES SELECTED FOR HIGH-NODULATION EFFICIENCY

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INTRODUCTION

The ability to common bean plants establish a symbiotic interaction with N_2-fixing Proteobacteria of the Rhizobiaceae family is an important evolutionary trait that has been considered in the Embrapa common bean breeding program. In previous stages of breeding *per se*, genotypes with high efficiency in nodulation were selected to be tested in field trials carried out to evaluate a series of traits of interest, prominently related to biological nitrogen fixation (BNF). Considering that ureides comprise up to 90% of the total N transported in the xylem of N_2-fixing tropical legumes (BARAL et al., 2016), its quantification becomes an important additional parameter to select potential parents for common bean breeding programs focused on BNF. This work presents results of the field evaluation of 15 common bean genotypes previously selected in a greenhouse screening which the ureides sap contents were quantified by contrasting the performance of those genotypes under N fertilization and under *Rhizobium* inoculation.

MATERIALS AND METHODS

The goal of this study was evaluating in field conditions the ureides sap content of 15 common bean genotypes previously selected for nodulation efficiency in a greenhouse screening (KNUPP et al., 2017). A field trial was carried out in the rainy growing season of 2013 in Santo Antônio de Goiás, GO, Brazil. The 15 genotypes and the control cultivars Pérola and Ouro Negro were evaluated under two N sources: seed inoculation with 10^9 CFUs. g^{-1} peat *Rhizobium* mix (strains SEMIA 4077, 4080 and 4088) or N fertilization (80 kg N ha^{-1}). A split plot design arranged in randomized complete block was used with N sources as main plots and genotypes as sub-plots. The quantification of ureides sap content was carried out as described by Hungria & Araújo (1994). Data were submited to a variance analysis and the averages were compared by the Tukey's test at 5% of significance.

RESULTS AND DISCUSSION

The statistical differences verified for ureides sap content are influenced by genotypes, N sources and the interaction between both (Table 1). The content averages obtained by the inoculated genotypes were higher than three times those obtained by the fertilized genotypes (61.94 and 18.14 nmol mL^{-1}, respectively) (Table 2). The genotype PI 313633, in addition to be among the highest performing genotypes for ureides sap content under fertilization, was the superior under inoculation, with 142.31 nmol mL^{-1} (Table 2), suggesting a strong ability to carry N in the form of ureide in sap. The ureides, which in the nodule has its origin in "de novo" synthesis of purines, in N-fertilized plants may have been synthetized from purines derived from nucleotide metabolism (BARAL et al., 2016). Ureides sap concentration were higher in inoculated plants (Table 2), indicating that it can be associated to the BNF and the internal N remobilization in fertilized plants.

Table 1. Analysis of variance for ureide sap contents of common bean genotypes sampled at flowering stage (R6) in field trial in Santo Antônio de Goiás (GO), Brazil (rainy growing season of 2013).

Variation source	DF	Ureides sap content
Blocks	2	6.60^{ns}
N source (S)	1	5.18×10^{4}**
Residue	2	26.99
Genotype (G)	17	3.32×10^{3}**
Interation GxS	17	3.18×10^{3}**
Residue	36	26.62

*, **, ***: Significant at the levels 5, 1 e 0,1% by F Test.

Table 2. Ureide sap contentes of common bean genotypes sampled at flowering stage (R6) under N-fertilization (40+40 kg N ha^{-1}) and *Rhizobium* inoculation, in a field trial carried out in Santo Antônio de Goiás (GO), Brazil (rainy growing season of 2013).

Genotype	Ureides sap content (nmol mL^{-1})	
	N-fertilization	*Rhizobium*
01. CNF 0011234	17.80 c	126.76 b*
02. CNF 0011559	38.06 a	15.03 gh*
03. CNF 0011239	16.49 c	91.72 c*
04. PI 209491	14.24 c	121.57 b*
05. PI 387865	33.58 ab	54.84 ef*
06. CNF 0011228	9.32 c	47.70 f*
07. CNF 0011026	15.98 c	83.96 cd*
08. CNF 0011137	18.69 c	51.30 f*
09. CNF 0011075	14.65 c	128.19 ab*
10. CNF 0011095	17.48 c	27.68 g*
11. CNF 0011240	15.28 c	9.81 h
12. CNF 0011028	9.75 c	27.68 g*
13. PI 325750	16.22 c	8.47 h*
14. PI 313633	33.66 ab	142.31 a*
15. CNF 0011086	14.59 c	70.02 de*
16. Pérola	10.05 c	81.30 cd*
17. Ouro Negro	12.08 c	15.80 gh
18. NORH 54	18.52 bc	10.71 h
Average	18.14	61.94*
CV (%)	13.02	

Averages followed by the same letter in the columns don't differ from each other at the 5% level by Tukey test. * Significant difference between N sources by F Test at the 5% level.

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MAIZE SILAGE PROTEIC AND MINERAL ENRICHMENT USING COMMON BEAN FRESH BIOMASS IN DURANGO, MÉXICO

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INTRODUCTION: In Durango, maize is the most important crop for silage production showing low levels for crude protein (8 %) compared to daily requirements of growing calves (16 %). Leguminous crop biomass have a relatively high protein content but this group of plants are difficult to ensile due to their low content for dry matter and water soluble carbohydrates. Legumes are used to enrich maize pastures with enhanced protein content (Goyal and Tiwana, 2016) and to produce large quantities of high quality silage mixtures at low cost. Traditional common bean climbing cultivars produce large quantities of biomass, which is considered as a desirable trait in order to increase ensiled forage production, silage quality and livestock productivity in Durango, México. The main objective was to assess the nutritional value of common bean fresh biomass as a protein and mineral source for maize silage enrichment.

MATERIALS AND METHODS: Commercial maize hybrid (Aspros®) plants were harvested at 20 cm from the ground level at 1/3 of the milk line stage (35 % for dry matter content) in Durango, Dgo. Common bean (cv. Río Grande) was harvested at the R_7 phase (pod setting). Above ground biomass of maize and common bean plants were chopped (minced) separately to a particle size of 2 to 4 cm. Sugar cane molasses was added to improve the silage fermentation process. A completely random design with a factorial arrangement (5 x 3) was used, including 15 treatments and three replications. Treatments consisted of five maize:common bean mixture ratios (1000:0, 750:250, 500:500, 250:750, 0:1000 g kg^{-1}) and three levels of molasses (0, 50, and 100 g kg^{-1}). Forage mixtures were homogenized before the addition of molasses and then packed in (10 kg) black polyethylene bags and put into 19 L PVC silos. The bags were sealed with a rubber band and the silos were closed and stored during 50 days period at room temperature. The silos were opened and silage was analyzed for crude protein, ash content (AOAC, 2016), Neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest *et al*., 1991). Readings for pH were performed in silage mixtures (potentiometer) and trypsin inhibitors were also determined (AOCS, 2009) and reported as trypsin inhibitor units per mL (TIU mL^{-1}).

Eight male calves (eight months old) with an individual average live weight (LW) of 327.2 ± 19.8 kg were used for the feeding trial. The feeding trial was performed under a Latin Square experimental design with four replications. Treatments included two complete mixed rations: 1) maize silage and 2) maize silage + 250 g kg^{-1} of common bean. An adaptation period was implemented, feeding trial started on the 54th day and bovines were fed with complete daily-based rations. The amount of consumed food was registered daily, freshwater was provided *ad libitum,* and the individual bovine LW was determined every 14 days. Analysis of variance was performed and when statistically significant differences were observed mean comparisons were performed by Tukey test ($P \leq 0.05$).

RESULTS AND DISCUSSION: Significant differences ($P \leq 0.05$) were observed for all the variables included in the chemical analysis. Higher ash values were found in silage mixtures containing 1000 g kg^{-1} of common bean biomass (Table 1). Common bean biomass also increased crude protein (CP) content (Table 1) among silage mixtures. Molasses addition caused significant reductions in CP level due to a dilution effect. Results suggest that common bean biomass is an essential source for protein and mineral enrichment in maize silage mixtures. Common bean biomass can contribute to reducing bovine feeding costs and supplementation requirements for raising calves.

Higher levels of neutral detergent fiber (NDF) were observed for maize silage with variations between 46.2 to 53.6 %, and significant reductions were registered according to increments in common bean biomass (43.2 to 52.5 %) and molasses addition level, favoring animal intake. High levels for ADF

($P \leq 0.05$) were observed in most of the molasses free silage mixtures (29.5 % to 36.4 %). Similar results were included in previous reports where NDF and ADF concentration were diluted by the additives and the ensiling process, due to the acids produced during fermentation (Bilal, 2008).

Lower pH levels were observed in maize silage (3.4 to 3.6) and higher values were related to common bean biomass (5.6 to 5.8) affecting silage quality. Considering protein content and the buffering capacity, low inclusion of common bean biomass (250 g kg^{-1}) is recommended during maize ensiling process. Significant ($P \leq 0.05$) increment for trypsin inhibitors (TI) was observed in silage mixtures including common bean. Higher levels for TI were registered in common bean silages (7.7 to 8.1 TIU mL^{-1}), showing stable response during fermentation and then affecting protein bio-availabilty. Similar values were observed in both complete rations for total LW gain, daily consume and daily LW gains (Table 2).

CONCLUSIONS: Common bean fresh biomass is an alternative source for protein and minerals in order to enrich maize silage. Reductions were observed for maize silage quality and bovine preference when common bean biomass was included in maize silage. Silage mixtures containing 250 g kg^{-1} of common bean biomass showed an adequate pH level and bovine preference in complete rations. High stability was observed for trypsin inhibitors affecting silage protein bio-availability. Ingredient combinations used in complete rations for calve feeding reduced negative effects observed when common bean biomass is used in silage mixtures. The use of common bean biomass for maize silage enrichment maintained live weight gains in rising calves and reductions for feeding costs could be also achieved.

Table 1. Component levels observed in maize:common bean silage mixtures.

Treatment M:CB[1] g kg^{-1}	Molasses (%)	Ash (%)	CP (%)	NDF (%)	ADF (%)	TI (TIU mL^{-1})
1000:0	0	6.4±0.4	7.3±0.4	53.6±2.5	32.2±2.5	1.9±0.03
	5	7.0±0.8	6.8±0.2	51.1±2.1	30.6±1.5	1.8±0.01
	10	6.9±0.3	6.9±0.9	46.5±2.3	29.6±2.2	1.5±0.04
750:250	0	7.0±0.2	8.6±0.8	52.5±2.5	29.5±2.3	2.5±0.05
	5	7.4±0.3	7.8±1.4	48.9±2.2	30.8±2.1	2.3±0.26
	10	7.3±0.4	7.6±0.6	44.9±2.3	28.9±2.1	2.6±0.21
0:1000	0	9.2±0.4	15.3±1.9	50.2±2.0	35.1±2.8	7.7±0.04
	5	9.1±0.5	12.4±2.1	46.7±1.8	31.6±1.8	7.8±0.45
	10	9.4±1.1	12.6±1.3	44.2±2.8	29.3±1.0	8.1±0.08

[1]M:CB = maize: common bean silage mixtures, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber, TI = trypsin inhibitor.

Table 2. Weight gain observed in two silage mixtures evaluated in bovine cattle feeding.

Trait	Maize: Common bean silage 750:250 (g kg^{-1})	Maize silage
Total Live Weight Gain (kg)	166.3±26.3	161.0±28.7
Daily consume on dry weight basis (kg)	9.0±0.03	9.2±0.10
Daily Live Weight Gain (kg)	2.0±0.6	1.9±0.7

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BEAN YIELD AND ITS COMPONENTS DEPENDING ON THE SHADING AND NITROGEN

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INTRODUCTION: The amount of solar radiation (SR) and nitrogen fertilization (NF), are decisive in the production of crops. Some species and cultivars to express higher growth and yield, require less intensity of SR. This intensity can be manipulated by shading with meshes, which prevents the temperature inside the canopy and the photoinhibition from rising (Bustamante, 2001). Escalante *et al*. (2013) in "Strike" snap bean under glasshouse, found 50 days after sowing, greater height of plant, leaf area and biomass with 50% shading and NF. Escalante *et al*. (1980) reported that 75% of shading, in the post-flowering stage, did not affected of grain yield of bean Michoacán 12A3. This suggests that the effect of the shading is in function of the cultivar and time of application. On the other hand, with NF is generated a greater leaf area, intercepted radiation, growth and yield of crops (Escalante and Rodriguez, 2010). Thus, the combination of shading and NF, could generate more appropriate conditions to express a greater growth and yield in some crops. The objective of the present study was to determine in snap bean cultivar "Strike", the effect of the 50% of shading and NF from planting on bean production and its components.

MATERIALS AND METHODS: The planting of the green bean cultivar "Strike" of determinate growth habit, was on 10 August 2015 to the density of 6.25 plants m^{-2} under field and rainy conditions, in Montecillo, Texcoco, State of México, Mexico (19 ° 29' N , 98 ° 53' W and 2250 meters of altitude) of temperate climate, in soil-clay, with 50 kg of N assimilable and pH of 7.0. The treatments were: 1) shaded with black mesh and N; 2) shaded with black mesh and without N; 3) without shading with N; and 4) without shading without N. The experimental design was randomized blocks with arrangement of split plots and four replications. The main plot was the shading and the minor the NF (100 kg N ha^{-1}). Twenty plants were harvested by treatment to register per m^2: the number of green beans (PN), bean yield (fresh weight of pod, PY) total of three harvest, and the final harvest was recorded the leaflet number (LN), raceme number (RN) and nodes number (NN). Were applied an analysis of variance and the mean comparison test (Tukey, 0.05). The maximum temperature (Tmax, ° C), minimum (Tmin, ° C) and rainfall (PP, mm) were recorded.

RESULTS AND DISCUSSION: The days to occurrence to phenologycal stages were similar between treatments. Thus the emergency and start of flowering was to 8 and 35 days after sowing, respectively. Low temperature during reproductive allowed only three harvest of pods to 55, 65 and 75 das. The Tmax and Tmin average during the vegetative stage was 36 ° C and 8° C and in the reproductive of 26° C and 6° C, respectively. The PP during the development of the crop was 412 mm (59% in the vegetative stage and 41% in the reproductive). The bean under shading presented 59% and 20% higher PY and PN than without shading, respectively. The NF increased at 28 and 58% the PY and PN (table 1). There were not effects of shading* NF interaction. The average PY was 220 g m^{-2} with 120 green beans m^{-2}, and is within the range of PY (195 to 483 g m^{-2}) reported by Salinas *et al*. (2012). The RN, LN and NN were not affected

by the treatments and was in average of 67,183 and 76 m^{-2}, respectively. These results indicate that shading 50% causes positive effect on the PY and PN of the bean "Strike", suggesting a possible reduction of the photoinhibition (Bustamante, 2001) and of the temperature inside the canopy, which decreases the fall of pods and with fertilization nitrogen bean production is increased.

Table 1. Snap bean yield, green beans number, raceme number, leaflet number and node number of Strike beans grown under conditions of shadhing and nitrogen ferilization. Montecillo, Texcoco, State of Mexico. Mexico. Summer 2015.

Treatment Shading (50%)	Pod yield (PY, g m^{-2})	Pod number (PN) m^{-2}	Racemes number (RN) m^{-2}	Leaflet number (LN) m^{-2}	Node number (NN) m^{-2}
S+	270 a	131 a	62 a	182 a	65 a
SO	170 b	109 b	72 a	184 a	88 b
TUKEY0.05	68	14	40	52	4
Nitrogen					
N100	305 a	147 a	71 a	194 a	77 a
N0	134 b	93 b	63 a	173 a	76 a
TUKEY0.05	29	11	16	81	21
Mean	220	120	67	183	76

S+ = Shading; S0 = no shading; N100 = nitrogen fertilizer; N0 = without nitrogen fertilizer.In columns values with similar letter are statistically equal.

CONCLUSION: The 50% shade with black mesh and the nitrogen fertilization increase the production of green beans in the "Strike" bean cultivar. The raceme number, leaflets number and node number are not affected by the shading and application of nitrogen.

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BREEDING AND SELECTION OF COMMON BEAN THROUGH MIXED MODELS FOR DROUGHT RESISTANCE

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) crop has an essential fundamental social and economic importance. This crop shows a prominent role as a cheap and efficient source of proteins, fibers, micronutrients, among other components, which are vital for human diet. The rainfall shortage is the main abiotic factor that directly affects the grain yield, reaching around 60% of world production (Urrea et al. 2009). Moreover, the study of the response of the plants submitted to a drought condition is an important strategy to understand the abiotic stress in common bean, since it is a limiting factor in the production at the global level. In different regions where common bean is cultivated, there are stresses due to the lack of water during key growth stages of the plant, which can be considered intermittent or terminal. Given the importance of common bean, the development of more productive and adapted cultivars to water deficit conditions is necessary. Therefore, this work had as objective to evaluated elite lines under conditions of water stress.

MATERIAL AND METHODS

A total of 169 $F_{2:5}$ and $F_{2:6}$ progenies, derived from the cross between IAPAR81 × LP97-27 were evaluated. The cultivars Juriti, Tangara, Guará, Talismã, Flor Diniz, BAT93, and Pérola were used as controls. Two experiments were conducted at Centro de Treinamento em Irrigação (CTI) of Universidade Estadual de Maringá during the 2014 and 2015 crop seasons. Each experiment consisted of one group without water deficit and the other with water deficiency at flowering. Each experimental unit was a 10 × 10 simple lattice with 1-m length, spaced 0.50 m apart, containing 12 plants of each progeny, by using a 10 × 10 Square Lattice design (Cruz 2013), with three replicates. To assess drought tolerance of the progenies, we evaluated the following agricultural features of interest: grain yield (GY), yield per day (PPD), number of pods per plant (NPP), weight of 100 seeds (W100), plant height (PH), days to flowering(DF), days to maturation (DM), and days to grain filling (DFILL). BLUP approach was adopted to predict genotypic values, using the estimates of variance components as random effects obtained by REML and to performance these analysis procedure we used the model 54 of the SELEGEN REML/BLUP software (Resende 2007, 2016).

RESULTS AND DISCUSSION

Joint analysis of deviance (ANADEV) revealed that only the trait **DGF did not show significant difference in both experiments**. Instead, through REML variance components, it was possible to select promising progenies derived from the cross Iapar 81 × LP97-27. BLUP approach (optimal selection procedure for additive genetic effects) and selection pressure of 15 for both experiments assisted us in the identification of superior progenies based on all agricultural traits evaluated (Figure 1). According to ANADEV, the progenies that achieved the best results without water deficit were 18, 19, 27, 57, 58, 91, 125, 130, 138, 156, 199, 219, 220, 248, 266, 284, 285, and 306. Further, the progenies that had the best performance under stress condition were 27, 29, 102, 123, 130, 176, 199, 201, 219, 224, 244, 245, 257, 273, 279, 287, 290, 311, 312, 340, and 344.

Progeny 19 produced 91.97 kg ha^{-1}more than control, commercial cultivar and susceptible progenitor Iapar 81 at experiment carried without water stress. Under water stress condition, progeny 130 was 24.83 kg ha^{-1} more productive than the control with best control performance (Guará commercial cultivar). Taking into consideration the GY, W100 and PH traits, the progenies 19, 57, and 156 revealed the best results in the experiment with water deficit; whereas under water stress the progenies 344 and 27 showed the best results. A combination of GY, W100 and NPP features demonstrated that without water deficit the progenies 19, 266, 156, 58, and 57 were the best ones, while under water stress the progenies 287, 312 and 27 achieved the best performance. The lines 19, 156, 57, and 27 under water stress exceeded the overall, highlighting the promising common bean lines that could be recommended for cultivation in the South region of Brazil. Our results demonstrate that it was possible to obtain genetic gains for the line 27, showing its high genetic potential.

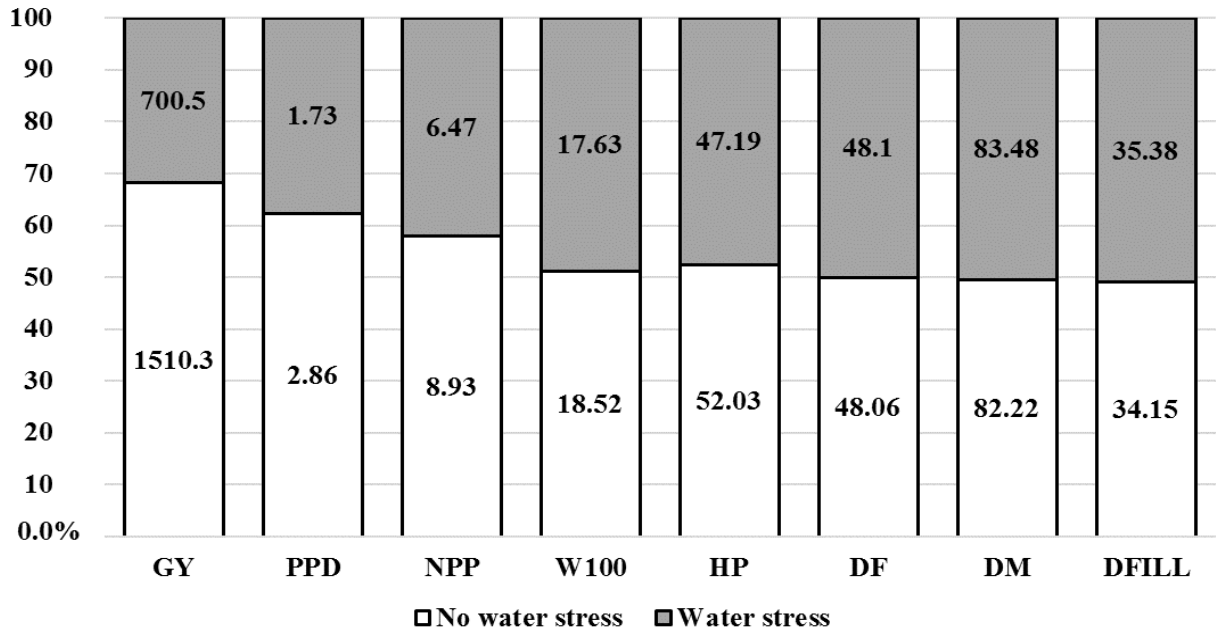

Figure 1. Comparison among means of evaluated variables in the experiments without water stress and with water stress, where: grain yield (GY), productivity per day (PPD), number of pods per plant (NPP), weight of 100 seeds (W100), height plant (HP), days to flowering (DF), days to maturity (DM) and days to grain filling (DFILL). Experiments were performed during 2014 and 2015 crop seasons.

ACKNOWLEDGEMENTS

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STARCH GRANULES IN COTYLEDONS OF DOMESTICATED AND WILD GERMINATING SEEDS OF *Phaseolus coccineus* L.

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INTRODUCTION: The starch granules of *P. vulgaris* L. seeds measure from 10 to 30 µm and are elliptical or spherical in shape (Kouichi, *et al*., 2002), while those of domesticated *P. coccineus* L. measure from 25 to 65 µm and are oval and round in shape (Bernardino-Nicanor, *et al*., 2017). Starch grains are used as tools to interpret the interaction, domestication, use and consumption of plants, as well as in the taxonomic identification of archaeological specimens (Babot *et al*., 2007). We set out to count and measure the starch grains in cotyledons of germinated seeds of domesticated and wild *P. coccineus* L. to know if the changes occurred in the carbohydrates, result of the domestication process, this is determined by modifications at the anatomical level.

MATERIALS AND METHODS: Two collections of domesticated *P. coccineus* L. were evaluated: an improved variety, Blanco Tlaxcala, and a native one collected in Tlayacapan, Morelos, Mexico; and two collections of wild *P. coccineus* L.: one from Ocotitlan, Morelos, and another from Teztcutzingo, Texcoco, Mexico. The starch content was determined with the enzymatic hydrolysis method. It included the starch overheating and the action of β-glucosidase (10 U, Sigma-Aldrich) and α-amylase (1 U, Sigma-Aldrich) enzymes, and sodium acetate. Number and dimensions of starch granules: four seeds of each variety were disinfected, scarified with a scalpel on the opposite side of the hilium, placed in 9 cm diameter Petri dishes with filter paper discs, moistened with distilled water and kept at a constant temperature of 25 °C. Once the radicle was visible, in each seed four cross-sectional histological cuts were made in the middle of the cotyledon. The slices were infiltrated, included in paraffin, dehydrated with graduated series of ethanol and stained with peryodic acid and Schiff's reagent (Johansen, 1940). Four 10 µm thick cuts, per seed, were made with a manual microtome, and semi-permanent preparations were made. With a Zeiss photonic microscope, model Axioscop 2, the characteristics of the starch grains were analyzed in a clear field at a 40x magnification. Images were taken with an Amscope digital camera, and in the Image J software, the quantitative attributes of the starch grains were examined. With the SAS program (SAS, 2012) an ANOVA and a comparison test of Tukey means (0.05) was performed.

RESULTS AND DISCUSSION: The cotyledon from domesticated seeds of *P. coccineus* L. showed 610 grains per mm^2, and that of wild seeds 956, significant difference (Tukey 0.05). The starch granules of the domesticated *P. coccineus* L. measured 25 µm long x 19.3 µm wide (average of 172 granules); and the wild ones measured 17.6 µm long x 13.3 µm wide (average of 170 granules) Significant difference (Tukey 0.05). The results suggested that lowest starch content per gram in cotyledons of domesticated beans, was related to changes occurred in the starch granules during the domestication process of the species. Also, we observed the presence of empty grains, so perhaps their content was already being used in the germination process (Table 1 and Figure 1), since upon completion of germination, the amyloplast membrane disintegrates and initiates the hydrolysis of the starch (Bewley *et al*., 2013).

Table 1. Starch content, number of starch grains, and length x width in domesticated and wild *P. coccineus*.

	Domesticated		Wild	
	Blanco Tlaxcala	Tlayacapan	Ocotitlan	Teztcutzingo
Starch (µmoles .g)	83.6 (71.3-95.7) 9.7 b	81.1 (60.0-103.8)17.7 b	200.9 (145.7-242.4) 32.6 a	174.7 (98.9-238.7) 66.0 a
Number of grains (mm^2)	845 (632-1088) 232 ab	375 (294-485) 80 c	1132 (912-1323) 194 a	779 (720-853) 57 b
Length (µm)	22.7 (12.6-34.8) 5.1 b	27.3 (11.4-49.4) 8.8 a	17.0 (8.9-26.4) 4.3 c	18.1 (10.8-35.8) 5.1 c
Width (µm)	16.7 (10.8-29.9) 3.5 b	21.9 (8.6-49.4) 7.3 a	13.4 (6.5-20.0) 3.0 c	13.2 (7.0-23.3) 3.7 c

Means (minimum value-maximum value) standard deviation. Different letters in the same line, indicate significant difference between genotypes (Tukey 0.05).

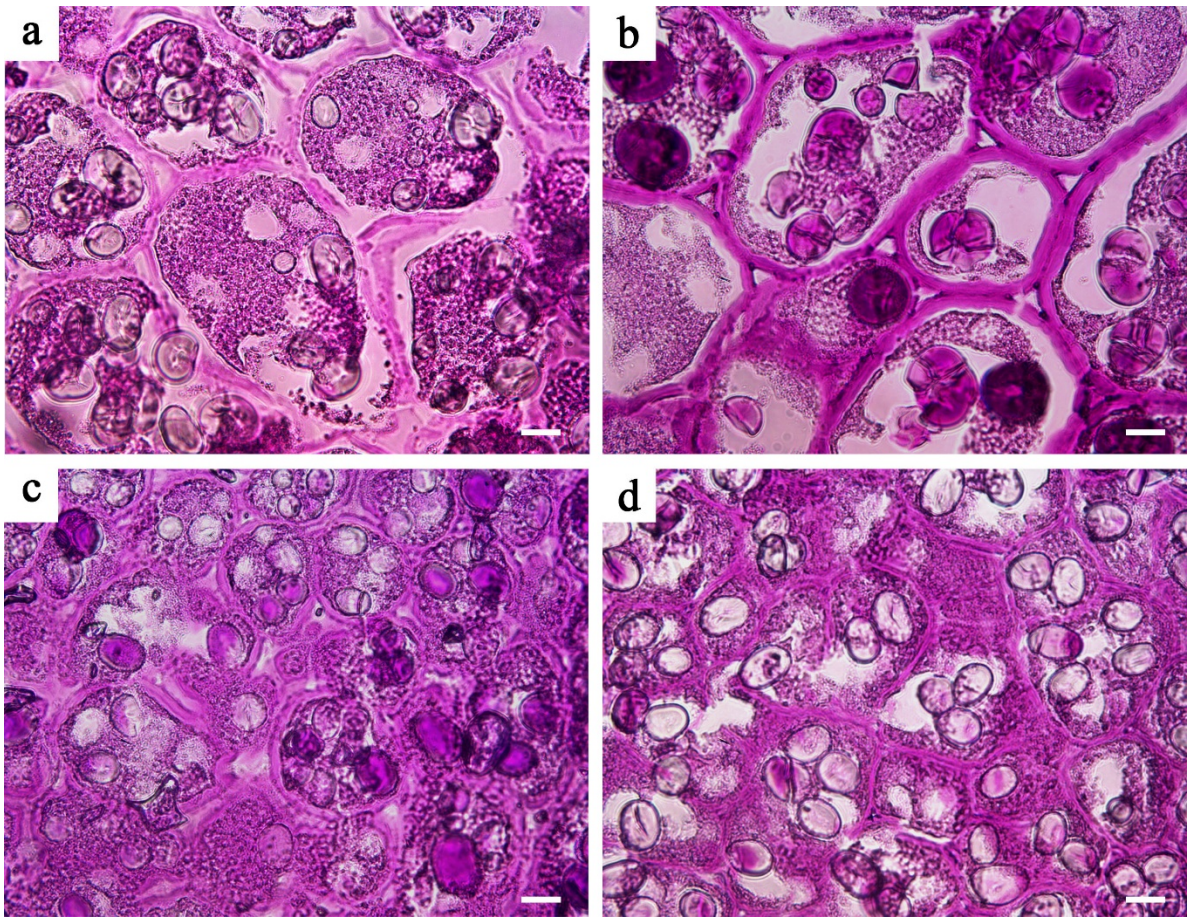

Figure 1. Starch granules of the domesticated and wild *P. coccineus* L. **a.** Blanco Tlaxcala; **b.** Tlayacapan; **c.** Ocotitlan; **d**. Teztcutzingo. Scale bar = 10 µm

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PHYSIOLOGICAL QUALITY OF "CARIOCA" BEAN GENOTYPES SEEDS

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INTRODUCTION: Carioca beans stand out among the different types of beans consumed and produced in Brazil. Thus, due to higher preference, breeding programs have introduced cultivars mainly with that type of grain. The highest yields are associated with the interaction between genotypes and environments, technological level, use of quality seeds and, mainly, the use of improved cultivars (MAMBRI *et al*., 2015; BARILI *et al*., 2016). Hence, the evaluation of the physiological quality of seeds, characterized by the seeds germination and vigor of the bean lines, can aid and assure the recommendation of new cultivars. Therefore, the objective of this work was to evaluate the germination and seeds vigor of common beans elite line of the Carioca class.

MATERIALS AND METHODS: The experiment was carried out at the Laboratory of Seed Analysis, at State University of Montes Claros, Campus de Janaúba-MG. The used seeds were from the Experimental Farm of the Federal University of Viçosa, Coimbra-MG, harvested in the autumn-winter of 2012. Bean seeds were used from 21 elite lines and four cultivars from the VCU of Carioca market class. The experimental design was a randomized block one with three replications. From each experimental plot, 50 seeds were randomly collected for the evaluations. The germination percentage (GER) (BRASIL, 2009) and seeds vigor, evaluated from emergence speed index (ESI), according to Maguire (1962), as well as the dry mass of seedlings (DM). The data were submitted to analysis of variance and when significant, the effects were studied by the Scott-Knott test, at 5% of significance

RESULTS AND DISCUSSION: The cultivars and evaluated elite lines showed similar germination (77.3 and 92%). There was a significant effect of the genotypes on seed vigor characteristics (ESI and DM). The lines EMB4, EMB9, EMB14, CNFC 10408, CNFC 10432, CNFC 10763, CNFC 11965, MAIV-15204, MAIV-18259, MAIV-18524, RCII-219, VC-18, VC-21, and VC-22, and the cultivar BRSMG Talismã, obtained the highest ESI values. These results demonstrate good physiological potential of the seeds with positive manifestations of vigor by emerge faster and evenly. The cultivars Pérola, BRSMG Talismã and nine lines obtained the highest values of dry mass of plantlets (Table 1). Therefore, these genotypes, for accumulating larger dry mass, have higher vigor of plantlets.

Table 1: Mean values of germination percentage (GER), emergence speed index (ESI), and dry mass of plantlets (DM) of Carioca bean genotypes

GENOTYPE	GER (%)	ESI	DM (g)
BRSMG Madrepérola	82.0 a	9.8 b	79.9 b
BRS MG Majestoso	87.3 a	10.4 b	72.8 c
Pérola	87.3 a	10.5 b	90.2 a
BRSMG Talismã	88.7 a	11.3 a	81.4 a
CVIII-2	77.3 a	10.5 b	83.1 a
CVIII-5	82.7 a	9.9 b	79.4 b
EMB4	86.7 a	11.2 a	84.2 a
EMB9	85.3 a	11.3 a	82.9 a
EMB14	84.0 a	11.8 a	79.2 b
CNFC 10408	79.3 a	10.8 a	73.8 c
CNFC 10432	85.3 a	11.1 a	72.2 c
CNFC 10763	84.0 a	10.9 a	72.5 c
CNFC 11965	87.3 a	11.8 a	71.1 c
MAIV-15204	83.3 a	10.7 a	87.3 a
MAIV-18259	88.0 a	11.5 a	90.3 a
MAIV-18524	77.3 a	11.0 a	87.1 a
P-18163	76.0 a	9.7 b	68.8 c
RCII-219	89.3 a	11.8 a	89.8 a
VC-17	92.0 a	10.4 b	75.2 c
VC-18	87.3 a	11.1 a	73.4 c
VC-19	84.0 a	10.2 b	69.5 c
VC-20	80.0 a	10.2 b	85.7 a
VC-21	90.0 a	10.8 a	76.7 b
VC-22	82.7 a	11.0 a	77.1 b
VC-23	90.7 a	9.2 b	89.1 a
CV(%)	8.61	6.93	5.77

[1] Means followed by the same letter in the column do not differ by Scott-Knott's test at 5% significance

CONCLUSIONS: The evaluated genotypes have similar seed germination. The cultivars BRSMG Talismã and the lines EMB4, EMB9, MAIV-15204, MAIV-18259, MAIV-18524 and RCII-219 present more vigorous seeds.

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ANATOMICAL COMPARISON OF SEED OF WHITE, *PERSISTENT COLOR* AND COLORED -SEEDED SNAP BEAN LINES

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INTRODUCTION

Most contemporary snap bean cultivars have white seed conditioned by *p*. Colored seed is unacceptable from a processing quality standpoint because the water soluble flavonoids present in the seed coat may discolor the processed product. On the other hand, colored-seeded types have better germination and emergence in the field than white-seeded cultivars. It is thought that the flavonoids act as antimicrobial agents similar to the action of fungicides applied to the seed coat to maintain high levels of germination and emergence in the field. Another category of seed coat color carried by approximately 40% of commercial snap bean cultivars grown in the U.S. is *persistent color* (*pc*) (Myers et al., 2018). *Persistent color* is a member of the stay-green gene family found in many crop species. Crops with stay-green are characterized by the plant vegetative and reproductive organs remaining green while undergoing senescence. In addition in common bean, dry seed of *pc* types are light green but have white cotyledons upon germination. Snap bean cultivars with *pc* are desirable for improved pod quality because the trait produces uniformly green pod color when compared to white-seeded types.

One deleterious effect of *pc* is reduced germination and emergence in the field compared to white-seeded genotypes. Fungicide treatment of the seeds will increase germination and emergence rates to levels comparable to fungicide-treated white seed (Al Jadi et al., 2016). This suggests the involvement of soil borne pathogens in seeking out and colonizing *pc* seeds before the bean seedling is established. One hypothesis as to why *pc* types exhibit reduced germination is that seeds release high levels of solutes in the surrounding rhizosphere, thereby attracting pathogens, which parasitize the seed before the plant can emerge. A possibility is that *pc* seed has higher levels of sugars and other pathogen attractants than do other forms of seed. Another is that *pc* seed is more fragile and prone to damage during seed harvest and handling, thereby increasing leakage during imbibition. A third possibility related to the second is that *pc* seed is more susceptible to imbibitional cracking during germination. The latter two possibilities might implicate differences in seed coat structure of *pc* compared to other types of seed.

The objective of this study was to examine the anatomical structure of *pc* seeds and compare to white and colored seeded snap beans.

MATERIALS AND METHODS:

The seed anatomy of 'Pascal', 'OR 91G', and GRI 2-1 were compared. Pascal is a flageolet type and similar to the original source of *pc* introgressed into snap beans. OR 91G is a bush blue lake green bean with white (*p*) seeds, while GRI 2-1 is a backcross line to OR 91G that has had the p^{gri} allele introgressed but otherwise is genotypically similar to OR 91G. The p^{gri} allele is a leaky version of *p* and allows partial expression of underlying color genes (Bassett, 1994). Seed used in this study was produced in the same greenhouse growing environment in 2017. Seed, cotyledon color and pod color were all verified before, during and after seed production.

For anatomical comparison, seeds were fixed under vacuum in formalin acetic alcohol (FAA) for 24 hours. Then, the samples were dehydrated in a graded alcohol series and infiltrated

in 2:1, 1:1, 1:2 (95% ethanol:Plastic Infiltration Solution Technovit 7100) followed by straight infiltration solution. Samples were embedded in Technovit 7100 glycol methacrylate plastic (Electron Microscopy Services) and sectioned with a steel knife at 5-7 microns on a rotary microtome. Sections were stained in 0.5% Toluidine Blue O in Citrate Buffer (pH 4.2) and mounted on glass slides. Seed testa layer measurements were repeated 10 times per genotype per tissue layer.

RESULTS:

Figure 1

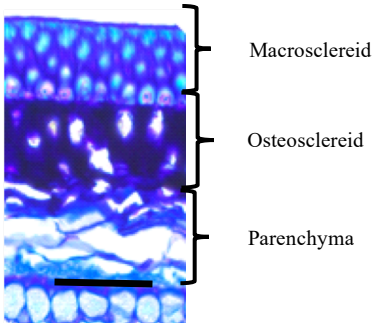

Figure 2

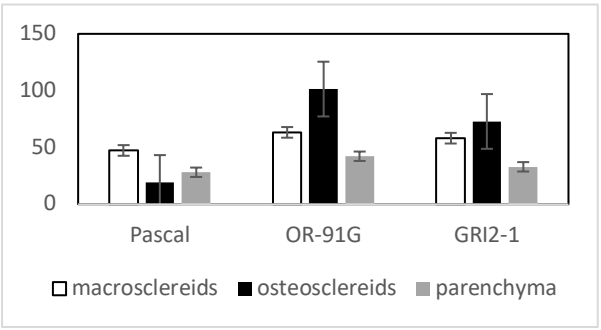

Fig. 1: Transversal section of the seed coat of OR 91G, three layers of the testa were measured: 1: The outer macrosclerid layer, 2: osteosclereids forming the hypodermal layer, 3: inner parenchyma layer. Bar equal to 100 µm.
Fig. 2 Thickness of outer testa layers of Pascal (*pp pcpc*), OR 91G (*pp PcPc*) and GRI 2-1 ($p^{gri}p^{gri}$ *PcPc*) common bean lines (Mean ± standard error).

Figure 1 shows a transverse cross-section through the seed of OR 91G with three layers above the testa being visible. The outermost was composed of macrosclereids (cuticle is not visible and was removed by the fixation process), a sub-epidermal layer of osteosclereids, and a parenchyma cell layer adjacent to the cotyledon (Fig. 1).

Comparison of seed testa thickness revealed that Pascal had a significantly thinner osteosclereid layer compared to other genotypes (Fig. 2). The macrosclereid and parenchyma layers were also thinner with Pascal significantly different from the others for the macrosclereid layer and significantly different from OR 91G but not GRI 2-1 for the parenchyma layer. The osteosclereids may play a role in imbibition and solute movement via cell expansion during imbibition (Harris 1984). This research suggests that the physical structure of the seed of *pc* types may be an important factor in pathogen colonization of seed. Additional *pc* genotypes need to be evaluated to determine whether this difference in seed coat thickness is characteristic of all *pc* types or just Pascal.

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AGRONOMICAL CHARACTERISTICS OF GREEN BEAN (*Phaseolus vulgaris* L.) CV. UEL-2 UNDER TWO TYPES OF SOILS AND DOSES OF MOLYBDENUM

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INTRODUCTION: Due to the natural deficiency of some soils in molybdenum (Mo) and its necessary requirement in green bean culture, the objective of this work was to study the response of increasing doses of this micronutrient in soils of different textures in some morphological variables of green bean and yield.

MATERIAL AND METHODS: The experiment was set up in a greenhouse at the State University of Londrina (known as UEL), in Londrina-Paraná, Brazil, from September to November 2018. Three-kg pots were filled with two types of soil: a) Clay soil (75% clay) and b) Sandy soil (10% clay). The soils used were submitted to previous liming, raising base saturation to 70%. After its incorporation, pots were irrigated to reach 70% of the water holding capacity of each soil, remaining incubated for 15 days. Before sowing, a basic fertilization was carried out, applying 60 and 30 kg ha^{-1} of P_20_5 and K_20, respectively. Green bean seeds cv. UEL-2, used in the experiment, were previously inoculated with a mixture of bacterial strains CIAT899 (*Rhizobium tropici*) and IPRF81 (*Rhizobium leguminosarium* bv. *phaseoli*). Two plants were cultivated per pot. Nitrogen cover-fertilization was performed 25 days after emergence (DAE) with a dose equivalent to 60 kg ha^{-1} of urea. During the experimental phase, water lost by evapotranspiration was replenished daily (by weighing the pots), aiming to maintain humidity at 70% water holding capacity of the soil. The experimental design was completely randomized in a 2 x 5 factorial scheme with three replications, in which the factors were: two types of soil and five doses of molybdenum (O, 30, 60, 90 and 120 g ha^{-1}), using sodium molybdate ($Na2MoO_4$) as source. The aerial part of the plants was collected by 53 DAE, measuring the variables plant's height, stalk diameter, number of productive pods and its fresh matter and aerial-part dry mass. Obtained data were submitted to analysis of variance, with comparison of means by Tukey's Test ($p<0,01$) when necessary.

RESULTS AND DISCUSSION: Productivity inferred by variables' measures were significantly higher in the clay soil (Table 1). According to the increasing molybdenum doses there were significant differences in yield and plants height. When molybdenum was applied, the highest yields were obtained with doses among 60 and 120 g ha^{-1}, although they did not differ from the control (Table 2). These results are in agreement with those presented by BERGER et al. (1993), who obtained maximum yield with 90 and 78 g ha^{-1} of molybdenum applied by foliar fertilizer, and by RODRIGUES et al. (1996), who obtained maximum grain yields with the foliar spraying of molybdenum in the range of 70 to 80 g ha^{-1}. In the present work, the highest height was obtained when 90 g ha^{-1} of molybdenum was applied (Table 2). ANDRADE et al. (1998) also verified augmentation of height related to foliar fertilization of 40 g ha^{-1} of the micronutrient in common bean cv. Carioca-MG. Regarding the number of productive pods per plant, a significant interaction was observed among soils and molybdenum doses, which reached its highest number in the clay soil added of 60g ha^{-1} of molybdenum. In the other hand, a dose of 120 g ha^{-1} of molybdenum was required for striking the maximum number of productive pods in sandy soil (Table 3).

Table 1- Productive pods yield, plant height, stalk diameter and aerial part dry mass.

SOIL	YIELD (Kg ha^{-1})		HEIGHT (cm)		STALK DIAMETER(cm)		DRY MASS	
CLAY SOIL	4424	A	51,63	A	0,45	A	16,99	A
SAND SOIL	2490	B	43,54	B	0,39	B	11,64	B

*Means followed by the same letter do not differ from each other by Tukey's Test ($p<0,01$).

Table 2- Fresh productive pods yield and stalk height for green bean UEL-2 related to different molybdenum doses.

DOSES (g ha^{-1})	YIELD (Kg ha^{-1})		HEIGHT (cm)	
0	3488	AB	43,28	B
30	2825	B	46,43	B
60	3663	A	46,58	B
90	3260	AB	52,92	A
120	4048	A	48,72	AB

*Means followed by the same letter do not differ from each other by Tukey's Test ($p<0,01$).

Table 3- Dry matter of productive pods in green bean UEL-2 depending on the soil.

SOIL	DOSES MO (g ha^{-1})									
	0		30		60		90		120	
CLAY SOIL	9,67	Aab	7,67	Ab	11,00	Aa	9,33	Aab	9,33	Aab
SAND SOIL	3,67	Bb	3,67	Bb	3,67	Bb	3,00	Bb	6,33	Ba

*Means followed by identical capital letters in columns and lowercase letters in lines do not differ by Tukey's Test ($p<0,01$).

CONCLUSION: The best results in the studied parameters were obtained in clay soil. The highest yields were obtained with doses of 60 and 120 g ha^{-1} of molybdenum for clay soil and sandy soil, respectively.

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GENOTYPE BY ENVIRONMENT INTERACTIONS OF FLAVOR TRAITS IN SNAP BEANS

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INTRODUCTION – Early studies on the production of flavor volatiles in snap beans focused on genetics and pod maturity as sources of variation (Stevens et al., 1967; Toya et al., 1974). They found that certain genotypes possessed characteristic levels of 1-octen-3-ol and linalool, and that volatile levels for both compounds peaked in the early stages of pod development and decreased with maturity. Gas chromatography mass spectrometry (GCMS) data from these early studies showed additional variability that may have been due to instrument error, but it may also have reflected environmental influences beyond pod maturity. If other environmental influences are present, the choices made by farmer's in the field could alter the quality traits of the beans irrespective of the genetic variation. This may have practical significance to the farmer to attain the highest pod quality. To address this question of environmental variation and G x E interactions, a replicated field study was conducted. The environmental factors of location, organic soil, and planting date were analyzed as were their interactions with genotype.

MATERIAL AND METHODS – Six cultivars were chosen representing three classes of snap beans. 'Ebro' and 'Bogota' represented Romano beans. 'OR5630' and a cultivar coded as 'PV-01' represented Blue Lake beans. Finally, 'Eagle' and 'Tendergreen' represented Midwestern Tendercrop beans. These six cultivars were grown at three locations with a May and July planting date in 2014. Two of the locations were in Corvallis, OR, with extremely similar climate and soil conditions, but one of these two locations was maintained using conventional farming practices and one was certified organic. The third location was in a commercially farmed, conventionally maintained, field in central Wisconsin. All genotypes and locations were planted in both May and July in a randomized complete block design. One gram of each sample was analyzed for linalool and 1-octen-3-ol content using head-space solid phase microextraction GCMS on a Shimadzu GCMS-QP2010 Ultra instrument (Kyoto, Japan). Deuterated linalool was used as an internal standard. Ion fragments were compared to a NIST11 spectral library for identity and relative peak areas were converted to concentrations using calibration curves and the standard addition method. All statistical analysis was conducted in R using base functions and the 'car' package. Residual plots, QQ plots, Shapiro-Wilk test for normality, and Fligner test for homogeneity of variances was conducted to test the assumptions of ANOVA. Normality, homogeneity of variances, and the coefficient of variation were all significantly improved with log transformation. ANOVA (sums of squares I) was conducted first with all interactions but was then conducted without interactions because the interactions were not significant. ANOVA analysis was conducted on the main effects of bean cultivar, location, and month of planting. A separate *t*-test comparison of the conventional vs. organic ground in Corvallis, OR, was also conducted. Tukey's multiple comparisons were done for linalool because genotype was significant for this compound.

RESULTS AND DISCUSSION – An ANOVA on linalool with factors for location, date of planting and genotype showed a significant main effect for genotype. No interactions were significant. When interactions were removed and the ANOVA was performed again, the date of planting main effect was also significant with an alpha of 0.05. Tukey's comparisons for linalool

showed that 'Eagle' was significantly different from nearly all other genotypes and 'OR5630' was also significantly different from 'Ebro' and 'Tendergreen'. An ANOVA on 1-octen-3-ol showed only one significant main effect for planting date. No interactions were significant. When interactions were removed and the ANOVA performed again, the location was also significant with an alpha of 0.05. The mean linalool value was 369.5 mg/L for May and 207.0 mg/L for July. Similarly, the mean 1-octen-3-ol value was 116.1 mg/L for the May planting but only 42.3 mg/L for the July planting. The mean 1-octen-3-ol level at the OSU Vegetable Research farm (Corvallis, OR) was 56.6 mg/L and 54.2 mg/L at the OSU Louis Brown farm (organic plots, Corvallis, OR) whereas a conventionally maintained commercial farm near Plover, WI had a mean value of 126.8 mg/L.

These results indicate that planting date may be critical to the pod quality trait of flavor. The earlier planting in May subjected pods to an average of 3 more days in the field to reach comparable maturity to the planting in July. The reduced temperatures and slower growth of the May planting may have allowed more linalool and 1-octen-3-ol to accumulate in pods. A comparison of closely matched organic and conventional plots in Corvallis, OR, showed no difference. Location was important to 1-octen-3-ol production. It is not clear what conditions at the Wisconsin location caused increased 1-octen-3-ol production. Future research needs to be conducted comparing multiple years of plantings for an additional main effect in the ANOVA for year and to validate the robustness of these results.

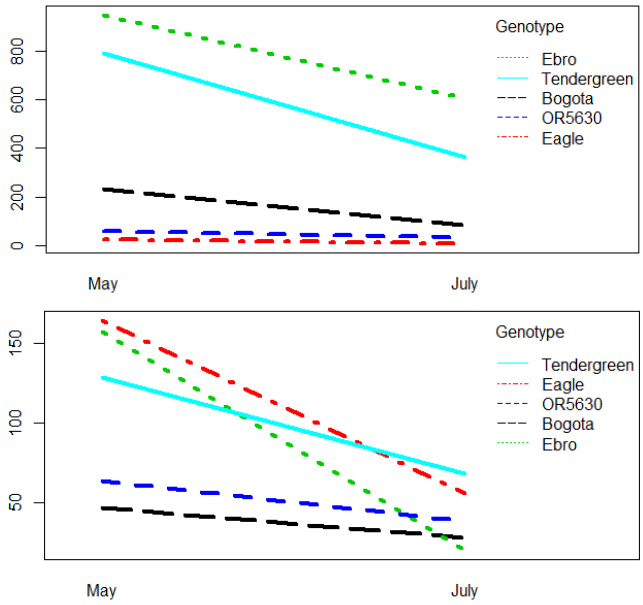

Figure 1: Interaction plots showing planting date effect on linalool production (top) and 1-octen-3-ol production (bottom). The y-axis is in mg/L.

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NUTRITION FOLIAR WITH CALCIUM, BORON, COBALT, MOLYBDENUM AND HORMONE IN SNAP BEAN AND INTERFERENCE OF YIELD

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INTRODUCTION: The snap beans are among the most popular vegetables, occupying thirteenth position in terms of economic importance and sixth in volume produced in Brazil. The culture has high nutrients demand, due to the small and shallow root system and short cycle. Mineral nutrition studies are very important, especially with soil fertility and crop fertilization. The objective of this study was to evaluate the effect of calcium, micronutrient and growth hormone-based products on the pod yield of bushing snap bean.

MATERIALS AND METHODS: The study was conducted in an Eutroferric Red Latosol with clay texture, in 2014 in Londrina-PR. The climate of the region is Cfa (classification of Koppen), characterized by well distributed rains and mild summers. Was used the bushing snap bean cultivar UEL 1. The soil was prepared with the use of a rotative mattock. In the fertilization of sowing, was used the equivalent of 400 kg ha^{-1} of NPK (04-14-08). Irrigations were made according to the water requirement of the crop. For weed control was performed manual weeding whenever necessary. Each experimental plot consisted of four lines of 4.5 m in length, spaced 0.50 m between lines. As a useful area, two central lines were considered, by subtracting 0.50 m from each end. The treatments tested were:

Table 1. Details of foliar fertilization applied in the snap bean cultivar UEL 1. Londrina, 2014.

Treatments	Description	Dose (L ha^{-1})	Phenological stage
1	Control	No application	-
2	Co-Mo®	0,12	R_5
3	Sett® + Co-Mo®	3,0 + 0,12	R_5
4	Stimulate® + Co-Mo®	0,25 + 0,12	R_5
5	Sett® + Stimulate® + Co-Mo®	3,0 + 0,25 + 0,12	R_5
6	Sett® + Co-Mo®	3,0 + 0,12	R_7
7	Sett® + Co-Mo®	1,5 + 0,12	R_5 - R_7
8	Sett® + Co-Mo®	3,0 + 0,12	R_5 - R_7

Sett® is a calcium and boron-based commercial product, while Stimulate® is a gibberellin, cytokinin and auxin-based commercial product. The commercial product Co-Mo® has 1,5% of cobalt and 15% of molybdenum. The productivity evaluation was performed by harvesting and measuring the green mass of the immature pods produced in each plot. The experimental design was randomized blocks with four replicates. The results were submitted to analysis of variance and means compared by the Tukey test at 5% of significance.

RESULTS AND DISCUSSION: Analyzing the obtained data, it was verified that the absolute average of the productivity of each treatment was higher than the control, but with no statistical difference (Fig.1), most probably due to the high coefficient of variations (CV) of the experiment. (CV = 60%). Thus, whereas the experimental errors are better controlled, with better knowledge of the area, planting at the appropriate season, efficient control of pests and diseases, as well as more efficient spraying methods, these foliar treatments can be effective in increasing productivity.

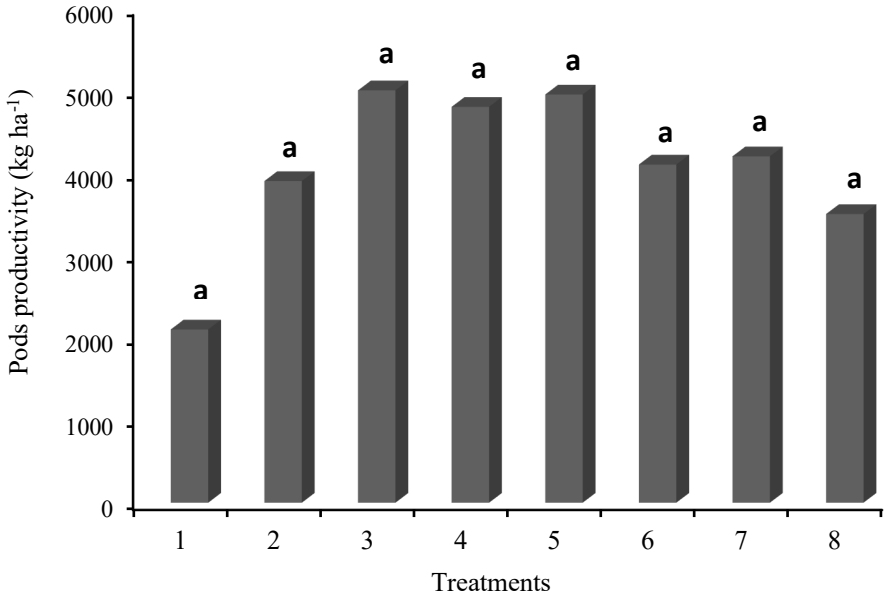

Figure 1. Pods productivity of bushing snap bean cultivar UEL 1 in response of foliar fertilization and application stadium. Londrina, 2014.

We observe that the mixture of the commercial products Sett® + Co-Mo® had greater efficiency when applied in phenological stage R_5 in the concentration of 3 L ha^{-1}. This same mixture when applied at R_7 (3 L ha^{-1}), it presented lower results than when applied at R_5, considering the same dose, and also when applied at R_5 (1.5 L ha^{-1}) + R_7 (1.5 L ha^{-1}). These results show the observation of Marschner (1995), that in the absence of calcium and boron there may be interference in the germination of the pollen grains and pollen tube growth, resulting in a reduction in the number of pods. It is preferable to apply the mixture Sett® (Ca and Bo) + Co-Mo® in R_5. The mixture of Sett®,

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APPLICATION OF SOILDOC KIT TECHNOLOGY FOR TAILORING FERTILIZER AND INPUTS RECOMMENDATIONS IN COMMON BEAN (*Phaseolus vulgaris*) FARMERS FIELDS

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INTRODUCTION: For many years soil analysis has been used as an aid to assess soil fertility and plant nutrient management options (Huising et. al., 2013). In Africa, low soil fertility is the major constraints in bean production followed with diseases (CIAT, 2003). Some bean diseases caused by root rot pathogens in common beans interact with soil fertility, impeding acquisition of plant nutrients from soil, and thus are especially severe when soil fertility is limiting (Abawi and Widmer 2000). Low soil fertility (N & P) and drought (PABRA, 2009) are abiotic constraints that can result in severe losses in common bean production. Low potassium, aluminum and manganese toxicities are of intermediate importance; sodium toxicity is important in some localities (Wortmann and Allen, 1994). We have been using wet and dry chemistry analysis which must be conducted in the soil laboratories, but currently we got another technique named SoilDoc. This is a portable, on-farm soil testing kit which provide numerous results that include unlimited and available on-farm extension services and increased farm production. It is used to test for Soil pH, EC, nitrogen, phosphorus, sulfur, and potassium, as well as active organic matter and active carbon (SoilDoc kit manual, 2014 and Adeoye and Agboola, 1985). The application of this precise soil inputs reduces costs and limit soil nutrient losses to the environment. This study focuses on soil audits, involving additional analyses and recommendations based on different approaches to the interpretation of analytical data being offered to farmers using SoilDoc Kit which allow us to advance quickly in fine-tuning agronomic and fertilizer practices to increase crop yields and increase resource use efficiency nutrients and water (David 1994).

MATERIALS AND METHODS: This study targeted northern zone in five districts i.e. Same, Moshi, Hai, Siha and Arumeru. Representative sites for soil sampling were selected through collaboration of researchers from Selian Agricultural Research Institute and respective District Extension officers. Using Android, in-built GPS recorder which records Longitude, Latitude and Altitude were used for determining specific farmer's field for soil sampling. The area of fields which soil samples collected were from a range of $2023.43m^2$ – $4046.86m^2$ and soil sampling was done as per SoilDoc kit protocol (SoilDoc kit manual, 2014). Labels for each sample included: Famer's name, Crop grown, Date of soil sampling, Names of soil sample collectors, Barcode number, Region, District, Village, Altitude, Latitude and Longitude. Soil samples collected were air dried, grinded and sieved to get a representative soil samples to determine Soil chemical and physical test using the recommended SoilDoc protocol. After making an analysis for each sample, an android tablet data entry was done using installed android-ODK collect software program – Version 1.4.11(1062). This was followed uploading of data from the android tablet to the server and downloading analyzed soil results from the server. Compilation of individual soil analysis results was done for each farmer's field which were involved in the soil sampling. Through using GPS coordinates, the digital map indicating soil sample sites was prepared so that to show the coverage area.

RESULTS AND DISCUSSION: Total of 114 soil samples were collected and analyzed in Same (26), Moshi (21), Hai (19), Siha (21) and Arumeru (27). The pH values were classified as optimum (5.10-6.10) for 78% of total samples and 22% high but not limiting with pH value of 6.1-7.1. For nutritive values, Nitrogen was very low (<21mg/kg) to 60% of total samples and excessive for only 8% with >120mg/kg. Phosphorus was extremely low <0.05mg/kg for 42% of total samples and excessive with >2mg/kg for only 20%. Potassium was very high of >60mg/kg for 77% of total samples. Sulphur was classified very low, low, medium and high with proportional of 11%, 32%, 34% and 23% respectively. Organic matter showed to range from extremely low with <150mg/kg to very high with >700mg/kg of 10% and 39% of total samples respectively. The established critical thresholds were, N: 42mgNO_3-N/kg soil, P: 0.30mg P/kg soil, K: 20mg K/kg soil, S: 10mg S/kg soil, Lime: pH $CaCl_2$ <5.10 (Huising, et al., 2013). From these samples, 75% indicated a need to apply N fertilizers; 54% P; 5% K; 43% S composed fertilizers and 22% organic matter. About 90% of soil samples analyzed had a pH ranging from 5.1 to 7.1 which was favorable to produce various crops including beans which have an optimum range of 5.5 to 6.5 (Sierra, 2014). Results for each parameter were analyzed, interpretation and recommendation based on each result were described for each site and shared with agricultural district officers so that they can advise on best management of their farm to increase production and apply costly fertilizers only where needed. Where levels of active carbon (AC) were very low, farmers were advised to incorporate into the soil any available crop residues, including straw, and organic wastes or use the recommended rate of farm Yard Manure (FYM) as one of remedial measure to increase carbon contents into the soil. Application of P fertilizer using of locally available fertilizes (TSP, DAP or Minjingu mazao) was also suggested especially in farms with low to extremely low levels of phosphorous.

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EVALUATION OF LOW PHOSPHORUS AND NITROGEN OF COMMON BEAN (*PHASEOLUS VULGARIS* L.) GENOTYPES FOR GRAIN YIELD AND YIELD COMPONENTS

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INTRODUCTION: Common bean is an important crop for food and income for smallholder farmers in Tanzania. Although the production appears relatively high, the actual yield per unit area is still low (0.81t/ha) compared to the potential yield of 2.5t/ha (FAOSTAT 2014). Low bean yields are mainly due to low soil fertility particularly phosphorus and Nitrogen (Atemkeng *et al*. 2011). Amijee and Giller (2000) reported that soils in major growing areas of Tanzania have very low concentration of extractable phosphorus ranging from 1.6 to 3.1 mg/kg soil. It is estimated that beans remove 12.5 kg P/ha which is higher than additions of phosphorus by resource poor farmers (Kaihura *et al*. 2001). The use of fertilizer in is very low as it is 1.9 kg P/ha of cultivated land (ICRA, 2002), in average fertilizer use is less than 10 kg/ha (IFDC, 2012). This is due to high cost of fertilizers, knowledge and transport. The objective of this study were to identify bean genotypes tolerance low P and N for improving bean productivity to low Phosphorus and Nitrogen soil status.

MATERIAL AND METHODS: Six bean genotypes consisted MR 14125-3, VTTT92/3-2-2-1, VTTT926/9-6, SAB 691, CIM RMOO POP 326 and BILFA Uyole (Control) were used with TSP 46% P_2O_5 and CAN 27% N. The experiment was established 2018 at Ivwanga village in split plot. The main plot was bean genotypes, the sub-plot was phosphorus and Nitrogen fertilizer levels 0N+ 0 P_2O_5 kg P/ha (control), 0N+ 30 P_2O_5 kg P/ha, 30N+ 0P_2O_5 kg P/ha, 30N + 60 P_2O_5 kg P/ha in form of CAN and TSP respectively. Soil samples were collected and analyzed at TARI Uyole. Agronomic data was collected and analyzed using GenStat statistical software version 15.

RESULTS AND DISCUSSION: The soil of the experiment field was characterized by low pH (H_2O) (4.7), low in available Extractable phosphorus (3.5mg/kg soil), total nitrogen (0.1%total N) and EC (9.3cmol/kg soil) suitable for the testing of bean genotypes for tolerance to low soil P and N. The results shows that, P and N treatments had significant ($P < 0.05$) effects on days to 50% maturity, number of pods per plant, plant biomass and yield. Days to mature above mean (81.06) on 30N+0 P_2O_5 and 30N+60 P_2O_5. Treatment 30N+60 P_2O_5 had higher pods per plant (9.31) than 0N+0 P_2O_5. The highest yields was 1.54 t/ha from 30N+60 P_2O_5, the lowest 0.82 t/ha was observed in control (Table 1). These results show that, variables increased with increase in P and N levels. There was significant ($P \leq 0.05$) variation among genotypes with respect to number of pods per plant, grain yields and 100 seed weight. Genotype SAB matured late 88.25 while genotype MR 14125-3 matured earlier 74.83 days (Table 2). BILFA Uyole had the highest mean (9.5) number of pods per plant, whereas genotypes VTTT92/3-2-2-1 had the lowest mean (6.5) number of pods per plant. Genotype VTTT926/9-6 and CIM RMOO POP 326 had higher grain yields that is 1.45 and 1.39 t/ha respectively while, MR 14125-3, VTTT92/3-2-2-1, and SAB 691 had the lowest grain yield that are 0.98, 0.83 and 0.91 t/ha respectively. There was significant difference on the interaction of fertilizer and genotypes on days to maturity Table 3, whereby genotype VTTT92/3-2-2-1, BILFA Uyole(control) both had many days to mature in treatments 0N+30 P_2O_5, 30N+0 P_2O_5 and 30N+60 P_2O_5, it seems fertilizer application contributes to late maturing and to high yields. SAB 691genotypes had many days to mature in all fertilizer treatments as a result of genetic composition.

Table 1: Effect of P and N treatments on days to 50% maturity, number of pods per plant and grain yields

Treatments	50% maturity	Pod/Plant	Biomass (gm)	Yield (t/ha)	100 Seed wt
0N+0 P_2O_5	77.83 a	6.17 a	0.48 a	0.82 a	41.83
0N+30 P_2O_5	80.67 ab	7.68 b	0.64 a	1.14 b	43.39
30N+0 P_2O_5	88.33 b	7.89 b	0.66 a	0.90 a	41.72
30N+60 P_2O_5	82.39 b	9.31 c	0.99 b	1.54 c	43.89

Grand Mean	81.06	7.76	0.69	1.10	42.71
CV (%)	5.8	24.5	6.9	0.0291	6.0
LSD	7.79	1.28	0.17	0.2143	4.24
p-value	**	***	***	***	NS

Table 2: Effect of bean genotypes on selected parameters and grain yields of six genotypes

Genotypes	50% maturity	Pod/Plant	Biomass (gm)	Yield (t/ha)	100 Seed wt
MR 14125-3	74.83 c	7.60 ab	0.56	0.918 b	44.67 a
VTTT92/3-2-2-1	82.17 b	6.50 b	0.68	0.831 b	46.50 a
VTTT926/9-6	77.83 bc	7.48 ab	0.80	1.45 a	42.17 b
SAB 691	88.25 a	7.92 ab	0.73	0.91 b	40.08 b
CIM RMOO POP 326	79.75 bc	7.55 ab	0.71	1.39 a	41.75 b
BILFA Uyole	83.50 ab	9.50 a	0.68	1.10 ab	41.08 b
Grand Mean	81.06	7.76	0.69	1.10	42.71
CV (%)	5.8	24.5	6.9	0.0291	6.0
LSD	3.9	1.56	0.21	0.263	2.12
p-value	***	NS	NS	***	***

Table 3: P, N and bean genotypes interaction effect on 50% maturity of six genotypes

S/N	Genotype	Fertilizer treatments			
		0N+0 P_2O_5	0N+30 P_2O_5	30N+0 P_2O_5	30N+60 P_2O_5
1	MR 14125-3	72.67	77.67	75.00	74.00
2	VTTT92/3-2-2-1	73.33	81.33	86.00	88.00
3	VTTT926/9-6	79.00	74.00	79.67	78.67
4	SAB 691	89.67	89.67	90.00	83.67
5	CIM RMOO POP 326	75.00	73.00	84.00	87.00
6	BILFA Uyole (control)	77.33	88.33	85.33	83.00
	Grand Mean	81.06			
	CV	5.8			
	LSD	7.8			
	P value	**			

CONCLUSION: Genotypes VTTT926/9-6 and CIM RMOO POP 326 seems tolerant to Low P and N but the data is of one season, therefore, there is a need for further evaluation to come up with conclusive results for documenting and to advance the best lines for breeding trials. Further evaluation is needed to confirm results for documenting and advancing the identified low P and N genotypes for breeding work.

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PERFORMANCE OF MESOAMERICAN BEANS IN A LOW FERTILITY SOIL

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The performance of 27 Mesoamerican bean (*Phaseolus vulgaris* L.) lines from the Bean Adaptation to Stress Environment (BASE) 120 trial was evaluated over a period of four years at Isabela, Puerto Rico. Soil P was low in the fields where the 2016 and 2018 trials were planted (7.4 and 4.2 ppm, respectively). Soil pH ranged from 5.5 to 6.6. Because the trials were planted in June, the lines were exposed to moderately high temperatures (maximum daily temperatures were > 32° C and minimum temperatures > 20° C) during flowering and pod filling. Although the trials were not fertilized, the seed was inoculated with a peat-based inoculant of *Rhizobium tropici* (CIAT 899) and *Rhizobium etli* (CIAT 632). Supplemental irrigation was used to avoid drought stress and insecticides were used for pest management. Root nodulation, based on samples excised from each row, were evaluated 40-45 days after planting using the CIAT 1-9 scale (Table 1). The experimental design was randomized complete blocks with five replications. The experimental unit was a single 2-m row spaced at 0.76 cm between rows. ANOVAs were conducted for seed yield and nodulation scores using planting date means as replications. Mean seed yields and nodulation scores were compared using Least Significant Differences ($P < 0.05$).

Significant differences were observed between lines for mean seed yield and mean nodulation scores over growing seasons (Table 1). Several bean lines produced seed yields significantly higher than the checks 'ICA Pijao' and BAT 477. TARS MST-1 had the highest mean seed yield (1,528 kg/ha). This black bean was released for tolerance to multiple stresses including resistance to common bacterial blight, root rots and tolerance to high temperature [Porch et al. (2012) J. Plant Reg. 6:75-80]. The small red breeding line BIOF 2-106 from the Zamorano bean breeding program in Honduras was ranked second in mean seed yield (1,490 kg/ha). The black bean breeding line B12724 from Michigan State University produced a mean yield of 1,479 kg/ha. This line had the most consistent performance, producing seed yields > 1,300 kg/ha in all planting dates. The black bean line PR1418-15, which produced a mean yield of 1,458 kg/ha, had been selected in Puerto Rico for root rot resistance. 'Bella' was the highest yielding white bean in the trial producing a mean seed yield of 1,351 kg/ha [Beaver et al. (2018) J. Plant Reg. 12:190-193]. The highest yielding lines in the trial produced intermediate nodulation scores ranging from 5.7 to 6.1. 'INTA Centro Sur' (ICB 301-204) from Zamorano had mean seed yields > 1,000 kg/ha across planting dates and was ranked second in mean root nodulation score (5.2).

Small-scale farmers in Central America and the Caribbean (CA/C) often plant beans in low fertility soils. The use of fertilizer is limited due to lack of access and the high cost of this input. The highest yielding lines in the BASE 120 trials planted at the Isabela Substation have been used as parents to develop breeding populations for CA/C. Some of the lines adapted to the low fertility soils at Isabela, Puerto Rico possess other valuable traits including resistance to *Bean Golden yellow mosaic virus*, *Bean common mosaic necrosis virus*, common bacterial blight and root rot and greater tolerance to high temperature.

Table 1. Mean seed yields (kg/ha) and nodulation scores of Mesoamerican lines in the Bean Adaptation to Stress Environment (BASE) 120 trials planted at Isabela, Puerto Rico over four growing seasons.

Line	BASE 120 entry	Mean seed yield (kg/ha)					Nodulation score[1]
		June 2015	June 2016	June 2017	June 2018	Mean	Mean
B12724	6	1699	1362	1521	1332	1479	6.1
BAT 477	10	853	1310	1130	815	1027	6.0
Beniquez	12	433	1493	784	684	849	5.4
BIOF 2-106	19	1154	1796	2018	991	1490	6.8
CENTA Pipil	30	1246	1404	903	960	1128	6.5
FBN 1203-43	36	1291	1476	1037	1205	1252	6.6
FBN 1203-47	37	919	1190	1294	1199	1151	5.9
ICA Pijao	43	396	887	695	771	687	5.0
INTA Centro Sur	44	1039	1393	1045	1338	1204	5.2
Lenca Precóz	53	427	834	537	546	586	6.0
Paisano	54	942	1516	1280	1292	1258	7.0
MHN 322-49	55	807	1095	882	1148	983	5.6
Paraisito	59	562	765	1380	789	874	6.5
PR1147-8	67	1540	1249	1586	889	1316	7.0
PR1165-3	68	353	1472	975	921	930	6.9
Bella	70	1652	1574	1221	955	1351	5.7
PR1418-15	71	1606	1732	1606	889	1458	5.8
PR1483-105	72	1418	1725	1493	978	1404	5.9
Sayaxché ML	78	1326	1502	1727	908	1366	6.2
SEF 16	92	347	1202	1251	677	869	6.3
SER 118	98	441	1681	1501	1005	1157	6.7
CENTA EAC	102	672	1429	1095	993	1047	6.3
SB2-170	109	1391	1304	844	1097	1159	7.3
TARS LFR-1	110	1011	1379	1550	897	1209	6.2
TARS MST-1	111	1083	1729	2261	1039	1528	6.6
Verano	117	420	1492	1280	710	976	6.2
Sankara	118	341	1401	1226	899	967	4.9
Mean		940	1385	1263	960	1137	6.2
L.S.D. (0.05)						388	1.3
C.V.(%)						24.2	14.7

[1] Rated using the CIAT 1-9 scale where 1 = > 80, 3 = (41-80), 5 = (21-40), 7 = (10-20) and 9 = < 10 nodules per plant.

COMMON BEAN MONOCROP EFFECTS ON SOIL CHEMICAL DEGRADATION IN DURANGO, MÉXICO

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INTRODUCTION: Common bean (*Phaseolus vulgaris*) is considered one of the most important cash crops in the State of Durango, México. After farmers harvest common bean, residual biomass (known as paja) is collected and then domestic livestock are introduced into the field in order to use vegetal residues as an animal food. As a result, low organic matter content and chemical degradation is observed in most of the soils (60 %) where mainly common bean and maize are grown. An integral diagnosis is necessary in order to improve agricultural productivity and sustainability in Durango, returning soil nutrients to their natural level and balance. The objective of this study was to evaluate the effects of common bean monocrop on chemical degradation of agricultural soils in Durango.

MATERIALS AND METHODS: Soil samples were obtained across main common bean production area (23° to 25º N and 103° to 106º W) in the state of Durango. Study area included nine municipalities: Durango, Canatlán, Pánuco de Coronado, Guadalupe Victoria, Peñón Blanco, Cuencamé, Poanas, Nombre de Dios and Vicente Guerrero. Sampling sites were randomly selected at the intersection points of square cells, 4 km by side. Sample size calculation was determined using equation: $n= [z^2 \times (p \times q)] /e^2 + [z^2 \times (p \times q)/N]$, where n = sample size (147), z = confidence level 95 % = 1.96, p = proportion with (0.50), q = proportion without (0.50), e = error (0.05) and N = total cell number (237). At each site composite samples were obtained at 0-30 cm in depth. Samples were analyzed for organic matter (Walkley-Black), nitrogen (Castellanos *et al*., 2005; Rivera *et al*., 2013), phosphorus (Olsen) and pH (potentiometer).

Descriptive statistics were used and then data was subjected to geostatistical analysis for each variable. Initially the data tabulated in X, Y and Z coordinates were processed with the GS Plus program (Version 9.0), in order to obtain the parameters of the semivariogram with better fit and then spatially correlated according to each variable. Geostatistical parameters were used to interpolate data by means of the ordinary Kringing method, using the QGIS program (V3.4.1) with GRASS (V7.4.2) complement. The adjustment obtained with the use of the QGIS program was also evaluated to determine the most accurate option in the visual representation (maps) of the area with different levels of soil fertility. Fertility maps were validated in order to estimate the validity of the representations (Henríquez *et al*., 2013).

RESULTS AND DISCUSSION: The average value for the content of organic matter was 1.4 %, considered as "low" and variation between very low (0.3 %) to medium (3.3 %) levels was observed (Table 1). The highest frequency was observed for the low level (62 %), followed by the medium (33 %) and very low (5 %) level. Nitrogen content averaged 59.7 kg ha^{-1}, with variations between 14.2 to 144.6 kg ha^{-1} and similar distribution frequency to those observed for biomass. Phosphorus content showed an average value of 8.7 mg kg^{-1} and fluctuation between 0.7 mg kg^{-1} and 44.5 mg kg^{-1} (Table 1). Intermediate proportion (43 %) was registered for sites showing low levels for phosphorus content, while the medium (29 %) and high (28 %) levels were less frequent. Variations were related to soil type, chemical degradation of soils, fixation of phosphorus in clay particles and its precipitation in Fe compounds and calcium phosphates compounds, commonly observed in calcic soils (tecnicoagricola, 2013). Low fertility, observed in soils, was also favored by the moderately alkaline pH observed in most of the soils (84 %). The alkalinity of the soil and the presence of calcium phosphates affected the availability of some plant macro-nutrients, especially phosphorus.

Table 1. Descripive statistics for soil traits evaluated in Durango.

Statistics	Organic Matter (%)	N (kg ha^{-1})	P (mg kg^{-1})	pH
Average	1.4	59.7	8.7	7.5
Minimum	0.3	14.2	0.7	6.2
Maximum	3.3	144.6	44.5	8.1
Std. Deviation	0.5	23.0	7.31	0.3

n = 147

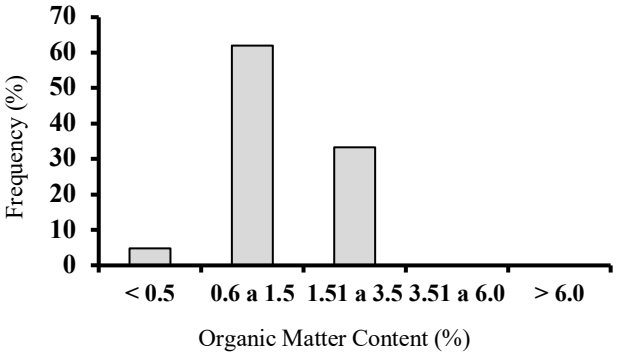

Figure 1. Soil frequencies based on organic matter levels.

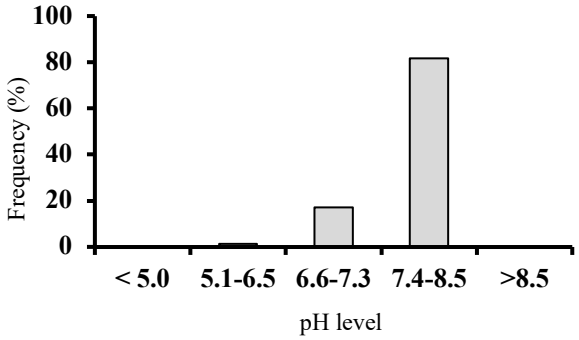

Figure 2. Soil frequencies based on pH levels.

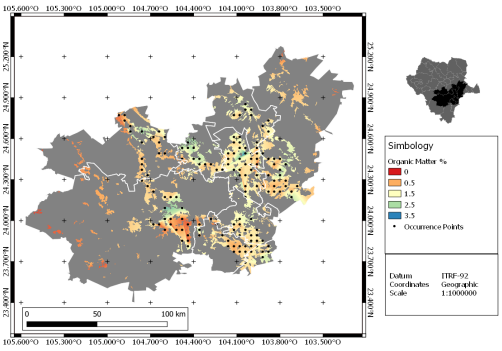

Figure 3. Soil fertility map based on organic matter levels.

Fertility maps permitted visualization of biomass, compost, manure and Bocashi requirements in agricultural soils of Durango (Figure 3), where very low and low values for organic matter were observed. Some sites showed intermediate values for organic matter due to government programs supporting amendments application to agricultural soils (compost, Bocashi and manure).

CONCLUSIONS: In Durango, organic matter application is required in order to reinforce soil fertility and to stabilize pH levels favoring natural release of soil nutrients. Increased yield and sustainable agricultural production would be also achieved.

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EARLY SELECTION OF BRAZILIAN SNAP BEAN CULTIVARS FOR ALUMINIUM TOLERANCE

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INTRODUCTION

Snap bean (*Phaseolus vulgaris* L.) is produced in the tropics and sub-tropics mainly by small farmers where abiotic factors limit the yield potential. Among abiotic factors, aluminum (Al) toxicity is a serious environmental problem that limits crop production at commercial level (Singh et al., 2017). Thus, the aim of this study was to evaluated eight snap bean cultivars for aluminum tolerance.

MATERIALS AND METHODS

Experiments were carried out in a greenhouse at Londrina State University (UEL) in Londrina, Paraná, Brazil. A total eight snap bean cultivars were evaluated (Macarrão Baixo, Manteiga Baixo, Macarrão Napoli, Top Seed, Preferido, Macarrão Brasília, Macarrão Trepador, and Favorito). Experimental design was a randomized block design with four repetitions per treatment. Two Al conditions for distinguishing tolerant and sensible cultivars were applied: with 6 mg L^{-1} Al (+Al) and without Al (–Al) considered is a control, using nutrient solution proposed by Hoagland and Arnon (1950). Seedlings were transplanted to hydroponic boxes with 30 liters of nutrient solution and one plant of each treatment. A total of eight hydroponic boxes were used. Nutrition solution was aerated permanently with pH maintained around to 4.0±0.2. After 14 days, cultivars had their roots scanned and the following traits were evaluated using the GiaRoots (Galkovskyi et al., 2012) software: root volume, root surface area, total number roots, maximum root length, total root length, and mean root diameter. Then, shoot length, dry shoot biomass, and dry root biomass were evaluated. The data were analyzed statistically using analysis of variance to test the hypothesis of differences among cultivars, Al concentrations, and cultivars × Al interaction. Heatmap was performed using standard Euclidean distances. Cultivars were classified for Al tolerance using the selection index under +Al conditions, which calculates the distances of each genotype to an ideal genotype (Wricke and Weber, 1986). The R software (https://www.r-project.org) was used to perform all the statistical analyzes.

RESULTS AND DISCUSSION

Analysis of variances revealed significant effect (P<0.01) for cultivars × Al interaction for all tested traits, indicating a differential behavior of the cultivars in both Al conditions. The visual representation by heatmap allowed to distinguishing clearly the behavior of the cultivars in both Al conditions (Figure 1), indicating that under +Al the average of all traits were lower when compared to –Al and that this condition affected the plants physiology and morphology. In relation to selection index, the cultivars Macarrão Napoli, Top Seed, and Mateiga Baixo were classified as the most tolerant to Al.

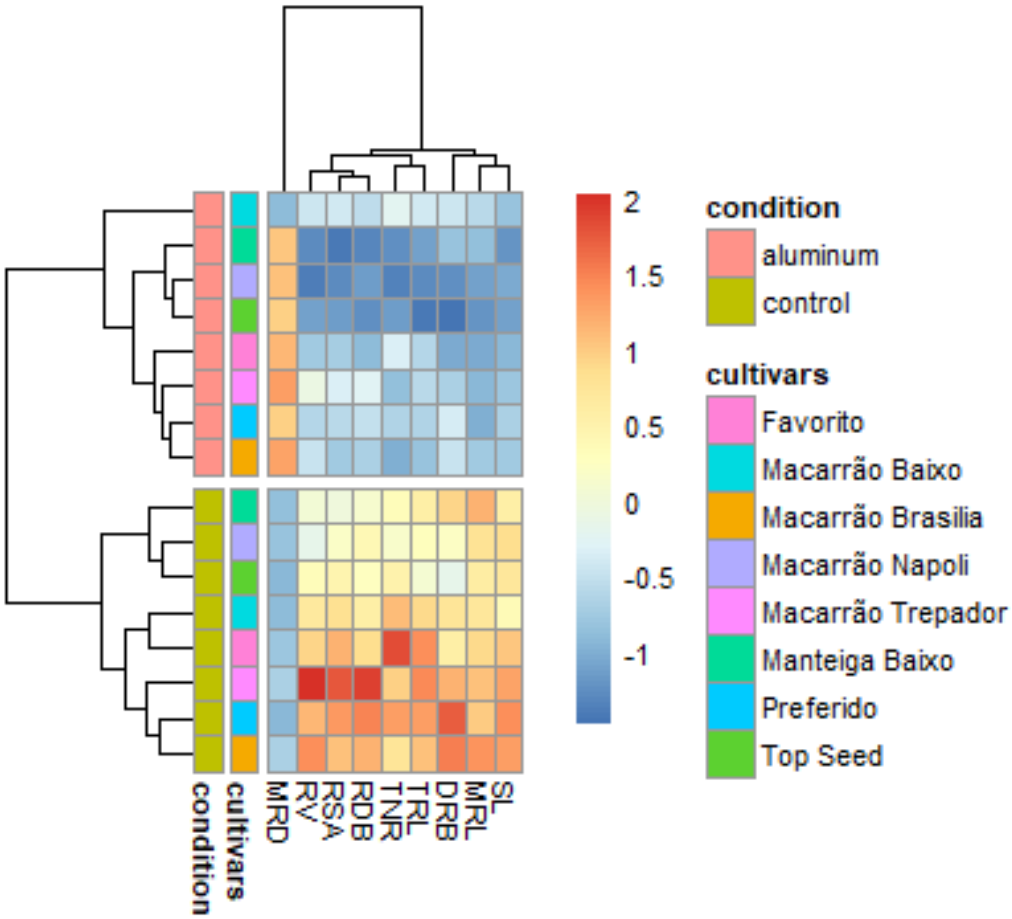

Figure 1. Heatmap of relationships among root volume (RV), root surface area (RSA), total number roots (TNR), maximum root length (MRL), total root length (TRL), mean root diameter (MRD), shoot length (SL), dry shoot biomass (DSB), and dry root biomass (DRB) under presence (6 mg L^{-1}) and absence (control) of aluminum in nutrient solution.

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EFFECT OF SOURCES AND DOSES OF NITROGEN ON THE CONTENT OF BEAN CHLOROPHYL

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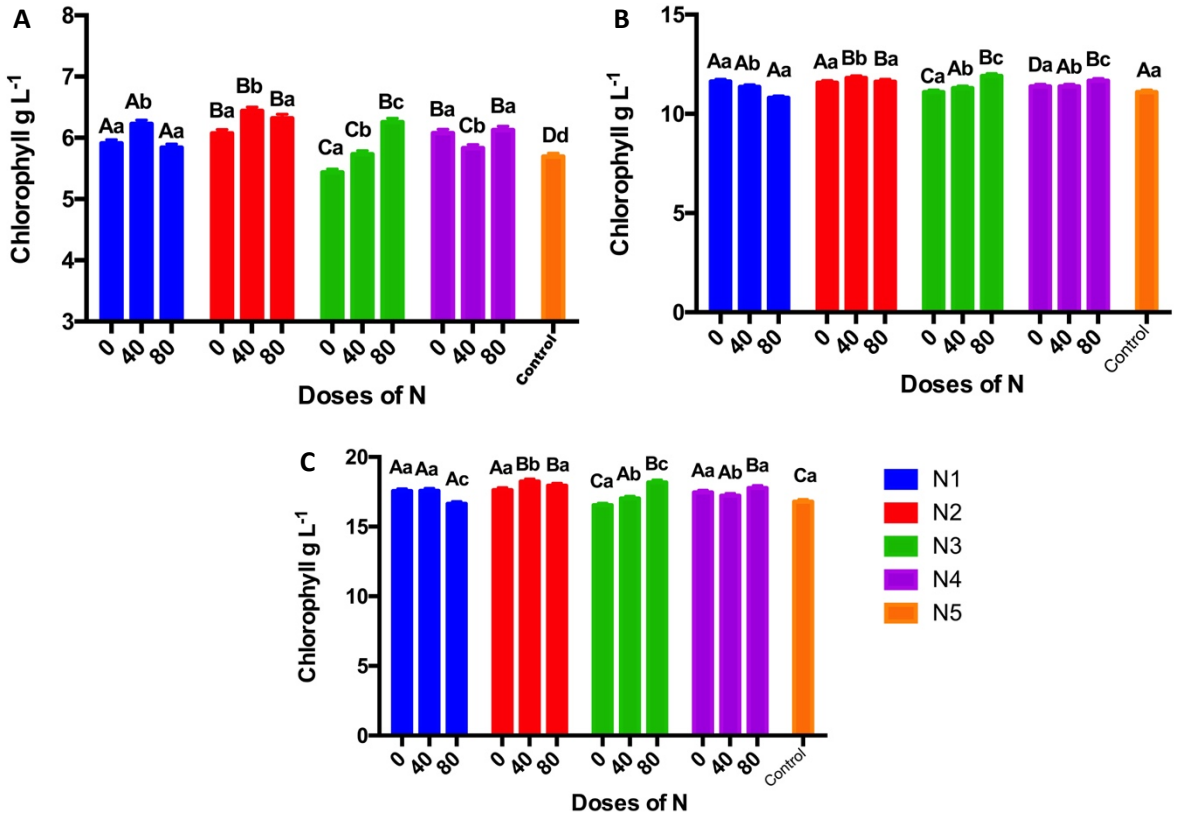
INTRODUCTION: Nitrogen (N) is the nutrient absorbed in the highest amount by common bean (*Phaseolus vulgaris* L.). Plants with deficiency of this element are atrophied and leaves exhibit pale green to yellow coloration symptoms, which begins in older leaves and are related to the role of N in the chlorophyll molecule's structure. The aim of the present work was to estimate the chlorophyll content in common bean plants submitted to three doses of four types of nitrogen fertilizers**.**

MATERIAL AND METHODS: The work was developed in an experimental area of the Agronomic Institute of Paraná (known as IAPAR) (23'21 "S, 51'10" W 585m), in a red dystroferric latosol. The results of soil chemical analysis were $H^{+}+Al^{+3}$ = 3.68 $cmol_c$ dm^{-3}; organic matter = 2.90 g dm^{-3}; K+ = 0.33 $cmol_c$ dm^{-3}; P mehlich = 5.4 $cmol_c$ dm^{-3}; Mg^{+2} = 3.59 $cmol_c$ dm^{-3}; Ca^{+2} = 5.75 $cmol_c$ dm^{-3} e Al^{+3} = 0.00 $cmol_c$ dm^{-3}. A completely randomized design with 14 treatments and four replications was used. Each experimental plot consisted of an area of 24 m^2 (4m x 6m) with 9 rows spaced of 0.50m and population density of 18 plants m^{-1}. In the experiment, four nitrogen sources were used: Urea (N1), ammonium sulfate (N2), nitrocalcium (N3) and ammonium nitrate (N4) in three different doses (O, 40 and 80 kg ha^{-1} of N) of fertilization. In the sowing fertilization, 60 kg ha^{-1} of P_20_5, 40 kg ha^{-1} of K_20 and 20 kg ha^{-1} of the source used for side-dressing nitrogen fertilization were applied. One last treatment consisted of no nitrogen fertilization at all (N5). Seeds of common bean cv. Carioca-80 were sowed on April 4th 2018, emerging eleven days later. Coverage was performed 20 days after emergence (DAE). In the experiment, biweekly irrigation was used, complementing the amount of rain up to 25 mm. In the last stage, chlorophyll-a, chlorophyll-b and total chlorophyll (a + b) were determined using the spectrophotometric method by N-N-dimethylformamide (DMF) extraction.

RESULTS AND DISCUSSION: Since the carbon content is considered to be very high above 1.4% and the irrigation system supplied all culture's water needs, as N is absorbed in higher content by mass flow (MALAVOLTA et al., 1997), distinct nitrogen sources influenced the chlorophyll content in leaves, probably due to the high content of organic matter determined in the soil analysis, which was 2.9%. The content of chlorophyll-b presented a mean value of 11.52 g L^{-1} in leaves of common bean, which was approximately twice the chlorophyll-a content, that presented an average content of 6.01 g L^{-1}. Results obtained by LIMA FILHO (1995) in stevia (*Stevia rebaudiana*), presented an average content of 8.20 to chlorophyll-a, being greater than chlorophyll-b, which presented an average content of 6.1 g L^{-1}. Total chlorophyll average content in stevia was 14.8 g L^{-1}, whereas it was 17.53 in common bean. This higher value of bean crop is probably related to the fact that it is a legume, which fixes nitrogen symbiotically. On the other hand, FERNÁNDEZ-LUQUEÑO et al. (2010) obtained higher yields on common bean plants grown in wastewater sludge-amended soil than in un-amended soil or soil with urea, suggesting that application of organic waste products had better impact on growth and yield of bean plants compared to those amended with inorganic fertilizer. According to BLOOM et al. (2015), once a plant root absorbs NH^{4+} or NO_3 from the rhizosphere, these forms can undergo several paths and either be stored or assimilated into aminoacids in the root, or translocated to shoots, where they

might, again, assume different roles. Authors also infer that mechanisms on how plant respond to different N sources are still little known, and say that despite high costs and time, specific research are necessary.

Figure 1 - A) Average chlorophyll-a content, B) Average chlorophyll-b content, C) Average chlorophyll (a+b) content in common bean submitted to three doses of five nitrogen fertilizers.



CONCLUSION: Different nitrogen sources and its doses directly influenced chlorophyll contents on common bean.

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VEGETATIVE GROWTH OF COMMON BEAN cv."BRSMG MADREPÉROLA" CULTIVATED ON SOIL CONTAMINATED WITH CHROMIUM

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INTRODUCTION – Chromium (Cr) is a chemical element found in several components in the biosphere. In the biotic environment, the metal can be found since in unicellular organisms until complex beings such as animals and plants. It may in some cases be part of metabolic processes which are vital to organisms. Cr is found in the environment in the oxidation states III and VI, being the last one with more severe toxic effects to humans and plants. The effects of Cr (VI) are more severe, due to its higher mobility in soil, its greater ability to penetrate tissues and in addition, to the oxidative and mutagenic potential. Cr (VI) has degenerative effect on cells, promoted by the reaction with nucleic acids of unstable compounds, which are formed inside the cells during the reduction process of Cr. Considering the toxic effect of Cr and the agricultural potential of cv. BRSMG Madrepérola, this study aimed to evaluate the effect of Cr on the vegetative growth of bean.

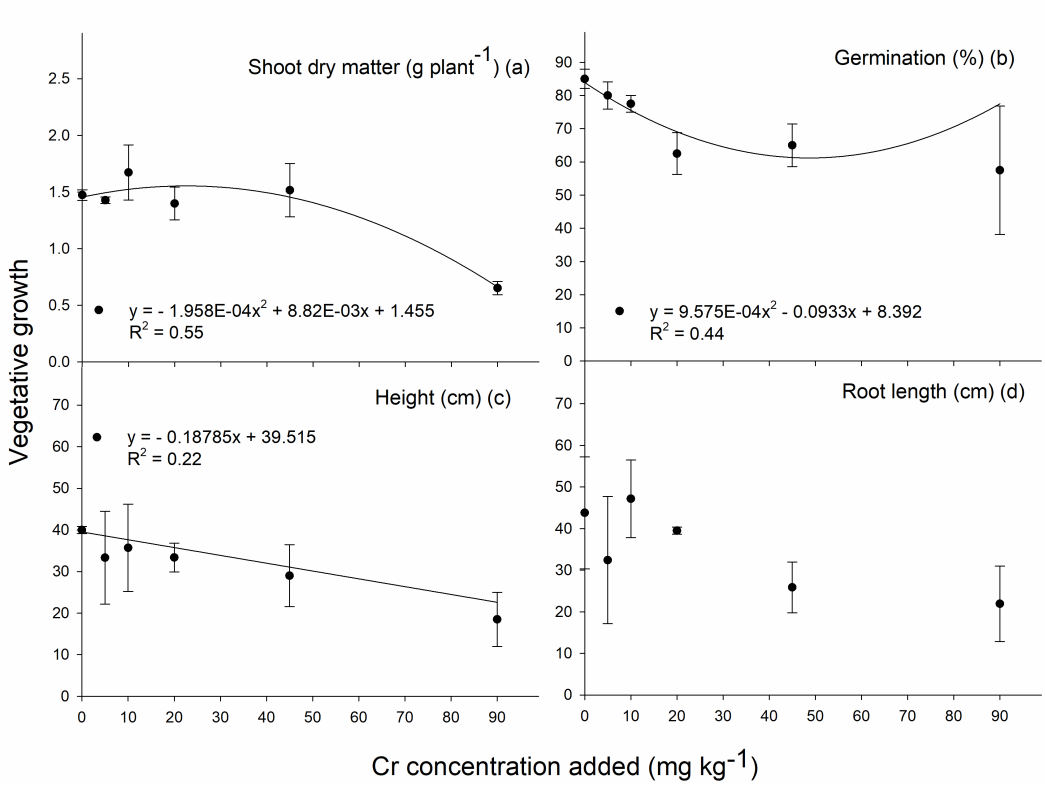
MATERIALS AND METHODS – The experiment was carried out in a greenhouse with 500 cm^3 pots. Samples were collected from the 0-20 cm layer of a Red-Yellow Dystrophic Latosol. Chromium doses were: 0; 5; 10; 20; 45; 90; 200 and 400 mg kg^{-1} and they were applied to the soil as potassium dichromate solutions ($K_2Cr_2O_7$). The experimental design was completely randomized, with eight treatments and four replications. Fertilization was performed according to recommendations for pot experiments. Ten seeds were seeded in each pot 24 hours after application of $K_2Cr_2O_7$. After emergence, the number of plants per pot was reduced to five. The experiment lasted 21 days after the emergence of 50% of the seedlings in the control pot. The crop selected was the common bean (Phaseolus vulgaris L.) BRSMG Madrepérola, with 95% pure and 97% germination.
The number of germinated seeds was counted. At the end of the experiment, the height of the plants was measured and they were harvested, dried and weighed. Data were submitted to variance analysis (ANOVA, $p < 0.05$) and those that presented statistical difference were submitted to the regression analysis ($p < 0.05$).

RESULTS AND DISCUSSION – A significant decrease in bean germination was observed when it was submitted to increasing doses of Cr (Figure 1). In treatments with higher Cr doses, the seeds did not germinate at all. The control treatment obtained at least 8 plants per pot. From the dose of 20 mg kg^{-1} there were significant differences. Concerning the root length, it was significantly affected with increasing Cr doses. On average, in the control treatment, the root length of the bean plants was 57 cm, so that this species was able to withstand the maximum dose of 90 mg kg^{-1} of Cr in soil. It is known that trace elements accumulate in the roots and represent a large part of the elements accumulation in roots. On the other hand, Cr absorbed and translocated to the aerial part could cause damage to its growth. Concerning height, the control treatment was, on average, 36 cm, while at the dose 90 mg kg^{-1} the plants grew around 20 cm, being this dose critical for plants growth. It is interesting to notice that at 5 mg kg^{-1} there was an increase in plant height. This effect called hormesis describes a phenomenon associated to toxic

compounds that at low doses stimulate the exposed organism. The shoot dry matter was also significantly affected. In the control treatment, the average production per plant was 1.47 g of biomass. Plants revealed higher sensitivity at 90 mg kg^{-1}. It was observed hormesis effect also for shoot dry matter at the first doses.

The decrease on shoot dry matter production and shoot height is due to direct and indirect effects, but the latter is more significant. The indirect effect is the reduction of the root system and the reduction in the absorption and translocation of water and nutrients. The direct effect is due to the impact on the cells metabolism which reduces cell divisions, due to the reducing effect on the palisade parenchyma, due to the increase of the number of vacuoles along the xylem and phloem walls and due to the reduction of gas exchange, the chemical composition and the internal morphology of the plant.

Figure 1. Shoot dry matter, germination, height and root length of bean plants submitted to Cr in LVAd. The standard error of the mean is represented by bars (n = 4).



CONCLUSIONS – At higher doses of Cr in soil (above 45 mg kg^{-1}), there is a decrease in germination and development of the aerial part of the cv. BRSMG Madrepérola. Chromium was toxic to this common bean cultivar and when present in soil at greater doses it can be inferred that it will affect the plant development and productivity.

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MICRONUTRIENTS LEVELS IN COMMON BEAN cv."BRSMG MADREPÉROLA" ON SOIL CONTAMINATED WITH CHROMIUM

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INTRODUCTION – Some elements, such as chromium (Cr), are essential for animals and are not for plants. Cr is found in the environment in the following oxidation states: III and VI. Cr (III) is essential for animals because it acts on glucose metabolism and is even used in food supplementation in humans. The Cr (VI) has greater oxidative power and greater permeability in the cellular membrane, being then more toxic than Cr (III). In plants, Cr appears to be more toxic to roots than to shoots, severely reducing root growth. It affects the germination, growth and plants development in general, as it promotes oxidative stress and nutritional imbalance. The oxidation state and the mobility of Cr in soil are influenced by factors such as organic matter, clay content, Al, Fe, Mn and pH. These relationships influence the dynamics of the metal in soil and contribute to lower risk conditions, as the less toxic oxidation state is favored by the environmental conditions.

Bean (Phaseolus vulgaris L.) is highly consumed in Brazil. There is an estimate that seven Brazilians out of ten consume beans every day in the country and, on average, the annual consumption can reach 19 kg of beans. Common beans are grown throughout Brazil under a variety of environmental conditions and are extremely relevant in the internal market. The cv. BRSMG Madrepérola produces high quality "Carioca" grains, is semi-precocious and takes 85 days from the emergence of seedlings until physiological maturation. Thus, adequate nutrition of common bean plants becomes important and disturbances regarding the availability of micronutrients can lead to severe losses of productivity, such as contamination of the soil by toxic elements. This work aims to evaluate the influence of Cr concentrations in soil on Fe, Mn, Zn and Cu micronutrients in common bean plants.

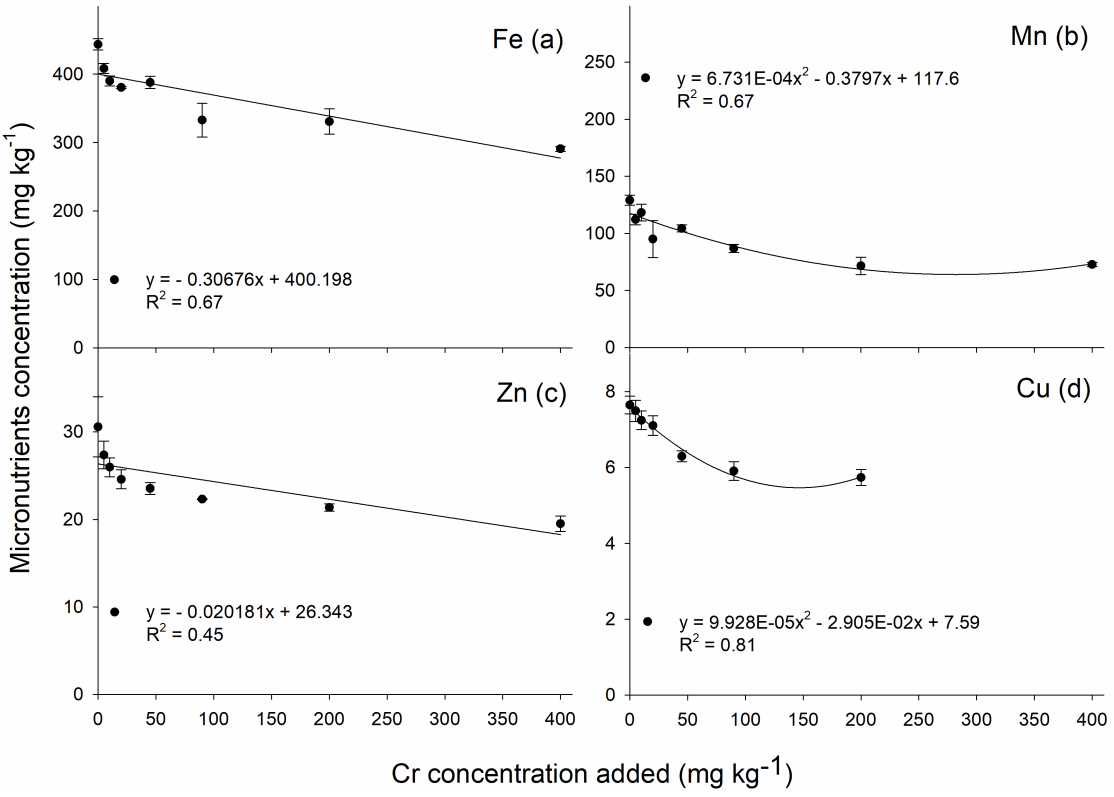
MATERIALS AND METHODS – The experiment was carried out in a greenhouse with 500 cm^3 pots. Samples were collected from the 0-20 cm layer of a Dystrophic Haplic Cambisol (Cxbd) with medium texture and 0.8 % of organic matter . Chromium doses were: 0; 5; 10; 20; 45; 90; 200 and 400 mg kg^{-1} and they were applied to the soil as potassium dichromate solutions ($K_2Cr_2O_7$). The experimental design was completely randomized, with eight treatments and four replications. Fertilization was performed according to recommendations for pot experiments. Ten seeds were seeded in each pot 24 hours after application of $K_2Cr_2O_7$. After emergence, the number of plants per pot was reduced to five. The experiment lasted 21 days after the emergence of 50% of the seedlings in the control pot. The crop selected was the common bean (Phaseolus vulgaris L.) BRSMG Madrepérola, with 95% pure and 97% germination.

After the experiment, plants were harvested and dried in order to have concentrations of Fe, Mn, Zn and Cu determined, according to the well established procedures. Data were submitted to variance analysis (ANOVA, $p<0.05$) and regression analysis ($p<0.05$).

RESULTS AND DISCUSSION – Contents of Fe, Mn, Zn and Cu decreased with increasing Cr content in soil. Iron content in beans cultivated in CXbd did not reduced significantly in shoots. However, Mn content decreased with increasing Cr from the dose of 5 mg kg^{-1}. Chromium can

induce the deficiency of Fe and Mn, as it competes in the absorption and transport of these nutrients, impairing the oxidation processes in the metabolism, photosynthesis and in the chlorophyll synthesis. Concerning Zn contents in the vegetal tissue, it also demonstrated reduction already at the first doses. The correlation between the increase in Cr concentration and Zn reduction is possibly due to a blockage at the absorption sites. In addition, Zn acts as a component and an enzymatic activator and the presence of a potentially toxic element can affect its role in the plant. In beans, there was no significant decrease in the Cu content of shoots of the plants grown in CXbd. Cu contents in shoots reduced significantly from 45 mg kg $^{-1}$ of Cr. The presence of Cr could affect the uptake and transport of Cu and also could be responsible for a decrease in gas exchange and for damages in enzymes that are responsible of protecting plants from reactive oxygen species, indicating low biomasss development.

Figure 1. Micronutrients concentration of common beans cv. BRSMG Madrepérola grown in Cambissolo Háplico Tb distrófico with increasing Cr concentrations. The standard error of the mean is represented by bars (n = 4).



CONCLUSIONS – The presence of Cr was crucial in the nutritional imbalance of beans. Micronutrients were reduced as the dose of Cr increased in the soil, which impaired the biomass production and productivity. A competitive inhibition could have occurred between Fe and Mn due to the great content of Fe in dry matter. Zinc and copper absorption was also damaged with increasing concentrations of Cr.

ACKNOWLEDGMENTS - The authors would like to thank CAPES, CNPq, and FAPEMIG for the financial support.

PROLINE LEVELS IN COMMON BEAN cv."BRSMG MADREPÉROLA" ON TROPICAL SOILS CONTAMINATED WITH MERCURY

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INTRODUCTION – Mercury is one of the most problematic elements due to its high toxicological risk. The dynamics of Hg in soils depends on the solubility and adsorption on the organic and mineral phases. The clay content is one of the most relevant attributes that influence Hg sorption. This metal can be absorbed rapidly and leads to changes in the physiology and concentration of many metabolites in the plant. Among these disorders, it can be observed a significant change in the proportions of amino acids and frequently increase in the concentration of proline promoted by proteins hydrolysis. Proline acts as osmoprotectant and also controling reactive oxygen species (ROS). On the other hand, the increase in the proline content can affect the vegetal metabolism, altering the water potential of the leaves.

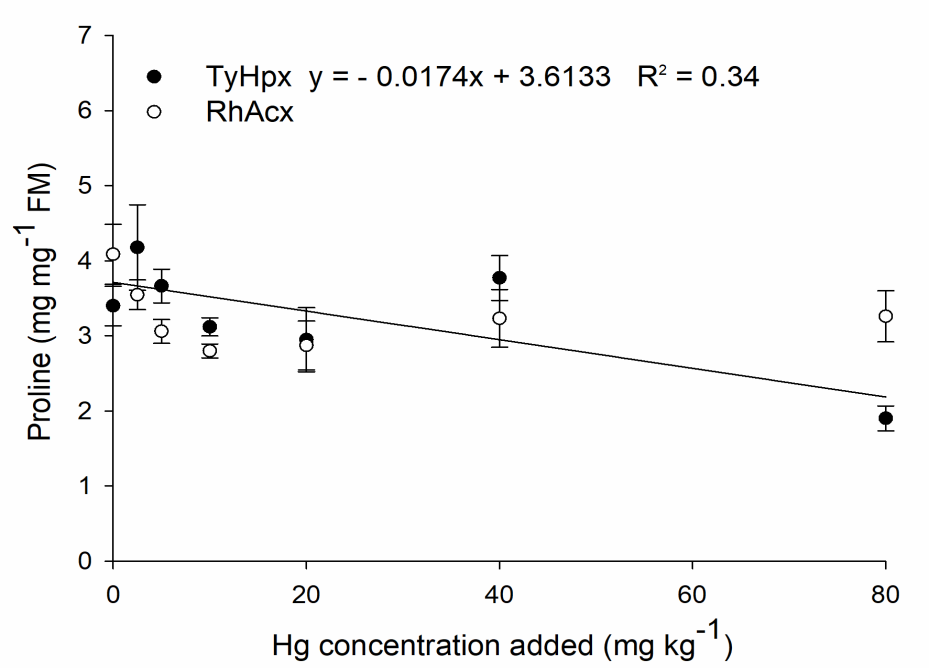
It is important to evaluate the effect of Hg on cultivated plants, emphasizing on highly consumed food crops as common bean (*Phaseolus vulgaris* L.). "Carioca" bean represents 79% of the consumption of this grain in Brazil. The BRSMG Madrepérola cultivar is highly favored due to its high quality, semi-precocious cycle (85 days). This work aims to study the effects of Hg on proline production in cv. BRSMG Madrepérola at the beginning of the crop cycle on two tropical soils.

MATERIALS AND METHODS – The experiment was carried out in a greenhouse. Samples (0-0.2 m) from Typic Hapludox (TyHpx) and Rhodic Acrudox (RhAcx) were used. The TyHpx has 31% clay and 2% organic matter (OM) and RhAcx 68% clay and 4% OM. Base saturation was increased to 60% and pH stabilized at 6. Soil was fertilized according to the recommendations for plant experiments in pots. Nitrogen, P, K, and S were applied through mono-ammonium phosphate and K-sulfate, while B, Cu, Zn, and Mo were supplied through boric acid, Cu-sulfate, Zn-sulfate, and ammonium molybdate, respectively. It was chosen the common beans (*Phaseolus vulgaris* L.) cv. BRSMG Madrepérola. Mercury concentrations of 0; 2.5; 5.0; 10.0; 20.0; 40.0 and 80.0 mg kg^{-1} were applied to 500 cm^3 of dry soil in the form $HgCl_2$ solutions. A completely randomized design was set, with seven treatments and four replications. Ten seeds were sown in each pot 24 hours after the $HgCl_2$ application. After emergence, the number of plants per pot was thinned to six. The experiment lasted 21 days after 50% of seedling emergence in the control pot.

For the analysis of proline, the extract was obtained by grinding 200 mg of the shoots dry matter in 3% sulfosalicylic acid. Then, the samples were shaken at room temperature for 60 minutes and the extract was filtered. For quantification, 100 μL of the extract was added in tubes with 1.9 mL of distilled water, where they were shaken and taken to the water bath at 100 °C for 60 minutes. Samples were cooled in ice bath and the proline contents were determined after analysis at spectrophotometer ELISA. The results were submitted to analysis of variance (ANOVA, $p < 0.05$) and those that presented a significant difference, also submitted to the regression analysis ($p < 0.05$).

RESULTS AND DISCUSSION – The proline content in TyHpx was increased at the concentration of 2.5 mg kg^{-1} of Hg comparing to the control treatment. At concentrations between 5 and 40 mg kg^{-1}, Hg was not capable to induce significant differences in proline content compared to the treatment without Hg. However, at the highest concentration (80 mg kg^{-1} of Hg), the proline content suffered a significant decrease compared to the control treatment. The regression curve, although statistical significant, had a very low determination coefficient. In the RhAcx at all tested concentrations of Hg, the proline content had a tendency of decreasing compared to the treatment without addition of Hg. however, the treatments did not show statistical difference (ANOVA, $p < 0.05$).

Figure 1 Proline in common bean cv. BRSMG Madrepérola after cultivation with increasing concentrations of Hg in Typic Hapludox (TyHpx) and a Rhodic Acrudox (RhAcx).



A common occurrence observed in plants under the effect of trace elements is the hormesis effect, in which low concentrations of the element can be responsible for an increase in certain physiological effect, with proline contents also increasing. However, at high concentrations, the contrary happens, the deleterious effect of the element promotes the decrease in many biological variables such as in proline contents, which was verified at TyHpx. This indicates that Hg began to cause negative effects on the proline content in TyHpx, suggesting toxicity in this soil, from the maximum concentration of Hg tested. It is worth mentioning that TyHpx possesses half of clay content and organic matter of RhAcx. Hence, the complexation and adsorption ratios can interfere in the fixation of Hg in soil and thus TyHpx can reveal greater availability to the plants.

The increased proline content in the first tested concentration of Hg in TyHpx results in a greater hydration of these plants, therefore, at the leaves, since a higher proline content has an osmoprotective effect, due to the formation of hydration shells around enzymatic proteins, allowing the correct structuring and functionality of the same.

In addition, there is a well-established relationship between proline content and the elimination of ROS and the inhibition of lipid peroxidation. Due to the increase in proline content, the oxidative stress commonly observed by the presence of trace elements such as Hg, can be controlled, indicating the tolerance of this metal at the concentration of 2.5 mg kg^{-1} of Hg in TyHpx. At the concentration of 80 mg kg^{-1} in TyHpx, where had a decrease in proline content, and at all tested concentrations of Hg in RhAcx, where proline content had a tendency of decreasing when compared to the control treatment, suggesting that proline no longer acts controlling the ROS that are formed by the presence of Hg, indicating toxicity promoted by this element.

CONCLUSIONS – The hormesis effect may occur with the application of Hg in TyHpx for, at the concentration of 2.5 mg kg^{-1} of Hg, there is an increase of proline content. The opposite occurs at higher concentrations (80 mg kg^{-1} of Hg), indicating toxicity. In RhAcx, Hg was deleterious even at the lowest concentration (2.5 mg kg^{-1} of Hg).

MICRONUTRIENTS IN COMMON BEAN cv."BRSMG MADREPÉROLA" ON TROPICAL SOILS CONTAMINATED WITH MERCURY

Lima, F.R.D.[1], Silva, A.O.[1], Vasques, I.C.F.[1], Oliveira, C.[1], Martins, G.C.[2], Engelhardt, M.M.[1], Dos Reis, R.H.C.L.[1], Pereira, P.[1], Oliveira, J.R.[1], Guilherme, L.R.G.[1], Marques, J.J.[1]

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INTRODUCTION – Toxic elements in the environment may interfere on the mineral nutrition and metabolic pathways when absorbed by plants. Mercury, due to its high toxicity and biaccumulation capacity, it is one of the most harmful elements to the biota. Mercury can modify the physiology and metabolism of the plant, due to the changes in nutrient absorption and accumulation in the plant tissues.

Brazil is the largest producer and consumer of common bean (*Phaseolus vulgaris* L.) in the world, with approximately 3.2 million tons per year. Among the several cultivars developed, cv. BRSMG Madrepérola is very well adapted, producing high quality semi-precocious "carioca" grains, with resistance to the main diseases, including the common mosaic virus and various types of anthracnose. This work aimed to evaluate the influence of high concentrations of Hg on the content and accumulation of micronutrients in BRSMG Madrepérola cultivated in two tropical soils, artificially contaminated.

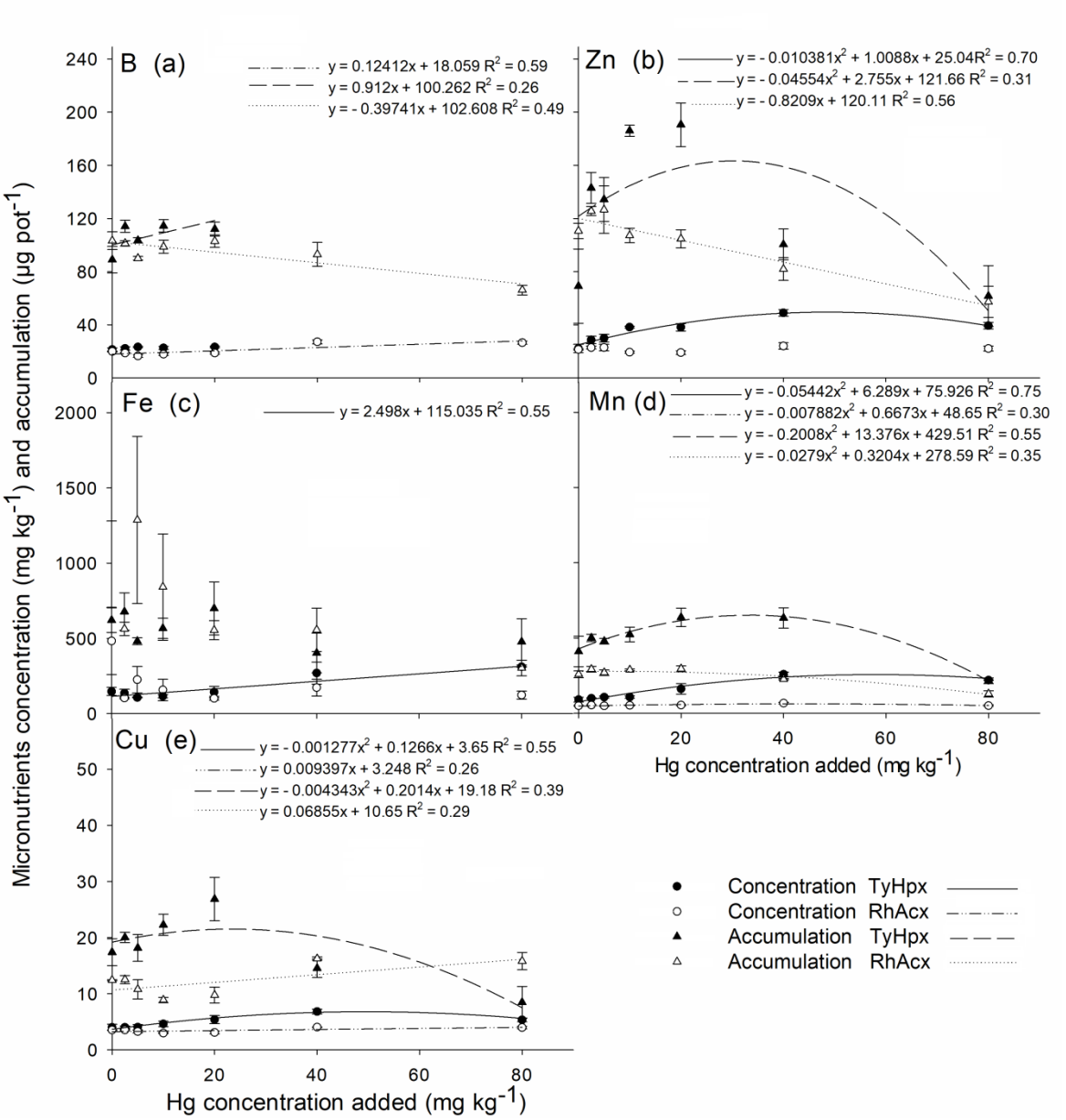
MATERIALS AND METHODS – The experiment was carried out in a greenhouse. Samples (0-0.2 m) from Typic Hapludox (TyHpx) and Rhodic Acrudox (RhAcx) were used. The TyHpx has 31% clay and 2% organic matter (OM); and the RhAcx 68% clay and 4% OM. Base saturation was increased to 60% and pH stabilized at 6. Soil was fertilized according to the recommendations for plant experiments in pots. Nitrogen, P, K, and S were applied through mono-ammonium phosphate and K-sulfate, while B, Cu, Zn, and Mo were supplied through boric acid, Cu-sulfate, Zn-sulfate, and ammonium molybdate, respectively. It was chosen the common beans (*Phaseolus vulgaris* L.) cv. BRSMG Madrepérola. Mercury concentrations of 0; 2.5; 5.0; 10.0; 20.0; 40.0, and 80.0 mg kg^{-1} were applied to 500 cm^3 of dry soil in the form $HgCl_2$ solutions. A completely randomized design was set, with seven treatments and four replications. Ten seeds were sown in each pot 24 hours after the $HgCl_2$ application. After emergence, the number of plants per pot was thinned to six. The experiment lasted 21 days after 50% of seedling emergence in the control pot.

After the experiment, the plants were harvested, dried and weighted. Micronutrients in the shoot dry matter (SDM) were analyzed according to standard procedures. Nutrient accumulation was obtained by multiplying concentration by SDM. Data were submitted to analysis of variance (ANOVA, $p < 0.05$) and those that presented a significant difference, also submitted to the regression analysis ($p < 0.05$).

RESULTS AND DISCUSSION – The increase of Hg levels in the studied soils promoted changes in the concentration and accumulation of the micronutrients B, Zn, Fe, Mn and Cu in the SDM of cv. BRSMG Madrepérola (Figure 1). For Zn and Mn (Figure 1b and 1d), there was a decrease for both concentration and accumulation. This behavior was also found for Cu when the plants were grown in TyHpx (Figure 1e). Boron and Cu increased when grown in RhAcx (Figure 1a and 1e). The higher percentage of OM (4%) and clay (68%) in the RhAcx may have influenced the higher complexation and adsorption of Hg in the soil, providing lower binding sites with Cu in the OM, thus increasing the absorption of this element by the plants. Concerning Fe, effect was only observed at TyHpx which was an increase in the concentration of this element in the SDM (Figure 1c).

The reduction of the absorption and accumulation of some nutrients in the plants may be due to the blockade promoted by Hg which restricts the entrance of cations in the root system. In addition, it is known that the physiological transport of nutrients is element-specific in plants. Trace elements such as Hg can compete with nutrients during transport by occupying the transmembrane transporters which causes nutritional imbalance.

Figure 1 Concentration and accumulation of micronutrients B (a), Zn (b), Fe (c), Mn (d) and Cu (e) in common beans cv. BRSMG Madrepérola grown in Typic Hapludox (TyHpx) and a Rhodic Acrudox (RhAcx) with increasing Hg concentrations (± mean standard deviation, n = 4).



CONCLUSIONS – The presence of Hg in soil at concentrations greater than 40 mg kg^{-1} caused the concentration and accumulation of the micronutrients B, Zn, Fe, Mn, and Cu in cv. BRSMG Madrepérola. This accumulation was more pronounced in the soil with lower content of clay and organic matter (TyHpx).

ACKNOWLEDGMENTS - The authors thank the CAPES, CNPq, and FAPEMIG for financial support.

INHERITANCE OF THE PRESENCE OF PURPLE STRIPES LOCATED AT THE EXTERNAL SURFACE OF THE FLOWER STANDARD AND ITS RELATIONSHIP WITH SEED COAT COLOR IN BADILLO/PR1144-5 COMMON BEAN POPULATION

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INTRODUCTION: The presence of colored stripes (e.g., purple or pink) at the external surface of the flower standard is a morphological characteristic observed in the Middle American gene pool of common bean (*Phaseolus vulgaris* L.) (Singh et al., 1991). The genetics of their inheritance in common bean genotypes of different market classes and their association with seed traits need to be evaluated. Our objectives were to determine: (1) the number of gene (s) involved in the presence of purple stripes and (2) the relationship of the presence/absence of these purple stripes with the seed coat color.

MATERIALS AND METHODS: Light-red kidney 'Badillo' (Beaver et al., 2010) was crossed with black common bean PR1144-5 (Beaver, 2017; personal communication). Badillo is a common bean cultivar with lilac flowers and lacks colored stripes at the external surface of the flower standard (Figure 1A). PR1144-5 is a breeding line derived from *P. coccineus* L. and has a salmon flower with purple stripes (Figure 1B). Fifty F_1 hybrids, 102 F_2, and 236 F_3 seeds, and their parents were evaluated in greenhouses at the Isabela and Lajas Substations at University of Puerto Rico in 2017 and 2018. The presence/absence of purple stripes at the external surface of the flower standards were noted. Also, seed harvested from the F_2 were classified as seeds with dark or light color seed coat. F_2 and F_3 were subjected to a chi-square test to identify the type of segregation and number of gene (s) involved with the presence of purple stripes. Moreover, a correlation analysis was conducted in the F_2 between flowers with and without purple stripes and seed with light red seed coat or a different color. Scores of 1=absence of purple stripes and light-red seed, and 2=presence of purple stripes and lack of light red seed were used.

RESULTS AND DISCUSSION: All F_1 had salmon flowers with purple stripes and produced black seed indicating dominance over light purple flowers with non-purple stripes and light red seed coat traits, respectively. The F_2 segregated into a 3:1 proportion ($\chi 2 = 0.05$; $P \geq 0.05$) for the presence of purple stripes vs. their absence on the standard of petals despite of the flower color observed (Table 1). The F_3 corroborated the 3:1 ratio observed in the F_2 ($\chi 2 = 1.51$; $P \geq 0.05$). Furthermore, plants with the dominant and recessive homozygous alleles (AA and aa, respectively) were noted in the F_3 and fit the expected ratio, perfectly (Table 1). Thus, a single dominant gene is responsible for the purple stripes in the Badillo/PR1144-5 population. A different pattern of dark seed coat color (e.g., black, red, and brown) was noted in seeds harvested from plants with purple stripes on their flowers. In contrast, flowers without purple stripes only produced light red and brown seed coat colors. Furthermore, a positive correlation ($r^2 = 0.92$; $P \leq 0.01$) was noted between flowers without purple stripes and light red seeds, suggesting that this recessive gene might be linked and cosegregating with the gene/QTL responsible of the light red seed coat. However, further research is necessary to identify the molecular position of this gene and the genetic relationship with other gene/QTL involved in red seeds (Bassett, 1998). It has been well

documented that the *P* locus is responsible of seed coat color in different common bean populations (Bassett, 2003; McClean et al., 2002). Likewise, QTL on Pv08 and Pv11 were involved in the seed coat color in common bean crosses between black and white genotypes (Zhu et al., 2017). In practical terms, the absence of purple stripes at the external surface of the flower standard might act as a morphological marker to select light red seeds before harvest. This practice would reduce labor, greenhouse/field and crop management expenses at reproductive stages in common bean breeding programs. Also, it would be useful to corroborate if flowers without colored stripes might be used as criteria to select seeds with light coat colors (besides red) in other common bean populations.

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Table 1. Segregation ratio of the presence/absence of purple stripes located at external surface of the flower standard in Badillo/PR1144-5 common bean population. Puerto Rico, 2017 and 2018.

Genotype	No. plants/ flowers	No. flowers with purple stripes	No. flowers with non-purple stripes	χ^2	*P*
Badillo	30	-	30	-	-
PR1144-5	30	30	-	-	-
Badillo/PR1144-5					
F_1	50	50	-	-	-
F_2	102	78	24	0.05	≥0.05
F_3[a]	49	49	0	0.00	≥0.05
F_3[a]	89	62	27	1.51	≥0.05
F_3[b]	98	0	98	0.00	≥0.05

[a] F_3 from F_2 plants with flowers with purples stripes (AA or Aa genotypes).
[b] F_3 from F_2 plants with flowers without purples stripes (aa genotype).

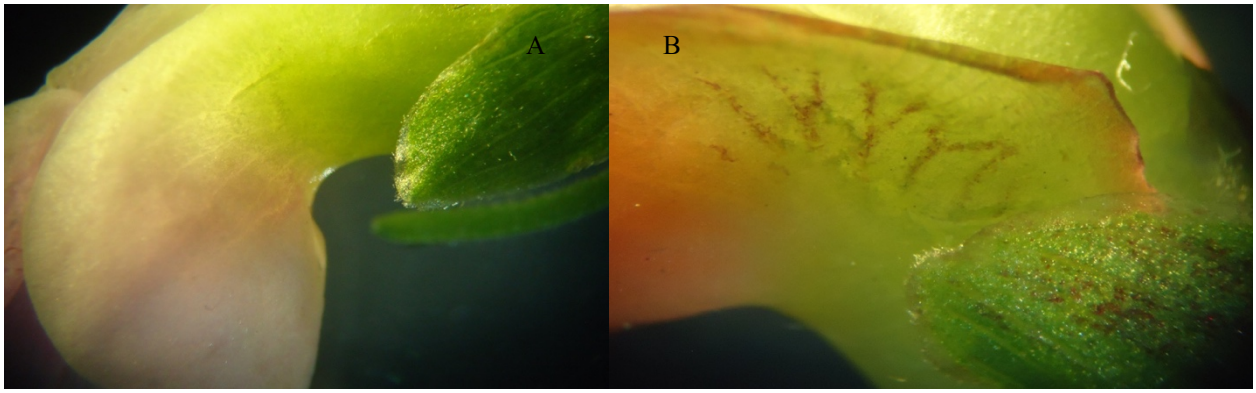

Figure 1. (A) absence of colored stripes observed at the external surface of the flower standard in cultivar 'Badillo'; (B) presence of purple stripes in breeding line PR1144-5.

GENOTYPING THE *EX SITU* GENETIC RESOURCES OF WILD AND CULTIVATED TEPARY BEAN

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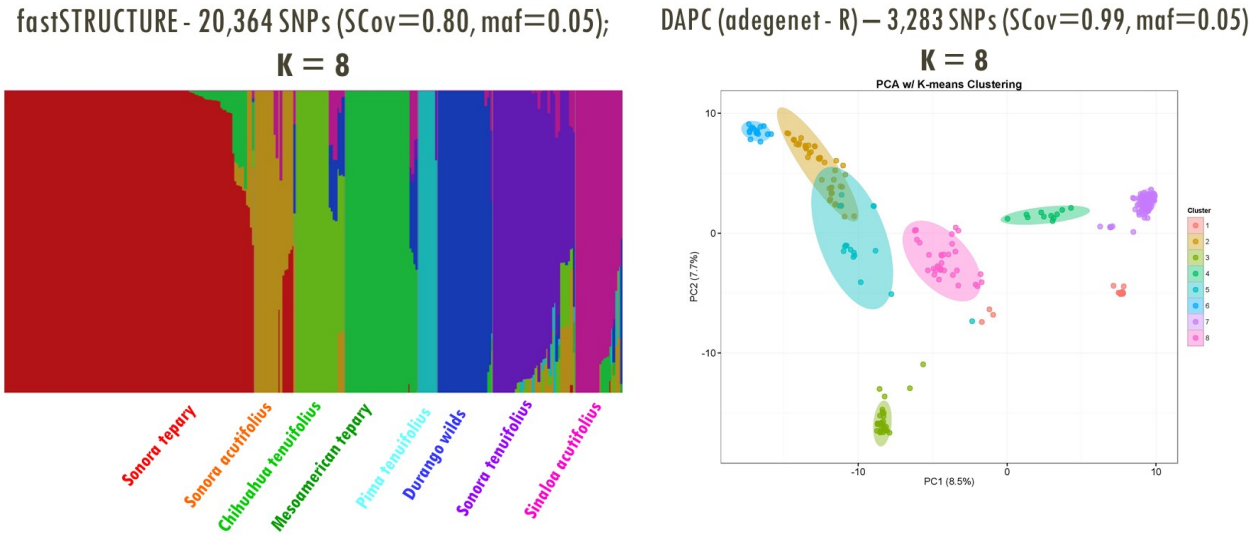
INTRODUCTION: The tepary bean (*Phaseolus acutifolius* A. Gray) is a relatively untapped source of novel allelic diversity both as a donor for common bean improvement, and as an intrinsically stress-tolerant and nutritious food crop. Given the present and future socioeconomic and climatic scenarios for Phaseolus bean production and the immense potential that tepary beans may offer for adaptation to these scenarios, further characterization of the *ex situ* genetic resources is timely. The objectives of this research were to assemble, increase, and genotype all of the available wild and cultivated tepary bean accessions held by the USDA, CIAT, and TARS germplasm collections, and to investigate the genetic diversity and population structure of this germplasm as it relates to domestication status, morphological classification, and geographical distribution. These objectives are part of an overall objective to advance the conservation, utilization, and deployment of the genetic diversity of tepary beans.

MATERIALS AND METHODS: An initial total of 314 accessions (158 wild, 156 cultivated) were obtained and increased in the screenhouse at TARS as single plants. The 156 cultivated accessions were further increased in the field and have been phenotyped extensively. These 314 accessions were genotyped as part of a 384-plex *Ape*KI genotyping-by-sequencing (GBS) library (Elshire et al., 2011; Hart and Griffiths, 2015) by submitting this library to 4 lanes of 101-cycle sequencing on an Illumina HighSeq 2500 at the Weill Cornell Genomics Resources Core. The resulting GBS tags were processed with the GBS Discovery Pipeline for species with a reference genome in TASSEL v3.0 (Bradbury et al., 2007), aligned to the *P. vulgaris* v1.0 reference genome (Schmutz et al., 2014), and SNPs were called and filtered with the TASSEL v3.0 Discovery SNP Caller (Glaubitz et al., 2014).

RESULTS AND DISCUSSION: This genotyping effort resulted in 3.2 million unique GBS tags of which 50% could be aligned to the reference genome with the Burrows-Wheeler Alignment (BWA) tool (Li and Durbin, 2009), or 64% with Bowtie 2 (Langmead and Salzberg, 2012). After alignment with BWA we were able to discover and genotype 20,364 SNPs (with MAF $\geq$ 0.05) that were present in at least 80% of the accessions. When the wild germplasm was considered exclusively, this number changed to 23,070 SNPs and was in sharp contrast with the cultivated germplasm where only 7,642 SNPs were discovered. This is another indicator of the severely reduced diversity in cultivated tepary germplasm. We used the dataset for all of the accessions to investigate population structure with fastSTRUCTURE (Raj et al., 2014) and 3,283 SNPs that were called in all accessions for discriminant analysis of principal components (DAPC) (Jombart et al., 2010). The results of both analyses suggested that the number of subpopulations present in the germplasm is equal to eight (Figs. 1 and 2), and that the membership of each accession in the eight subpopulations is structured based on domestication status, geographic origin, and

morphological variation. These preliminary results confirm the strong bottleneck caused by tepary domestication, identify subpopulations of tepary germplasm in both the wild and cultivated genepools according to geographical origin, and present extensive opportunities for further research into the evolution, domestication, diversity, and improvement of tepary bean.

Figures 1 & 2: Results of fastSTRUCTURE analysis (left) and DAPC (right) with 314 wild and cultivated tepary germplasm accessions.



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SEED YIELD OF WILD, DOMESTICATED BEANS AND THEIR LINES

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INTRODUCTION

Mexico has one of the main centers of plant diversity in the world, particularly common bean (*Phaseolus vulgaris* L.) and their wild relatives have great diversity. Beans are consumed as a snap beans and dry beans. Efforts towards the improvement of the domesticated form continue to this day. The aim of the present work was to assess the seed yield, its components and the modified harvest index of a domesticated form of common bean, of a wild form bean and several of their inbred lines.

MATERIALS AND METHODS

The study was conducted under greenhouse conditions at Montecillo, State of Mexico (19º 29´ N and 98º 53´ W; 2250 m above sea level). Seven bean genotypes provided by Dr. Jorge Acosta Gallegos of Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) were employed. Wild (G23429 S13) (CIAT, 2017) a type IV (indeterminate growth) and domesticated Negro Tacaná (NT) type II were mated to obtain the inbred lines 118b, 53b, 51b, 3.3 and 11.1. The seeds were sowed (May 13th 2014) in 250 mL polyurethane pots filled with "tezontle" (inert volcanic cinder) and watered with distilled water. After six days the plants were watered with Steiner solution (Steiner, 1984) using tap water added micronutrients (Fermil®). Ten or twelve days after sowing (das) the plants were transplanted one per pot of 19 kg of capacity filled with "tezontle". A completely randomized experimental design with eight replications was used. Each inbred line and each progenitor constituted a treatment. The experimental unit was one plant per pot. The abscissed organs were collected to determine the modified harvest index (Kohashi *et al*., 1980). A variance analysis was used with SAS (SAS Institute, Inc. 2012).

RESULTS

There were statistical differences among progenitors and inbred lines (Table 1). Lines 118b and 3.3 exhibited the highest seed yield; wild S13, the domesticated NT and the lines 11.1 and 51b showed intermediate seed yield and the line 53b showed the lowest yied. S13 exhibited the highest number of pods and seeds per plant, characteristic of wild plants (Ross and Lembi, 2009). The knowledge of the characteristics of wild and inbred lines can be useful in a plant breeding program.

Table 1. Seed yield and its components of wild, common bean and their inbred lines grown under greenhouse and watering with Steiner solution.

Progenitor/ Inbred line	Seed yield (g plant^{-1})	Pods per plant	Seeds per plant	Seeds per pod	100 seed weight (g)	†Biomass (g plant^{-1})	MIH (%)
118b	226.5 a	296 b	1641 b	5.8 abc	13.8 c	489.5 ab	46.2 a
3.3	224.5 a	229 bc	1526 cb	6.6 a	14.7 b	514.4 a	45.4 a
NT	156.4 b	103 d	553 e	5.8 abc	28.3 a	320.7 c	48.9 a
11.1	154.2 b	201 c	1108 cd	5.5 bc	13.9 c	333.4 bc	47.3 a
S13	153.6 b	817 a	3513 a	4.4 d	4.4 e	493.3 ab	31.2 a
51b	138.1 bc	192 c	974 de	4.9 cd	14.2 bc	311.0 c	45.6 a
53b	98.6 c	171 cd	1088 cd	6.5 ab	9.1 d	288.4 c	39.6 a
CV	18	19	20	19	1	18	24
$DSH_{0.05}$	47	87	466	1	1	168	24

Mean values with the same letter within columns are statistically similar ($\alpha \leq 0.05$).
† = shoot biomass (include shoot and shoot fallen organs); MHI = Modify Harvest Index.

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ESTIMATORS OF THE LEAF AREA IN BEANS

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INTRODUCTION

The main photosynthetic machinery of a crop is the leaf area (LA), so its measurement is of importance, since it is related to the growth and production of dry matter. In general, the measure of LA is made in instruments such as the area integrator that provides accurate measurements. However, the equipment is expensive, which limits its availability. Thus, the search for estimators is based on the leaflet dry weight (Escalante, 1980). Others using non-destructive methodologies such as length and width of leaf blades such as the case of Pandey and Singh (2011), who report a coefficient to estimate the LA of 0.75 in maize; Stickler *et al*. (1961), of 0.747 for grain sorghum; of 0.660 and 0.750 for sugar cane, (Brito *et al*., 2003). To estimate the LA, we use mathematical models that allow the estimation of the dependent variable as a function of independent variables. In the present study to estimate the LA in bean cultivars, the number and dry weight of leaflets (LN and DWL, respectively) were used. The objectives were to determine for three bean cultivars: a) which of the LN or DWL variables it is the most appropriate estimator of LA; b) if the determination coefficient is greater when using a model that includes both variables; and c); if the precision is greater when generating models for each cultivar.

MATERIALS AND METHODS

Planting bean cultivars (*Phaseolus vulgaris* L.) Cacahuate 72 (Cacahuate) of determined growth habit type I, Michoacán 12-A-3 (Michoacán) and Bayo Madero (Bayo) both of indetermined shrub growth type II, was made under field conditions and rainfall in a clay loam, with pH of 7.6, on June 10, 2015, at a population density of 4.16 m^{-2} plants (80 * 30cm) in Montecillo, Texcoco, State of Mexico, Mexico (19 ° 29'N and 98 ° 53'W and 2250 meters of altitude) of temperate climate. It was fertilized with 100 kg of N ha^{-1} (50% applied before sowing and the rest to the first weeding) and 100 kg of P_2 O_5 applied before planting. At flowering (42, 50 and 53 days after sowing (das) for Cacahuate, Bayo Madero and Michoacán, respectively), 20 plants were taken and the LA was registered with an area integrator, the NL and DWL were counted. Using the SAS package, a simple and multiple regression analysis was applied to look the model that best estimated the leaf area.

RESULTS AND DISCUSSION

The emergence of the cultivars was 8 das. During the development of the crop, the minimum and maximum average temperature was 8.2 and 28.6 °C, the PP of 380 mm. In Table 1, which presents the linear adjustment model for the three cultivars, it is observed that the DWL is a better estimator of LA than the LN as indicated by R^2 of 0.87 ** and 0.50 *, respectively. Likewise, when involving both variables and applying multiple regression, an R^2 of 0.94 ** was observed, which indicates greater precision in the estimation.

Table 1. Models that estimate the leaf area (LA) in three bean cultivars (*P. vulgaris* L.) according to the number and dry weight of the leaflets. Montecillo. Texcoco. State of Mexico. Mexico. Summer 2015.

Independiente Variable	Model	R^2
Dry weight leaflets (DWL,g)	LA = 0.41 + 0.6 DWL	0.87**
Leaflets number (LN)	LA =0.66 + 0.03 LN	0.50 *
DWL and L N	LA = -0.26+0.33 DWL+0.024LN	0.94 **

In table 2, it is observed that R^2 is higher when looking for the best estimation model for each cultivar, than when using the information of the three cultivars. Also with the multiple regression model that involves the DWL and the LN, it is more accurate than when only the LN is used. However, this does not exceed the model LA = a + b DWL. This indicates that the DWL is a more reliable estimator of LA than the LN. Similar trends were reported by Escalante (1980) for the cultivar Michoacán 12A3.

Table 2. Models that estimate the leaf área (LA) in each bean cultivar (*P.vulgaris* L.) according to the number and dry weight of the leaflets. Montecillo. Texcoco. State of Mexico. Mexico. Summer 2015.

Cultivar		
Cacahuate	Model	R2
Independiente variable		
Dry weight leaflets (DWL,g)	LA= -0.04 + 0.73 DWL	0.99**
Leaflet number (LN)	LA= - 19 + 0.57 LN	0.92 **
DWL and LN	LA = -0.26+0.33DWL+0.024 LN	0.93 **
Michoacán 12-A-3		
Dry weight leaflets (DWL,g)	LA = 0.23 + 0.64 DWL	0.96**
Leaflet number (LN)	LA = 0.33 + 0.04 LN	0.87*
DWL and LN	LA = 0.36+0.56 DWL+0.002 LN	0.94 **
BAYO	Model	R2
Dry weight leaflets (DWL,g)	LA = 2.1 + 0.2 DWL	0.93**
Leaflet number (LN)	LA = 0.15 + 0.035 LN	0.86 **
DWL and LN	LA = -0.36+0.59 DWL+0.009LN	0.96 **

CONCLUSIONS

For the growth conditions of the cultivars Cacahuate, Michoacán 12A3 and Bayo Madero, the leaflets dry weight is a better estimator of the leaf area than the leaflet number. Likewise, the R^2 is raised with the multiple regression model that involves the number and dry weights of leaflets The precisión of the estimate is higher when models are generated for each cultivar.

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GRAIN YELD OF LIMA BEAN (*Phaseolus lunatus*) GENOTYPES FROM SUBTROPICAL BRAZIL

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INTRODUCTION: When we consider the construction of more sustainable agroecosystems, we find that the process requires, at first, the reduction in the use of synthetic chemical inputs, such as N. Thus, it is affirmed that leguminous plants play a very important role, since they are able to fix atmospheric nitrogen. In addition, the improvement of the chemical, physical and biological attributes of soils the implementation of adequate techniques of soil preparation, maintenance of soil cover and increment of green fertilization. Another factor to be mentioned is that many of these legumes, besides being used as soil cover and green manure, are used for human and animal food as fodder or grains, and thus are considered as multipurpose legumes, which is inserted *Phaseolus lunatus* (VIEIRA, 1992).

In the different regions, associated with family farming systems, especially those with an ecological base, these species occupies a prominent place because of its rusticity and adaptability to low fertility soils, a fact that is directly related to the survival capacity of these materials creoles, besides being a source of protein for human and animal feeding. Embrapa Clima Temperado counts on a Bean Germplasm Bank (BAG-Embrapa) that presents more than 500 accessions of landraces of leguminous of multiple purpose. In this way, cataloging them and characterizing them is of extreme importance to obtain information that can contribute to family farmers in the construction of more sustainable agroecosystems (PINHEIRO et al, 2017). The present work aims to analyze the agronomic characteristics of lima bean.

MATERIALS AND METHODS: The site where the experiment was carried out presents the soil with the following characteristics: Solubic Hapludic Planosol, Pelotas mapping unit (Santos et al., 2006), with poor drainage and the following physico-chemical characteristics: 1.2% of (P), 35 mg kg^{-1} of potassium (K), 20% of clay and pH 5.8.

The area was fertilized with a mixture containing natural phosphate, tungue pie and agromineral granodiorite in the dosage of one t ha^{-1}, applied manually and after incorporation to the soil one week before sowing. Seeding was carried out for all genotypes on January 14, 2013. Four rows of each genotype, spaced 0.65m, each row containing 4 meters long with a seed density of 6 seeds per linear meter were sown.

RESULTS AND DISCUSSION: Were evaluated about forty genotypes of Lima bean in which it was possible to observe up to six seed collections, spaced between 7 and 10 days, making two months of production. Among them there are materials especially indicated for the production of grains while others are of dual purpose, also including the production of fodder for animals (FRAZÃO et al, 2010).

The emergence occurred in all genotypes within 8 days. It is verified that all the genotypes present indeterminate habit of growth, however the cycle ranged from 48 to 62 days, being considered early genotypes for that case those that presented DEF below 52 days, which occurred in a single case for the G 349. In contrast, it was considered as late genotype those with

DEF above 62 days, which happened for G 196, the other genotypes presented the duration of their cycle around the mean.

When we verified the DFH, it was verified that superior genotypes, that is, those that had the longest periods between flowering until the first harvest, were those that presented DFH greater than 77 days, which occurred for G 349, in contrast G125 presented lower DFC. Early genotypes with few days to harvest provide a more rapid supply of protein or fodder to farmers, which can optimize the adoption of their strategies and better adaptation in the production unit. Both genotypes presented between 2 and 3 harvests, in addition it is verified that the genotype G 125 that presented lower DFC was the most productive, as well as presented 3 harvests. The lowest productivity occurred in G 349, which also obtained three harvests, and was the earliest.

Table 1 - Grain color, growth habit (GH), number of days from emergence to flowering (DEF) and flowering to the beginning of harvest (DFH) and grain yield (kg ha^{-1}) of the different harvests (1st to 3rd Harvest) in genotypes of lima bean BAG of Embrapa Clima Temperado.

Genotypes	Grain color	GH	DEF days	DFH days	1ªH	2ª H	3ª H	Grain Yield
G 120	white	U**	58	65	167	156	-	323
G 195A	Red/white	U	58	65	133	61	133	327
G 125	White/black	U	58	61	241	155	141	537*
G 196	Violet	U	62*	75	183	303	-	486
G 349	Red/black	U	48*	81*	49	82	46	177
Media			57	69				370
Std			4,7	7,4				128
Media +std			61	77				498
Media -std			52	62				242

*Genotypes differ of media plus or less standard deviation; **growth habit indeterminado (U)

CONCLUSION: The different lima bean genotypes of BAG of Embrapa Clima Temperado vary in cycle and yield of grains, aspects of extreme importance, since due to the heterogeneity of familiar agricultural units, these genotypes can adapt in different ways and allow new strategies for the sustainable agro-ecosystems.

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ADDITIVE MAIN EFFECT AND MULTIPLICATIVE INTERACTION ANALYSIS OF GRAIN YIELD OF BLACK COMMON BEAN GENOTYPES

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INTRODUCTION: The INIFAP bean-breeding program for southeastern Mexico has used the univariate model proposed by Eberhart and Russell (1966) to determine the stability of seed yield of tropical black bean genotypes. In recent years, the AMMI (Additive Main Effects and Multiplicative Interaction) multivariate model has been applied, due to its effectiveness in estimating stability, since it allows to describe and interpret the effects of the GxA interaction, in addition to being more useful in characterizing the response of genotypes to different environments (Vargas et al., 2016). In the present research work, the AMMI model was used to determine the yield stability of a group of black bean genotypes evaluated over two yr (2016 and 2017) in 10 environments in the states of Veracruz and Chiapas, Mexico.

MATERIALS AND METHODS: We evaluated 12 elite lines that were previously selected for their productive performance and adaptation or tolerance to one or more of the following limiting factors: acid soil of poor fertility, drought and bean diseases. Commercial varieties Negro Grijalva and Negro Comapa were used as controls. Table 1 shows location, cropping season and environmental conditions in which elite lines were evaluated.

Table 1. Experimental sites, cropping season and environmental conditions where bean genotypes were evaluated in the states of Veracruz and Chiapas, México.

Site/State	Cropping season	Environmental conditions
Villaflores, Chiapas	Summer 2016	Rainfed – acid soil + dolomite (pH>5.6)
Villaflores, Chiapas	Summer 2016	Rainfed – acid soil (pH<4.4)
Ocozocoautla, Chiapas	Fall-winter 2016-17	Residual moisture
Orizaba, Veracruz	Fall-winter 2016-17	Residual moisture
Medellín, Veracruz	Fall-winter 2016-17	Residual moisture
Rodríguez Clara, Veracruz	Fall-winter 2016-17	Residual moisture - acid soil + dolomite (pH >6.1)
Rodríguez Clara, Veracruz	Fall-winter 2016-17	Residual moisture - acid soil (pH <4.7)
Tlapacoyan, Veracruz	Fall-winter/2016-17	Residual moisture
Medellín, Veracruz	Winter-spring 2017	Full irrigation
Medellín, Veracruz	Winter-spring 2017	Terminal drought (imposed at flowering)

The experimental design used was an RCBD with three repetitions and three-row, 5.0 m long plots. During physiological maturity, grain was harvested and seed yield determined, data were statistically analyzed so that individual ANOVA was performed for each trial as well as the combined analysis (genotypes-environments) across the 10 test environments. The AMMI model was also used to identify outstanding genotypes for their productive performance and less interaction with the environment (Vargas and Crossa, 2000).

RESULTS AND DISCUSSION: Figure 1 shows that the Jamapa Plus / XRAV-187-3-1-8 (G10) breeding line, in addition to having obtained high average yield (1,437.3 kg ha-1), had the least interaction with the environment, which indicates that it has adaptation in all test environments; therefore it would be a good candidate to register as a new improved cultivar. N. Citlali / XRAV-187-3-1-6 (G5) and Papaloapan / SEN 46-7-7 (G3) also showed low interaction with the environment, but their average yield performance was lower; these breeding lines could be used in breeding programs as a source of wide adaptation to environments with tropical conditions. On the other hand, Jamapa Plus / XRAV-187-3-1-2 (G11) breeding line, which obtained the highest average seed yield (1,504.3 kg ha-1), was the one that interacted the most with the environment, which showed specific adaptation to acid soil of low fertility (Villaflores, Chiapas) and terminal drought (Medellín, Veracruz) environmental conditions in which it obtained significantly outstanding seed yields. In turn, check cultivar Negro Grijalva (G14) also interacted strongly with the environment and showed specific adaptation to irrigation in Veracruz and acid soils of low fertility in Chiapas.

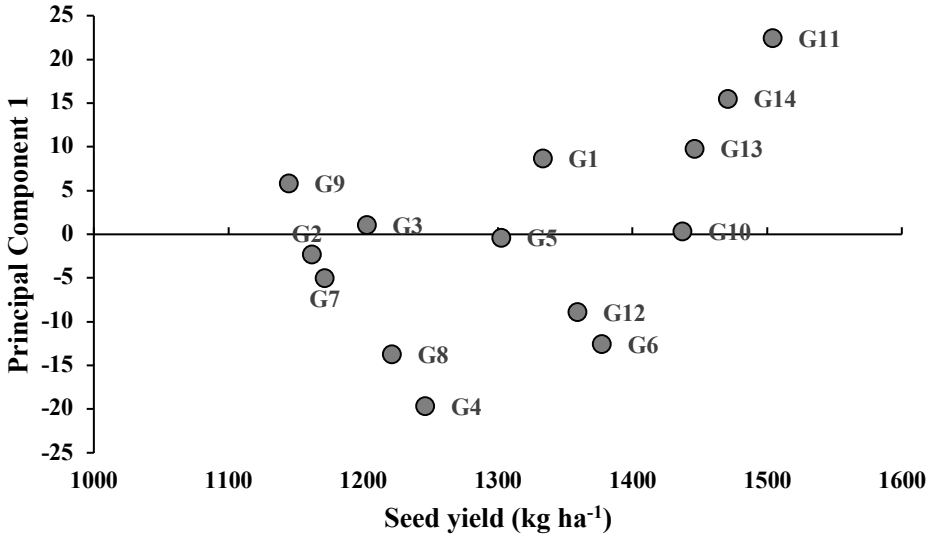

Figure 1. Main effects and interaction observed for seed yield of 14 black bean genotypes. G1 = Papaloapan/SEN 46-3-7; G2 = Papaloapan/SEN 46-6-6; G3 = Papaloapan/SEN 46-7-7; G4 = Papaloapan/SEN 46-7-11; G5 = N. Citlali/XRAV-187-3-1-6; G6 = N. Citlali/XRAV-187-3-1-8; G7 = N. Citlali/XRAV-187-3-14-6; G8 = N. Citlali/XRAV-187-3-14-7; G9 = N. Citlali/XRAV-187-3-16-7; G10 = Jamapa Plus/XRAV-187-3-1-8; G11 = Jamapa Plus/XRAV-187-3-1-2; G12 = Jamapa PlusX/RAV-187-3-4-4; G13 = Negro Comapa; G14 = Negro Grijalva.

CONCLUSIONS: Jamapa Plus / XRAV-187-3-1-8 was one of the breeding lines with the least interaction to the environment, and had a high average seed yield (1,437.3 kg ha-1). Whereas the breeding line Jamapa Plus / XRAV-187-3-1-2 produced the highest seed yield (1,504.3 kg ha-1), but its adaptation was specific in certain environments mainly those with abiotic stresses due to soil acidity of low fertility of Chiapas and terminal drought of Veracruz.

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RELATIONSHIP OF ROOT BIOMASS WITH TOTAL PLANT BIOMASS AND SEED YIELD IN BLACK BEANS

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INTRODUCTION: Scully and Wallace (1990) investigated the relationship of aboveground biomass, and phenology to seed yield in common bean. We wished to determine if root biomass, along with shoot biomass, was related to seed yield in a sample of black beans grown in tropical Veracruz, Mexico and Michigan, USA. Bean cvs. were grown in root tubes in a cooled glasshouse in Riverside, California, in spring 2018.

MATERIALS AND METHODS: A group of 11 commercial cvs. of black bean grown in Mexico and the USA was evaluated for their root and shoot biomass traits. Some cvs. were type III growth habit, Puebla-152 with long vine and Jamapa with short vine, others of type II either upright with long vine such as Huasteco-81, INIFAP, Veracruz, Papaloapan and Comapa, or of short vine, which included Tacaná and Verdín from Mexico and Zenith and Zorro from the USA. Seeds were germinated and seedlings of similar morphology were planted into the sand in 110 cm plastic sleeves supported in 1 m PVC tubes in a rack in a cooled glasshouse. Turface was not included in the medium; otherwise, the method followed that of Waines et al. (2018). Seedlings were planted on March 30, and plants harvested 87 days later on June 25, 2018. There were four replications of accessions arranged in a RCBD.

RESULTS AND DISCUSSION: ANOVA (Table 1) indicated there were highly significant differences among the cvs. for all the measured plant traits except seed weight (significant), and harvest index (ns).

Table 1. ANOVA of plant biomass traits of black bean cultivars grown in sand tubes in a cooled glasshouse.

Source of variation	DF	Mean Squares								
		Root biomass	Aboveground biomass	Seed wt.	100 seed wt.	Above total biomass	Total plant biomass	Number of seeds	R/S ratio	H.I.
Reps	3	0.961466	11.740886	21.504557	0.94401	40.787762	52.078125	408.177094	0.000452	0.003228
Cultivars	10	6.445422**	18.532129**	11.129492*	40.589844**	55.319530**	76.405472**	537.862488**	0.004622**	0.001539ns
Error	30	0.534432	3.551172	4.868099	2.275846	10.96	13.582292	103.615623	0.000438	0.001
Total	43									
C.V. (%)		12.76	11.84	11.17	6.76	9.28	8.90	14.40	12.97	6.330

*, ** Statistical significance at 0.05 and 0.01 level of probability; ns = Non-significant.

Landrace Puebla-152 produced the highest root biomass 8.5 g pl^{-1}, followed by Zenith (7.8 g pl^{-1}), whereas Comapa had the lowest (4.1g pl^{-1}). Older cvs. e.g. Puebla-152 and Jamapa with weighty root systems (8.5 and 6.2 g pl^{-1}) tended to produce higher seed yields, even though the biomass distribution between them was different (Figure 1). Jamapa produced similar seed yield (22.2 g pl^{-1}) to Puebla-152 (22.4 g pl^{-1}) but had less biomass in roots and total aboveground biomass, which resulted in a lower R/S ratio (0.153) than Puebla-152 (0.20). Zorro and INIFAP with much less root biomass (5.4 and 5.0 g pl^{-1}) and above-ground biomass (16.6 and 16.8 g pl^{-1}), with R/S (0.15 and 0.13) that was close to that of Jamapa, but different from Puebla-152, yet they produced almost the same seed yields 20.4 and 21.5 g pl^{-1}, respectively as Jamapa and Puebla-152. In contrast,

Zenith, which showed the second highest root biomass (7.2 g pl^{-1}) produced intermediate values for traits of aboveground biomass (14.2 g pl^{-1}) and seed yield (18.4 g pl^{-1}). As a result, the R/S for Zenith was higher (0.22) than that of Puebla-152. The most recently released cvs. Papaloapan and Verdín, that produced good root biomass (6.0 and 6.1 g pl^{-1}) similar to Jamapa, did not necessarily produce high seed yields. Verdín with an R/S (0.20) similar to that of Puebla-152 was the lowest seed producer (17.9 g pl^{-1}) among the cvs. The remainder of the cvs., which included the newest, have medium to low root biomass production and intermediate seed yields. Although the relationship of root biomass to aboveground biomass and seed yield was not clear in this experiment, there was a tendency of cvs. with larger total biomass to have high seed yields. Root biomass correlated significantly with total plant biomass (r= 0.62*), R/S ratio (r= 0.82**), harvest index (r= 0.86**) and negative with seed number (r= -0.60*).

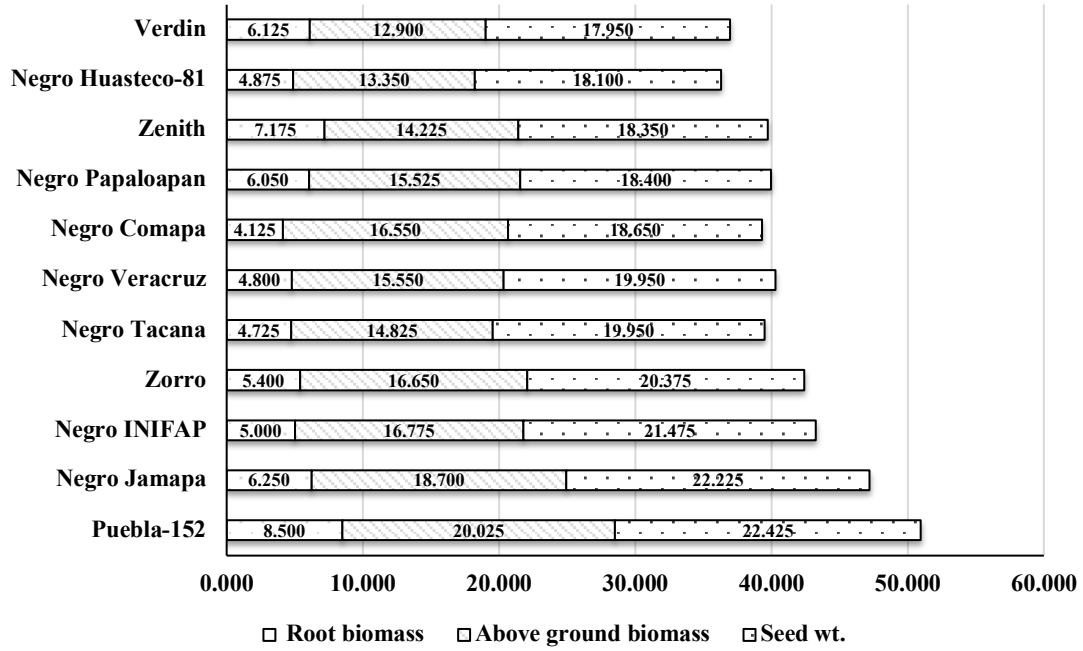

Figure 1. Plant biomass (g $plant^{-1}$) distribution of black bean cultivars grown in sand tubes under glass house conditions. UC Riverside, CA. 2018.

CONCLUSIONS: Breeders may wish to observe root characteristics of advanced lines in root tubes before releasing new cultivars in drought-prone or rainfed or irrigated environments.

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GENETIC VARIABILITY IN QUANTITATIVE DESCRIPTORS IN LIMA BEAN SEEDS

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INTRODUCTION: In Brazil, the lima bean (*Phaseolus lunatus* L.) presents socioeconomic importance in States of the Northeast region and in the north of Minas Gerais. It's a source of vegetable protein, whose green or dry grains are used in the human diet, and green pods and leafy in the animal diet. Genetic variability can only be used efficiently if properly evaluated, and the description of accesses is fundamental for the maintenance and exploration of the potential of the collections. In lima bean, the morphological characters of the seeds are among the main criteria to estimate the genetic diversity. The objective of this work was to evaluate the genetic variability in lima bean accessions of the *Phaseolus* Germplasm Bank (BGP) of the Federal University of Piaui (UFPI).

MATERIALS AND METHODS: The experiment was carried out in the Genetic Resources and Plant Breeding Laboratory of UFPI, PI, in 2018. The genetic material was 52 lima bean accessions, registered in the BGP - UFPI from six Brazilian States. In the morphological characterization of the seeds, quantitative descriptors were evaluated, according to Bioversity International (IPGRI, 2001), in ten seeds taken at random from each accession. The SmartGrain software determined seed measurements using scanned images (Tanabata et al., 2012), such as: area size, perimeter length, length, width, length/width ratio and seed circularity. besides the seed thickness with a digital pachymeter, and the weight of one hundred seeds (P100S) in gram, with an average of three replicates. The Tocher and UPGMA methods were used to estimate the genetic diversity. We adopted as a dissimilarity measure the average Euclidean distance. Statistical-genetic analyzes were performed using the GENES Program (Cruz et al., 2013) and R (R Core Team, 2015).

RESULTS AND DISCUSSION: The Tocher and UPGMA clustering methods formed four groups (Table 1 and Figure 2). In both methods, we observed variation of the accessions regarding the area size, length of the perimeter, length, width and weight of 100 seeds. In the Tocher method, there were variations in the size of the area (158.47 to 206.28 mm²), perimeter length (48.21 to 56.19 mm), length (17.01 to 20.19 mm), width (11.80 to 14.13 mm) and weight of 100 S (60.67 to 86.9 g) in group I. In the group II, there were variations in the size of the area (61.95 to 117.17 mm²), perimeter length (30.14 to 42.04 mm), length (10 to 14.75 mm), width (8.02 to 10.94 mm) and weight of 100 S (11.08 to 53.33). Group III included 11 accesses with variability in area size (117.53 to 148.08 mm²), perimeter length (41.74 to 47.55 mm), length (13.95 to 16.86 mm), width (10.78 to 11.83 mm) and weight of 100 S (48.47 to 63.27 mm). The group IV was represented by accessions UFPI 1109 and UFPI 1167. In UPGMA method, group I presented 30.77% of the accessions, which varied in size (61.95 to 101.07 mm²), perimeter length (30.14 to 39.11 mm), length (10 to 13.67 mm), width (8.02 to 10.02) and weight of 100S (11.08 to 47.28). This group corresponds to the seeds with the smallest dimensions in relation to the others. In group II, only the UFPI-1109 accession was inserted. It presented the largest dimensions in area size (236.27 mm²), perimeter length (60.32 mm), length (21.30 mm) and width (14.25 mm). In the group III, the variations also occur to the area size (159.20 to 199.50 mm²), perimeter length (49.97 to 57.17 mm), length (17.01 to 20.41 mm), width (11.84 to 14.13 mm) and weight of 100S (65.98 to 103.5 g). In this group was the accession with greater weight of 100S, the UFPI - 1167. In group IV, the area size (100.39 to 158.47 mm²), perimeter length (37.87 to 49.69 mm), length (12.24 to 17.32 mm), width (10.06 to 11.97 mm) and weight of 100S (43.05 to 67.22 g) had the third highest mean values. It is of great importance to observe the relative contribution of the characters to the divergence, thus being able to identify those who have the greatest contribution and help in discarding those

who do little or no contribution, thus reducing costs, time and labor. The quantitative traits that most contributed to the genetic divergence among the accessions evaluated were the seed area, the perimeter length, the length, the width and the weight of one hundred seeds.

Table 1 - Grouping of 52 lima bean accessions by the Tocher method, based on ten quantitative descriptors, using Mahalanobis dissimilarity matrix. Teresina - PI, 2018.

Grupos	Acessos
I	1007, 1012, 893, 1166, 1168, 976, 810, 997, 923, 929, 1165, 804, 807, 988, 1157, 889, 1169, 928
II	1004, 1014, 1005, 847, 873, 1016, 957, 844, 1015, 1002, 866, 849, 828, 902, 998, 922, 1003, 987, 1017, 1113
III	946, 1161, 1010, 862, 892, 1159, 1164, 1006, 1170, 827, 868
IV	1109, 1167

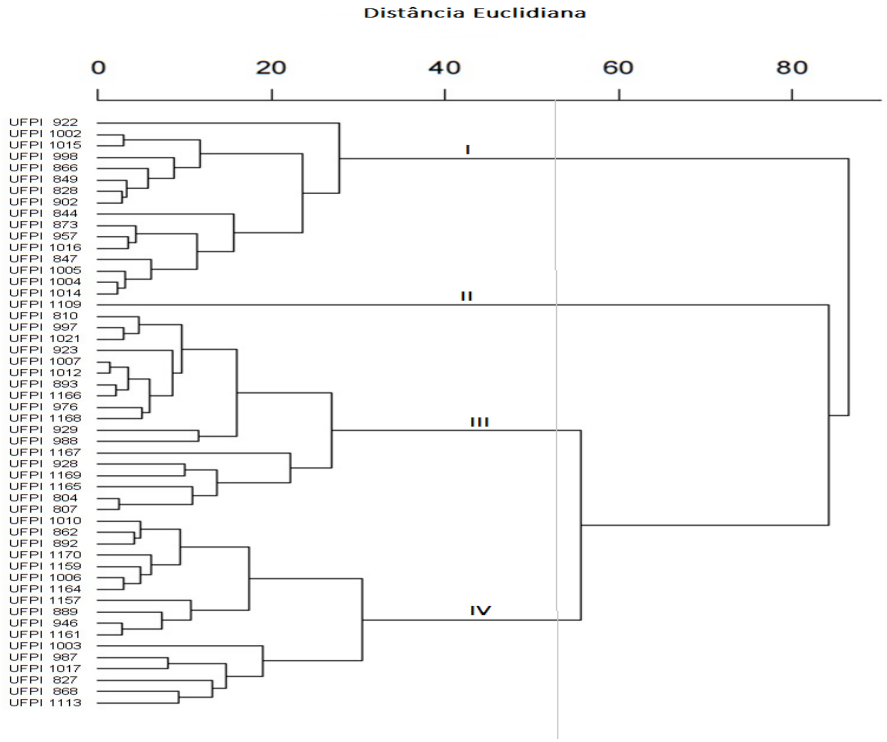

Figure 1. Dendrogram of dissimilarity of 52 accessions of lima bean generated by the UPGMA grouping method, based on the mean Euclidean distance for quantitative characters. Teresina, PI, 2018.

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GENETIC IMPROVEMENT OF COMMON BEAN ACCESSIONS

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) plays essential role in the diet of several countries, especially those in tropical and subtropical regions, such as Asia, Africa, Europe, and Americas (Broughton et al., 2003; McClean et al., 2008). Brazil is reasoned as a secondary center for diversity, as common bean accessions are distributed along its territory with valuable source of genes (Andean and Mesoamerican). Consequently, evolution studies and breeding programs benefit from these genes (Burle et al., 2010). The present work evaluated accessions from the Active Bean Germplasm Bank (BGB) for different agronomic traits in order to show their feasibility and encourage their use in breeding programs of Nupagri (UEM).

MATERIALS AND METHODS

We evaluated 121 accessions (116 landraces) and five controls (which correspond to commercial cultivars) from active BGB during the 2018 crop season. The experimental design was an 11 × 11 simple lattice, with plots of one 2-m row, spaced 0.5 m apart, with three repetitions. We assessed physiological features of the accessions by measuring the following agricultural traits: mass of 100 seeds (M100S); grain yield (kg ha^{-1}); days to maturation (DM); days to flowering (DF); mean number of pods per plant (MNPP); mean number of seeds per pod (MNSP); mean height of the first pod insertion (MHFPI), and plant height (PH). Analyses of selection indices were performed based on desired-gain (DG) (Pesek and Baker, 1969) and on sum of ranks - (SRMM) (Mulamba and Mock, 1978). We conducted statistical analyses with the Genes software (Cruz, 2013).

RESULTS AND DISCUSSION

The study of the morphological and quantitative traits revealed high heritability (Table 1). Grain yield (98.22%) and plant height (89.87%) showed the highest values, whereas mean number of seeds per pod (67.69%) and mean height of the first pod insertion (40.09%) exhibited the lowest values. These results indicate the possibility of genetic gain with selection and the presence of wide genetic variability among the evaluated accessions. On the subject of genetic gain through DG index (23.16%), it was observed that days to flowering (10.36%) and plant height (8.33%) were the traits with greater gain (Table 1). On the other hand, small genetic gain were noted for features such as grain yield (5.46%), days to maturation (5.0%), and mean number of seeds per pod (2.16%). Therefore, we could predict that selection based on desired-gain index would result in plants with early flowering (optimal height for automated harvesting) and maturation, and high grain yield. According to DG index, the following accession were selected: BGF 9, BGF 15, BGF 23, BGF 25, BGF 27, BGF 28, BGF 45, BGF 49, BGF 57, BGF 58, BGF 69, BGF 112, BGF 113, BGF 117, BGF 121, BGF 138, BGF 155, BGF 161, BGF 197, and BGF 201.

Table 1- Heritability (h^2 %) and genetic gain of 121 bean accessions from Active Bean Germplasm Bank of the Núcleo de Pesquisa Aplicada a Agricultura (Nupagri - UEM)

Characteristics	h^2 (%)	DG (%)	SRMM (%)
Mass of 100 seeds	83.94	-5.15	-8.90
Grain yield	98.22	5.46	50.74
Days to maturation	75.90	5.00	7.77
Days to flowering	84.74	10.36	10.39
Mean number of pods per plant	77.82	-1.46	30.57
Mean number of seeds per pod	67.69	2.16	7.06
Mean height of the first pod insertion (cm)	40.09	-1.54	2.69
Plant height (cm)	89.87	8.33	17.4
Total gain	-	**23.16**	**117.72**

DG (%): Index based on Desired-Gain (Pesek and Baker 1969); SRMM: Index based on Sum of Ranks - Mulamba and Mock (1978).

In relation to SRMM index, accessions BGF 23, BGF 24, BGF 25, BGF 26, BGF 27, BGF 30, BGF 32, BGF 33, BGF 34, BGF 60, BGF 72, BGF 85, BGF 95, BGF 105, BGF 117, BGF 138, BGF 168, BGF 201, and BGF 205 were selected(Table 1). Predictive genetic gain by SRMM index resulted in a total gain of 117.72%, underscoring the highest gains for grain yield (50.74%) and mean number of pods per plant (30.57%) traits. Interestingly, according to both DG and SRMM indexes, the accessions BGF 23, BGF 25, BGF 27,BGF 117, BGF 138, and BGF 201 exhibited superior and desirable genotypes (Table 1). In conclusion, all selected accessions expressed better agronomical traits in the field. Thus, they exhibit a potential to be inserted in cross-breeding blocks in order to obtain new cultivars.

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CANONICAL CORRELATIONS OF YIELD COMPONENTS IN *PHASEOLUS VULGARIS* L.

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INTRODUCTION

Development of new common bean lines with great genetic potential for higher yield is the main goal of breeding programs. For that reason, the success of such programs relies on different approaches. One of the most important ones is the study of the correlation between variables. Canonical correlation analysis method offers advantages, because it allows a study with more than one dependent variable (Hair Junior, 2005). By that, genetic plant breeding efforts can be focused on high heritability features of easy measurement and low complexity (Coimbra et al., 2000). The present study performed canonical correlations analyses to assess interrelations patterns between primary and secondary components of grain yield of $F_{2:6}$ common bean lines derived cross between LP 97-28 × IPR-Uirapuru.

MATERIAL AND METHODS

Experiments were conducted during the 2018 summer crop season at Centro de Treinamento em Irrigação (CTI -UEM), where a total of 190 $F_{2:6}$ lines derived from cross between LP 97-28 × IPR-Uirapuru and six commercial cultivars were evaluated. F_1 and F_2 populations were subjected to breeding until $F_{2:6}$ generation through SSD method (Brim, 1966) at the facilities of Nupagri (UEM). The experimental design row was a 14 x 14 simple lattice with 2.0 m length, spaced 0.5 m apart, with four repetitions. Correlation and canonical pairs (Hair Junior et al., 2005) were estimated between the following characteristics: mass of 100 seeds (M100S); total mass of seeds (TMS); days to maturity (DM); days to flowering (DF); mean number of pods per plant (MNPP); mean number of seeds per pod (MNSP); mean height of the first pod insertion (MHFPI). We carried out statistical analyses with software Genes (Cruz, 2013).

RESULTS AND DISCUSSION

Higher values for canonical loading, the more important is the characteristic to derive the canonical variate (Hair et al., 2009). The results revealed that canonical coefficients for MNPP, MNSP, TMS, M100S, and DM traits exhibited high values for the three canonical pairs (Table 1). The first canonical pair underscored positive correlation between MD, TMS, and MNPP. The second group revealed that M100S, MNSP, MHFPI were significantly and positive correlated, while DF was negatively correlated. Thus, it is plausible to assume that when DM, MT, NMVP, M100S, NMSV, AIPV show high values, DF will exhibit lower values. The second canonical pair, based on correlation between groups, shows that higher values for DM, DF, NMSV, and AIPV, implies lower values for MT, NMVP, and M100S. For the third and last canonical pair, we observed that higher mean values for NMVP and NMSV entail lower values for MD, TMS, DF, M100S and MHFPI.

Table 1. Canonical coefficients for the pair between Group I and Group II for seven characteristics of common bean

Group	Characteristics	Canonical pair		
		1°	2°	3°
I	Days to maturity	0.6382	0.7660	-0.077
	Total mass	0.8633	-0.498	-0.083
	Mean number of pods per plant	0.2850	-0.062	0.9565
II	Days to flowering	-0.059	0.5528	-0.311
	Mass of 100 seeds	0.7754	-0.591	-0.118
	Mean number of seeds per pod	0.1199	0.1178	0.9690
	Mean height of the first pod insertion	0.6335	0.6055	-0.094

According to coefficients of structural matrix (Table 2), intergroup associations were established especially because of a collection of influences of the first pair of canonical correlation (r=0.61), which associates plants with great performance related to days to maturity (late vegetative cycle), mass of 100 seeds, number of pods per plant, number of seeds per pod and yield. The second pair of canonical correlations (r=0.44) described plants with late growth cycle, but with higher number of pods per plant, number of seeds per pod, and height of the first pod insertion. Nonetheless, it was also associated with plant with lower yield and mass of 100 seeds.

Table 2. Canonical correlation coefficient for canonical pairs estimated between Group I and Group II for seven characteristics of common bean

Group	Characteristics	Canonical pair		
		1°	2°	3°
I	Days to maturity	0.499	0.8805	-0.092
	Total mass	0.7547	-0.656	-0.267
	Mean number of pods per plant	0.1052	0.0141	1.015
II	Days to flowering	0.1332	0.4935	-0.237
	Mass of 100 seeds	0.7787	-0.524	-0.106
	Mean number of seeds per pod	0.1958	0.1579	0.9364
	Mean height of the first pod insertion	0.6008	0.6581	-0.07
Correlation (r)		0.61	0.44	0.06

Correlation data for primary and secondary grain yield components reported in this study (Table 2) will assist in the selection of more promising $F_{2:6}$ lines derived from cross between LP 97-28 × IPR-Uirapuru for the common bean program of UEM, in order to develop productive and suitable cultivars for automated harvesting.

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VARIATION IN POD SHATTERING BETWEEN MARKET CLASSES OF COMMON BEAN

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INTRODUCTION: Pod shattering is an issue in many dry bean production regions. Pod shattering can cause extensive yield losses and constrains when harvest can occur, as yield losses increase considerably after crop maturity. Pod shattering in legumes is greatly exacerbated by atmospheric heat and dryness and is most problematic in regions where these conditions are prevalent. Aridity is predicted to increase in coming decades (Sherwood and Fu, 2014), furthering the need for a thorough investigation of pod shattering. Extensive variation exists in common bean susceptibility to pod shattering, but little information is available to growers and breeders regarding the level of pod shattering resistance in the major economic groups of common bean.

MATERIALS AND METHODS: Diversity panels of Andean and Middle American common bean were grown on the UC Davis Plant Sciences Field Facility in California's Central Valley to investigate patterns of pod shattering variation in common bean. In summer 2016, 98 members of major market classes in the Andean Diversity Panel (ADP, Cichy *et al.* 2015) produced pods in an unreplicated field trial. A sample of these pods (mean n=35) was harvested from each accession. These pods were desiccated at 65ºC for seven days and re-equilibrated to room temperature for a minimum of seven additional days (method modified from Funatsuki *et al.* 2014). The proportion of pods shattering in this treatment was recorded. In summer 2017, 265 accessions belonging to major market classes in the BeanCAP Middle American Diversity Panel (Moghaddam *et al.* 2016) produced pods in a field-grown trial. Two plot replicates were grown per genotype, and a sample of pods (mean n/replicate=15) was harvested from each replicate. Pod shattering was assessed using the same method as in the ADP.

RESULTS AND DISCUSSION: Market classes showed considerable variation in pod shattering susceptibility. In the Andean gene pool, cranberry types displayed the greatest susceptibility to pod shattering (mean=41%) while purple speck/mottled types were relatively resistant (mean=3%, Table 1). Kidney, red, and yellow/canario types were intermediate in this group. In Middle American beans, a strong pattern between ecogeographic race and pod shattering susceptibility was observed, as reported recently (Parker *et al.* 2019). The highest shattering levels were found in market classes of race Mesoamerica, such as black beans (mean=18%) and navy/small white types (mean=15%), while members of race Durango, such as pinto (mean=1%) and great northern (mean=1%) types, were highly resistant to the process.

Race Mesoamerica is adapted to humid lowland environments, where pod shattering is masked by environmental conditions. In this group, pod shattering may have never been sufficiently problematic to warrant selection against it. In these environments, a tendency for pods to dehisce easily could even be desirable, as it could improve the threshability of pods. Race Mesoamerica has previously been noted for its ease of pod threshing (Singh *et al.* 1991). In contrast, Race Durango is native to the semi-arid climates of northern Mexico and the southwestern United States, where pod shattering is expressed readily. Selection against shattering was strongest In these environments, leading to resistance in these groups today.

Market classes with resistance to pod shattering will be of great value for growers working in hot, dry environments where pod shattering is most problematic. Since these conditions are predicted to become more widespread in coming decades, breeders will need to develop new varieties adapted to these areas. The strong pod shattering resistance found in specific market classes of common bean will be of great value to breeding efforts in the 21st century.

Table 1. Pod shattering after desiccation, by market class, gene pool, and ecogeographic race

Market class	Gene pool	Race	Mean PS (%)	Median PS (%)	St. dev. (%)	n
Cranberry	Andean	Nueva Granada	41.43	46.29	29.86	24
Kidney	Andean	Nueva Granada	21.09	13.89	18.32	43
Purple speck/mottled	Andean	Nueva Granada	3.11	0	5.84	17
Red	Andean	Variable	7.54	5.51	8.1	22
Yellow/canario	Andean	Variable	8.45	3.04	10.54	14
Great northern	Middle American	Durango	0.94	0	2.12	31
Pink	Middle American	Durango	2.48	0	6.37	23
Pinto	Middle American	Durango	0.74	0	2.38	93
Black	Middle American	Mesoamerica	17.63	19	13.22	43
Navy/small white	Middle American	Mesoamerica	15.2	8.5	16.62	46
Red/small red	Middle American	Variable	9.59	4	14.7	29

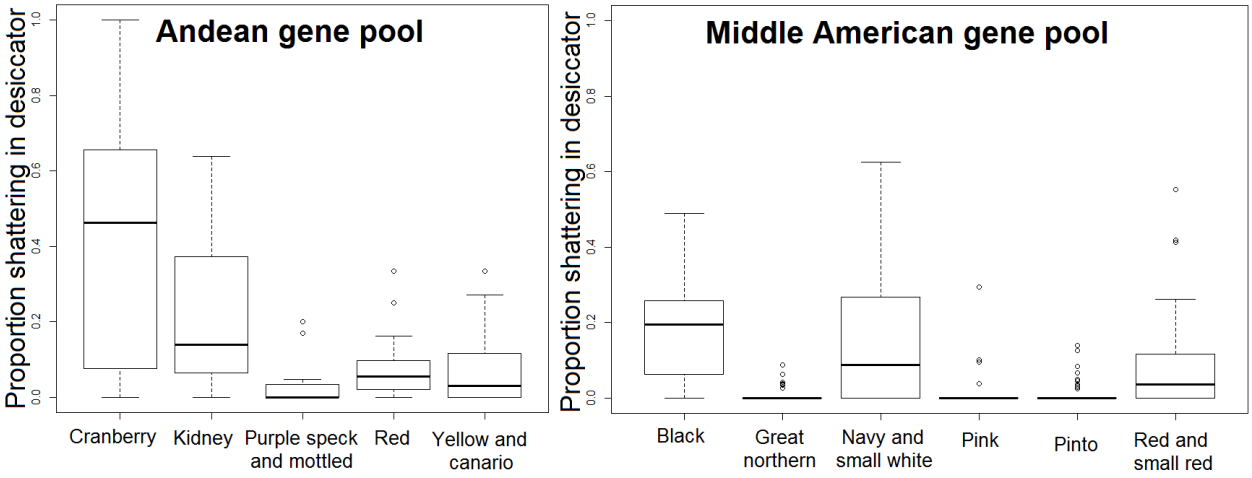

Figure 1. Variation in pod shattering between major commercial market classes of common bean.

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SELECTION OF COMMON BEAN LINES BASED ON AGRONOMIC PERFORMANCE AND HIGH GRAIN YIELD

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INTRODUCTION

Common bean (*Phaseolus vulgaris L.*) is one of the most important cultivated and consumed legumes worldwide. It also plays a major role in the Brazilian diet, as it is considered the main source of proteins, carbohydrates, fibers, vitamins, and essential nutrients such as zinc, iron, and calcium (Broughton et al., 2003; Campos-Vega et al., 2013). In addition, common bean crop contributes to the national socio-economic background because it constitutes part of income of several people, from family farming to corporate business. In this context, it is necessary to develop productive cultivars with plant architecture that promotes farming practices and harvesting. This work evaluated new common bean lines derived from cross between LEC01-10 × Juriti in order to obtain cultivars with high productive potential and erect plant architecture, favorable features for automated harvesting.

MATERIAL AND METHODS

Experiments and evaluations were conducted at Núcleo de Pesquisa Aplicada a Agricultura (Nupagri) and Centro de Treinamento em Irrigação (CTI) from Universidade Estadual de Maringá (Paraná, Brazil). Segregant populations derived from cross between LEC01-10 × IPR Juriti were generated by Single Seed Descend method (Brim, 1966). A total of 150 lines from $F_{2:4}$, $F_{2:5}$, and $F_{2:6}$ generations were previously selected and assessed under field conditions. We used the approach of augmented block design (Federer, 1955) with controls (Pérola, Flor Diniz, LEC01-10, Guará and Juriti). The experimental plots were composed of rows (0,5 m width 2 m length) spaced 0.5m apart. The following characteristics were evaluated: vegetative growth, height of plant, height of insertion of the first pod, mass of 100 seeds, and grain yield. Statistical analyses were carried out with Genes software (Cruz, 2013). Scott-Knott test was applied for comparison of mean values with 5% level of significance.

RESULTS AND DISCUSSION

Results showed that twenty out of the 150 common bean lines underscored. They not only exhibited a grain yield superior to 4,800 kg ha^{-1}, but also showed great results for the other traits evaluated (Table 1). Lines 23, 138, 105, 137, 27, 25 revealed the best grain yield mean with the following values, respectively: 6,031; 5,969; 5,946; 5,694; 5,576; 5,534 kg ha^{-1}. It is important to mention that other lines also demonstrated good results for grain yield and great potential for automated harvesting based on their height of insertion of the first pod, which was superior to 15 cm (Table 1). Given these points, it is possible to assume that these lines exhibit high potential to be included in value for cultivation and use (VCU) trials.

Table 1. Vegetative growth (VG), height of plant (HP), height of insertion of the first pod (HIFP), mass of 100 seeds M100S), and grain yield (GY) mean values from the 20 common bean lines with the best results and controls

VG		HP		AIPV		M100S		GY	
Line	**(days)**	**Line**	**(cm)**	**Line**	**(cm)**	**Line**	**(g)**	**Line**	**(Kg ha^{-1})**
48	76 e	4	94 a	10	23a	105	34a	23	6,031a
28	76 e	8	94 a	8	22a	38	33a	138	5,969 a
29	77 e	129	94 a	9	22a	26	33a	105	5,946 a
27	78 e	7	93 a	89	21a	140	32a	137	5,694 a
87	78 e	119	91 a	110	20 b	25	32a	27	5,576 a
89	78 e	82	91 a	45	20 b	37	32a	25	5,534 a
90	78 e	33	90 a	46	20 b	109	31a	136	5,247 b
88	78 e	5	90 a	122	20 b	148	31b	143	5,145 b
25	79 e	15	90 a	123	20 b	91	31b	85	5,136 b
9	79 e	22	90 a	7	20 b	47	31b	110	5,040 b
10	79 e	31	89 a	142	20 b	48	31b	116	4,999 b
36	79 e	115	89 a	144	20 b	119	31b	13	4,970 b
86	79 e	87	89 a	145	20 b	30	31b	67	4,929 b
26	80 e	86	88 a	34	19 b	67	31b	6	4,924 b
24	80 e	88	88 a	103	19 b	90	31b	50	4,900 b
110	80 e	66	88 a	49	19 b	8	31b	90	4,891b
91	80 e	62	87 b	128	19 b	95	31b	65	4,850 b
119	81 d	150	86 b	6	19 b	24	31b	106	4,848 b
85	81d	3	86 b	40	18 c	7	31b	83	4,817 b
47	82 d	49	86 b	14	18 c	45	31b	64	4,815 b
LEC01-10	89 b		75 c		15 e		26 e		3,092 d
Juriti	93 a		70 d		15 e		25 d		3,177 c
Flor Diniz	87 c		72 d		15e		26 e		3,502 c
Guará	90 b		79 c		16 d		26 e		3, 418 c
Pérola	91 a		76 c		16 d		28 c		3,246 c

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

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EVALUATION OF GENETIC DIVERGENCE IN COMMON BEAN (*PHASEOLUS VULGARIS* L.) CULTIVARS AND LINES BY MULTIVARIATE ANALYSES

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INTRODUCTION: The common bean breeding programs are based almost exclusively on hybridization of cultivars and obtaining superior lines from segregating populations (Tsutsumi et al. 2015). Knowing that registered cultivars and lines of common bean are used in several common bean breeding programs because they have favorable characteristics (Veloso et al. 2015). This research aimed to estimate the genetic divergence between cultivars and lines of common bean and to indicate contrasting and superior parents to produce segregating populations with high variability.

MATERIALS AND METHODS: Were evaluated 25 common bean cultivars/lines, of which, 11 with characteristics of early cycle (1 - Novo Jalo, 2 - Carioca Similar, 3 - Carioca Pitoco, 4 - Jalo Precoce, 5 - Goiano Precoce, 6 - Iraí, 7 - BRS Radiante, 8 - Carioca, 9 - Bambuí, 10 - CNF 6911 and 11 - CNFM 7119) and 14 of the carioca group (12 - Pérola, 13- Magnífico, 14 - BRS Pontal, 15 - IAPAR 81, 16 - CNFC 10408, 17 - CNFC 10410, 18 - CNFC 10429, 19 - CNFC 10431, 20 - CNFC 10432, 21- CNFC 10438, 22 - CNFC 10444, 23 - CNFC 10455, 24 - CNFC 10467 and CNFC 10470 ceded by Embrapa Arroz e Feijão.

The agronomic characters evaluated were: Number of days for flowering (FLOWER), Cycle (CYCLE), Average plant height (HP), Average height of first pod insertion (AHFPI), Average longitudinal length of pods (LLP), Average number of pods per plant (NPP), Average number of seeds per pod (NSP), Average number of seeds per plant (NSPL), Average weight of 100 seeds (W100) and grain yield (GY).

The obtained data were submitted to analysis of variance, later genetic divergence was estimated based on the generalized distance of *Mahalanobis*, then performing the agglomerative methods of tocher and hierarchical optimization "UPGMA". The level of preservation of the genetic distances in the dendrogram was verified from Cophenetic Correlation Coefficient (CCC). All analyzes were performed using the computational resources of the Genes software (Cruz, 2013).

RESULTS AND DISCUSSION: The analysis of variance revealed significant differences at the 1% level for the characteristics FLOWER, CYCLE, HP, AHFPI, LLP, NPP, NSPL, NSP, W100, allowing to infer about the existence of genetic divergence in the genotypes analyzed in the present research, according to Cruz et al. (2014). Genetic diversity is fundamental to obtain genetic gains in breeding programs. Only the characteristics GY did not present statistical difference, this result indicates the need of development of new cultivars.

Using the generalized distance of *Mahalanobis*, the 25 cultivars/lines were similarly grouped into four groups, by Tocher's optimization method (Table 1), and by the UPGMA method (Figure 1). This equivalence in the clustering of cultivars/lines by methods is evidence of consistency in results.

Table 1 – Group of common bean genotypes with similar patterns, established by the Tocher method, using Generalized Distance of *Mahalanobis* as a measure of dissimilarity evaluated in the city of Cáceres-Mato Grosso, Brazil.

Groups	Cultivars/lines
I	Carioca Similar; Carioca Pitoco; Carioca; Bambuí; CNFC 6911; Magnífico; BRS Pontal; Iapar 81; CNFC 10408; CNFC 10410; CNFC 10429; CNFC 10431; CNFC 10432; CNFC 10438; CNFC 10444; CNFC 10455 and CNFC 10467.
II	Pérola and CNFC 10470.
III	Novo Jalo; Jalo Precoce; BRS Radiante and Iraí.
IV	Goiano Precoce and CNFM 7119.

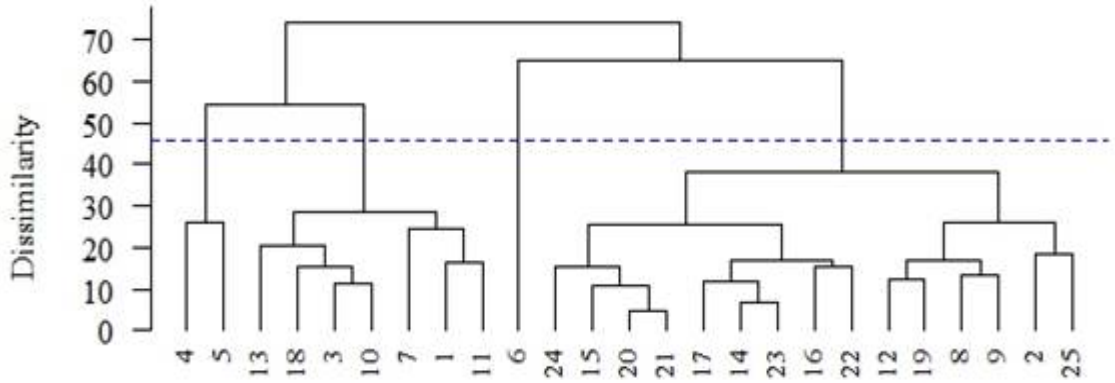

Figure 1 - Dendrogram of the genetic divergence among 25 common bean cultivars/lines obtained by the average linkage method (UPGMA), using the generalized distance of *Mahalanobis* as a measure of dissimilarity. Cophenetic Correlation Coefficient is significant for UPGMA (0.90 **) by t test.

The evaluated cultivars / lineages present genetic dissimilarity regarding the evaluated agronomic characteristics, hybrids with greater heterotic effect can be obtained from the crosses between Iraí × Goiano Precoce, Iraí × Pérola, Goiano Precoce × CNFC 10467 e IAPAR 81 × Pérola.

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GENETIC IMPROVEMENT OF BLACK BEANS FOR THE SOUTHEAST OF MEXICO (1954-2018)

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INTRODUCTION: In the southeast of Mexico, the great majority of bean plantings are made with black-seeded cultivars, due to its high commercial demand throughout the region. Bean production is carried out in two well-defined crop seasons; the most widely used is the fall-winter (September-December) under residual moisture regime, and in the summer (June-September), during the rainy season. Yields obtained are generally less than 700 kg ha^{-1}, since the crop is affected by various biotic and abiotic factors, among them are fungal and viral diseases, such as rust, angular leaf spot and bean golden yellow bean mosaic virus (BGYMV), acid soils of low fertility and terminal drought, which commonly occurs during the reproductive stage of the crop. The INIFAP bean breeding program for the southeast of Mexico has generated over the years several improved tropical black bean cultivars with tolerance and adaptation to these limiting factors, so the objective of this work is to show the advances of this program.

MATERIALS AND METHODS: The breeding program for tropical black beans began in 1954 in the Cotaxtla Experiment Station (CECOT), using mainly black bean landraces collected in the central region of Veracruz, Mexico. In this first stage, the breeding methods used were mass selection, individual selection as well as individual-mass selection (multiline) using the bean landrace germplasm. These methods continued to be used until the late '70s. During the early '80s, an intense selection program began by selecting breeding lines in both, early and advanced generations from genetic populations derived from hybridizations coming from different sources, either from CIAT's bean breeding program, or the INIFAP national program of bean improvement and the regional bean breeding program of INIFAP for southeastern Mexico. During this period, the breeding methods mostly used were the pedigree and the modified-pedigree, which continue to be used up to date. The breeding scheme using the bean landraces was firstly selection then field-testing of germplasm, adaptation trials, field yield trials, registration, and release for commercial use of the new cultivar. The hybridization improvement scheme included multiple crosses, selection, adaptation nurseries, preliminary yield trials, uniform yield trials and validation plots in farmer´s fields at the semi-commercial level prior to the registration and release of the new improved cultivar.

RESULTS AND DISCUSSION: Based on the work carried out, four cultivars were released in the first years of bean improvement, Negro Veracruz, cultivar obtained by mass selection and three by individual selection, Negro Actopan, Negro Antigua and Negro Jamapa a multiline composed of 15 lines derived from landrace Veracruz 87 (Voysest, 2000). This last cultivar is still valid, for its wide adaptation and high performance in different production zones of Mexico. Selection of

genetic material was based on yield, adaptation, disease resistance and commercial quality of grain. In the early '80s, an incidence of the bean golden mosaic virus caused serious economic damages in the tropical lowlands of Mexico including the Huasteca region (south of the state of Tamaulipas and north of Veracruz), central-south of Chiapas and the Yucatan Peninsula, Mexico. Therefore, the bean breeding program at CECOT introduced improved bean germplasm from CIAT with resistance to golden mosaic virus then DOR-145 was selected, an improved breeding line that gave rise to Negro Huasteco-81 released as the first Mexican cultivar tolerant to golden mosaic virus. As a result of crosses made in CECOT, during 1991 and 1992 improved cultivars Negro Cotaxtla-91 and Negro INIFAP were registered and released as highly tolerant to golden mosaic virus, the first carried also resistance genes to rust and angular leaf spot, the second with high performance in acid soils of low fertility. In 1993, the CIAT breeding line DOR-390 was introduced to Mexico; it was intensively evaluated in different environments and gave rise to Negro Tacaná, a cultivar with greater yield potential and tolerance to the golden mosaic virus than previous cultivars. From 2000 to 2007, a series of cultivars were released including Negro Medellín, a cultivar derived from a single cross made in CECOT and Negro Tropical and Negro Papaloapan, both breeding lines derived from multiple crosses made in CIAT. Negro Medellín is resistant to angular leaf spot with specific adaptation to central Veracruz. Negro Tropical (DOR-500) is a cultivar tolerant to golden mosaic virus and angular leaf spot with wide adaptation to climatic transition zones and Negro Papaloapan (DOR-454), a cultivar with wide adaptation to tropical lowlands of Mexico, highly tolerant to golden mosaic virus, rust, angular leaf spot and high performance in acid soils. From 2010 to 2015, breeding lines CIAT-103-21 and SEN-70 were selected and gave rise to Negro Comapa and Verdín, respectively. Negro Comapa is a cultivar with wide adaptation to tropical southeastern Mexico that can be sown at any time of the year, in a wide range of environments, whether under irrigation, rainfed or residual moisture regimes. On the other hand, Verdín is an early-season cultivar with wide adaptation to tropical conditions, highly tolerant to terminal drought and BGYMV and resistant to BCMV (Rosales *et al*., 2004).

CONCLUSIONS: The improved black bean cultivars developed by INIFAP has helped to solve a great deal of the problems of low yields caused by biotic and abiotic factors, which limit bean productivity in southeastern Mexico. The collaboration of the National Bean Breeding Program of INIFAP and the Bean Program of CIAT (Colombia)-PROFRIJOL has allowed the development of new improved bean cultivars. The exchange of improved germplasm from different sources and the introduction of genes to Mexican bean genetic material for earliness, resistance to diseases, tolerance to drought and acid soils of low fertility has been extremely useful to maintain the national and regional bean breeding programs of INIFAP in Mexico.

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ADVANCEMENT IN COMMON BEAN BREEDING AND FUTURE PROSPECTS IN ETHIOPIA

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INTRODUCTION: Common bean (*Phaseolus vulgaris* L.) is believed to have been introduced to Ethiopia in the 16th century, and since then it is predominantly produced by more than 10 million smallholder farmers annually (CSA, 2016). It is one of the most important pulse crops which contribute to food and income security for the growers and for generating foreign exchange earnings for the country. Though, there is large genetic diversity grown by farmers, the bush type is commonly grown in the country and the most commercialized type of bean is the small white pea, speckled, small and large red bean used for canning industry. Ten year ago, the main production constraints contributing to the low productivity of beans (0.8 t/ha) included susceptibility to foliar bacterial diseases, moisture stress, low yielding potential of varieties and low soil fertility. To address these production limitations, the bean research program have been aimed to develop and promote high yielding, disease tolerant/resistant varieties with good canning quality for export market and local consumption that are adaptable to moisture stress lowland areas of the country.

BEAN IMPROVEMENT APPROACH: The bean program uses experimental material or germplasm through introduction of fixed lines from the International Center for Tropical Agriculture (CIAT) through the Pan Africa Bean Research Alliance (PABRA see http://www.pabra-africa.org/), local collection and hybridization. The program mainly uses recurrent selection and pedigree method of breeding. Early generation germplasm are tested at nursery stage and followed through into replicated multi-location national yield trials for two to three years, using RCBD or triple lattice designs. Superior performing lines based on yield, disease performance, and stability across years and location are selected and released by an independent variety release committee. Early generation seed of the released varieties is multiplied and promoted using different approaches including, on farm demonstrations, field days, using different promotional materials (fliers, brochures, posters) and media (TV, Newspapers and radio). Finally, more seed is produced for scaling up/out throughout the bean growing areas for wider farmer use in the country in collaboration with multi-stakeholders along the bean value chain by formulating bean promotion and innovation multi-stakeholder platform. The status of bean production and challenge along the value chain are monitored by team of bean value chain actors. Continuous variety replacement and promotion of new varieties is also done to sustain bean production and export in the country.

MAJOR ACHIEVEMENTS: Since the inception of bean research program, more than 50 bean varieties from major seven market class have been released for production (Table 1) (MoA, 2017). The major focus of the breeding program was targeting the small white, small red, and mottled beans but, recently based on the export market demand large white, speckled beans have been included on the programs priority list. The released varieties have been promoted to end users and the current production area is estimated at >0.5 million ha/annum and average productivity per

hectare is 1.6t/ha resulting in average export earnings of >100 million USD/annum. Through the bean trader's and government investment, bean trade (small white and red) is currently marketed under the Ethiopian Commodity Exchange Market (ECX) that is a modern market system. The ECX system facilitates quick information flow between the seller and the buyer, provides quality control checks, warehouse service and provides a market platform for exporters. In addition to the enhanced employment opportunities along the bean value chain as a result of the transformed bean sector, great financial benefits have been accorded to the growers and the country.

Table 1: Common bean varieties released in Ethiopia for local consumption and export market

No	Name of Variety	Number of released varieties	Adaptation Altitude (m) a.s.l	Rainfall (mm)	Date of maturity	Range of Productivity (t/ha)	
						Research field	Famers field
1	Small white	9	1100-2100	500 -1100	85-94	2.0-3.1	1.6-2.7
2	Large and medium white	3	1300-1900	400-750	84-90	2.0-2.5	1.8-2.2
3	Small red	14	1100-2200	350-1000	86-120	2.0-3.5	1.5-3.0
4	Large and medium red	6	1300-2250	400-1100	80-120	1.7-3.2	1.5-2.5
5	Large and medium Speckled	4	1100-1950	400-1100	80-105	1.9-2.7	1.6-2.4
6	Large mottled	8	1300-2200	500-1200	90-105	2.0-3.0	2.0-2.5
7	Cream/yellow	9	1100-2000	350-700	75-110	2.0-3.5	1.5-3.0

Source: MoA, 2017

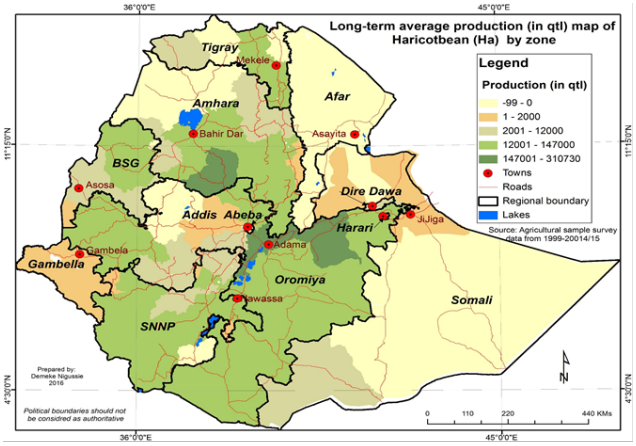

Figure 1: Common bean production) areas (EIAR, 17)

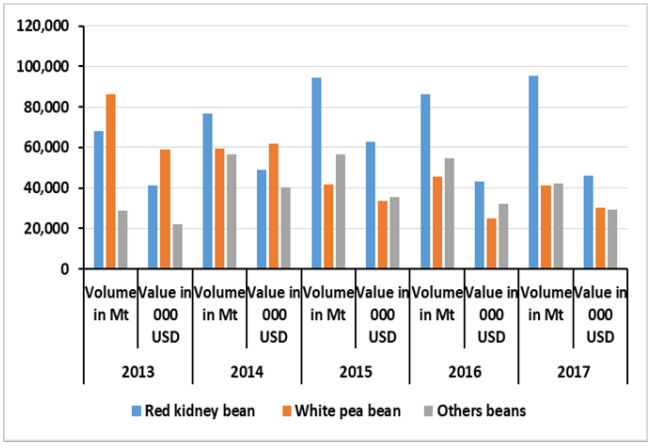

Figure 2: Common export by bean colors (MoT, 2017)

FUTURE PROSPECT: In the future, the bean program of Ethiopia has plans to modernize the breeding program so as to have enhanced annual genetic gains that are demanded by the growing market. The program is aiming at developing varieties with multiple constraint tolerance/resistance, suitable for mechanized production systems, faster cooking and with superior canning quality and adaptable to different agroecologies of Ethiopia. The breeding program will be supported with modern molecular and biochemical breeding tools to enhance the quality of breeding and to speed up the time of variety development.

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NOTICE OF NAMING AND RELEASE OF RUBÍ, A NEW HIGH YIELDING OPAQUE BLACK COMMON BEAN CULTIVAR FOR TROPICAL AND SUBTROPICAL AREAS OF VERACRUZ AND CHIAPAS, MEXICO

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INTRODUCTION: The Cotaxtla Experiment Station (CECOT) of the National Institute for Forestry, Agriculture and Livestock Research (INIFAP) announces the naming and release of Rubí, an improved high-yielding black bean, with small seed size (17.5 g 100 seeds^{-1}), shape and appearance (opaque seed coat) characteristics that meet the standards of the black bean seed class of domestic markets. This new bean cultivar exhibits the type-II upright short vine (indeterminate) growth habit (Singh, 1982). Plants average 42.5 cm in height, produce purple blossoms, flowers at 36-40 d, matures at 69-73 d, pods are yellow-cream in color. Rubí is well adapted to tropical and subtropical production areas of Veracruz and Chiapas in southeastern Mexico, and exhibits tolerance to terminal drought and low incidence of *Bean golden yellow mosaic virus* (BGYMV), since it carries the *bgm*-1 gene that confers resistance to the leaf chlorosis. It possesses the single dominant hypersensitive *I* gene that conditions resistance to the seed-borne *Bean common mosaic virus* (BCMV), while the majority of the landraces together with Negro Jamapa commonly used by farmers are highly susceptible.

MATERIALS AND METHODS: Rubí, tested as Jamapa Plus/XRAV-187-3-1-8 breeding line was derived from the cross Jamapa Plus/XRAV-187-3, made in 2007 at Campo Experimental Bajío (CEBAJ), Guanajuato, Mexico. The aim of the cross was to introduce BGYMV resistance into high-yielding semi erect drought-tolerant black bean breeding lines. Jamapa Plus was used as the parent with wide adaptation to tropical conditions and its commercially accepted grain characteristics, and XRAV-187-3 as a source of resistance to BCMV, BCMNV and BGYMV. The XRAV-187-3 line was derived from the PR0003-124 / Raven cross. Parental line PR0003-124 carries the *bgm*-1 gene and the QTL SW12, both resistance genes to BGYMV, as well as the *I* gene, which confers resistance to BCMV (Beaver *et al*., 2014). Rubí was obtained by mass selection made in the F_2 and F_3 conducted at CEBAJ, and in the F_4 and F_5 conducted at CECOT, Veracruz, Mexico. Individual selections were made based on plant type, pod load, disease reaction and seed size to derive F_{5-6} lines. Mass selection was conducted in the F_7 and F_8 generations for seed yield performance and disease reaction in Rincón Grande, Orizaba, Veracruz, Mexico. In 2013, the selected lines from the Jamapa plus/XRAV-187-3 population were coded. In the 2014-2015 cropping seasons the selected breeding lines were evaluated in a regional adaptation bean nursery in different environments of Veracruz and Chiapas, either under conditions of natural presence of bean diseases (bean rust, angular leaf spot, BCMV and BGYMV), or terminal drought and acid soils of low fertility. During yr 2015, Jamapa Plus/XRAV-187-3-1-8 breeding line along with 67 other bean genotypes, was phenotyped by artificial inoculation with the BCMNV NL-3 and BGYMV-MX strains and genotyped with molecular markers SW13, ENM, SBD5 and SR2 associated with the *I*, *bc*-3, *bc*-1^2 and *bgm*-1 resistance genes, respectively (Anaya *et al*., 2018).

Jamapa Plus/XRAV187-3-1-8 was field tested for two yr (2016 to 2017) across 10 different environmental conditions in Veracruz and Chiapas, Mexico. Rubí was planted on farmer´s fields as part of validation plots conducted during the summer season yr 2018 in two locations, one in Veracruz (Rincón Grande, Orizaba) the other in Chiapas (Rancho Sebastián, Villacorzo).

RESULTS AND DISCUSSION: During two yr (2015 and 2016), Rubí, as part of an adaptation nursery, was assessed together with two commercial regional cultivars used as checks in seven different environments: rainfed conditions, residual soil moisture, residual moisture in acid soils, irrigation and terminal drought. In most cases, Rubí was superior in seed yield performance than check cultivars; under rainfed conditions, the new variety obtained a seed yield similar to Negro Comapa (1825 kg ha^{-1}) but 23.9% higher than Negro Grijalva. Under residual moisture, Rubí obtained 9.2 and 17.9% higher seed yield than the same check cultivars, respectively. In the presence of terminal drought, seed yield (661 kg ha^{-1}) of Rubí was also much higher (87.5 and 117.8%) than that obtained by Negro Comapa and Negro Grijalva. Rubí was also field evaluated as part of the regional uniform yield trial conducted during two yr (2016-2017) to assess seed yield, disease reaction an adaptation across 10 environments of Veracruz and Chiapas. According to the combined analysis of variance, Rubí, along with four other breeding lines obtained significant outstanding average seed yield (1437 kg ha^{-1}) across the test environments. In the validation plots, Rubí produced 61.3% higher seed yield than T-39 but 20.7% lower than Negro Jamapa in Veracruz, while in Chiapas Rubí produced higher seed yield than both commercial bean cultivars. The overall average seed yield (1416.5 kg ha^{-1}) of Rubí was 22.8% higher than the average obtained by the two regional check cultivars (Table 1).

Table 1. Seed yield (kg ha^{-1}) of Rubí and two commercial black common bean cultivars used as checks evaluated in validation plots conducted in farmer´s fields in Veracruz and Chiapas, Mexico. Summer rainfed season, 2018.

Location/State	Rubí	Negro Jamapa	T-39	Check average
Rincón Grande, Orizaba, Veracruz	1,602	2,023	993	1,508
Seed yield increase respect to checks (%)		-20.7	61.3	6.2
Rancho Sebastián, Villacorzo, Chiapas	1,231	746	852	799
Seed yield increase respect to checks (%)		65.0	44.5	54.1
Overall average	1,416.5	1,384.5	922.5	1,153.5
Seed yield increase respect to checks (%)		2.3	53.5	22.8

CONCLUSIONS. These results allowed confirming that Rubí, a new black bean, is a high yielding cultivar with small seed size and opaque seed coat, highly resistant to BGYMV and BCMV, with a wide adaptation to tropical and subtropical areas of Veracruz and Chiapas, Mexico.

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2018 FINANCIAL STATEMENT
BEAN IMPROVEMENT COOPERATIVE

BALANCE AS OF January 1, 2018 **$ 11,886.39**

INCOME

		2018
2018 and 2019 Dues	$	6540.00
Extra Reports	$	0.00
2020 Dues prepaid	$	120.00
Back Issues	$	105.00
BIC 2017 Meeting excess	$	**26,735.00**
Bank Interest	$	150.18
TOTAL INCOME	$	33,560.18

EXPENSE

Labor charges	$	425.00
Postage, Copy Charges and Office Supplies	$	790.00
Book editing and publishing fees	$	1324.00
PayPal Fees	$	312.57
TOTAL EXPENSE	$	2851.57

BALANCE AS OF December 31, 2018 **$ 42,595.00**