ANNUAL REPORT OF THE

BEAN IMPROVEMENT COOPERATIVE

BIC

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

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BEAN IMPROVEMENT COOPERATIVE

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Cover Photo: Andean Diversity Panel (ADP) accessions courtesy of Stephen Ausmus, USDA-ARS

THE 59TH ANNUAL REPORT OF THE BEAN IMPROVEMENT COOPERATIVE

The Bean Improvement Cooperative (BIC) celebrated the twenty-eighth Biennial Meeting in Niagara Falls, Ontario. This BIC meeting had 154 registrants including participants from Africa, Asia, Europe, Mexico, and Central & South America which contributed to a vibrant and exciting atmosphere. The meeting began with the Frazier-Zaumeyer Distinguished Lectureship '*From Phaseolin to SNPs: Genetic Diversity of Common Bean and Application to Breeding*' which was presented by Dr. Paul Gepts, Professor, Bean Geneticist and Evolutionary Crop Biologist, at UC-Davis. This lectureship kicked off the symposium '*Applied Bean Genomics*' which highlighted the exciting and innovative genomic research the bean community is conducting. The meeting ended with the session 'Bean Nutritional Quality and Effects on Human Health' with topics ranging from seed mineral and dietary fiber concentrations in beans to effects of bean on reducing colitis-associated tissue damage. Overall there were 43 oral and 71 poster presentations.

The meeting received generous support from numerous donors - Platinum: ADM Edible Bean Specialties Inc., Applied Bean Genomics Project, Hensall District Cooperative, Ontario Bean Growers, Thompsons Ltd., Syngenta; Gold: Alberta Pulse Growers Association, Bayer Crop Science, Saskatchewan Pulse Growers Association, Seminis; Silver: ProVita Inc., Plant Agriculture Department, University of Guelph; Bronze: Crites Seed Inc., Pure Line Seeds Ltd., Seneca Foods Corp., Treasure Valley Seeds, and Trinidad Benham Corp. On behalf of the BIC, I wish to acknowledge the substantial role of the organizing committee, Peter Pauls, Greg Perry, Chris Gillard, Tom Smith, and staff, and would like to thank them, the sponsors and the participants for making the meeting a success.

At the Awards Banquet, the Frazier-Zaumeyer Lecturer was recognized, and Distinguished Achievement Awards were presented to Dr. Karen Cichy and Dr. Juan Osorno. Six graduate student awards were presented for the best oral (three) and poster (three) presentations, and travel awards were presented to 12 graduate students.

The BIC Coordinating Committee directed the President to: i) continue to set more stringent publication standards, and ii) move toward an electric online version for the annual report by establishing a password-protected CD version on the BIC website and to enable book publishing by a third party for members interested in receiving a hard copy. BIC Coordinating Committee changes included Dr. Karen Cichy replacing Dr. Talo Pastor-Corrales as the USDA-ARS representative; Dr. Jennifer Trapp filling the Industry Representative positon vacated by Dr. Ron Riley after 20 years of service, and Dr. Thiago Souza accepting the International Representative positon vacated by Dr. Antonio de Ron after 18 years of service. Dr. James Myers replaces Dr. Steve Noffsinger on the BIC Awards Committee. Dr. Kirsten Bett continues to chair the BIC Genetics Committee. The BIC community applauds this service which contributes to sustained success of the organization.

The next BIC Biennial Meeting is planned for East Lansing, MI in October/November 2017. The local organizing committee consists of James Kelly, Karen Cichy, Greg Varner, and Martin Chilvers. As the 2017 BIC meeting approaches, details will be posted on the BIC Web page www.css.msu.edu/bic.

Wishing you a successful year …….

Dr. Phillip Miklas, BIC President

BIC COMMITTEE MEMBERSHIP - 1957 TO 2016

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

BIC Genetics Committee Meeting Minutes
During the 2015 BIC Meeting

Meeting location: Niagara Falls Marriott Gateway Hotel, Niagara Falls, ON. Salon A
Date: Nov. 4, 2015
Time: 8:00 – 9:15 AM

Committee Members – all present:
Kirstin Bett Chair
Paul Gepts
James Kelly
Phillip McClean
Tim Porch
Carlos Urrea
Juan Osorno
Kal Kalavacharla
Juan Jose Ferreira
Celeste Gonçalves-Vidigal
Phil Miklas BIC president

1. Old Business:

1. **Approval of the Genetics Committee meeting minutes at the DoubleTree Hotel, Portland, Oregon on Oct 30, 2013.**

AIF

2. ***Co-16* gene symbol** (Maria C. Gonçalves-Vidigal)

Dr. Goncalves-Vidigal presented an updated version of her data supporting a new anthracnose resistance gene locus on Pv04. This locus appears to be distinct from *Co-3* based on mapping but there really needs to be additional segregation data for multiple races from RILs or F3 progenies to complete the hypothesis testing.
Motion: allow the use of *Co-16* as a temporary gene symbol for now. AIF
Dr Goncalves-Vidigal is encouraged to conduct further testing on RILs and or F3 progenies with multiple races to confirm *Co-16* is a separate locus from *Co-3*.

2. New Business

a) Update "List of Genes- *Phaseolus vulgaris*"

- *Co-17* – anthracnose resistance gene from SEL1308 on Pv03 (Trabanco, Campa and Ferreira, 2015). AIF
- ALS gene symbols (Thiago Lívio P. O. Souza) – reviewed at meeting in S. Africa (AIF)
    - *Phg-1* on Pv01; from AND277 (A) (Goncalves-Vidigal et al. (2011)
    - *Phg-2* on Pv08 from Mexico 54 (MA) (Queiroz et al., 2004); and *Phg-2*^{2} from BAT332
    - *Phg-3* on Pv04 from Ouro Negro (MA)(Goncalves-Vidigal et al., 2013)
    - ALS 4.1 QTL (*Phg-4*) on Pv04 from G5686 (Mahuku et al., 2009; Keller et al., 2015)
    - ALS 10.1 QTL (*Phg-5*) on Pv10 from G5686 and CAL143 (Oblessuc et al., 2013)
- *Pkp-1* - resistance to soybean rust (SBR), caused by the fungus *Phakopsora pachyrhizi.* From PI 181996 (T.L.P.O. Souza et al. 2014)

b) Reusing defunct symbol names (e.g. *Co-9* renamed *Co-3^3*) – absolutely not!
c) Nomenclature issues:
   - Genotype ID ontology (Bodo R); information on attempts to clarify this within the CG system. Stay tuned.
   - SNP nomenclature (Jim Kelly) – brief discussion about trying to unify SNP nomenclature based on the genome assembly.
   - Acceptable abbreviations for traits (Jim Kelly)
     - crop ontology.org: Some of us need to work with them to tweak their symbols.
     - Bodo Ratz to send out link to the committee; we would then put the link on the BIC Genetics page and encourage people to follow it
d) GBS data – Discussion regarding where/how it can be stored for all to have access to it.

3. Membership: Ted Kisha added as *ex officio*

4. Next meeting: Pullman WA, August 2016 with the next W3150. Date TBA.

THE BEAN IMPROVEMENT COOPERATIVE

Proudly Presents the

Frazier - Zaumeyer Distinguished Lectureship

to

Paul Gepts

Professor
Department of Plant Sciences
University of California
Davis, California

Distinguished Achievement Award

to

Karen A. Cichy

USDA-ARS
East Lansing, Michigan

Juan M. Osorno

North Dakota State University
Fargo, North Dakota

In recognition of outstanding accomplishments relating to bean (Phaseolus) improvement

PAUL GEPTS

Dr. Paul Gepts received his B.S. degree from the Faculté des Sciences Agronomiques, Gembloux, Belgium in 1976, and his Ph.D. in 1984 in Plant Breeding and Genetics from the University of Wisconsin, Madison where he worked with Dr. Fred Bliss, a noted bean geneticist. The genetics of methionine content and F_1 hybrid weakness in common bean was the focus of his graduate research. After receiving his Ph.D., Paul completed a postdoctoral fellowship at the University of California, Riverside working with Dr. Michael Clegg on molecular variation in pearl millet.

In 1987, Paul joined what is now the Department of Plant Sciences at the University of California, Davis, rose to the rank of full professor in 1995, and continues his professional career there. At UC, Davis, he trained 13 M.S. students and 17 Ph.D. students, while mentoring eight postdoctoral fellows and 36 visiting scientists. Paul's active, world-wide research program has resulted in 171 refereed journal articles, chapters, and conference proceedings. Paul is a fellow of both the American Society of Agronomy (2001) and the Crop Science Society of America (2005). He was awarded the Distinguished Achievement Award (1991) and Meritorious Service Award (2003) by the Bean Improvement Cooperative.

From a research perspective, Paul is well known for his early career research that convincingly established the presence of two unique wild and domesticated gene pools in common bean and described the dissemination pathways of beans throughout the world. Critically, these discoveries supported the hypotheses of all subsequent efforts that focused on the population genetics of bean. From the results of his research group we now have a description of the location of the Mesoamerican domestication of bean, and an understanding of the importance of gene flow, especially from the wild to the domestication form of the crop, in establishing and maintaining genetic diversity. Other recent research has established the critical role of farmers in maintaining diversity by their variety selection processes.

From a genetic perspective, Paul's research team developed a BAT93 x Jalo EEP55 recombinant inbred population that has been used to develop a primarily DNA-based molecular map that has been used as a world-wide resource to map many simple and quantitatively-inherited genes and as a resource to link other mapping populations into a community-wide composite molecular map. Paul's vision of a clearinghouse for bean molecular markers spurred his interest in developing the PhaseolusGenes database for bean breeders and geneticists. This is the central repository for over 100,000 molecular markers that have been described in the literature or mined from sequence data. The database also describes the genetic location of all of the QTL found in common bean research publications. Breeders and geneticists utilize the resource to discover polymorphic markers linked to their trait of interest, and apply the marker data along with the common bean reference genome sequence for candidate gene discovery. Due to Paul's leadership, the database was a major output of the USDA Common Bean Coordinated Agricultural Project.

Recently, Paul assumed the duties as the head of the UC, Davis bean breeding program that develops common and lima bean, as well as garbanzo bean varieties. The major goals of the program are high yield, insects, nematodes, and disease control based on natural genetic resistance, and an increase of seedling vigor to achieve uniform stands to control weeds. Finally, Paul is the scientific advisor to the African Bean Consortium, a group of researchers funded by the Kirthouse Trust. In this role, he follows breeding efforts in Ethiopia, Kenya, Rwanda, Tanzania, and Uganda as they develop multiple disease resistance varieties with acceptable agronomic performance. The BIC and the entire bean community are honored to have Dr. Paul Gepts as an active contributing colleague dedicated to the improvement of beans throughout the world!!

KAREN A. CICHY

Dr. Karen Cichy is a USDA-ARS research geneticist working on the genetics of bean quality at Michigan State University. Dr. Cichy is a native of Pennsylvania and graduated with a BS degree from Penn State in 1998 with a major in Horticulture and a minor in International Agriculture. She entered graduate school at Michigan State University and received M.S. in Plant Breeding and Genetics in 2002 under the direction of Dr. George Hosfield, working on phosphorus and zinc content in beans. She completed Ph.D. studies at MSU in 2006 in Plant Breeding and Genetics, studying root traits in low phosphorus soils and her co advisors were Drs. Sieg Snapp and Jim Kelly. During this period she was awarded a J. William Fulbright Foreign Scholarship to conduct part of her doctoral studies at CIAT, Colombia under the direction of Dr. Matthew Blair. While at MSU she was also supported by C.S. Mott Pre-Doctoral Fellowship in Sustainable Agriculture. Following graduation she received a USDA postdoctoral position where she studied the genetics of seed phosphorus quantity and low phytic acid mutants in barley at the USDA lab in Aberdeen, ID from March 2007 to May 2009 under the supervision of Dr. Victor Raboy. In July 2009 she returned to MSU and assumed a position as research geneticist in the USDA-ARS Sugarbeet and Bean Research Unit where her duties were to develop and lead research to improve the bioavailability of nutrients and consumer acceptance and utilization of dry bean as a food source. She continues in that role today.

As the lead scientist for CRIS project "Genetic Enhancement of Dry Bean Nutritional and Processing Qualities" Dr. Cichy's assigned area of responsibility is the genetic characterization and improvement of the nutritional quality and the consumer acceptance and utilization of dry bean as a food source, including improving bean seed nutritional value, seed mineral bioavailability, digestibility, antioxidant capacity, cookability, flavor, seed coat color, and canning quality. She evaluates and characterizes germplasm, identifies genes and molecular markers related to food quality traits, and works with plant breeders to introgress important genes influencing food quality into adapted and usable cultivars. She interacts, cooperates, advises, and consults with researchers from ARS, universities, and industry and has an impressive publication record. Her research activities involve the breeding of dry bean germplasm for increased nutrient density and for decreased phytic acid in dry bean seeds, and identification of the genes involved in these traits; and the determination of the genetic control of, and develop molecular markers for, dry bean germplasm with decreased cooking time, improved canning quality and color retention traits.

Dr. Cichy has published two book chapters, one bulletin, and 23 peer reviewed articles many in high recognized journals such as PlosOne. She lists six invited talks, eight papers/presentations delivered and 15 reports among her accomplishments. She is co-PI on eleven grants many of which are international in scope including the Feed the Future Legume Innovation Lab project in Uganda. She has actively participated in eight variety releases providing the critical information on canning quality, color retention and cooking time depending on the bean market class. She is an active collaborator with USDA-ARS colleagues, fellow bean scientists, nutritionists, and she keeps current with local and national industry leaders in the bean community. She has been active in the W-2150 Multistate Research Project. She currently advises three PhD and two Master students and has graduated one PhD and one Master student. In addition she has hosted three Borlaug Fellows and visiting scientists in her lab. Despite her young years she has considerable depth of knowledge of bean genetics as it relates to culinary and quality traits, so important in today's healthy conscious society.

JUAN M. OSORNO

Dr. Juan M. Osorno is an Associate Professor and Dry Bean Breeder/Geneticist in the Department of Plant Sciences at North Dakota State University (NDSU). Dr. Osorno studied at the Universidad Nacional de Colombia, Palmira, Colombia. Dr. Daniel Debouck at the CIAT Genetic Resources Unit supervised his undergraduate thesis research which won a national award for the best thesis in agricultural sciences. He graduated in 1997 with a B.S. degree in Agronomy. After graduation, he worked for the CIAT bean breeding programs of Dr. Shree Singh and Dr. Steve Beebe as a young investigator supported by the Colciencias-BID program. This provided Juan a wide range of experience conducting bean research. In 2000, Dr. Osorno received support from the Bean/Cowpea CRSP to pursue a M.S. degree in Plant Breeding and Genetics at the University of Puerto Rico under the direction of Dr. James Beaver. Two genes from *Phaseolus coccineus* that confer resistance to Bean Golden Yellow Mosaic Virus (BGYMV) in common bean were identified as part of his thesis research. BGYMV resistant germplasm lines were also released as part of this research. In 2003, Juan received a Pioneer Hi-Bred International Scholarship to pursue a Ph.D. degree in Plant Breeding and Genetics at North Dakota State University as a Graduate Research Assistant for the NDSU Corn Breeding Program led by Dr. Marcelo Carena. His dissertation research focused on the identification of groups of genetic diversity of maize for grain quality.
In 2007, Dr. Osorno joined the faculty in the Department of Plant Sciences at NDSU where he assumed the leadership of the dry bean breeding program. He took advantage of the breeding lines and populations initially developed by Dr. Ken Grafton to release navy, pinto, kidney, and small red bean cultivars for the largest bean production region in the U.S. In the next few years, the NDSU bean breeding program expects to release cultivars of several different market classes with enhanced levels of disease resistance and greater tolerance to abiotic stress such as waterlogging. During the past three years, Dr. Osorno has served as the Principal Investigator for a Legume Innovation Lab project focused on the improvement of climbing beans for the highlands of Guatemala. He has developed populations that have been used to model yield and development of common beans and response to drought. He has also been actively involved in collaborative research that developed a reference genome for common bean. Dr. Osorno has conducted research to identify best management practices for direct harvesting beans in North Dakota. He has also participated in the screening of bean germplasm and breeding lines for resistance to numerous diseases of economic importance in North Dakota including bean rust, halo blight, and root rots.

Dr. Osorno co-authored a review paper in *Crop Science* describing progress in breeding beans for increased seed yield and a separate review in *Euphytica* describing the current status of bean breeding. He has been invited to make more than 15 domestic and 8 international presentations at scientific meetings and has contributed to 18 refereed publications. He is an active member of the Crop Science Society of America, the Regional Hatch project W-2150, and a frequent contributor to the Annual Report of the Bean Improvement Cooperative. He has been very successful in obtaining external funding to support a wide range of bean research topics. Dr. Osorno is committed to the training of students in plant breeding. In addition to teaching genetics at NDSU, he has participated in the development of learning material and has supervised an intern program to promote interest in plant breeding. He has served as a major advisor for ten graduate and numerous undergraduate students and interns at NDSU. Dr. Osorno enjoys frequent interaction with bean growers from North Dakota and Minnesota.

IN MEMORY OF M. WAYNE ADAMS

M. Wayne Adams died September 9, 2015 at the age of 97. Born in 1918, in Catlin, Indiana, he graduated first in his class from Purdue University where he was editor of the Purdue Agriculturist, he received his Ph.D. from the University of Wisconsin, and was hired by South Dakota State University where he was the alfalfa breeder for twelve years. In 1959, Dr. Adams joined the faculty in Crop and Soil Sciences at Michigan State University as a bean breeder and geneticist. He left a successful career in forage breeding in South Dakota because he wished to work with a food crop and he choose to work with dry beans. In a distinguished career that lasted thirty-seven years he developed many varieties too numerous to mention but among them were the landmark varieties Seafarer navy and Montcalm dark red kidney bean. He was among the first breeders to select for defined market class and food quality traits. Dr. Adams is recognized for his many innovative publications on the genetic improvement of beans but among the most significant was one on yield component compensation and another on ideotype breeding.

Early in his career at MSU, Dr. Adams felt that if he was to make future genetic gain and improvements in beans he needed to find new germplasm. He successfully applied for a Rockefeller Grant to identify and introduce new bean germplasm from Central America. His vision in doing so led to two very significant achievements in his career that have benefited everyone in the local and international bean community today. The first achievement was to change the architecture of the bean plant. Using bean varieties from Central America as parents, Dr. Adams developed an upright plant type, similar to soybean that was suitable for direct harvest. Today over 80% beans in Michigan are direct harvested at considerable savings in time, labor and equipment for growers. The characteristic has helped sustain the industry in Michigan which otherwise may have been lost to alternative crops.

His most significant achievement was the establishment of international bean research programs that have impacted many worldwide. Following his work with Rockefeller, Dr. Adams realized the need to share research findings with the rest of the bean world particularly in developing nations. He went to Washington and with assistance from colleagues he secured a major grant from USAID directed at improving beans and cowpeas. The program was called the Bean/Cowpea CRSP – Collaborative Research Support Program and the management office was set up at MSU in 1980. A testament to the success of this program is the fact that the program still exists today after 35 years as one of USAID Legume Innovation Labs. He established research relationships between major US universities and programs in developing countries largely in Latin America and Africa. As a result numerous foreign students have been trained many with advanced degrees who are running successful research programs in their own countries. In addition the sharing of research has resulted in release of scores of improved varieties and technologies that contribute to improved bean and cowpea production in these nations. His inspiration and foresight in identifying local needs and providing the insight and solutions to meet those needs was second to none. Many around the world have benefited from his efforts and knowledge.

Dr. Adams was a member of Bean Improvement Cooperative and attended many of the meetings in the course of his career. In 1975 he received the Meritorious Service Award from the BIC in recognition of his significant accomplishments in bean improvement.

IN MEMORY OF JAMES RONALD BAGGETT

Dr. James 'Jim' Ronald Baggett, died on Jan. 21, 2016 in Corvallis, Oregon. Jim was born April 24, 1928, to James and Laura Baggett in Boise, Idaho. His early years were centered on farming in southern Idaho. After high school, Jim enlisted in the Navy for two years, and served as a Yeoman while stationed in Guam. In 1948, Jim returned and enrolled in Horticulture at the University of Idaho. Upon receiving his B.S. in 1952, he started graduate school at Oregon State College (renamed Oregon State University or OSU) under Dr. Walt 'Tex' A. Frazier. Jim helped initiate pea, bean, cabbage, and broccoli breeding programs at OSU. Upon completion of his Ph.D. in 1956, he was hired as an instructor at OSU and continued breeding pea, broccoli, cabbage, and disease resistance aspects of green beans. When Dr. Frazier retired in 1973, Jim became responsible for all breeding projects.

From the beginning of the 20th century up until about 1978 the Oregon processed green bean industry was based on production of pole Blue Lake (BL) cultivars. Because of their superior quality, BL cultivars were and are nationally renowned. However, a bush bean harvester was introduced by Chisholm Ryder Co. in the Midwest in 1950 and the Oregon industry faced strong competition on price. The principal objective of the OSU bean breeding program was to develop mechanically harvestable bush beans with BL pod type and quality. Lines with BL pod quality were selected through a backcross program, but plant habit was poor. It took until 1970 when 'Oregon 58' was released to provide the processing industry with a cultivar that had satisfactory bush habit and BL quality. The 1972 release, 'Oregon 1604', became very important in the Oregon processing industry. It was replaced during the mid-1980's by 'Oregon 91G', which became the standard cultivar in western Oregon until the mid-2000's. 'OSU 5630', developed from crosses originally made by Dr. Baggett was released by his successor in 2005, and since has become the predominant bush BL cultivar in Oregon. Jim released 'Oregon Trail', a long-podded bush BL, and 'Cascade Giant', a pole bean resembling 'Oregon Giant', for home gardeners. Another processing release, 'Oregon 54', became popular in the home garden trade as 'Blue Lagoon'. Over his career, Jim released 13 green bean cultivars.

Jim was a prolific breeder of other vegetables including 25 shell, snap and snow pea cultivars and germplasm lines, approximately 30 broccoli and cabbage inbreds, 15 tomato cultivars, four carrot inbreds, two Delicata squash cultivars and one germplasm line, three peppers, two ornamental corn open pollinated cultivars, and one crisphead lettuce. As a plant breeder, Jim possessed an excellent memory and exceptional observation skills. These two traits were critical to his success in creating high quality cultivars that are still in the commercial trade. Jim made only public releases and was a strong opponent of intellectual property protection of varieties.

As an academic and a scholar, Jim mentored 33 graduate students during his career at OSU. Many of his students became leaders in the vegetable breeding industry. He published over 80 peer refereed articles on breeding and genetics of various vegetable crops. He had over 200 non-refereed papers, as well as self-publishing a history of the Oregon BL processing industry. Jim was recognized for his many professional contributions and service through various awards (including the BIC and NAPIA), but crowning recognition of his achievements came in 1996 with the establishment of the Baggett-Frazier Vegetable Breeding Endowed Professorship at OSU.

IN MEMORY OF HUBERT BANNEROT

Hubert Bannerot died on August 29, 2014, at the age of 85. Born in Nancy, in a rural area of eastern France, he discovered agriculture as a child, during World War II. He was married and raised 3 children.

Hubert Bannerot received his Baccalaureat in Science and was selected for the National School of Agronomy in Paris from which he graduated in 1953. After his military service, he accepted a position at the National Institute of Agronomy (INRA) in Versailles, where he stayed all his professional life. During his career, as a geneticist and plant breeder, he devoted his interests to several crops. It started with wheat, and continued with apples, asparagus, cabbage and common bean. Thus he was called to work with a highly autogamous plants such as *Phaseolus* as well as with strict allogamous monoecious plants such as asparagus. This led him to interact with lots of colleagues both in the public (INRA, Universities, International institutions…) and private (Vilmorin, Clause) sectors. This is probably the reason why he was so open to new ideas and concepts, very curious and enthusiastic researcher.

As a bean breeder/geneticist, Hubert Bannerot searched for solutions for private breeders concerned with several important diseases, mainly anthracnose, but also BCMV and halo blight. He was among the first in Europe to characterize reaction of genotypes against a range of purified strains of *Colletotrichum lindemuthianum*. He thus discovered that the ARE gene identified by Mastenbroek was protecting against all strains of *Colletotrichum* present in France. He reported these data in a meeting in London thinking he had the solution. This is when colleagues from South America requested the plant material and discovered, of course, that these genotypes were susceptible to their local strains of *C. lindemuthianum*. From that point onwards he started a long and successful collaboration and exchange with Daniel Debouck at CIAT and with other colleagues dedicated to *Phaseolus* breeding in South America and elsewhere.

France is known for its round, stringless green beans, which are a kind of specialty bean. Hubert spent some time to breed these special beans and showed that you can increase the length of the pod very substantially, which happened with the Fortex variety which possesses a pod of 35 cm. Hubert Bannerot is known for the development of at least 13 new varieties of beans, green beans as well as dry bean seeds for “Cassoulet” bean, grown in the south of France.

Hubert Bannerot was a member of the BIC and received the Meritorious Service Award in 1993 in recognition of his achievements to bean improvement in France. He probably is one of the few French scientists to receive this award during his professional career. Now in France, most of the breeding for *Phaseolus* beans is carried out by private breeders, something that Hubert Bannerot complained about toward the end of his career. His concern was that genetic variability would become reduced because private industry performs less diverse crosses to obtain new varieties, whereas the public sector is more able to conduct wider crosses even to cross two different species like *P vulgaris* x *P coccineus* to diversify bean germplasm. Hubert Bannerot has successfully broadened the genetic base of germplasm in close collaboration with Daniel Debouck and other colleagues around the world.

IN MEMORY OF MERION MARGARET LIEBENBERG

Merion Margaret Liebenberg sadly passed away on 30 November 2015 at the age of 69. Merion, better known in the South African dry bean industry as Tokkie, was born in Johannesburg on 20 May 1946 but grew up in Zimbabwe. After primary and high school she obtained a degree in social sciences at the University of Stellenbosch. There she met Andries and three children were born from the marriage. During this time, while being a stay-home mother, she studied psychology and obtained an Honours and Master's degree from UNISA and University of Free State, respectively.

Merion decided that she would like to persue a career in bean pathology once the children left home. Andries was the common bean breeder at ARC at that stage which stimulated her interest in beans. She studied part-time and obtained a BSc degree in Botany and Zoology from UNISA and a Honours degree in Plant Pathology at the Northwest University. She was appointed as plant pathologist at the Agricultural Research Council – Grain Crops Institute, Potchefstroom in 1992 where she worked until her retirement in 2011. During this time she obtained a M.S. degree from the Northwest University with her work on the angular leaf spot disease and a Ph.D. degree in 2003 from and University of Free State. Her highly informative disseration was about "Breeding for resistance to rust of dry bean (*Phaseolus vulgaris*) in South Africa".

The major focus of her research was on the rust disease. She was fortunate to spend some time in Dr. Rennie Stavely's lab early in her career which made her familiar with the techniques of identifying rust races and subsequent resistance. She identified many races of the bean rust pathogen that occurred in Southern Africa and other countries of Africa. The diversity of the pathogen that she discovered within the pathogen populations made it necessary for her to combine different resistance genes to obtain durable resistance. She identified and selected the best rust resistance genes in her breeding programme that gave broad resistance across Southern Africa and therefore the least likely to be overcome by new races. She described the resistance gene *Ur-13*, derived from Kranskop, that was accepted by the BIC Genetics Committee. Markers for this gene have also been developed. Rust resistant cultivars that were released from her programme included Teebus-RR 1, Teebus-RCR 2 (combined rust and CBB resistance) and CAR 2008. She had a dream to release a well adapted dark red kidney bean with resistance to rust and halo blight. Good progress was made and a number of improved lines from her programme are currently being evaluated for possible release

Apart from rust, Merion also made good progress in breeding for resistance to other bean diseases. Her research on angular leaf spot resulted in the release of three ALS and rust resistant red speckled sugar bean varieties, namely Sederberg, Tygerberg and Kamiesberg. She attempted breeding for resistance to root rot disease complex disease, but most resistant lines identified were not well adapted under local conditions. She supervised a student who studied the virulence diversity within anthracnose pathogen populations. A number of races in South Africa were identified, but she lacked time and manpower to breed for resistance to this disease.

During her career she contributed greatly to transferring technologies to farmers through articles in the local magazine 'SA Dry Beans', presentations at farmers' days and contributions to the Dry Bean Production Manual. She also had numerous presentations at conferences and workshops locally and abroad and enjoyed training researchers. Her research has been published in several journals and includes a broad review article on bean rust that serves as an excellent source of information for young scientists. She had close contact with bean scientists abroad who had a high regard for her accomplishments and collegiality.

IN MEMORY OF RONALD SHELLENBERGER

Ronald Gene Shellenberger of Kuna, Idaho died June 30, 2015 at the age of 71. He was born September 6, 1943 in La Junta, CO. Ronald attended school: at Goshen College from 1962-1966, receiving his Bachelor of Arts in Biology; Michigan State University from 1966-1970 receiving his Masters in Agronomy; and 1970-1975 at Texas A&M receiving his Ph.D. in Horticulture.

Ronald worked at Rogers Seed Company from 1975 - 1995, in Twin Falls and Boise, and was a pioneer in dry bean breeding for private industry. He developed a number of bean varieties in a range of market classes such as Beryl and Marquis great northern and Topaz pinto which are still grown today. In 1996, Ronald started his own dry bean breeding company, ProVita, Inc., in Kuna Idaho, and continued to work as a private dry bean breeder affiliated with major bean companies across the U.S. He enjoyed his work, owning his own business, and working with colleagues throughout the bean world.

Ronald's vision for dry bean development created a pathway for many private companies to participate in his various breeding efforts. This enabled the industry to move forward with his breeding efforts and promote them throughout North America. Some of Ronald's most notable contributions to the U.S. dry bean industry were the development of high yielding direct harvestable beans for the industry. He produced highly successful cultivars in most commercial classes including Medalist and Merlin navy beans; Loreto black bean; La Paz, Sinaloa, and Monterrey pinto beans; Aries and Taurus great northern beans; Ruby and Viper small red beans, Pink Floyd pink bean; and Big Red and Chaparral kidney beans. The increased yields and new harvest practices have helped maintain a competitive edge and superior commercial product for farmers throughout the U.S. and in turn supporting the need for clean disease-free dry bean seed from states such as Idaho. His efforts on dry beans also allowed the industry to remain competitive with other commodity crops.

Ronald took any opportunity he could to support the western dry bean seed industry. As a dry bean breeder, Ronald knew the great benefits that the western seed program on dry beans had for the industry and plant breeders in general. Living and having his base of operation in Idaho allowed him to bring well adapted and high yielding varieties to the Idaho growers. He also took the time to visit production fields throughout the U.S. where his varieties were being tested or grown and aided in advising what growers needed to do to correct any issues that might be happening in any given year.

Dr. Shellenberger was a member of the Bean Improvement Cooperative (BIC), served on the Coordinating Committee, and attended many of the meetings in the course of his career. He was a humble man of integrity who refused to be recognized by the BIC for his significant accomplishments in bean improvement. Ronald's legacy continues with his family running the business and exhibiting his same passion for dry beans.

Sclerotinia sclerotiorum isolates available for research

Results of the multi-year, multi-site white mold testing have identified the following isolates or Mycelial Compatibility Groups (MCGs) of isolates to be useful for screening for resistance and other research.

Highly aggressive isolates which consistently caused the most severe disease reactions as measured by the Straw test (Petzolt and Dickson, 1996) are isolates #s 698 (ST mean rating of 7.8), 705 (7.8), 708 (7.8), 717 (7.8) and 710 (7.9).

Those isolates causing the least severe disease reactions as measured by the Straw test include #s 841 (2.8), 591 (2.9), 765 (3.2) and 596 (3.3).

Isolates belonging to a single MCG group that is distributed widely across the United States include #s 473 (collected from Oregon), 698 (North Dakota), 803 (Michigan)and 889 (Colorado).

Isolates belonging to a single MCG group that was only collected from one location include #s 469 and 477, from Oregon.

All of the isolates of another single MCG group including #s 661, 668, 806, 815, 816 and 840 were collected from Michigan in the years 2005, 2008 and 2009.

Some of this information was previously reported in the 2010 Annual Report of the BIC, pp. 232-233.

Please contact Jim Steadman at jsteadman1@unl.edu or 402-472-3163 if you would like sclerotia from the above isolates or have use for any of our 366 characterized *S. sclerotiorum* isolates or isolates from hosts other than beans.

MAJOR LOCI CONTROLLING RESISTANCE TO THE ANGULAR LEAF SPOT OF COMMON BEAN

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Angular leaf spot (ALS) caused by the fungus *Pseudocercospora griseola* is currently one of the most widespread and destructive disease of common bean (*Phaseolus vulgaris* L.) in Latin America and Africa (Pastor-Corrales *et al.* 1998). Yield losses up to 80% have been reported in Brazil (Jesus-Junior *et al.* 2001). Genetic resistance is the most cost-effective, easy to use and ecological strategy to manage the disease. However, progress in breeding for resistance to ALS has been difficult. The high virulence diversity of *P. griseola* and the recurring discovery of new races of this pathogen are challenging for the development of cultivars with effective resistance to ALS (Sartorato & Alzate-Marin 2004; Abadio *et al.* 2012).

During the Common Bean Disease Workshop on Angular Leaf Spot and Root Rot, held in Skukuza, South Africa, in July 2015, a work group was established to review the progress in genetic analysis and breeding for ALS resistance. At that time, only three ALS resistance genes had been mapped and named following the guidelines for gene nomenclature proposed by the Bean Improvement Cooperative (BIC) Genetic Committee: *Phg-1* (AND 277) on chromosome Pv01 (Carvalho *et al.* 1998; Gonçalves-Vidigal *et al.* 2011), *Phg-2* (Mexico 54) on Pv08 (Sartorato *et al.* 2000), and *Phg-3* (Ouro Negro) on Pv04 (Corrêa *et al.* 2001; Gonçalves-Vidigal *et al.* 2013). However, in addition to these genes, unnamed major resistance loci have also been reported in different resistance sources used by common bean breeding programs in Uganda, Colombia and Brazil. Among these resistance sources are BAT 332, CAL 143 and G5686 (Table 1).

Caixeta *et al.* (2005) named tentatively additional ALS resistance genes in AND 277, Mexico 54, MAR 2 and Cornell 49-242. However, none of these studies included physical linkage information and their results or genetic hypotheses still need to be validated. Consequently, the gene names proposed by these authors were not accepted by the BIC Genetic Committee. Based on allelism test results by Namayanja *et al.* (2006), the ALS resistance gene in BAT 332 shall be considered as allelic to *Phg-2*, present in Mexico 54. A physical position analysis using the reference genome sequence of *P. vulgaris* (Schmutz *et al.* 2014) indicated that the ALS resistance genes in MAR 2, Cornell 49-242, G10474 and G10909 may also be alleles of *Phg-2*. The physical map developed using sequence information obtained from molecular markers linked to *Phg-2* and to the major loci controlling ALS resistance in these ALS resistance sources showed the presence of one single gene cluster on Pv08 (Figure 1). However, additional studies on the genomic characterization of *Phg-2* are necessary to better clarify the allelic relationship of *Phg-2* and the ALS resistant genes present in MAR 2, Cornell 49-242, G10474 and G10909, and guide the nomenclature of these genes.

It was also proposed that the major QTL ALS4.1^{GS,UD} on Pv04, present in G5686, and the ALS10.1^{DG,UC} on Pv10, identified in both G5686 and CAL143, shall be officially named as *Phg-4* and *Phg-5*, respectively (Table 1). This proposal considered that these major loci had consistent and significant effects across different environments and populations (Mahuku *et al.* 2009; Oblessuc *et al.* 2012, 2013; Keller *et al.* 2015). These QTLs have been physically mapped

on positions different from those of the ALS resistance genes *Phg-1*, *Phg-2* and *Phg-3* (Figure 1). This effort to better characterize and formally name the major ALS resistance loci identified so far will be useful to support common bean breeding programs for ALS resistance regarding to selection and use of resistance sources. In addition, it will also guide the characterization of new ALS resistance loci.

Considering all information presented above, in the last BIC Genetic Committee meeting held during the 2015 BIC Meeting, in Niagara Falls, Canada, in November 2015, the work group on genetic analysis and breeding for ALS resistance has proposed new gene symbols for unnamed major ALS resistance genes and QTLs previously reported, as summarized in Table 1. Based on the evidences presented, results from classical genetic studies, fine-mapping information and physical position analysis using the reference genome sequence of *P. vulgaris*, the BIC Genetic Committee has formally accepted the proposed new gene symbols.

Genetic and molecular evidences indicate that common bean loci controlling resistance to diseases caused by high variable pathogens are organized in clusters in which individual genes confer resistance to one specific isolate or race of the pathogen (Ferreira *et al.* 2013). For this reason, direct or indirect mapping using information from molecular markers linked to known ALS resistance genes and QTLs is recommended to support the characterization of new ALS resistance loci.

Table 1. Named and mapped loci that control resistance to the angular leaf spot of common bean.

Gene symbol		Resistance Source	Gene Pool[b]	LG[c]	Pathogen race	Reference
New[a]	Original					
Phg-1	*Phg-1*	AND 277	A	Pv01	63-23	Carvalho *et al.* (1998) Gonçalves-Vidigal *et al.* (2011)
Phg-2	*Phg-2*	Mexico 54	MA	Pv08	63-19 63-39	Sartorato *et al.* (2000) Namayanja *et al.* (2006) Mahuku *et al.* (2011)
Phg-2[2]	*Phg-?*	BAT 332	MA	Pv08	63-39	Namayanja *et al.* (2006)
Phg-3	*Phg-ON*	Ouro Negro	MA	Pv04	63-39	Corrêa *et al.* (2001) Gonçalves-Vidigal *et al.* (2013)
Phg-4	ALS4.1^{GS,UD}	G5686	A	Pv04	31-0 Field[d]	Mahuku *et al.* (2009) Keller *et al.* (2015)
Phg-5	ALS10.1^{DG,UC}	CAL 143	A	Pv10	0-39 Field	Oblessuc *et al.* (2012, 2013)
	ALS10.1^{DG,UC}	G5686	A	Pv10	31-0 Field	Keller *et al.* (2015)

[a] Highlighted are the ALS resistance genes previously mapped, named and accepted by the BIC Genetic Committee. Those that are not highlighted are the new gene symbols proposed based on results from classical genetic studies, fine-mapping information, and physical position analysis using the reference genome sequence of *P. vulgaris* (Schmutz *et al.* 2014), which have recently been accepted by the BIC Genetic Committee. [b] Andean (A) or Mesoamerican (MA) gene pool. [c] Linkage group (LG) or chromosome based on genetic mapping and genomic analysis information. [d] Resistant reaction under natural infection in the field.

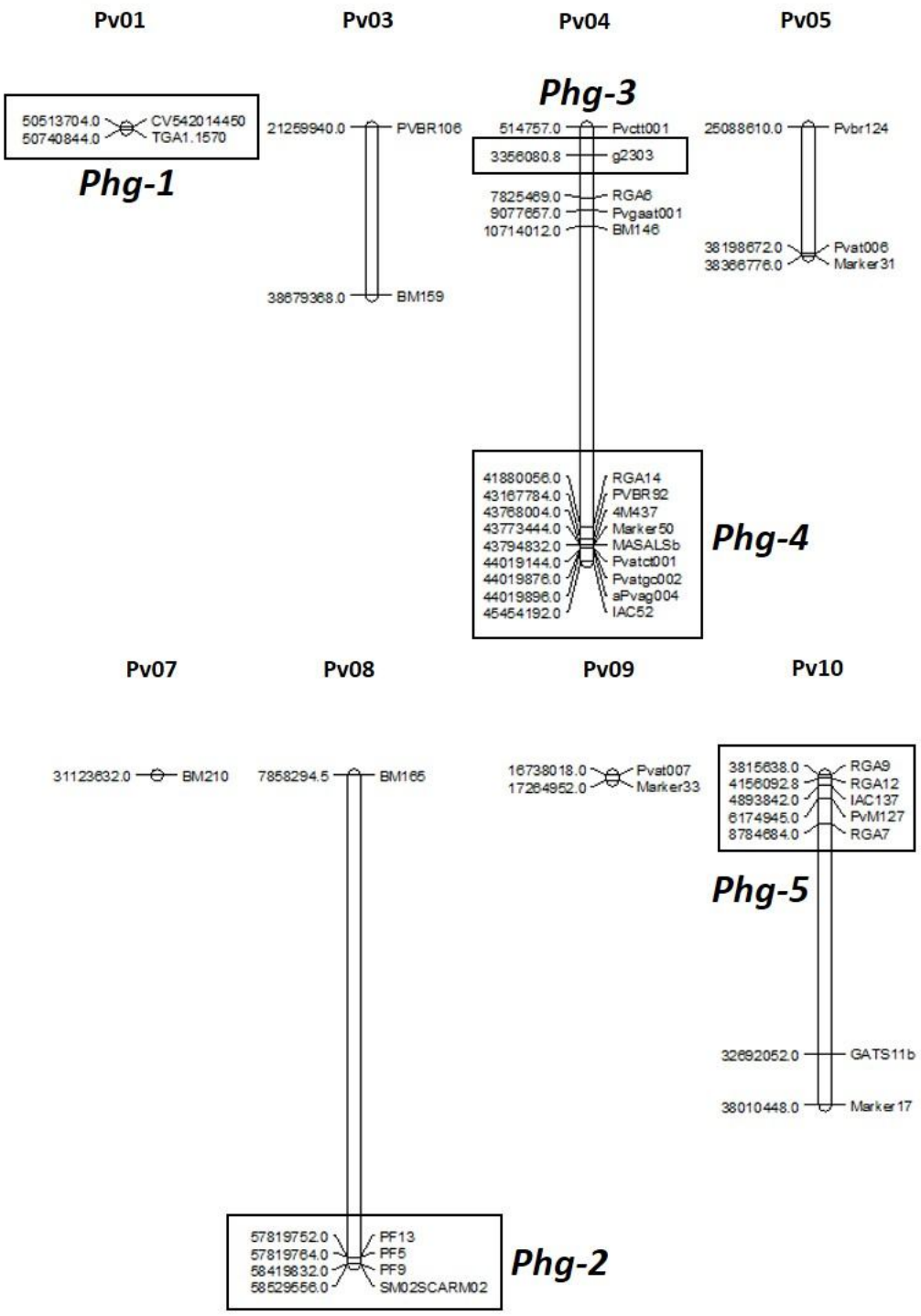

Figure 1. Linkage groups showing the physical position of the molecular markers linked to the major loci controlling resistance to the angular leaf spot of common bean. This physical map was developed based on sequence information obtained from the markers.

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HIGH DENSITY SNP GENOTYPING OF LINES WITH DIFFERENT STORAGE PROTEIN COMPOSITION REVEALS SITES OF INTERSPECIFIC INTROGRESSION AND MARKERS ASSOCIATED WITH PHASEOLIN OR LECTIN DEFICIENCY

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INTRODUCTION: The germplasm lines SARC1, SMARC1-PN1 and SMARC1N-PN1 integrate a progressive deficiency in storage proteins, phaseolin and lectins (Osborn et al. 2003). SARC1 contains arcelin-1 derived from a wild *Phaseolus vulgaris* accession, G12882. SARC1 had been crossed with MB11-29, lacking phaseolin due to an introgression from *P. coccineus* and L12-56, lacking major lectins due to an allele from Great Northern 1140. Progeny had been selected giving rise to SMARC1-PN1, lacking phaseolin, and SMARC1N-PN1, lacking both phaseolin and major lectins. The three lines share Sanilac as recurrent background.

MATERIAL AND METHODS: Parental genotypes Sanilac, G12882 and Great Northern 1140, as well as SARC1, SMARC1-PN1 and SMARC1N-PN1 were subjected to high density SNP genotyping using the BARC-Bean6K_3 BeadChip (Song et al. 2015). Also included were three phaseolin deficient *P. coccineus* lines (Pandurangan et al. 2016). Parent specific alleles were identified, and their distribution analyzed among progeny lines.

RESULTS: There were a limited number of *P. coccineus* specific alleles, present on chromosomes 2, 3, 7 and 11 in SMARC1-PN1 and SMARC1N-PN1 (Figure 1). On chromosome 7, six alleles flank the phaseolin locus, consistent with its introgression from *P. coccineus*. On chromosome 11, two alleles flank the albumin-1 locus, suggesting a possible origin from the interspecific parent. A methanol soluble albumin-1 (encoded by Phvul.011G205300.1/-400.1) had been shown to be present at higher levels in SMARC1N-PN1 than SARC1 (Liao et al. 2012). On chromosome 3, another *P. coccineus* specific allele is proximal to a Great Northern 1140 allele and to a pectin acetylesterase gene (Phvul.003g277600.1) whose transcript was elevated in developing seeds of SMARC1N-PN1 as compared with SARC1 (Liao et al. 2012). G12882 and Great-Northern 1140 specific SNP alleles are linked to the introgression of the arcelin-phytohemagglutinin-α-amylase inhibitor (APA) locus in SARC1/SMARC1-PN1 versus SMARC1N-PN1, respectively. The new markers could be useful to breed for bruchid resistance, and provide information on genomic introgression from *P. coccineus* into *P. vulgaris*.

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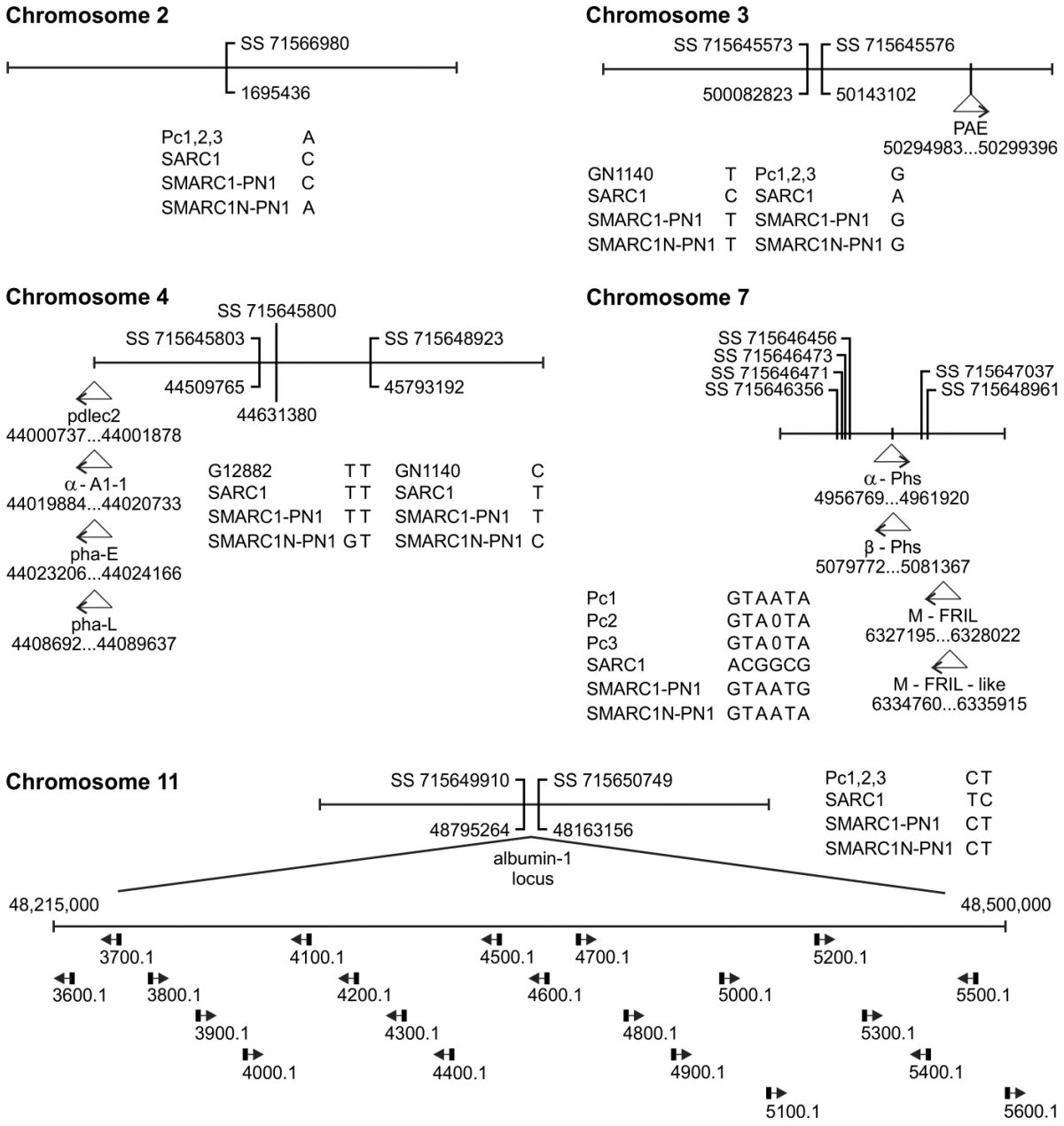

Figure 1. Localization of *P. coccineus* specific alleles and of parent specific alleles near the APA locus. Names above the horizontal bar refer to SNP markers on the BARCBean6K_3 BeadChip (Song et al. 2015). SNP positions on chromosome 7 are as follows: SS715646356, 3780517; SS715646471, 4063954; SS715646473, 4048409; SS715646456, 4277150; SS715647037, 5986657; and SS715648961, 6312942. Accession number of albumin-1 preceded by the prefix "Phvul.011G20-".

INSIGHTS FROM SYNTENY ANALYSIS BETWEEN BEAN AND SOYBEAN

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Synteny is the conservation of gene order in two chromosomal segments. It occurs at macro and micro scales and is based on the assumption that the syntenic regions are derived from an ancestral genomic region through duplication or speciation. The availability of whole genome sequences for common bean (*Phaseolus vulgaris* L.) and soybean (*Glycine max* L. Merr) has enabled detailed comparisons to be made of their genomes. We used *in silico* analyses to compare the genomic contexts of genes that function in the phenylpropanoid pathway, folate synthesis genes and genes associated with CBB resistance QTLs and yield in common bean and soybean. These studies confirmed that for most genes in common bean, two homologous genes occur in soybean. The DNA sequence correspondence we observed between common bean and soybean agreed with previously reported patterns of synteny between these two species and supported conclusions that the soybean genome was duplicated after the establishment of Phaseolus and Glycine from a common progenitor. The high degree of synteny between the two species facilitated the identification of gene function and allowed information from a highly characterized crop like soybean to be associated with specific loci and genes in bean.

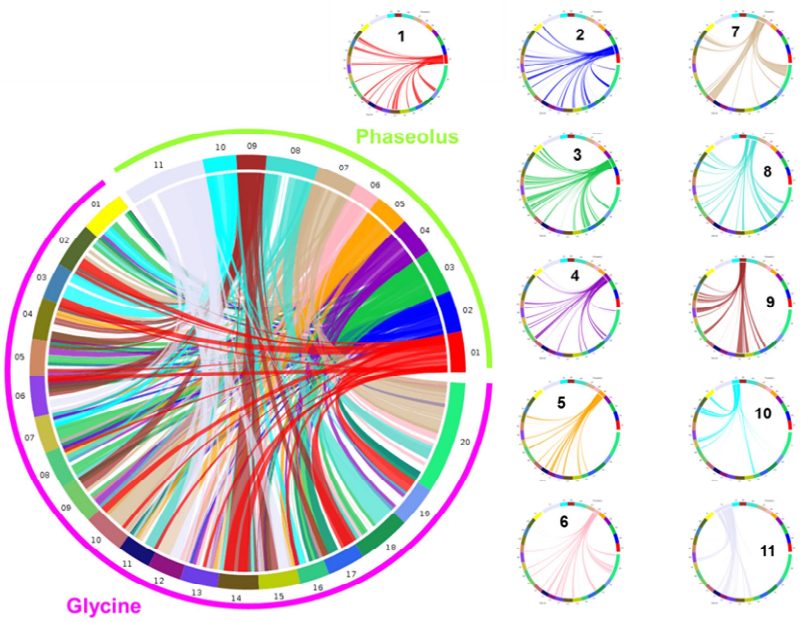

Figure 1. Syntenic relationships between bean and soybean A whole genome comparison is shown as well as individual bean chromosomes against the whole soybean genome.

A Plant Genome Duplication Database http://chibba.agtec.uga.edu/duplication/ comparison of the whole bean and soybean genomes shows extensive synteny that is highly fragmented. Each bean chromosome is made up of segments of multiple soybean chromosomes and *vice versa*.

Figure 2 shows gene identity and order for a segment of Phaseolus DNA, from the variety OAC Rex (Perry et al, unpublished) that contains a commonly used marker for resistance to common bacterial blight, namely SU91. It also contains genes that are unique to OAC Rex, which is resistant to common bacterial blight, including a divided form of the Niemann-Pick gene (at 59,432,373 nt) and unique R genes in a upstream R gene cluster (starting at 59,045,290 nt; Perry et al, 2013). The two

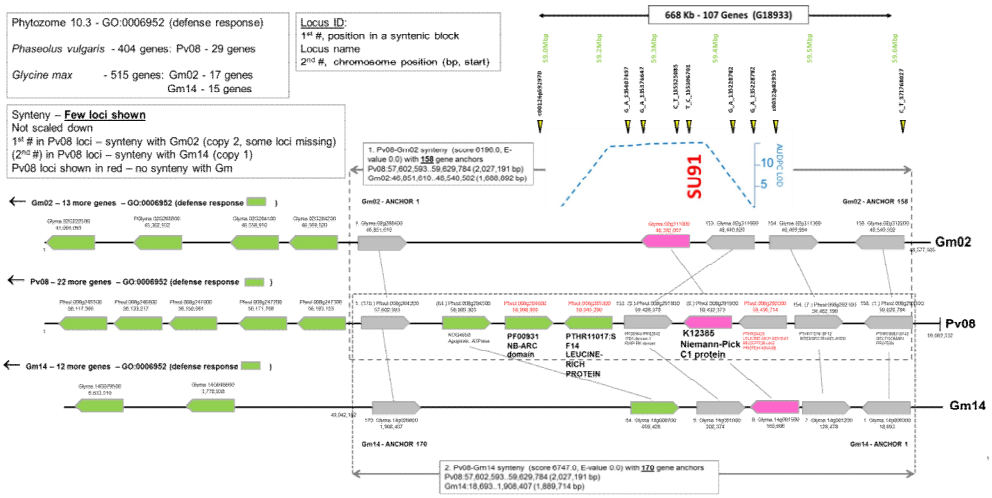

Figure 2. Comparison of a bean chromosome 8 genome segment (pv08) with the marker for common bacterial blight resistance (SU91) and the two regions of synteny regions from soybean (Gm02 & Gm14); pink genes are Niemann-Pick-like genes; green genes are resistance-related genes.

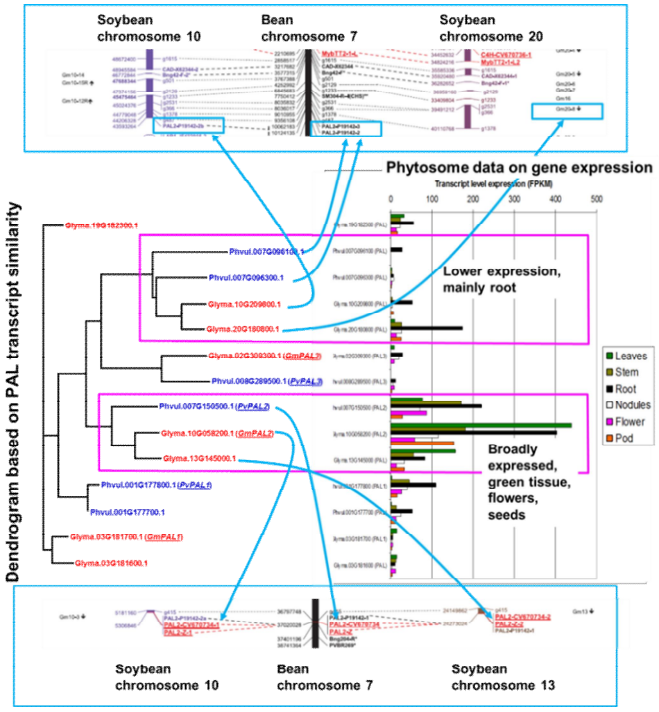

Figure 3. Phenyl ammonia lyase genes in bean and soybean. The dendrogram is based on cDNA similarity; gene expression levels are from Phytosome and boxes on the top and bottom show the positions of the genes in bean and syntenic soybean segments.

syntenic regions in soybean show similar arrangements of the Neiman Pick gene and clusters of R genes. Figure 3 shows the Phenyl Ammonia Lyase (PAL) gene family in bean and soybean. It codes for the first enzyme of the phenylpropanoid pathway that leads to lignin and pigment synthesis in plants. The dendrogram of PAL cDNA sequences identifies clusters of bean and soybean genes with similar coding sequences. The alignment of expression data (available at Phytozome) with the dendrogram shows that the clustered genes have similar tissue-specific expression patterns in bean and soybean. Furthermore, the comparison of the positions of the genes in the bean and soybean genomes illustrates that that the genes with similar sequences and expression patterns occur in syntenic blocks in these two species.

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CURRENT BEAN GERMPLASM COLLECTIONS AND ACTIVITIES IN SPAIN

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The common bean (*Phaseolus vulgaris* L.) has expanded through all the continents during the last centuries and the bean germplasm out of its regions of origin is more diverse than was previously thought and contains additional diversity that remains to be explored for its breeding value (Singh 2001, Santalla et al. 2002, Angioi et al. 2010, Santalla et al. 2010, De Ron et al. 2015). The common bean is a traditional crop in Spain and an important socioeconomic resource in many areas, playing a relevant role in the Spanish diet, therefore several traditional varieties have been awarded with European labels such "Protected Geographical indications (PGI)" or "Protected Designation of Origin (PDO) such as "Faba Asturiana" and "Faba de Lourenzá" (favada market class), "Judías del Barco de Avila", "Alubia de La Bañeza-León" (both regarding several types, including white kidney), "Judía del Ganxet" (hook), "Fesol de Santa Pau" (navy) or "Judía Tolosana" (black). In Spain, activities focus on the conservation of plant genetic resources started at the end of the 1970's of the 20th century; and common bean has been one of the species collected in all the Spanish regions and consequently a large collection has been established at the CRF (wwwx.inia.es/inventarionacional/). The high number of bean accessions of the Spanish collections makes their regeneration, multiplication, characterization and results communication a complex task. To overcome these constraints since 1994 a collaborative program has made possible the maintenance of these resources (table 1). In this paper we describe the current bean germplasm collections and activities of the Spanish genebanks network, whose active and base collections are located in the CRF that maintains 3442 accessions of *Phaseolus vulgaris*, some of them shared with other national institutions.

Table 1. Contributions by the partners of the Spanish network on bean germplasm

Institution / Code	Nr accessions multiplied /characterized	Years in the network
CRF / ESP004	154	11
MBG / ESP009	1315	18
NEIKER / ESP016	159	7
SERIDA / ESP032	557	16
UPC / ESP061	539	10
UNILEON / ESP151-ESP168	468	13
IMIDRA / ESP198	60	8

Using passport and seed morphological data, a core collection of 211 accessions was established in 2000 from the CRF collection. Later, SERIDA carried out a validation and update of this collection based on morphological and molecular data (Pérez-Vega et al. 2009) and UPC made a last revision lately (www.crf.inia.es/crfesp/paginaprincipaljudia.asp).

Collection at the MBG (since 1987): 2014 accessions (456 shared with the CRF). Origin of the accessions: Europe (17 countries), The Americas (15 countries), Asia (4 countries), Africa (1 country) and Oceania (1 country). The genetic stock includes about 500 breeding lines and RILs. A core collection was built in 2003 including 52 Spanish accessions (Rodiño et al. 2003).

Collection at the SERIDA (since 1991): 421 accessions of germplasm collected in northern Spain (395 shared with the CRF), 571 accessions in genetic stock including sources of resistance to biotic and abiotic stress, breeding lines, cultivar developed in the SERIDA and mapping populations.

Collection at the UPC (since 1992): 539 accessions (90 shared with the CRF). The accessions, all them collected in the Catalonia area (north east Spain), have been grouped according seed morphology and genetic background.
Collection at UNILEON (since: 1992): 308 accessions (199 shared with the CRF). The germplasm program includes the University of León (UNILEON, Spain), the CRF-INIA, and the Provincial Chamber and farmer organizations of León (Spain).
Collection at IMIDRA (since 1994): 78 accessions (54 shared with the CRF) (Lázaro et al. 2013).
Collection at NEIKER (since 1991): 128 accessions (34 shared with the CRF). All the accessions were collected in the Basque Country (Spain). The origin of the varieties is representative of the main growing areas highlighting varietal types Pinta Alavesa, Tolosana and Gernikesa.
The microclimate of the cultivation areas has played a strong influence on the evolution of the primitive landraces. The current Spanish landraces are the result of selective pressure and phenotypic selection by farmers and they are currently well adapted to the agroecological conditions under they have been grown for centuries. Therefore, during the last years, the bean accessions at the Spanish collections have been widely utilized in genetic studies and breeding programs:
Genetic and evolution studies (Santalla et al. 2002, Perez-Vega el al. 2009, Yuste-Lisbona et al. 2014)
Resistance to diseases (Monteagudo et al. 2006, Pascual et al. 2010, Trabanco et al. 2012, Campa et al. 2014)
Study of the symbiotic system bean-rhizobia (Santalla et al. 2001, Rodiño et al. 2011)
Evaluation of nutritional and sensory quality (Casquero et al. 2005, Rivera et al. 2013)
Generation of breeding populations (Pérez-Vega et al. 2010, Yuste-Lisbona et al. 2014)
Development and release of new varieties (Ruiz de Galarreta et al. 1998, Almirall et al. 2010, Ferreira et al. 2012)

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NUCLEOTIDE POLYMORPHISMS OF TWO NOVEL *DREB* GENES IN WILD MESOAMERICAN COMMON BEAN ACCESSIONS ARE STRUCTURED ACCORDING TO ENVIRONMENTAL VARIABLES

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INTRODUCTION

DREB (*DEHYDRATION RESPONSIVE ELEMENT-BINDING*) genes have been studied as candidates for abiotic stress tolerance improvement in many crops, but little has been done with this gene family in *Phaseolus vulgaris*. Just recently, we proposed a categorization of the *DREB* gene family in common bean, encompassing 54 genes distributed across six subgroups (A-1 to A-6), according to the nomenclature proposed for other model plants such as *Arabidopsis* and *Glycine* (Konzen et al., 2014). Two particular genes were isolated and named after their orthologs in *Arabidopsis*. *PvDREB2A* revealed to be drought-inducible in common bean genotypes. *PvDREB6B* was induced under drought and low-temperature (4^{o}C) treatments (Konzen et al., 2014). To verify the potential of these genes as candidates for abiotic stress tolerance breeding in common bean, we first investigated the nucleotide polymorphism within the open reading frame and intron regions of *PvDREB2A* and *PvDREB6B* and its structure in a set of common bean genotypes of Andean and Mesoamerican background. We further analyzed the structure of *PvDREB6B* polymorphisms in a wide range of Mesoamerican wild accessions and its possible association with a series of bioclimatic (temperature and precipitation-derived) and phenotypic variables (root and shoot depth and biomass). Hereby, we outline the potential of such genes and phenotypic variables as indicators of stress-tolerance, with emphasis on drought.

MATERIAL AND METHODS

First, we sequenced the entire open reading frame (ORF) and the first intron within *PvDREB2A* and the entire ORF of *PvDREB6B* from DNA samples of 17 common bean genotypes of Andean (Jalo EEP558, Midas, G19833, UCD-0801, UCD Canario 707 and CAL 143) and Mesoamerican (BAT 93, BAT 477, IAC-Carioca 80SH, Rosinha G2, IAC-Una, SEA-5, SxB 405 and ICA-Bunsi) background. The further step consisted on sequencing the ORF of *PvDREB6B* in a wild bean collection of 121 Mesoamerican accessions spanning the natural area of distribution of common beans, from northern Mexico (drier areas) to Colombia (higher humidity over the year). The wild beans were also evaluated in a greenhouse experiment consisting on an adaptation of the tube screening method of Rao et al. (2006). Root and shoot growth and depth, along with biomass and leaf area parameters were evaluated for all accessions treated with irrigation to field capacity and drought. Nucleotide diversity of *PvDREB6B* was used to infer the structure of wild bean populations based on BAPS (Bayesian Analysis of genetic Population Structure, http://www.helsinki.fi/bsg/software/) software. The genetic structure of *PvDREB6B* was correlated with a series of bioclimatic variables (http://www.worldclim.org/) retrieved for the location of each accession, as well as with the phenotypic data from the greenhouse experiment.

RESULTS AND DISCUSSION

From the first sequencing step, *PvDREB2A* revealed only two polymorphic sites within the ORF of the 17 genotypes. Higher variability was observed within the first intron, with 5 polymorphic sites. *PvDREB6B* exhibited much higher variability, with 17 SNP sites along its ORF. Thereby, it

was sequenced in the whole wild bean collection. High-quality sequences were obtained for 112 out of all accessions. In total, 33 polymorphisms were identified within the ORF when comparing the 112 genotypes. The population structure analysis of *PvDREB6B* polymorphisms allowed the identification of seven main subgroups of accessions (clusters C-1 to C-7) (Figure 1A). When the genetic structure was coupled with the bioclimatic variables, a principal component analysis on the correlation between those two groups of variables was able to partially distinguishing the genetic groups defined by *PvDREB6B* according to temperature, precipitation and location (Figures 1A and B). Most genotypes from C-5 subgroup belonged to the Durango area, in which the lowest precipitations and highest temperature variations are observed throughout the year. On the other hand, C-6 subgroup is represented by accessions located in the most humid areas. The greenhouse data also revealed significant phenotypic differences in root and plant growth according to the genetic clusters. Root deepening exhibited a trend to be higher in wilds originated from drier areas, especially under drought treatment. Our results provide insights for the application of *DREB* genes and phenotypic analyses for selecting wild beans for drought tolerance improvement.

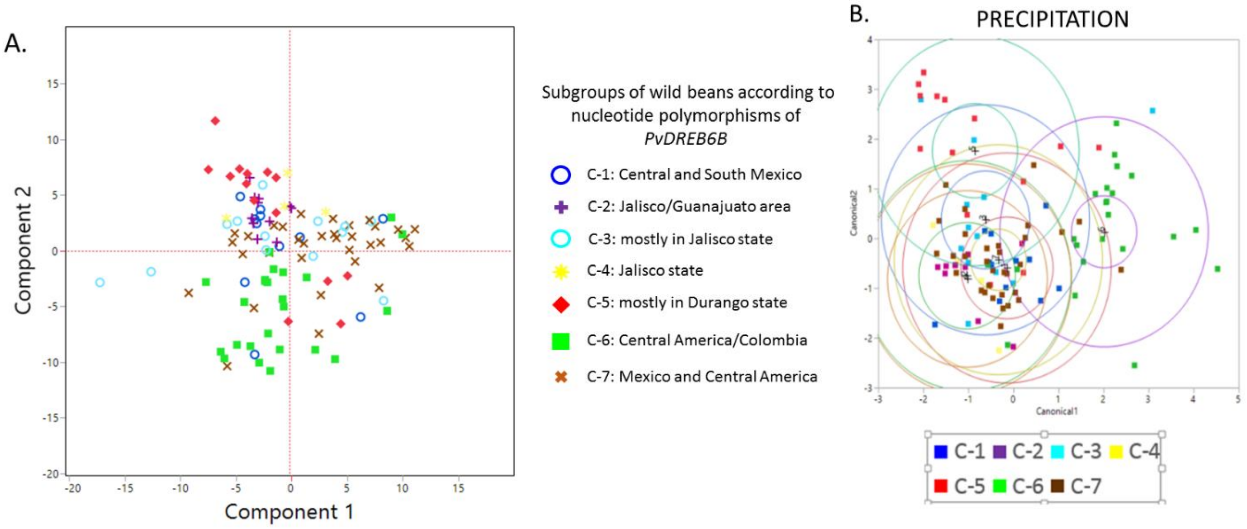

Figure 1 – Genetic structure of 112 wild common bean accessions of Mesoamerican origin as inferred by nucleotide polymorphisms within *PvDREB6B* and its association with phenotypic and bioclimatic variables. Genetic clusters (C-1 to C-7) were determined based on Bayesian inferences (BAPS). A – Combination of phenotypic data (root depth, plant height, leaf area and biomass under drought treatment) and bioclimatic variables (precipitation, temperature, altitude, location). B – Genetic structure as modulated by precipitation variables (annual precipitation and averages over each month) of the location of the wild bean accessions.

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INVESTIGATING THE ROLE OF CANDIDATE GENES IN DROUGHT TOLERANCE OF COMMON BEAN GENOTYPES

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Brazil is one of the world's greatest producers of common beans (*Phaseolus vulgaris* L.). For the years 2014/15, it is expected that the harvests will reach 3.2 million tons (CONAB, 2015). Despite its great economic, cultural and nutritional importance, being present as a valuable staple food in the daily diet of around 70% of its population, the culture still suffers with poorly technological input which is aggravated by intermittent drought episodes. Dry bean cultivation in the country is majorly conducted at the beginning of the dry seasons, therefore critically affecting flowering and pod-filling as water deficit intensifies. Because of this, improvement of drought tolerance for common bean has been for a long time a major objective for many breeding programs. Hence, in the last years our research group has focused on elucidating key molecular mechanisms that might be involved on drought tolerance responses in common beans. In our studies, the Mesoamerican advanced line BAT 447 was initially selected as a model due to its improved root architecture and superior performance in soils with low water potential. Molecular biology techniques have been applied in order to select candidate genes with potential for both improvement and selection of drought tolerance in local common bean varieties, such as the Carioca type.

In one of our initial studies, a Suppressive Subtractive Hybridization (SSH) cDNA library was constructed contrasting BAT 477 (drought tolerant) and IAC-Carioca 80SH (a drought-sensitive cultivar) roots transcriptome under a water deficit regime of 192 hours during the pre-flowering stage (R5) (Recchia *et al.*, 2013). A set of 1.632 clones were randomly sequenced, generating 1,572 valid reads (total of 1,120 unigenes). After gene annotation and biological functional classification, 148 reads were grouped as possibly implicated on to drought stress response: transcription factors (NAC, DREB, ABRE, WKRY, bZIP, MYB), transmembrane transporters like aquaporins, K+/H+ pumps and Ca^{+2} transporters, osmo-protectants and regulators, oxidative stress response, protein folding (Heat-shock proteins, chaperones) and degradation (ubiquitins).

A time-course RT-qPCR experiment (after 72, 144 and 192 h of water deficit, followed by a 24 hours of re-watering) was conducted for SSH library validation. For BAT 477, from the 31 selected transcripts, twelve genes underwent a gradual increase in up-regulation during water stress progression (*LEA5*, *NAC protein*, *EF-hand* Ca^{+2}*-binding motif*, *mapK*, *sucrose synthase*, *rhamnose biosynthesis enzyme 3*, *26S protease s10b subunit*, *ENOD18 factor*, *sinaptotagmin*, *raffinose synthase* and *cellulase*), and for IAC-Carioca 80SH, this pattern was observed for six of them (*LEA5*, *cationic peroxidase 2*, *rhamnose biosynthesis enzyme 3*, *sucrose synthase*, *ENOD18 factor* and *cellulase*). For BAT 477, some genes that proved to be actually down-regulated during all the periods analyzed (*aquaporin PIP2.a*, *trehalose 6-P synthase* and *calmodulin like kinase*) became suddenly up-regulated after rehydration; the same being observed for *calmodulin like-kinase*, *Ser/Thr protein phosphatase*, *cation:cation antiporter* and *GTP-binding protein* in IAC-Carioca 80SH, revealing possible mechanisms of re-acclimation.

Further characterization of the contrasting drought tolerance responses of BAT 477 and IAC-Carioca 80SH genotypes was achieved through a cDNA-AFLP approach applying six different primer combinations. This analysis allowed the identification of 100 transcripts derived fragments (TDF) specifically regulated on BAT 477, and 77 on IAC-Carioca 80SH. Among the transcripts identified in the tolerant genotype, 11 were listed with potential to be selected for future studies (*chlorophyll A-B binding protein*, *HSP40*, *HSP70*, *glycosyl hydrolase*, *serine/threonine protein kinase*, *trehalose-6-phosphate synthase*, *E3 ubiquitin ligase*, *fructose biphosphate aldolase*, *mediator*

complex subunit 13, *aquaporin nodulin MTN-3-related* and *TCP transcription factor*), and in the susceptible genotype, nine can be listed: *coatomer protein complex*, *monoamine-oxidase A repressor R1*, *synaptobrevin*, *haloacid dehalogenase-like hydrolase*, *ADP-ribosylation factor*, *mTERF*, *serine protease S1C HtrA-related*, *legume lectin and SWI/SNF-related chromatin binding* (Biazuzo *et al*., in preparation).

Recently, a lot has been argued about how symbiotic interactions established with beneficial soil microorganisms in the rhizosphere can improve plants physiological and adaptive responses against environmental factors, and the colonization of roots by natural abundant arbuscular mycorrhizal fungi (AMF) has revealed to be an excellent candidate on this matter. A whole transcriptome RNA-Seq analysis was conducted in 96 hours' drought-stressed roots of BAT 477 plants inoculated with a mixture of AMF. Differential gene expression analysis revealed the regulation 12,086 transcripts in AM plants, and 11,938 transcripts in no-AM treatments, during stress. Furthermore, 71 transcripts were selected as directly regulated by AMF inoculation in response to drought, between them: transmembrane transporters like aquaporins, transcription factors, epigenetic regulation factors, post-translation regulators and proteins associated to host-specific interaction. Laser-capture microdissection microscopy coupled with RT-qPCR analysis validated our whole-root transcriptomic analysis and helped in the localization of tissue-specific gene expression patterns of 23 selected transcripts. From these, seven genes were only detected in cortical cells, being two up-regulated in cells containing fungal structures, three down-regulated, and two (aquaporin *PIP2;*3 and *β 1,3 Glucosidase*) exclusively detect (Recchia et al., in preparation).

Dehydration Responsive Element-Binding (*DREB*) genes code for transcription factors (TF) that are induced in plants in response to a variety of abiotic factors like water deficit, salt, cold and heat. Thus, *DREB* genes were selected by our group as promising candidate genes for marker-assisted selection (MAS) aimed at drought tolerance in common beans. An initial *in silico* search on both Phytozome and NCBI public databases offered 54 putative *DREB* genes in *P. vulgaris* genome (Konzen *et al.*, 2014). Gene expression analysis of four of them, *PvDREB1*, *PvDREB2A*, *PvDREB5* and *PvDREB6B*, revealed both temporal and spatial variation among roots, stems and leaves of BAT 477 plants, and in relation to other common bean cultivars (Konzen *et al.*, 2014; Konzen et al., 2015). Additionally, the functional analysis of *PvDREB6A* revealed important prospects. This transcription factor was located at the cell nucleus, being successfully transferred and expressed in *Arabidopsis* (Pereira et al., in preparation). Four transgenic events with better expression of this gene were selected: Col-0/pFEC2.1 #1, Salk_020767C/pFEC2.1 #13.1, Salk_020767C/pFEC2.1 #19.7 and Salk_020767C/pFEC2.1 #23.7. Plants overexpressing *PvDREB6A* presented higher survival rates than non-transgenic events under drought conditions.

Taking the drought tolerant genotype BAT477 as a model and comparing against different genotypes with distinct histories of breeding and drought tolerance capacities, our group was able to identify a series of candidate genes possibly involved in key regulatory pathways of common beans response and adaptation to drought. Unraveling the role of these candidate genes might have important applications for future breeding and engineering strategies aimed at developing bean cultivars with improved performance under drought episodes.

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SOYBEAN CYST NEMATODE - A THREAT TO DRY BEAN PRODUCTION

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Soybean cyst nematode (SCN), *Heterodera glycines*, is a serious soybean pathogen that has spread throughout the US soybean production area since the initial discovery in 1954 in North Carolina. In 2003 SCN was found in southeastern North Dakota (Bradley et al. 2004), close to the major dry bean production areas of North Dakota and northern Minnesota where there are approximately 283,000 ha of dry bean. This area produces about 35 % of US bean production. Since then, SCN has spread rapidly and is now in at least 20 counties of eastern North Dakota from the border with South Dakota all the way to the border with Canada. SCN is also in a number of counties in northwestern Minnesota. SCN has recently been found in counties with high acreage of dry bean production which are primarily located in the Red River Valley of ND and MN.

Although *Phaseolus vulgaris* has been known as a host of SCN since the 1930's in Japan, SCN has not been considered a major threat to dry bean or snap bean production in the United States primarily because bean production has been in areas with limited or no occurrence of SCN. Only recently has there been an awareness of the potential threat of SCN to bean production in ND, MN, MI and Ontario where SCN has spread into bean production areas because of the production of soybean in those areas. There have been limited studies on the effects of SCN on dry and snap bean and until the report by Poromarto at al. in 2010, there had been no field studies conducted to document yield loss from SCN. Research in North Dakota has shown that SCN will reproduce on all of the most common bean types grown in the region such as pinto, navy and kidney beans. SCN can cause yield reductions between 30 to 56% depending on the egg density in the soil, environmental conditions and the specific bean type (Poromarto and Nelson, 2009; Poromarto et al. 2010) (Figure 1). In addition to the potential yield loss associated with SCN damage, another concern is the interaction of SCN with root rot pathogens which may increase overall damage to bean roots.

Because of the potential threat of SCN to dry bean production in the ND-MN region, a research program is underway at North Dakota State University to identify dry bean germplasm resistant to SCN, understand the genetics of resistance and incorporate resistance into breeding material for the eventual production of SCN resistant dry bean varieties. In addition, a strong outreach program is underway to educate dry bean growers on the biology of this disease and its management. Drs. Juan Osorno (bean breeder) and Phillip McClean (bean genetics) from Plant Sciences, and Shalu Jain (research scientist), Sam Markell (extension pathologist row crops) and Berlin Nelson Jr (soybean pathologist) from Plant Pathology are the principal scientists involved in this effort. The research program is being funded by the Northarvest Bean Growers Association and USDA specialty crop block grants.

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Figure 1. Effects of soybean cyst nematode (SCN) on growth of pinto dry bean plants. Plant on the left is growing in soil with no SCN while plant on the right is growing in soil infested with a high population of SCN. Notice the stunting and general poor growth of plant growing in the presence of SCN. Photo courtesy S. Poromarto.

TOWARDS AN UNDERSTANDING OF INTERACTIONS BETWEEN BACTERIAL VIRULENCE FACTORS AND GENES IN BEAN RESISTANCE QTL FOR BREEDING BEANS WITH DURABLE COMMON BACTERIAL BLIGHT RESISTANCE

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INTRODUCTION

Common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli* and its fuscan variant *X. fuscans* subsp. *fuscans,* is a damaging disease of common bean throughout the world. In order to understand the diversity of the CBB pathogen, 10 to 12 single colonies (lines), purified from each of four Ontario collected bacterial isolates, were genotyped with diagnostic marker of pathogenicity and phenotyped on susceptible cultivar Nautica. Seven selected pure bacterial lines with differential aggressiveness were characterized by genome sequencing. Differences in candidate virulence factors were identified that may be related to differences in aggressiveness between the bacterial lines through interactions with the promoters of target genes in bean resistance QTL.

RESULTS AND DISCUSSION

From the 10-12 colonies that were tested from each isolate, 100%, 8%, 30%, and 100% were pathogenic for isolates ISO18, ISO98, ISO12, and ISO118, respectively, indicating that non-pathogenic Xanthomonads existed in CBB pathogen populations. The X4c/X4e marker, a diagnostic tool for pathogenic CBB pathogens reported by Audy et al. (1994), was present in both pathogenic and nonpathogenic colonies of non-fuscans isolates (ISO18 and ISO98). In contrast, X4c/X4e marker was absent in pathogenic colonies of fuscans isolates (ISO12 and ISO118) (Fig. 1).

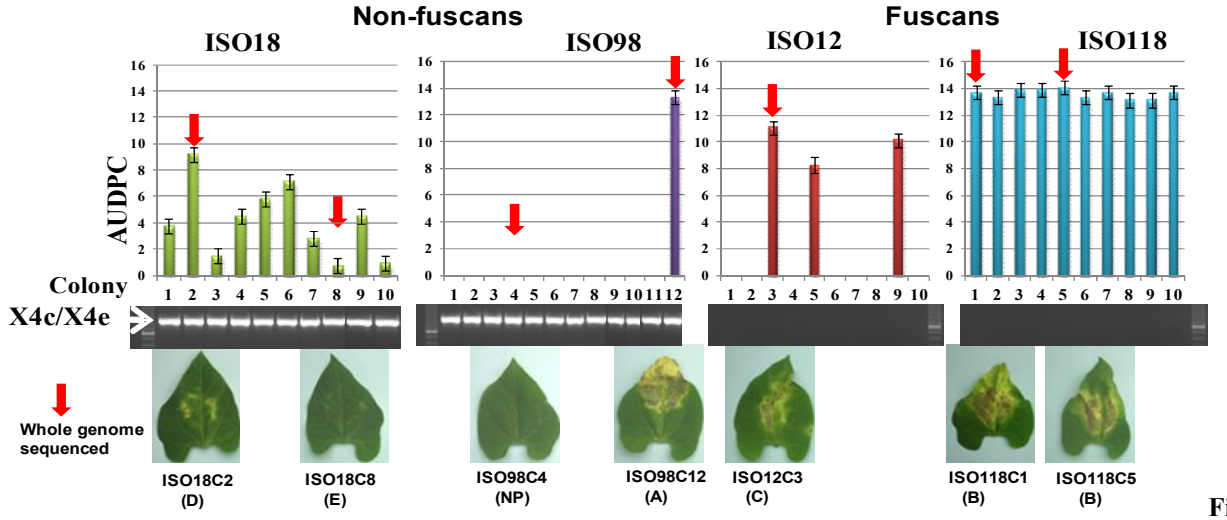

Fig. 1. Pathogenic diversity of four common bacterial blight isolates, collected from Ontario, Canada. Ten to 12 single colonies purified from each of non-fuscans isolates (ISO18, ISO98) and fuscans isolates (ISO12, ISO118) were tested on susceptible Nautica and were PCR amplified with X4c/X4e marker. Letters in parentheses of seven selected bacterial lines indicates the level of aggressiveness, based on their CBB tests on 13 bean genotypes, with A representing the most aggressive. ISO98C4 is a nonpathogenic (NP) *Xanthomonas* line. Red arrows indicate the colonies selected for whole genome sequencing.

Sequence alignment revealed that there was a segment of nucleotide sequence (86 bp) missing of marker X4c/X4e in fuscans lines ISO12C3, ISO118C1 and ISO118C5, explaining the absence of

X4c/X4e marker in their PCR amplification, and indicating that X4c/X4e might not be a good marker for differentiating pathogenic and non-pathogenic *Xanthomonas*.

Fully assembled genome sequences of seven *Xanthomonas* lines were obtained by de novo assembly. Genome sizes ranged from 5.32-5.36 Mbp (Table 1). Genomic comparison revealed that: there are sequence inversions in the middle of chromosomes between a non-fuscans line (ISO98C12) and a fuscans line (ISO118C1), and between our fuscans line ISO118C1 isolated from Ontario, Canada, with fuscans strain Xff str. 4834-R isolated from France (Darrasse et al. 2013) (Fig. 2).

Table 1. Genome sequences and type III effectors of seven pure bacterial lines

	X. axonopodis pv. *phaseoli*			Np *Xanthomonas* sp.	*X. fuscans* subsp. *fuscans*		
	ISO18C2	ISO18C8	ISO98C12	ISO98C4	ISO12C3	ISO118C1	ISO118C5
Pathogenicity	D	E	A	non-pathogenic	C	B	B
Genome size (Mbp)	5.33	5.32	5.32	5.33	5.36	5.36	5.36
Type III effectors	24	24	24	24	30	30	30
Transcription activator-like (TAL) effector	2	2	2	2	1	1	1

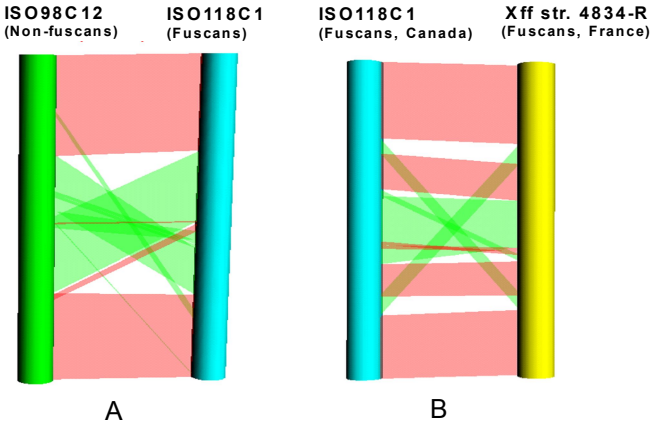

Fig. 2. Alignment of different genomes using Symap. **A** Alignment between non-fuscans line ISO98C12 with fuscans line ISO118C1. **B** Alignment between fuscans strain from Canada (ISO118C1) and fuscans strain from France (Xff str. 4834-R, Darrasse et al. 2013).

A Blastn search of the 66 known type III effector genes identified 24-30 effector genes in those bacteria lines, including 1 to 2 Transcription Activator-Like (TAL) effectors. Polymorphism in repetitive central domain of a TAL effector exists in those *Xanthomonas* lines with differential aggressiveness. Based on repeat-variable di-residues (RVD) of TAL effector proteins, multiple binding sites were identified for less aggressive bacterial lines but none for the most aggressive line ISO98C12, in promoter regions of QTL resistance candidate genes (Perry et al. 2013) on Pv8 of resistant cultivar OAC Rex. Our results suggested that polymorphism of TAL effectors might change the interaction between bean and CBB pathogens, and the expression of disease severity. This information will facilitate breeding bean cultivars with durable CBB resistance.

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DEVELOPMENT OF A BLACK BEAN LINE THAT COMBINES BRUCHID AND MULTIPLE VIRUS RESISTANCE

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INTRODUCTION

Bean golden yellow mosaic virus (BGYMV), bean common mosaic virus (BCMV) and bean common mosaic necrosis virus (BCMNV) threaten dry bean (*Phaseolus vulgaris* L.) production in the lowlands of Central America and the Caribbean. The common bean weevil (*Acanthoscelides obtectus* Say) can cause significant post-harvest losses.

MATERIALS AND METHODS

The black bean breeding line PR1464-4 was developed in Puerto Rico that combines resistance to the common bean weevil and the aforementioned viral diseases. PR1464-4 was derived from the cross 'AO1012-29-3/XRAV-40-4'. AO1012-29-3 is a red kidney line derived from the cross 'Rojo*3///SMARC-2-PN-1//ICA Pijao*2/G40199' made by Dr. Paul Kusolwa and Jim Myers that was selected in Puerto Rico for resistance to the common bean weevil. XRAV-40-4 is a black bean cultivar released that has resistance to BGYMV, BCMV and BCMNV (Beaver et al., 2014). Marker-assisted selection was used at the USDA-ARS Tropical Agricultural Research laboratory to determine the presence of alleles for resistance to BGYMV, BCMV and BCMNV. PR1464-4 has the *bgm-1* gene (SR2 SCAR marker) and the SW12 QTL that confers resistance to BGYMV and the *I* gene (SW13 SCAR marker) and the *bc-3* gene (ENM CAPs marker) for resistance to BCMV and BCMNV. The BCMNV resistance of PR1464-4 was confirmed in greenhouse evaluations using the NL-3 strain. PR1464-4 was screened in the laboratory for bruchid resistance. Seed samples (100 g) were infested with 25 adults of the common bean weevil. A Completely Randomized Design with four replications was used.

RESULTS AND DISCUSSION

At 58 days after infestation, almost 90% of the seed of PR1464-4 showed no damage whereas > 50% of the seed of the susceptible check 'Verano' was damaged (Table 1). Less than 5% of the seed weight of PR1464-4 was lost compared with 19.8% seed weight loss for the susceptible check cultivar 'Verano'. PR1464-4 has the molecular markers reported by Kusolwa et al. (2009) for the APA locus from the wild tepary bean (*Phaseolus acutifolius*) accession G 40199. PR1464-4 was harvested at 78 days after planting at Isabela, Puerto Rico. Seed yield of PR1464-4 (1,230 kg/ha) in a drought-stressed trial planted in Puerto Rico in February 2015 was not significantly different from the check cultivars 'XRAV-40-4' (1,579 kg/ha) and 'Verano' (1,322 kg/ha). PR1464-4 has an opaque black seed with a 100 seed weight of 18.7. Seed samples were analyzed by the University of Missouri Agricultural Experiment Station Chemical Laboratories. Crude protein of seed of PR1464-4 was 23.0% compared with 21.3% for 'XRAV-40-4' and 24.2% for 'Verano'. (Table 2). The amino acid profiles of the bruchid resistant and susceptible lines were similar. Trials are currently being conducted at Zamorano, Honduras to evaluate the resistance of PR1464-4 to the Mexican bean weevil (*Zabrotes subfasciatus*) and different eco-types of the common bean weevil.

Table 1. Seed damage caused by *Acanthoscelides obtectus* Say in a laboratory evaluation conducted at Isabela, Puerto Rico.

Line	Seed type	% seed damaged at 58 days after infestation	% seed weight loss at 58 days after infestation
PR1464-4	Black	10.3	4.6
PR1464-6	Black	8.6	5.1
Verano (Susc.)	White	51.0	19.8
AO1012-29-3-3A	Red Kidney	8.2	4.9
AO1012-29-3-6B	Red Kidney	6.3	4.4
Badillo (Susc.)	Light Red Kidney	59.1	11.5
Mean		23.9	8.4
LSD (0.05)		6.0	3.4
C.V. (%)		16.8	27.4

Table 2. Crude protein (%) and amino acid profiles of bruchid resistant lines PR 1464-4 and PR 1464-6 and bruchid susceptible cultivars 'Verano' and 'XRAV-40-4'.

Amino Acid	PR1464-4 W/W%	PR1464-6 W/W%	Verano W/W%	XRAV-40-4 W/W%
Aspartic Acid	3.03	2.93	2.97	2.69
Threonine	1.13	1.11	1.01	0.95
Glutamic Acid	3.30	3.05	3.86	3.28
Proline	0.92	0.90	0.93	0.79
Glycine	0.94	0.89	1.00	0.89
Alanine	1.02	0.98	1.01	0.94
Cysteine	0.22	0.21	0.24	0.21
Valine	1.28	1.22	1.26	1.14
Methionine	0.28	0.26	0.33	0.28
Isoleucine	1.04	1.02	1.04	0.93
Leucine	1.80	1.68	1.92	1.72
Lysine	1.66	1.58	1.73	1.53
Total	16.62	15.83	17.30	15.35
Crude Protein[1]	23.02	23.26	24.15	21.37

[1] Percentage N X 6.25. W/W%= grams per 100 grams of sample.

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MAPPING THE *CO-1* LOCUS CONDITIONING ANTHRACNOSE RESISTANCE IN COMMON BEAN

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INTRODUCTION

The dry bean (*Phaseolus vulgaris*) breeding program at Michigan State University (MSU) has a long history of breeding for resistance to *Colletotrichum lindemuthianum*, the causal agent of bean anthracnose. The first anthracnose resistant navy bean cultivars released were Sanilac in 1957 (Andersen et al., 1960) and Seafarer in 1968 (reviewed by Kelly, 1999). Both carried major gene resistance to alpha race 17 but the gene was never fully characterized. Future cultivars with the same resistance gene were later threatened by the emergence of new delta race 23 that appeared in Ontario in 1970-80s (Wallen, 1976; Tu, 1988). Race 23 overcame the resistance in most navy bean cultivars and virulence data suggested that the defeated resistance gene in the MSU navy beans was the *Co-1* (formerly *A* gene) gene on chromosome Pv01 (Kelly and Young, 1996; Kelly and Vallejo, 2004). Major shifts in the breeding program that focused on plant architectural improvements in 1980-1990s (Kelly, 2000) resulted in a loss of the anthracnose resistance in many of the first upright navy and black bean cultivars released by MSU. With the appearance of new anthracnose races 7, 73 and 65 in Michigan in 1990s (Kelly et al., 1994; Balardin and Kelly, 1996), research was undertaken to introgress anthracnose resistance into the new upright germplasm and verify if the gene previously deployed in the MSU breeding program was the *Co-1* gene as this gene is widely believed to be of Andean origin and resides in many North American kidney bean cultivars (Kelly and Vallejo, 2004). Crosses between Seafarer and Montcalm kidney bean revealed allelism for resistance to race 17 (alpha) in segregating populations (Young and Kelly, 1996) suggesting that the *Co-1* resistance gene had been introgressed into navy beans. Further studies revealed that the resistance gene in the related black bean cultivar Raven was in fact the *Co-1* gene and was independent of other resistance genes *Co-3, Co-4* and *Co-5* on Pv04, Pv08 and Pv07, respectively (Young and Kelly, 1997).

The objective of the current study was to confirm if the gene conditioning resistance to race 73 in the current black bean cultivar Jaguar (Kelly et al., 2001) is the *Co-1* gene and conduct mapping studies to verify its position and identify linked markers for breeding purposes.

MATERIALS AND METHODS

Reaction to anthracnose was investigated in an $F_{4:6}$ Middle American black bean population consisting of 95 recombinant inbred lines (RIL) developed from a cross between Jaguar assumed to possess the *Co-1* gene with resistance to race 73 (Kelly et al., 2001) and Puebla 152 (landrace cultivar known to be susceptible to race 73). The RIL population, along with the parents, was screened for reaction to race 73 and genotyped using an Illumina BARCBean6K_3 BeadChip with 5398 SNPs (Song et al., 2015).

RESULTS AND DISCUSSION

A major putative QTL for resistance to race 73 was identified on Pv01 adjacent to SNP ss715645251 at 50.30 Mb within the 58 kb region (50.26-50.32 Mb) where the *Co-x* was mapped (Richard et al., 2014). This region likely corresponds to the major resistance cluster consisting of the five alleles at the Co-1 locus.

Further confirmation came from a separate study conducted to confirm resistance gene locations in a subset of 226 bean accessions from the Andean Diversity Panel (ADP; Cichy et al.,

2015) that was screened with eight races of anthracnose to identify and map new sources of resistance using a genome-wide association study (GWAS). Output from the GWAS detected a major QTL for resistance to race 73 on Pv01 that was linked to the same SNP marker ss715645251 at 50.30 Mb (Zuiderveen, 2015). An InDel marker NDSU_IND_1_50.2219 was also identified that was linked at 3.2 cM from the *Co-1* gene in the RIL population. The InDel marker (50.22 Mb) was present in genotypes possessing different resistant alleles except *Co-1*^{5} (Widusa) at the Co-l locus including the *Co-1* allele in Jaguar and the *Co-x* in Jalo EEP558. The InDel marker should be useful for breeders in third world countries as it can be utilized to integrate different resistance alleles at the Co-1 locus using marker assisted breeding in labs with limited resources.

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EFFECT OF SEED-TO-SEEDLING TRANSMISSION OF *COLLECTOTRICHUM LINDEMUTHIANUM* IN DRY EDIBLE BEANS UNDER FIELD CONDITIONS

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INTRODUCTION

Anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib., is a devastating seed-borne disease of dry bean (*Phaseolus vulgaris* L.), causing substantial yield loss under conducive environmental conditions (Schwartz et al. 2005). Seed-to-seedling transmission of anthracnose has been demonstrated to be the most important factor in disease development, providing an early source of inoculum (Dillard et al. 1993; Tu, 1983). The objective of this study was to determine the effect of varying levels of seed-borne anthracnose on foliar disease severity, yield and discoloration in harvested seeds under field conditions.

MATERIALS AND METHODS

Field trials were conducted in 2014 and 2015 in Morden, Manitoba in a randomized complete block design with four replicates. Comparisons were made using; 1) disease-free seed and seed grown from anthracnose infected plants sorted into three categories including 2) seeds that had no visual symptoms of anthracnose and seed with 3) slight and 4) moderate discoloration.

RESULTS AND DISCUSSION

In 2014, weather was generally dry until approximately 65 days after planting. Plots planted to disease-free seed displayed only a very low level of disease at the end of the season, most likely from inter-plot spread (Fig. 1A). Planting seeds grown from infected plants, but displaying no visible symptoms (symptomless), resulted in disease, but severity was less than when symptoms of anthracnose were visible on the seed planted (slightly discolored and discolored). Yield and the level of discolored seeds harvested when symptomless seeds were grown were not different than when disease-free seed was grown; however, yield was significantly lower, and discolored seeds harvested were significantly higher, when seeds planted had visual lesions (Fig. 2A and B). In 2015, rain and high relative humidity were much more frequent, resulting in higher anthracnose severity in all plots (Fig. 1B), including plots grown from disease-free seed. Anthracnose severity in plots grown from symptomless seed was similar to seed with visual symptoms. Yield decreased significantly with increasing discoloration of planted seeds (Fig. 2A). As was observed in 2014, discoloration in harvested seeds was significantly different in symptomless compared to discolored seeds; however, in 2015, discolored seeds were harvested from all diseased seed categories at a significantly higher rate than disease-free seed (Fig. 2B).
These results had not been previously demonstrated under field conditions and provide a very vivid illustration of the dangers of planting seed that was produced in a field were anthracnose was present, even if seeds appear disease-free. These trials also reinforce the impact conducive weather can have on spread and development of this devastating disease.

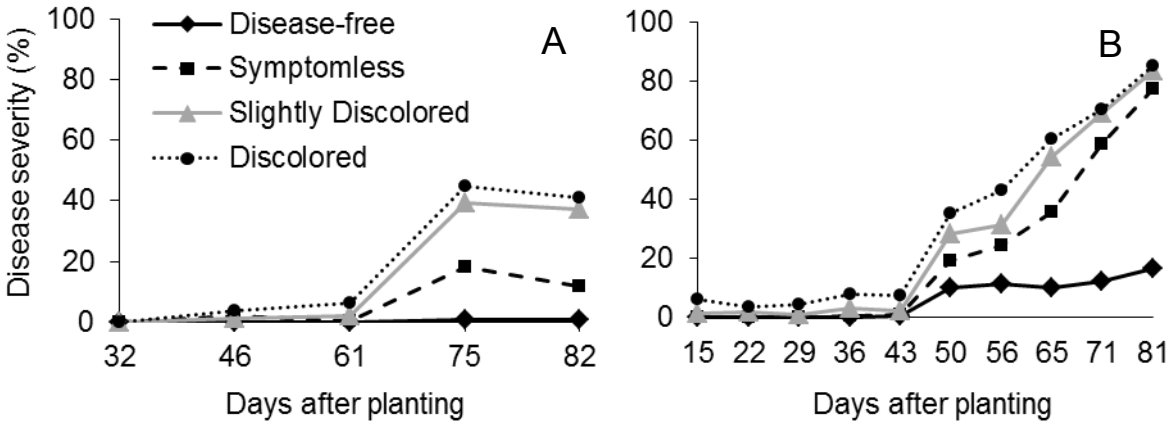

Figure 1. Disease severity (%) within plots during 2014 (A) and 2015 (B) field trials across asymptomatic and symptomatic seed categories.

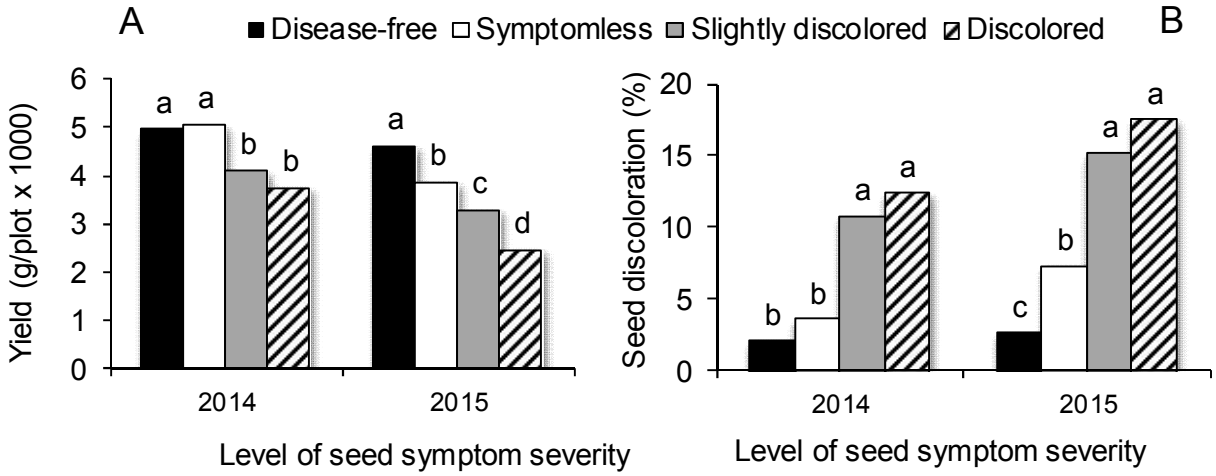

Figure 2. Yield (A) and percent seed discoloration (B) across asymptomatic and symptomatic seed categories in field trials conducted in 2014 and 2015. Bars of the same color with the same letter above are not significantly different based on Fisher's protected least significance difference test (α = 0.05).

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MOLECULAR CHARACTERIZATION OF *BEAN COMMON MOSAIC VIRUS* ISOLATE OVERCOMING THE *BC-3* ALLELE IN COMMON BEAN

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INTRODUCTION

In common bean (*Phaseolus vulgaris* L.) six recessive alleles have been found to control the resistance against *Bean common mosaic virus* (BCMV). Among these alleles, *bc-3* was identified as the mutated *eIF4E* allele and able to provide the most effective protection against all BCMV strains and against other potyviruses, when present in a homozygous state and in combination with the *bc-u* helper allele (1). Because of its ability to confer complete, strain non-specific resistance against BCMV and other legume-infecting potyviruses, the *bc-3* allele has been introduced by breeders into common bean germplasm to protect different market classes of dry and snap beans against BCMV. In 2013, we found an isolate of BCMV, named 1755a, able to replicate in the cultivar IVT7214 harboring *bc-u*, *bc-2*, and *bc-3* alleles, and hence overcome the *bc-3* resistance allele. Here, we molecularly characterized this BCMV isolate 1755a that was able to replicate in *bc-3* containing cultivar IVT7214. We also confirmed the presence of the intact homozygous *bc-3* CAPS marker and corresponding mutations in the *eIF4E* allele in IVT7214. The whole genome sequence of 1755a was determined and found recombinant between NL1, US1 (both PG-I), and a yet unknown BCMV strain. NL1 and US1 shared large 3'-proximal sections of the genome with BCMV isolates 1755a (PG-VIII), indicating that P1/HC-Pro cistrons of BCMV strains may interact with most resistance genes.

MATERIALS AND METHODS

The RNA extractions, RT-PCR, cloning and sequencing steps of 1755a were performed according to the protocol described elsewhere (2). The complete viral genomes were assembled using SeqMan (DNASTAR, Madison, WI). All sequences were initially analyzed using the BLASTn 2.2.17 tool available at the National Center for Biotechnology Information (NCBI). Open reading frames (ORFs) were identified using the ORF Finder program available at the National Center for Biotechnology Information (NCBI). Complete sequences of BCMV isolates were aligned using ClustralX Ver. 2.0 (Conway Institute, UCD, Dublin). Further analysis was conducted with the Recombination Detection Program v.4.16 (RDP4). Genomic DNA (gDNA) was extracted from 0.1 g of young leaf tissue using the CTAB methodology as described in Naderpour et al. (1). For CAPS analysis, a fragment of 541 bp of eIF4E was amplified by PCR (1), using ENM-FWe and ENM-RVe primers (1). The 541-bp fragments amplified from the gDNA extracted from bean cultivars SGR and IVT 7214 were cloned into the T-Easy plasmid vector (Promega, Madison, WI). Three independent clones for each recombinant plasmid were selected and subjected to sequencing at the Genewiz laboratory (South Plainfield, NJ) from both ends of the cloned insert using plasmid-specific primers.

RESULTS

Using the primer pair ENM-FWe/RVe, PCR fragments of 541-bp were amplified from both susceptible genotype SGR and *bc-3* carrying genotype IVT7214. Digestion with *Rsa*I of PCR products derived from IVT7214 generated 381-bp and 160-bp fragments, as expected for the resistant, *bc-3* carrying genotypes. The PCR products derived from SGR were not cleaved by *Rsa*I, suggesting susceptible genotype. These 541-bp PCR fragments were cloned and sequenced from both genotypes. This partial *eIF4E* sequences from both genotypes (SGR/IVT7214) were aligned and one deletion and four codon differences affecting amino acid were found in this genomic area: del/Thr(32), Asn/Lys(126), Phe/Tyr(161), Ala/Glu(194), Asp/Gly(299). The nucleotide sequence change at 161 (T/A) introduced an *Rsa*I cleavage site into IVT7214. Except for the deletion in the SGR sequence at nt 32, all other changes were consistent with data published from previous study (1).

The 1755a genome was found to be 10,064-nt long, excluding the poly (A) and encoded a single polyprotein of 3,222 aa. The whole genomes for US1, and NL1 both from PG-I, together with 1755a (KT175570, PG-VIII), were aligned using CLUSTALX and further analysis was conducted with the RDP4 program package. Based on the RDP4 analysis, the 5'- terminal sequences of isolates US1 and NL1, between nt 1-2090, shared more similarities to each other (98% identity) than to 1755a isolate (84% identity). The sequences of the downstream segment, between nt 2091-6850 (position in alignment) in the NL1 and 1755a genomes were found to have close similarities to each other (96% identity), while US1 was found to have 89% identities to the NL1 and 1755a sequences in this area. The 3'-terminal genome segments between nt 6851-10076 (position in alignment), were found very similar among all three isolates (97-98% identity) (Fig.1).

CONCLUSIONS: We confirmed that IVT7214 has the CAPS marker for *bc-3*, and carries the mutated *eIF4E* allele associated with resistance to potyviruses. Our data also suggests that 1755a was a recombinant derived from NL1, US1 (both PG-I), and a yet unknown BCMV strain. The large 3'-proximal sections of the genome shared with these BCMV isolates indicates that P1/HC-Pro cistrons of BCMV strains may interact with most resistance genes.

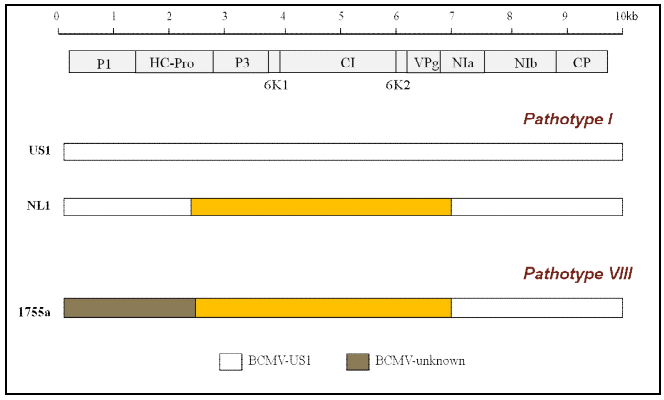

Fig. 1. Schematic representation recombination structure of 1755a, US1, and NY15P isolates of BCMV. Similar shading designates homologous regions in the genomes of three different pathotypes of BCMV.

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BREEDING COMMON BEAN FOR RESISTANCE TO WHITE MOLD

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INTRODUCTION

White mold is among the most severe diseases of common bean in North America and similar environments elsewhere. Under favorable cool and humid conditions 100% crop loss may occur on susceptible cultivars (Schwartz and Singh, 2013; Singh and Schwartz, 2010). Low levels of white mold avoidance/resistance occur in the Middle American common bean. However, higher levels of resistance occur in the Andean common bean and the highest levels in *P. coccineus*, *P. polyanthus*, and *P. costaricensis*. White mold resistance and avoidance traits are quantitatively inherited with low to moderate heritability, and controlled by >20 QTL. Also, major dominant and recessive resistance genes in common bean, and a single dominant gene in *P. vulgaris*/*P. coccineus* interspecific populations were reported (see review by Schwartz and Singh, 2013).

MATERIALS AND METHODS - BREEDING STRATEGY

A three-pronged breeding strategy comprising (1) introgression of resistance from distantly related germplasm, (2) combining together or pyramiding resistance from across *Phaseolus* species for high levels of broad-spectrum resistance, and (3) combining high levels of resistance with other desirable traits into high yielding high quality cultivars simultaneously was carried out between 2003 and 2015. Recurrent and congruity backcrossing for resistance introgresion, use of multiple-parent crosses for pyramiding resistance and cultivar development, and use of multiple *Sclerotinia sclerotiorum* isolates of different aggressiveness, multiple-inoculations/plant, multiple evaluations between 7 and 60 days post-the first inoculation, and verification of the resistance response at harvest was used. This was the severest greenhouse screening test ever applied in *Phaseolus* beans for white mold resistance breeding.

RESULTS - RESPONSE OF BREEDING LINES DEVELOPED

We introgressed white mold resistance from *P. coccineus* into interspecific breeding lines VCW 54, VCW 55, and VCP 13 (Singh et al., 2009a, b, 2014a) and from *P. costaricencis* into VRW 32 (Singh et al., 2009a, 2012). We developed pinto breeding line PRP 153 and Andean breeding lines PRA 152, PRA 154, and PRA 155 (Table 1, Singh et al., 2014a, b). To the best of our knowledge the five newly developed breeding lines (PRA 152, PRA 154, PRA 155, PRP 153, and VCP 13) posses the highest levels of broad-spectrum resistance to white mold developed to date. Also, we combined white mold resistance with resistance to other major diseases for common bean cultivars of four major market classes between 2003 and 2015.

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ACKNOWLEDGEMENTS

The authors gratefully acknowledge partial support by the USDA-ARS National Sclerotinia Initiative from 2003 to 2009, and much needed complementary support from the Idaho and Colorado Agricultural Experiment Stations. We also thank Drs. Phillip Miklas, James Steadman, and James Myers for generously supplying parental germplasm and/or *S. sclerotiorum* isolates.

Table 1. Mean white mold disease scores for 2015 national Bean White Mold Nursery, selected common bean checks, donor parents, and new Andean breeding lines PRA 152, PRA 154, and PRA 155 with pyramided resistance for four to seven *Sclerotinia sclerotiorum* isolates, evaluated at 60 days post the first-inoculation in the greenhouse at University of Idaho, Kimberly in the fall of 2015.

	***Sclerotinia sclerotiorum* isolates**						
Genotype	**ARS15T**	**ARS14D**	**ND710**	**NY133**	**CO467**	**ARS12D**	**ARS14M**
***2015 National Bean White Mold Nursery*†**							
Middle American							
031-A-11	7.7‡	7.3	7.8	7.3			
039-A-5	9.0	9.0	8.2	8.5			
ARS1003	8.7	8.7	6.5	8.3			
B12724	9.0	9.0	9.0	9.0			
Beryl	9.0	9.0	9.0	9.0			
ICA Bunsi	9.0	8.0	8.7	9.0			
Lighthouse	7.8	9.0	7.7	8.8			
Mist	8.8	9.0	8.2	8.3			
N13140	8.8	9.0	9.0	9.0			
P14815	8.7	9.0	7.7	9.0			
R12844	9.0	9.0	9.0	8.8			
R13752	9.0	9.0	8.2	9.0			
USPT-WM-12	7.2	8.2	8.5	8.3			
Andean							
A195	7.7	7.2	6.0	6.5			
G122	7.0	8.3	5.7	6.3			
VA19	7.0	7.3	6.0	5.3			
New common bean breeding lines with high levels of broad-spectrum white mold resistance							
Andean							
PRA152	3.0	3.0	3.0	3.0	3.0	3.0	3.0
PRA154	3.0	3.5	3.0	3.0	3.0	3.0	3.3
PRA155	3.0	3.2	3.0	3.0	3.0	3.0	3.8
Middle American							
PRP153	3.3	3.0	3.3	3.0	3.0	3.0	3.0
VCP13	3.0	3.3	3.0	3.2	3.2	3.0	3.7
Mean	7.1	7.2	6.7	6.9	3.3	3.0	3.1
LSD ($P \leq 0.05$)	1.1	1.3	1.4	1.2	0.8	0.9	0.6

†After inoculation with the first four *Sclerotinia sclerotiorum* isolates there were no live plants left to inoculate with other isolates.

‡WHITE MOLD DISEASE SEVERITY WAS SCORED ON A 1 TO 9 SCALE, WHERE 1 TO < 4 IS RESISTANT, 4 TO < 7 INTERMEDIATE, AND 7 TO 9 SUSCEPTIBLE

COLOR RETENTION IN CANNED BLACK BEANS

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INTRODUCTION

Canning quality is a priority to the U.S. dry bean industry since most beans are delivered to the market as a canned product. In black beans, color retention after processing is a major component of canning quality. The objective of this research was to assess genetic variability for color retention in a diverse set of black bean breeding lines and to test the ability of Vis/NIR reflectance spectroscopy on dry seeds to predict canned bean color retention.

MATERIALS AND METHODS

Visible and near-infrared (Vis/NIR) reflectance spectroscopy scans. A laboratory Vis/NIR spectrophotometer (Model 6500, Foss NIR Systems Inc., Silver Springs, MD, USA) was used to acquire reflectance spectra from intact dry beans in the range of 400 to 2,500 nm collected at increments of 2 nm, yielding each spectrum of 1,050 wavelengths (Mendoza et al., 2014). **Canning Quality:** Dry seed of black bean lines were scanned using the sensing methods described above. The lines included a RIL population of Shiny Crow x Black Magic (Cichy et al., 2014), 70 U.S. black bean breeding lines contributed by the public bean breeders, and a comparison of three varieties, Eclipse, Zorro, and Zenith across different planting dates and harvest aid treatments (Goffnett et al, under review). They were evaluated for canning quality according to the methods of Hosfield et al. (1984). Approximately one month after canning, visual appeal and color were evaluated by 18-20 trained panelists. **Model Development**: The analysis of Vis/NIR data was based on preprocessed reflectance spectra. The prediction performance of applying first derivative, second derivative or wavelet transform preprocessing methods were computed and compared. The best parameters were selected for predicting the quality traits based on multivariate regression and classification analysis. The correlation coefficients (R) and standard error of prediction (SEP) are presented.

RESULTS AND DISCUSSION

In a set of 70 black bean breeding lines from the U.S. public bean breeding programs, significant variability for canned bean color retention was observed. The average color score (as rated by a sensory panel) was 3.4, where 1 is very light brown and 5 is dark brown. The lowest score was 1.7 and the highest was 4.9. The ranges in colors and the sensory scores are shown in Fig 1. Using Vis/NIR spectroscopy it was possible to use dry seed to predict canned bean color scores at an R value of 0.87 when evaluating across 70 genotypes and at an R value of 0.97 when evaluating across three genotypes (Fig 2.). These findings show the need to evaluate color of canned black beans in the breeding program to assure acceptable color scores and the possibility of using VIS/NIR to predict canned color scores on dry seed.

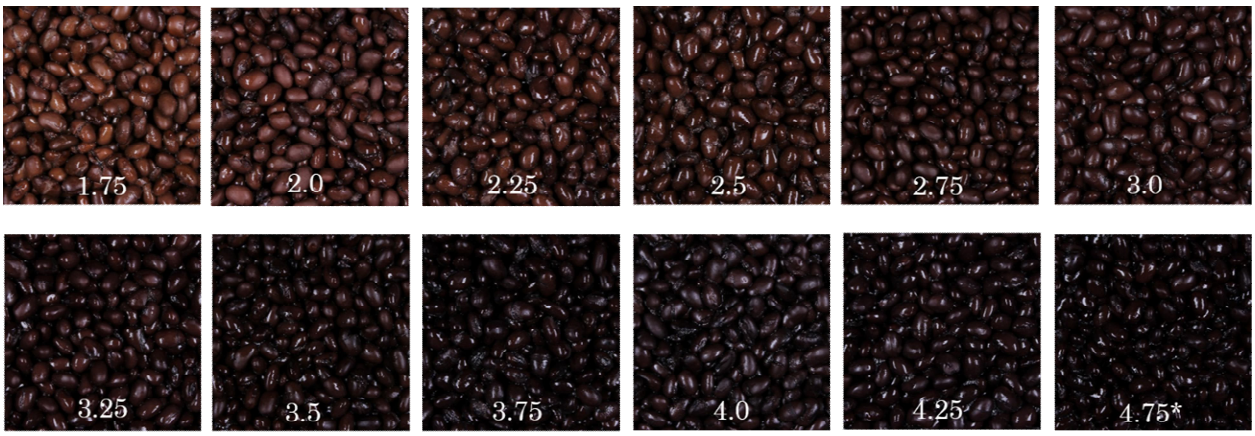

Figure 1: Phenotypic variability for canned bean color retention in U.S. black bean breeding lines and associated color score on a scale of 1 to 5 where 1 is very light brown and 5 is very dark. * indicates a line with a shiny seed coat.

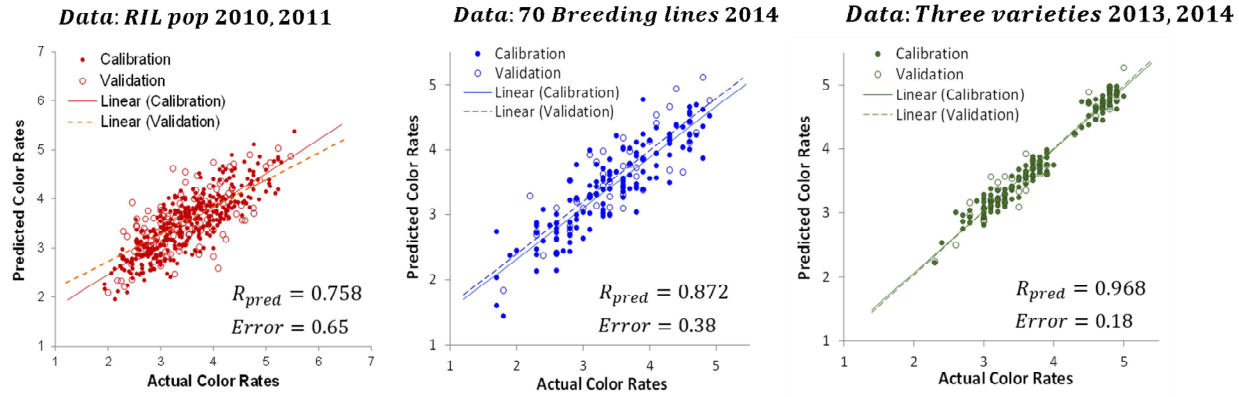

Figure 2: Color Score Prediction in Canned Black Beans via Vis/NIR Spectroscopy

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MAPPING THE NON-DARKENING TRAIT FROM WIT-ROOD IN A PINTO BEAN POPULATION USING SNP MARKERS

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INTRODUCTION

The seed coat darkens after harvest in some market classes of dry beans, including pinto bean. This process is known as postharvest darkening (PHD) or after darkening. Several factors have been associated with postharvest darkening in dry beans that including the environment, changes in chemical composition of the seed coat during seed development and after harvest, and genetics. In particular, higher levels of polyphenols, proanthocyanidins (PAs) and total flavonols (kaempferol derivatives), have been implicated in PHD. Despite their beneficial effects on plants and human health, polyphenols may be regarded as anti-nutritional compounds in colored beans. These compounds are known to strongly affect iron bioavailability and have negative effects on digestibility of proteins. Moreover, phenolic and polyphenolic compounds have been associated with the hard-to-cook (HTC) defect in different studies. As a result, darkened seed coats are discounted in the market place, since they are believed to be associated with lower nutritive quality, increased cooking time, and a decreased palatability. To obtain pinto bean varieties that retain their light background seed coat color, the ND trait was transferred from Wit-rood into the genetic background of pinto bean with the objective of developing high yielding ND varieties adapted for production in Ontario, Canada.

In spite of our knowledge about the genes involved in the expression of colors (*P, C, R, J, D, G, B, V,* and *Rk*) and patterns (*T, Z, J, Bip,* and *Ana*) in common bean, and information that brown or red-brown seed coat pigmentation may be caused by the accumulation of PAs and their oxidation products, little is known about the role of color genes in biochemical reactions of phenylpropanoid pathway in common bean. However, it is believed that a dominant allele at the *J* locus is responsible for PHD phenomenon, while the homozygous recessive *jj* is associated with the non-darkening phenotype (Elsadr et al. 2011). McClean et al. (2002) developed a STS marker, $OL4S_{500}$, that was associated with the *J* gene on linkage group 10. The purpose of the current study was to map the ND trait from the plant introduction Wit-rood, crossed into a pinto bean background.

MATERIALS AND METHODS

A mapping population consisting of 128 F_6 recombinant inbred lines (RILs) was developed from a cross between Wit-rood and 1533-15. Wit-rood is a plant introduction (PI439540) from the Netherlands with the appearance of a very pale cranberry bean. 1533-15 is a slow-darkening pinto bean line from the Crop Development Center (CDC), University of Saskatchewan. In order to obtain the segregation ratio for the trait, darkening trait was induced in $F_{2:3}$ seeds based on an accelerated darkening protocol (Junk-Knievel et al. 2007). Afterwards, two different methods for phenotyping were used to assign each genotype to its respective phenotypic group: (1) qualitative phenotyping which refers to visual screening of UV-irradiated seeds and placing each genotype into its respective phenotypic classes based on the rate and extent of formation of brown pigments in the seed coat and (2) quantitative phenotyping for which a colorimeter apparatus (Konica Minolta CM-3500D, Osaka, Japan) was used to quantitatively assess the variation among genotypes for seed coat pigmentation, before and after exposure to UV light.

Quantitative phenotyping increases the accuracy of phenotyping which lead to precise detection of QTL. Colorimeter readings were done for three seeds of each F_6 recombinant lines, before and after exposure to UV light thus 6 variables were obtained. In order to test the null hypothesis (H_0) that the observed phenotypic ratio follows the expected phenotypic ratio, a Pearson's chi-square test was conducted using *Proc Freq* in SAS 9.3 (SAS Institute, Cary, NC). Using principal component analysis (PCA), a biplot was generated to select a numerical variable which explains as much of the variation as possible within the colorimeter observations. A genetic map was constructed using JoinMap 4.0 and QTL analysis was done using MapQTL 6.

RESULTS

Three distinct phenotypes of darkening were identified: (1) regular darkening (RD) which darkens quickly with aging, (2) slow darkening (SD) which darken to a lesser extent than RD beans, and (3) Non-darkening (ND) which never darken during storage. A phenotypic ratio of 9RD: 3SD: 4ND was obtained for the trait indicating a recessive epistasis between *j* and *Sd* loci.

Among 6 variables measured with a colorimeter, the a* value after exposure of the seeds to UV light was selected to be included in QTL analysis. A major QTL underlying the ND trait was mapped to a region between 36.8 cM and 41.57 cM on chromosome 10. The SNP marker ss715647913 was tightly linked to the QTL with the LOD score 25.56 which explained up to 50.5% of phenotypic variance. Within this region 27 candidate genes were identified which will be examined for their possible effects on postharvest darkening phenomenon.

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DISSECTING PHENYLPROPANOID PATHWAY IN COMMON BEAN (*PHASEOLUS VULGARIS*) BY RNA-SEQ BASED TRANSCRIPTOME PROFILING

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INTRODUCTION

Besides having high levels of nutritional components, common bean (*Phaseolus vulgaris*) also contains a substantial amounts of antioxidants, present in polyphenolic compounds that are synthesized via the phenylpropanoid pathway (PP). To date, no study has been done to profile the expression of PP genes in common bean market classes.

MATERIALS AND METHODS

An RNA-Seq analysis was performed from immature seed samples (1-3 mm) of 19 different common bean classes of different seed colour, shape and germplasm sources using Illumina deep sequencing.

RESULTS AND DISCUSSION

A total of 27726 genes, 29665 CDS, 37287 TSS, and 73235 isoforms were identified. A dendrogram developed from relative expression of 7000 differentially expressed genes showed two distinct groups, one clearly contains only Andean landraces (SVMTH, Montcalm, OAC 03-D1, Litekid, OAC 04-L1, Prim, PI 432687, Pompadour 1014) and the other one contains mostly Mesoamericans (OAC Rex, AC Compass, Ica Pijao, Othello, PI 207210, 9438-140, Bat 93, Vax 4, LHX 3073) along with two Andean landraces (Jalo EEP558, XAN 159) (Fig 1).

The levels of transcripts between low and high antioxidant rich common bean classes identified ~ 260 differentially expressed PP-related genes and also their transcription factors (TFs). Among them, 51 were known PP genes and their TFs in the core phenylpropanoid pathway, and 20 were known genes were from other PP-linked pathways, such as genes in the shikimate pathway, carbohydrate synthesis and fat metabolism.

Expression correlations were observed between the target genes and their transcription factors. For example, MYBL-2 and TT2-2 were found to be the possible transcription factors for almost all the target genes, such as: C4H, F3H, PAL-1, ANR, PvDFR-1, PvDFR-2, 4CL, CHS-1, PAL-2, 4CL-3, F3'H. RNA-Seq data was validated by qPCR.

These findings may facilitate the identification of candidate genes involved in the synthesis of beneficial phenolic compounds in common bean and facilitate breeding lines with higher levels of antioxidants.

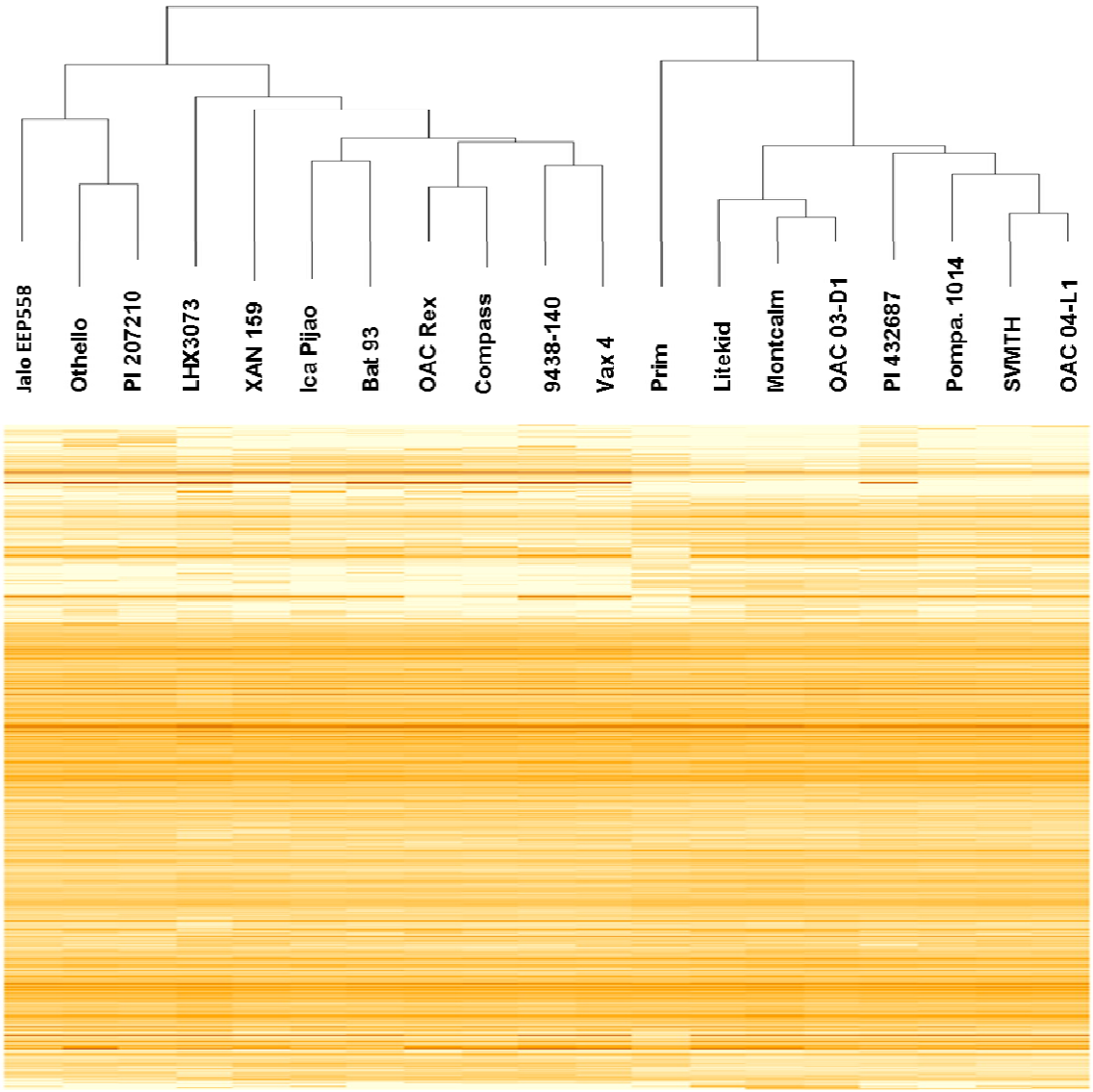

Fig 1. A heat map of ~7000 differentially expressed genes (DEG) in 19 different common beans.

UNDERSTANDING AND IMPROVING FLAVOR IN SNAP BEANS: SCREENING THE USDA *PHASEOLUS* CORE COLLECTION FOR POD SUGAR AND FLAVOR COMPOUNDS

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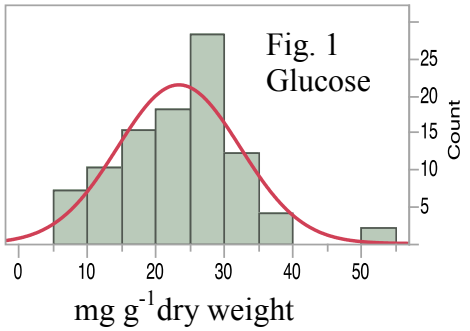
The objective of our research is to gain knowledge regarding variation in sugar and flavor content among a sample of dry bean and green pod-type accessions from the USDA *Phaseolus* Germplasm Core Collection, Pullman, WA. Knowledge of the variation will allow better utilization of germplasm resources in the development of new bean cultivars with more desirable sugar and flavor profiles. The results of this project could be used to market product quality and offer unique opportunities to expand market share to an increasingly health conscious population.

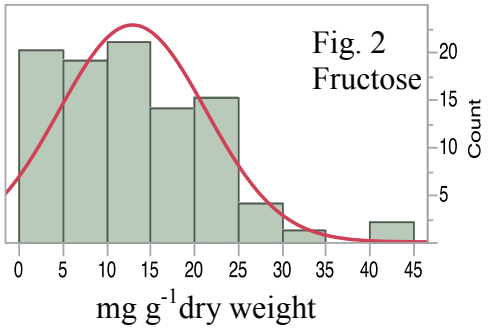
We developed a diverse sub-core of 94 Plant Introductions (PI) characterized as snap beans, Romano-types, and other beans eaten as edible immature pods, and 20 dry bean PI accessions. In addition checks included a kidney bean (Montcalm, Andean gene pool) as well as 8 cultivars (e.g. Caprice, Huntington, 04-88, OSU5402, OSU5630, Masai, Slenderpack, Tapia) representing the various market classes consumed as edible green pods currently grown commercially in the United States.

Accessions were grown in replicated trials at the West Madison Agriculture Experiment Station, Madison, WI and five pods sampled from each plot when the pods were sieve size 4 (8.33-9.52 mm) 90^{o} off the suture.

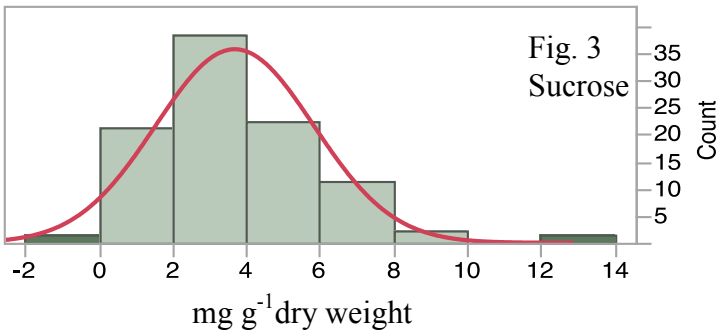
A large positive correlation (r=0.79**) was observed between the simple sugars glucose and fructose. In contrast a large negative correlation was observed between the disaccharide sucrose with both mono-saccharides, glucose (r=-0.37) and fructose (r=-0.43). Glucose concentration had a mean of 19.96 mg g^{-1} dry weight, and ranged from near zero to over 40mg g^{-1} dry weight (Fig. 1) P.I accessions with high concentrations of sucrose were generally heirloom and modern commercial snap beans cultivars, e.g. Provider, Eagle, Cascade, Hystyle and BBL47. Fructose concentration had a mean of 19.9 mg g^{-1} dry weight, and ranged from near zero to over 50mg g^{-1} dry weight (Fig. 2).

Distribution of Glucose (Fig. 1) and Fructose (Fig. 2) mg g^{-1} dry weight.





Sucrose had a much lower mean concentration of 3.7 mg g^{-1} dry weight, and ranged from near zero to over 14 mg g^{-1} dry weight (Fig 3).



The range and concentrations of mono- and disaccharide sugars is consistent with those previously observed by Vandenlangenberg et. al. (2012 a and b) in a recombinant inbred line population derived from a cross between a black dry bean cultivar Puebla 152 and the white seeded commercial snap bean cultivar, Eagle. The magnitude of variation for sugar concentrations among both accessions from the core collection and the high heritabilities previously identified the RIL population by Vandenlangenberg et. al. (2012 a and b) suggests that selection should be effective in developing genotypes with high or low levels of mono- and disaccharide sugars. The negative correlations between mono- and disaccharide sugars may preclude high levels of all.

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BREEDING FOR A FAST COOKING BEAN: A STUDY OF GENOTYPES ACROSS ENVIRONMENTS TO DETERMINE STABILITY OF THE COOKING TIME TRAIT IN *PHASEOLUS VULGARIS*

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INTRODUCTION

Dry beans (*Phaseolus vulgaris*) are an inexpensive and nutritious food containing high amounts of protein, fiber, vitamins, and minerals [2]. Beans are consumed in many parts of the world including the US, South America, and East Africa; however, beans require a long time to cook. In addition, countries in Africa primarily use firewood as fuel for cooking.

Harvesting and burning of firewood on a weekly basis not only increases pollutants such as CO, CO_2, and particulates, but also results in local deforestation that typically would have a mitigating effect on pollution. As women and children are often responsible for cooking in African countries (spending an average of 25.6 hrs/wk on this task alone), they tend to have a higher exposure to these pollutants and, thus, more likely to have health issues [4]. Development of faster cooking beans would address these issues. This study compared two genotypes of brown dry bean in different environments to assess effects on cooking time. Previous research has explored environmental effects, but a dearth of research exists on a genetic component to cooking time differences. This work represents the beginning of a larger project that will ultimately elucidate areas of the genome associated with this trait.

MATERIALS AND METHODS

Previous experiments revealed significant variation within the Andean Diversity Panel [1]. Two of these genotypes (TZ-27 [Incomparable] and TZ-37 [W616488]), from Tanzania, had contrasting cooking times (slow and fast, respectively). These genotypes were grown in 8 different locations, harvested, and transported to Michigan State University.

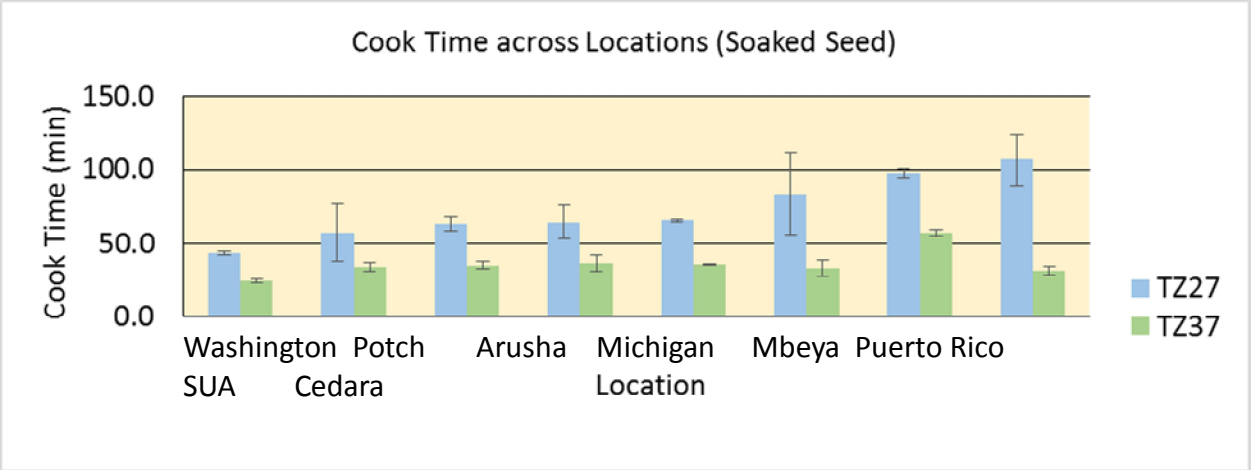
A sample of 30 seeds for each treatment was weighed before and after soaking (if applicable) as well as after cooking. Seeds were soaked in deionized water for 12 hours at room temperature. Cook time was measured on 25 seeds with a pin drop cooker in boiling deionized water [3]. Cook time was recorded when 80% of the pins pierced through the beans. Cooking time of each sample was measured on soaked and/or unsoaked seed.

RESULTS AND DISCUSSION

Soaking significantly lowered cook time in both genotypes compared to not soaking prior to cooking. Though cooking time varied across different environments, two trends were observed. When soaked, TZ-37 always cooked faster than TZ-27. The other trend was that the percent difference in cooking time between TZ-27 and TZ-37 was remarkably stable across nearly every tested environment. When soaked, a 45% cook time difference in TZ-37 compared to TZ-27 was observed, but when unsoaked, this difference dropped to 10%. This is consistent with the hypothesis that in addition to environmental conditions, there is a genetic component influencing cooking time in dry beans. When unsoaked, cooking times, generally, were similar across

genotypes and locations. These results suggest that some physiological change occurs during the soaking process resulting in contrasting cooking times between TZ-27 and TZ-37.

a.



b.

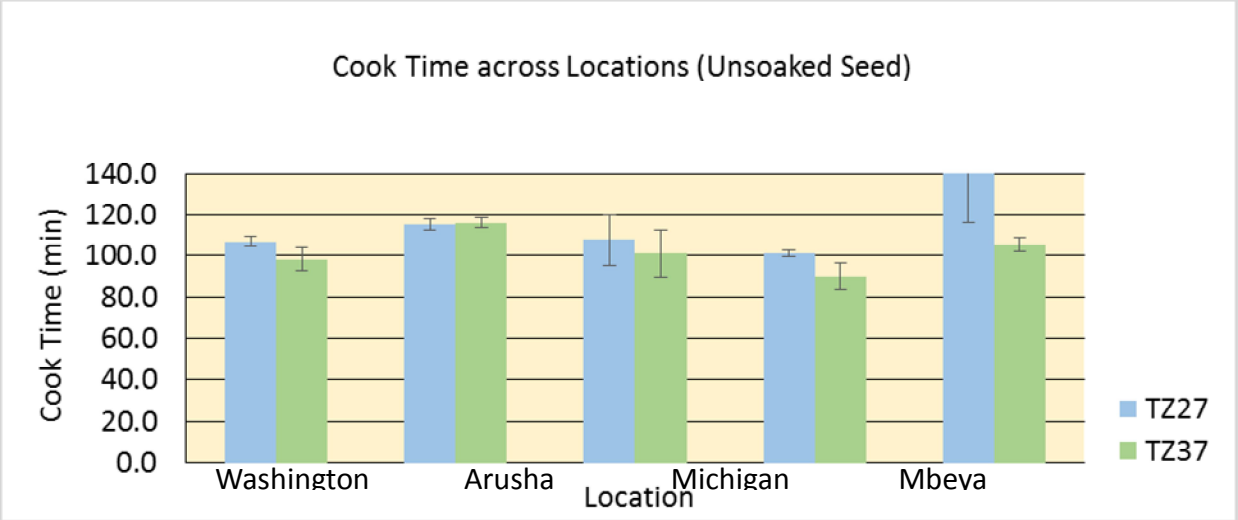


Figure 1. Comparisons of cooking time between TZ-27 and TZ-37 across multiple environments. Soaked seed of TZ-27 was compared to soaked seed of TZ-37 across 8 different locations [SUA – Sokoine University of Agriculture] (a). Unsoaked seed of both TZ-27 and TZ-37 was compared across 5 locations (b). Error bars were generated using two replicates from each site performed in duplicate.

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POSTERS

PHYSIOLOGICAL RESPONSES OF TWO DROUGHT-TOLERANT COMMON BEAN CULTIVARS

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INTRODUCTION

Brazil is the main global producer and consumer of common beans (*Phaseolus vulgaris* L.). Currently, the country produces around 3.5 million tons with average productivity slightly more than 1,000 kg ha^{-1}, but the yield potential of the crop could be higher if the occurrence of adverse environmental conditions was minimized during cultivation. Drought is the main cause of yield reduction, since 60% of common bean crops are in drought prone areas (FAO, 2009). The intensity of the damage caused by water restriction depends on the crop development stage and on the severity of drought period. The major reductions occur during the reproductive phase, with losses that could reach 92% (Acosta-Díaz, 2009). Currently, the selection for drought tolerance in common beans has been carried out through assessment of the characteristics associated with yield, but it requires a long process, what limits the improvement on breeding efficiency. However, despite of the efforts to find secondary characteristics (related to plant physiology) associated with drought tolerance, few are validated and can be used in tolerant cultivar selection. The aim of this study was characterizing the physiological responses of two drought-tolerant common beans during the reproductive period.

MATERIAL AND METHODS

The experiment was conducted from June to September of 2014 under controlled greenhouse conditions, located in the experimental area of the Agronomic Institute of Paraná State - IAPAR, Londrina - PR, Brazil, using BAT 477 and IAPAR 81 drought tolerant cultivars (Beebe, et al., 2013; Moda-Cirino, et al., 2001). The experimental design was randomized blocks, with split-split plots and six replications. The plots were consisted by with and without water deficit, the sub plots by the cultivars and sub-sub plots by four water deficit periods (0, 4, 8 and 12 days), starting when the plants were in R5 phenological stage and ending up at the R8 stage. Mock plants were grown under 80% of pot capacity, while the plants under water deficit were cultivated under 30% of pot capacity. During water deficit periods rate of photosynthesis, transpiration, stomatal conductance, internal concentration of CO_2 and leaf temperature were assessed, using the infrared gas analyzer (IRGA) instrument. In addiction, instantaneous carboxylation efficiency and water use efficiency were also estimated. Data were subject to Scott-Knottt test ($p<0,05$) for classes grouping.

RESULTS AND DISCUSSION

In the early drought periods (4 days), the cultivars showed low reduction rates in all physiological variables evaluated. IAPAR 81 showed tolerance until eighth day of drought (Figure 1B and F), presenting drought tolerance mechanisms more efficient than observed for BAT 477 in all evaluated variables (Figure 1). Probably the tolerance is caused by the absence of sharp drop in the amount of internal carbon in the early days of drought (Figure 1). It indicates that in this cultivar, Rubisco is maximizing the efficiency of CO_2 usage and hence reducing the

drop rate of photosynthesis. BAT 477 showed the best mechanisms for maintaining photosynthesis rate when subjected to 30% of pot capacity after 12 days of drought (Figure 1A). This water regime is not a limiting factor for maintenance of photosynthesis rate in BAT 477 cultivar. IAPAR 81 presented constant leaf relative water content (RWC), showing no differences between stressed and irrigated plants (Figure 1J). It could be an osmotic adjustment mechanism, since plants can maintain high WRC values, associated with low water availability in the soil. Based on the results it is possible to conclude that both cultivars presented distinct strategies for drought-tolerance: BAT477 presented higher gas exchange homeostasis, while IAPAR81 has better RUBISCO usage and leaves turgidity constancy.

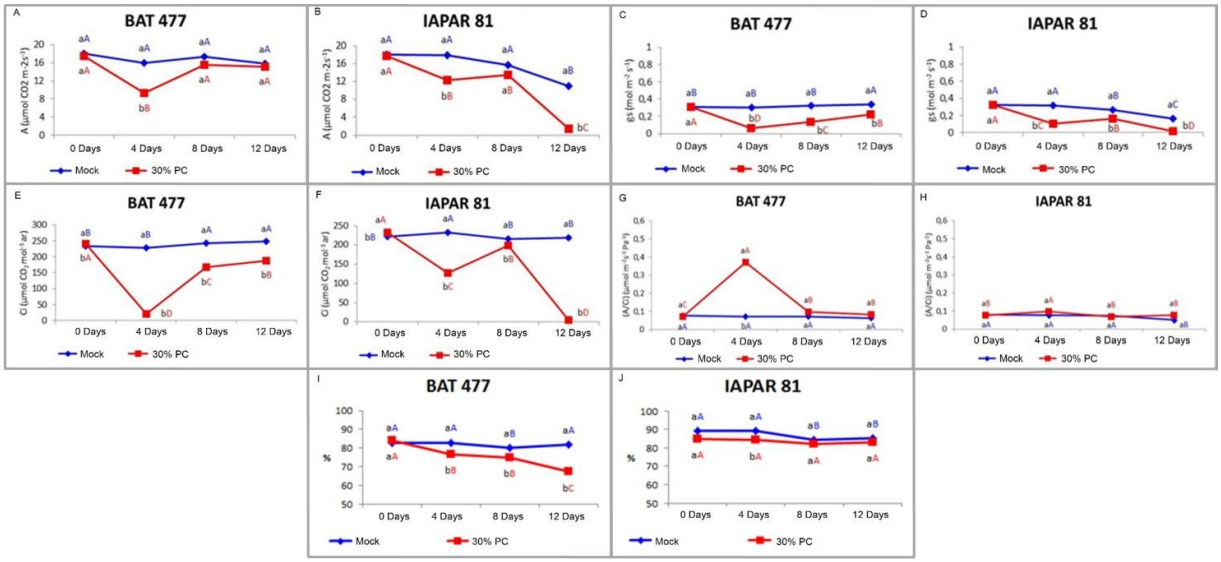

Figure 1. Monitoring of gas exchange and leaf relative water content of two common bean cultivars submitted for 12 days to two water regimes during the reproductive stage: without deficit (mock: 80% of pot capacity) and with deficit (30% of pot capacity). **A and B.** Photosynthesis rate of BAT 477 and IAPAR 81 cultivars. **C and D** Stomatal conductance of BAT 477 and IAPAR 81 cultivars. **E and F.** Internal carbon content of BAT 477 and IAPAR 81 cultivars. **G and H** Internal efficiency of carboxylation of BAT 477 and IAPAR 81 cultivars. **I and J.** Leaf relative water content of BAT 477 and IAPAR 81 cultivars. Lower case letters represent average groupings by Scott-Knottt test ($p<0,05$) among water regimes in the same evaluation period. Capital letters represent the average groupings among means in the same water regime in different evaluation period.

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MINERAL DISTRIBUTION IN THE SEED COAT, COTYLEDONS AND EMBRYONIC AXIS IN COMMON BEAN CULTIVARS FROM DIFFERENT CENTERS OF ORIGIN

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INTRODUCTION

Information about the genetic variability in the distribution and accumulation of minerals in the different structures of common bean seed is important, since there is evidence that the embryo and seed coat have different bioavailability of some nutrients essential to the human diet. The objective of this study was to evaluate the genetic variability for the accumulation of the minerals K, P, Ca, Mg, Cu, Zn, B, Mn, Fe and S in the seed coat, cotyledons and embryonic axis, in the seeds of common bean cultivars, belonging to different commercial groups from Andean and Mesoamerican origin.

MATERIAL AND METHODS

Ten common bean cultivars were studied (BRS Radiante, IPR Garça, Red Hawk, Jalo Precoce, Hooter, IPR Gralha, IPR Siriri, IPR Juriti, IAPAR 31, IPR Uirapuru). The seeds analyzed were obtained from field experiments carried out in rainy season of 2007/2008, in Londrina Paraná State (lat 23° 22', long 51° 10', 585 m asl). The experimental design used was randomized blocks with four replications. The control of pests, diseases and weeds were made according to technical recommendation for culture. After harvest, the seeds were collected and stored in cold chamber (5,6ºC and humidity abouth 33%). The mineral levels (K, P, Ca, Mg, Cu, Zn, B, Mn, Fe and S) were determined in the different fractions of the seed (cotyledons, embryonic axis and seed coat). In the laboratory, the seeds were washed with tap water and then in distilled water. Then, 1,000 seeds were placed in a becker with a liter of distilled water. After an hour maceration, the tegument, embryonic axis and cotyledon were separated. The tissues was dried at 60ºC during 48 hours. The tissues were then ground and homogenized in a Wiley mil, and 0.4 g samples were removed and subjected to nitropercloric digestion with 3:1 ratio HNO_3: $HClO_4$ solution (Miyazawa et al., 1999). The mineral content was determined with na atomic emission spectrophotometer (Thermo Jarrell Ash ICAP 61E). The data were subjected to analysis of variance (ANOVA) by applying the F test at a 5% and 1% probability level.

RESULTS AND DISCUSSION

The cultivars showed genetic variability for the levels of K, Ca, Mg, Cu, Mn and Fe. The seed fractions (seed coat, embryonic axis and cotyledon) showed different mineral compositions. The seed coat represents 8.5% of the total dry weight of the seed, the cotyledon 90.3% and the embryonic axis 1.2%. These values were similar to the ones reported by Ariza-Nieto et al., (2007). The seed coat is variable and depends on the type of beans. In this study it was found that the seed coat represented 7.7% (IAPAR 31) to 10.4% (Hooter) of the total dry matter of the seeds. The proportion of minerals in the different fractions of the seed must be taken into consideration in the nutritional value of processed grains. If during grain cooking the seed coat is removed, the mineral nutrients that are present in greater proportion in this fraction can be lost. The minerals show differentiated accumulation in the fractions of the seed. Higher percentage of

the minerals K, P, Mg, Cu, Zn, B, Mn and Fe were found in the cotyledon, except for the mineral Ca that is present in greater proportion in the seed coat (Figure 1).

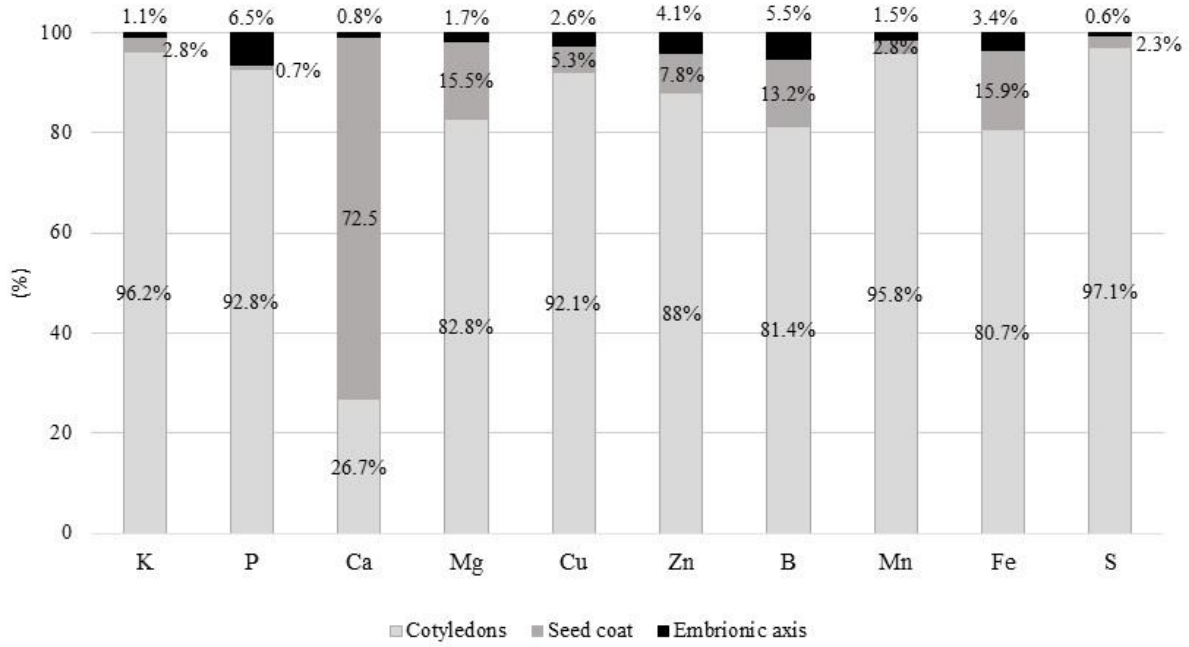

Figure 1. Percentage average of ten minerals in the different fractions of the seed (cotyledons, embryonic axis and seed coat) evaluated in seed samples of ten common beans cultivars. Londrina - PR, rainy season of 2007/2008.

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AN EFICIENT CULTURE MEDIUM FOR INCREASING SPORULATION OF *PSEUDOCERCOSPORA GRISEOLA*

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INTRODUCTION

Angular leaf spot (ALS), incited by *Pseudocercospora griseola* (Sacc.) Crous & U. Braun, is one of the most widespread diseases of common bean (*Phaseolus vulgaris* L.). ALS cause significant loss of common bean yield in tropical and subtropical regions, mostly in areas with moderate temperatures and high humidity (Pastor-Corrales et al. 1998). The fungus *P. griseola* grows slowly in artificial culture medium (Correa-Victoria et al. 1989), and obtaining satisfactory amount of inoculum still one of the major challenge related to the management of this fungus in laboratory. Therefore, the objective of this work is introduce a new culture medium based on green bean for increasing sporulation of *P. griseola*.

MATERIAL AND METHODS

This work was developed at the Núcleo de Pesquisa Aplicada à Agricultura (Nupagri) of the Universidade Estadual de Maringá (UEM), Paraná state, Brazil. The new culture medium protocol consisted of: 200 g green bean, 17 g agar, 3 g $CaCo_3$ and distilled water to complete 1000 mL. An experiment was carried out to test the efficiency of the new medium for increasing sporulation of *P. griseola.* Two different media were used: the new green bean medium and the V8 medium (200 mL V8 juice, 17 g agar, 3 g $CaCo_3$ and distilled water to complete 1000 mL). Evaluation of the number of spores was performed at 12, 15, 19 and 21 days after seeding the spores of *P. griseola* on both media, following the methodology described by Sanglard et al. (2009): a mycelium fragment of 0.5 cm in diameter of the *P. griseola* isolate PG002 was transferred to a test tube containing 1 mL of sterile water. The mycelium was macerated with a sterile wooden stick and the suspension was transferred to Petri dishes containing fresh medium. The plates were kept in BOD in absence of light at 25°C until the evaluation. The experiment was conducted in a completely randomized design with eight treatments arranged in a 2×4 factorial, corresponding of two media (green bean medium and V8 medium) evaluated at 12, 15, 19 and 21 days after seeding the spores on the media, with four replications. Each replication was constituted of one petri dish containing the corresponding medium. The number of spores was determined by adding 10 mL of sterile water to each plate surface and the conidia and mycelium fragments were scrapped with a sterile spatula. The suspension was filtered through gauze and the spore concentration was determined through the average of spores in 16 independent measures on the corner squares of hemocytometer. Analysis of variance for a completely randomized design was performed to test the significance of medium, time and medium × time interaction effects, and the effect of time was analyzed by regression.

RESULTS AND DISCUSSION

This is the first attempt and success on cultivating angular leaf spot fungus in artificial medium made of green bean, and results showed that the medium is quite efficient in increasing the number of spores. According to the analysis of variance for number of spores of *P. griseola*, there is no interaction between the two tested media and days after plate inoculation (Table 1). However, significant difference on amount of spores between the green bean and V8 media was

observed. The average of number of spores of *P. griseola* when the fungus was grown on green pod medium was 28.0 spores, while the number of spores of the fungus was grown on V8 medium was 22.4 spores. The data demonstrate that the culture medium based on green bean produced higher amount of spores of the ALS pathogen than the traditional V8 medium, the mostly used medium to induce sporulation of this pathogen.

Table 1. Analysis of variance for number of spores of *P. griseola* obtained from green bean and V8 media at 12, 15, 19 and 21 days after inoculating the plates

Source	DF	Sum of Square	Mean Square	F Value	Pr > F
Medium	1	668.41	668.41	10.89	0.003
Time	3	3883.13	1294.37	21.08	<.0001
Medium × Time	3	480.66	160.22	2.61	0.0748
Error	24	1473.48	61.39		
Total	31	6505.68			

The number of spores evaluated at 12, 15, 19 and 21 days after plate inoculation adjust to a cubic function (Figure 1). The higher sporulation of *P. griseola* for both green bean and V8 media occurred at 12 to 15 days, with severe reduction of the number of spores at 19 and 21 days. As reported, *P. griseola* has low growth rate *in vitro*, what might be a genetic characteristic of the fungus (Stenglein et al. 2006). However, a significant increment on the spores production was observed when the fungus were grown on green bean medium. Additionally, we observed that spores grown on green bean culture medium showed better formation and bigger size compare to the spores grown on V8 medium. This new protocol for increasing sporulation of ALS pathogen has been efficiently used in Nupagri's laboratory to obtain high quality inoculum and improve ALS evaluation on common bean plants.

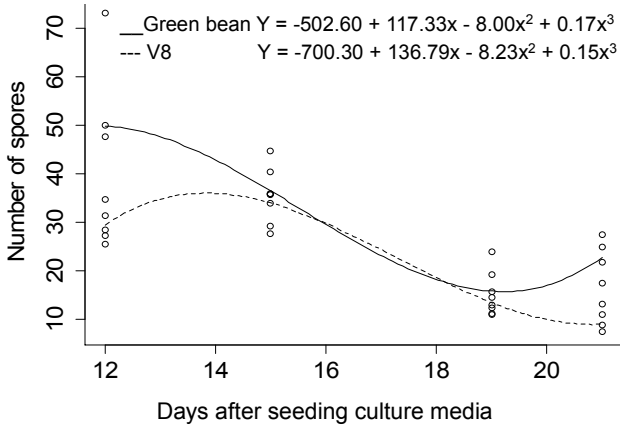

Figure 1. Regression analysis of number of spores of *P. griseola* obtained from green bean and V8 media at 12, 15, 19 and 21 days after seeding the spores of the pathogen on the medium

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VIRULENCE AND GENETIC DIVERSITY OF *PSEUDOCERCOSPORA GRISEOLA* ISOLATES FROM PARANÁ STATE, BRAZIL

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INTRODUCTION

The angular leaf spot, caused by the pathogenic fungus *Pseudocercospora griseola,* is one of the most widespread diseases in common bean (*Phaseolus vulgaris* L.) producing areas. The use of resistant cultivars is indicated as an effective strategy to control the ALS pathogen (Miklas et al. 2006). Various resistance genes, named *Phg*-, conferring race specific resistance to different races of *P. griseola* have been identified. However, the strength of the resistance is complex, and a pathogen such *P. griseola* shows high diversity in its virulence (Pastor-Corrales and Tu 1989). In this work, the differential cultivars of angular leaf spot were used to characterize *P. griseola* isolates from infected common bean plants from Ponta Grossa, Paraná state, Brazil. In addition, these isolates were characterized through sequencing of the internal transcribed spacer (ITS) region.

MATERIAL AND METHODS

This work was conducted at the Núcleo de Pesquisa Aplicada a Agricultura (Nupagri) at the Universidade Estadual de Maringá (UEM), Paraná state, Brazil. Leaves with angular leaf spot symptoms from common bean infected plants were collected in Ponta Grossa, located in the center of Paraná state. Monosporic cultures of isolates of the fungus *P. griseola* were performed as described by Sanglard et al. (2009). A total of five isolates were obtained and the cultures were replicated in Petri dishes containing green bean medium (200 g of green bean, 17 g of agar, 3 g of $CaCo_3$ and distilled water to complete 1000 mL) and kept in BOD in absence of light at 25°C for 12 days. The race identification was performed using the set of 12 ALS differential cultivars proposed by Pastor-Corrales and Jara (1995). Ten seeds of each differential cultivar were sown in trays containing soil in trays and kept in greenhouse condition for 17 days. The first trifoliolate leaf of each plant was inoculated with a spore suspension adjusted to 2×10^4 spores.mL^{-1} and transferred to a chamber for 72 h at 22°C and humidity >95%. The evaluation of the symptoms was performed according the scale proposed by Inglis et al. (1988). The genomic DNA extraction was performed from the mycelial mass previously stored at -20ºC, using a CTAB protocol. The ITS1-5.8S-ITS2 regions of the rDNA were amplified by PCR using the primers ITS1F (5' CTTGGTCATTTAGAGGAAGTAA 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3') and the PCR products were visualized in a 0.8% agarose gel. The PCR product was purified using the PureLink PCR Purification Kit (Invitrogen) and the sample were sequenced at the Centro de Estudos do Genoma Humano e Células-Tronco CEHG-CEL of the Universidade de São Paulo (USP), São Paulo state, Brazil. The analysis of the DNA sequences and construction of the phylogenetic tree were performed using BioEdit 7.2.5 and MEGA 5.2.2 software. For the phylogenetic tree construction, nine sequences of the ITS1 and ITS2 regions of *Pseudocercospora* spp. from the GenBank database were selected, based on their similarity to the isolates from Paraná state (Figure 1).

RESULTS AND DISCUSSION

All *P. griseola* isolates using the ALS differential cultivars allowed the identification only the race 63-63. This is the first report of the occurrence of 63-63 race of *P. griseola* in Paraná state. Race 63-63 showed high virulence, overcoming the resistance of all differential cultivars. This race is broadly distributed through the Brazilian producing areas and its presence was confirmed in Santa Catarina, Minas Gerais and Goiás states (Nietsche et al. 2001, Sartorato 2002). Phenotypic and molecular data on pathogen variability have supported the separation of *P. griseola* into two major groups: Andean (*P. griseola f. griseola*) and Mesoamerican (*P. griseola f. mesoamericana*) (Guzmán et al., 1999). Therefore, the compatibility of race 63-63 to the Andean and Mesoamerican cultivars Don Timóteo, G 11796, Bolón Bayo, Montcalm, Amendoin, G 5686, PAN 72, G 2858, Flor de Mayo, Mexico 54, BAT 332, Cornell 49-242, revealed a tendency of the isolates belong to the Mesoamerican gene *pool*. The phylogenetic tree based on the DNA sequences of the ITS-rDNA regions evidenced the presence of two major groups (Figure 1). A group of isolates recovered from species *P. vitis, P. eucalyptorum* and *P. paraguayensis*, and a group including the *P. griseola* isolates. The five isolates from Ponta Grossa (PG001-PG005) show high similarity to the *P. griseola* f. *mesoamericana* isolates retrieved from the GenBank database, confirming the Mesoamerican origin of the isolates collected in Paraná. On the other hand, the Mesoamerican group was clearly divergent from the Andean isolates previously described in the literature.

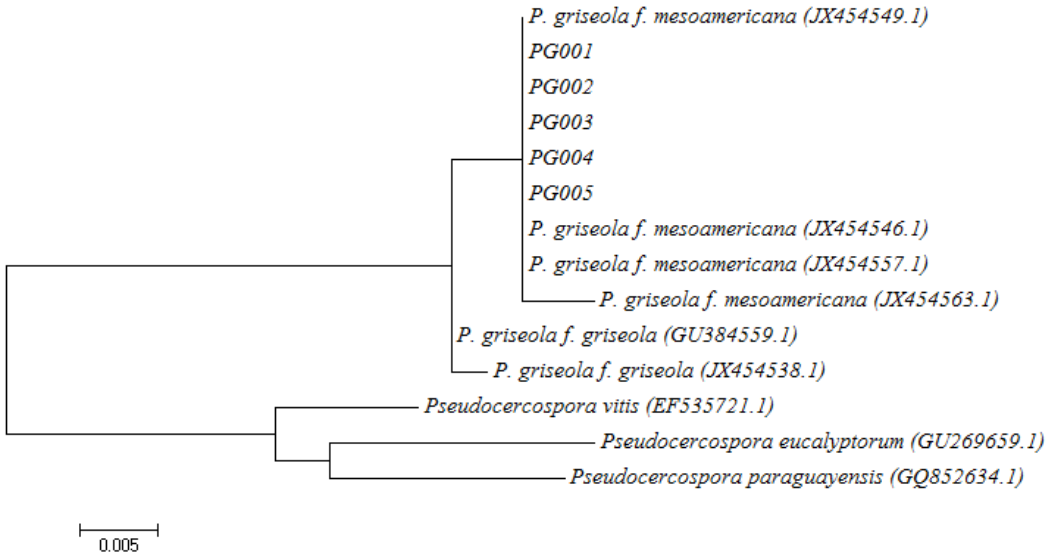

Figure 1. Phylogenetic tree of 14 isolates of *Pseudocercospora spp.*, based on DNA sequences of ITS1-5.8S-ITS2 genomic region.

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A PROTEIN BINDING IS INVOLVED IN THE RESPONSE TO POWDERY MILDEW IN THE COMMON BEAN CULTIVAR PORRILLO SINTETICO

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INTRODUCTION

Powdery mildew (PM) is a widespread plant disease caused by Ascomycete fungi, including a broad range of genera and species within order *Eryshiphales*. Geographic distribution of this disease is increasing rapidly in different parts of the world. This expansion has been related to global climate change (Glawe 2008). Qualitative resistance have been described in common bean, being indirectly located two genes on linkage groups (LG) Pv04 and Pv11 (Pérez-Vega et al. 2013). The objective of this study was to identify the gene/es conferring total resistance response against PM in the cultivar Porrillo Sintetico, combining genetic, genomic and transcriptomic analyses.

MATERIAL AND METHODS

A population of 160 $F_{2:3}$ families obtained from the cross X2776 x Porrillo Sintetico was used. X2776 is a breeding line developed in SERIDA. Porrillo Sintetico is a resistance source against PM. A local isolate of PM, maintained on plants of susceptible bean cv. Xana in spore-proof chambers, was used. Resistance tests were carried out according to Trabanco et al. (2012). Plant responses were recorded as infection type (IT) following a 0-4 scale, where IT0 is no visible symptoms, and IT4 abundant mycelial development on leaves and profuse sporulation (Trabanco et al. 2012). Mapmaker V2.0 software was used for linkage analyses (Lander et al. 1987). Relative gene expression was estimated using quantitative reverse-transcription polymerase chain reaction technique and the $2^{-\Delta\Delta Ct}$ method (Livak et al. 2001).

RESULTS AND DISCUSSION

Line X2776 showed an intermediate resistance response against PM (IT3), and Porrillo Sintetico showed a total resistant response (IT0). Observed segregation of PM resistance in the $F_{2:3}$ population was: 37 families with IT0 response, 69 families having seedlings with IT3 and IT0 response, and 44 families with IT3 response. This ratio fitted the expected for one gene ($\chi^2_{1:2:1}$= 1.61; p= 0.45). The gene conferring IT0 response was mapped on LG Pv04 (Fig. 1A), flanked between INDEL markers IND4_00798 (0.4 cM) and IND4_02324 (1.8 cM), whose physical positions on chromosome 4 are 79.827 bp and 232.391 bp, respectively. Microsatellite markers were developed within this region on the basis of the common bean sequenced genotype G19833 (www.phytozome.net). Fig. 1B shows in detail the linkage map obtained for this region. A co-segregation was observed between the PM resistance gene and SSR markers Chr4_SSR4, Chr4_SSR8, Chr4_SSR10, and Chr4_SSR15. The PM resistance gene was flanked by markers Chr4_SSR4 and Chr4_SSR25, with a physical position of 84.202 bp and 218.664 bp, respectively.

In the reference genome G19833, seven predicted genes and a gap of 68.873 missing values, have been reported in this chromosome region (Fig.1C). Considering their functional annotations, three genes were discarded since no functional annotations for them had been described. The remaining four genes are candidate to be involved in the resistance response against PM.

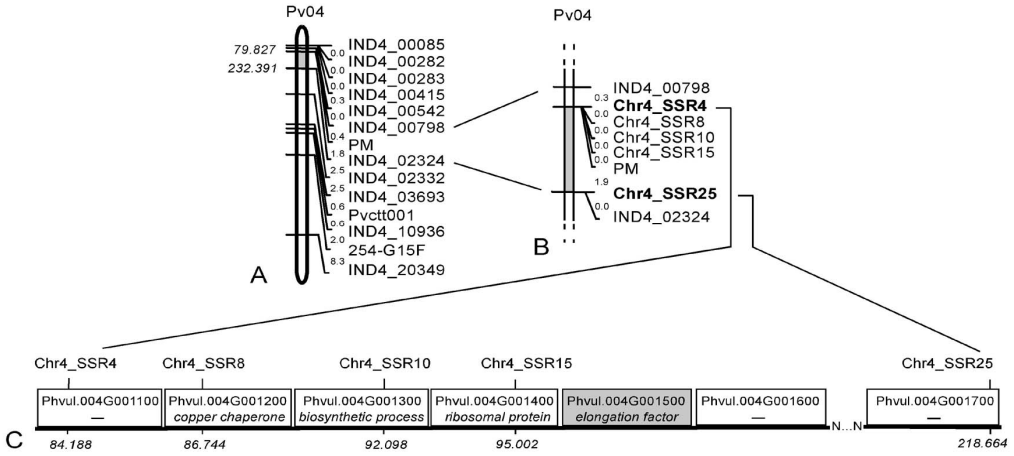

Fig. 1. (A) Genetic linkage map obtained for LG Pv04, including the gene conferring IT0 response against PM (PM, powdery mildew resistance gene). Map distances are expressed in centiMorgans using the Kosambi mapping function. **(B)** Detail of the genetic linkage map between 79.827-232.391 bp of chromosome 4. **(C)** Genes predicted between 84.188 and 218.664 bp of chromosome 4 in the bean sequenced genotype G19833 (www.phytozome.net). The annotated function for each locus is indicated in italic. "-", no functional annotations; "N", missing base pair values.

Relative expression levels of each one of the four candidate genes were evaluated in both parental genotypes under two conditions, without inoculation, and 72 hours after inoculation. Only the gene Phvul.004G001500 was significantly up-regulated in the resistant genotype Porrillo Sintetico 72 hours after PM infection (Fig. 2), suggesting that is involved in the resistant response. The functional annotation for this gene is an elongation factor, having a molecular function of protein binding. Currently, Phvul.004G001500 intragenic variation between genotypes X2776 and Porrillo Sintetico is being studied in order to identified putative changes that could support their different response against PM infection.

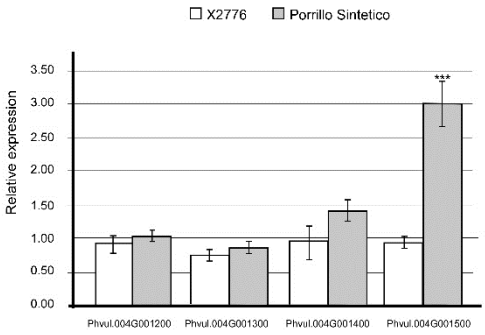

Fig. 2. Relative expression levels 72 hours after PM inoculation for genes Phvul.004G001200, Phvul.004G001300, Phvul.004G001400 and Phvul.004G001500 in the genotypes X2776 and Porrillo Sintetico using the $2^{-\Delta\Delta Ct}$ method. Data points are average values of three biological independent experiments, and four replicates per sample in each experiment. Standard error is showed. ***$p < 0.001$, statistical significance calculated with Student´s t-test.

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PHENOLIC COMPOSITION AND BIOACCESSIBILITY AS AFFECTED BY PRESSURE COOKING AND *IN VITRO* GASTROINTESTINAL DIGESTION OF REGULAR- AND NON-DARKENING CRANBERRY BEANS (*PHASEOLUS VULGARIS* L.)

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INTRODUCTION

Cranberry beans from regular (RR) and nondarkening (CND) genotypes were pressure cooked, and free, conjugated, and bound phenolics were analyzed. Simulated in vitro gastrointestinal digestion was used to assess the bioaccessibility of these phenolic fractions.

MATERIALS AND METHODS

A pressure-soaking and -boiling method was employed consisting of a 30 min soak under pressure followed by a 15 min cook under sustained pressure. This method was able to quickly cook the beans with minimal soaking time required. The beans had also preserved their structural integrity following thermal processing. Various methods were used to quantify the loss of phenolic content following pressure cooking, including the folin-ciocalteu method which was used to determine total phenolic content (TPC).

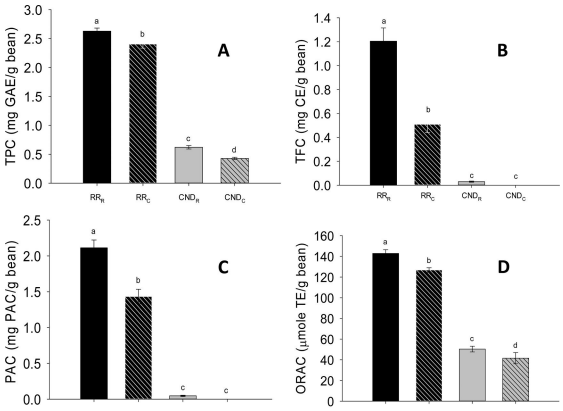

Figure 1. Effect of pressure cooking on phenolic content (A, TPC; B, TFC; C, PAC) and antioxidant activity (D, ORAC) of 70% MeOH crude extract except for PAC, which was extracted by 65% acetone. Bar data are expressed as the mean ± SD, n = 3. Bars marked with the same letter above are not significantly different ($p < 0.05$). Regular Red Rider (RRC) and nondarkening (CNDC) cranberry beans were pressure soaked for 30 min and pressure cooked for 15 min (10.2−11.6 psi).

RESULTS AND DISCUSSION

TPC was significantly decreased for both cooked RR and CND by 8.54 and 31.20%, respectively. Cooked RR had higher TPC (2.40 mg GAE/g) compared to cooked CND (0.43 mg GAE/g). Flavonoids were much more susceptible to degradation following pressure cooking, as evidenced by a loss of 57.79 and 100% in cooked RR and CND, respectively. Total proanthocyanidins (a subclass of polymeric flavonoids), quantified using the DMAC method, decreased by 32% following pressure cooking in RR and were undetected in raw and cooked CND. The decrease in overall phenolic content contributed to a significant decrease in antioxidant capacity measured with the oxygen radical absorbance capacity (ORAC) assay. HPLC-DAD, used to characterize and quantify free (FP), conjugated (BHP) and bound phenolics (BPB) in RR and CND before and after pressure cooking, showed that the FP fraction consisted of: flavan-3-ols; procyanidin, catechin, and epicatechin; and phenolic acids; p-coumaric and ferulic acids (Figure 2A). The flavan-3-ols were exclusive to RR; however, both lines contained the phenolic acids p-coumaric and ferulic acid. Following pressure cooking, all flavonoids monitored in the FP fraction decreased, while the phenolic acids remained unchanged in RR and increased in CND ($p < 0.05$). Conjugated phenolics in the BHP fractions included p-coumaric,

ferulic, and sinapic acids and were present in both lines, however, in higher concentrations in RR. Pressure cooking significantly decreased the concentrations of these phenolic acids ($p < 0.05$). Flavan-3-ols in the bound BPB fraction increased following cooking in RR, this mainly included catechin and procyanidin. In both RR and CND, bound ferulic and sinapic acids also increased significantly following cooking ($p < 0.05$). *In vitro* gastrointestinal digestion, (simulated by the Englyst method) was used to determine bioaccesibility of polyphenols to the small intestine. When comparing to solvent extracted results, the bioaccessible polyphenols represented 8.75 and 14.69% of total phenolic content in RR and CND, respectively and 60.97 and 53.91% of phenolics from RR and CND, respectively, were bioaccessible to the small intestine. These results show that although CND possess significantly less phenolic content compared to RR, this difference is not reflected in the bioaccesibility of polyphenols, suggesting that a large portion of phenolics are not accessible and are in fact insoluble or non-digestible and may be released by microbial hydrolysis in the large intestine.

We postulated that colonic bacteria in the large intestine can secrete carbohydrate-hydrolyzing enzymes capable of releasing bound phenolics. A series of cellulolytic enzymes (Viscozyme, Cellulase and Pectinase) were used on cooked bean residues (which should only have bound phenolics) obtained after triple extraction with 70% methanol to release all free, soluble phenolics. The treatments extracted ferulic, sinapic and p-coumaric acids from both RR and CND, but at concentrations of bound phenolics were significantly lower when compared to that released by alkaline hydrolysis in BPB. However, the RR and CND bound phenolics released by enzymatic hydrolysis did not differ significantly, suggesting that in physiological conditions, the differences in bound phenolic contents between that of RR and CND are much smaller than assessed by alkaline hydrolysis methods. Additional differences between CND and RR cranberry beans were observed by differential scanning calorimetry, which measures the enthalpy of the melting of the amylose-lipid complexes, even though no differences in starch content (including, rapidly digestible, slowly digestible and resistant starches) were observed. These could be due to increased presence of phenolics in RR which has been shown to cause the degradation of amylose-lipid complexes. Further studies are warranted in order to assess the overall health benefits between regular and non-darkening cranberry beans.

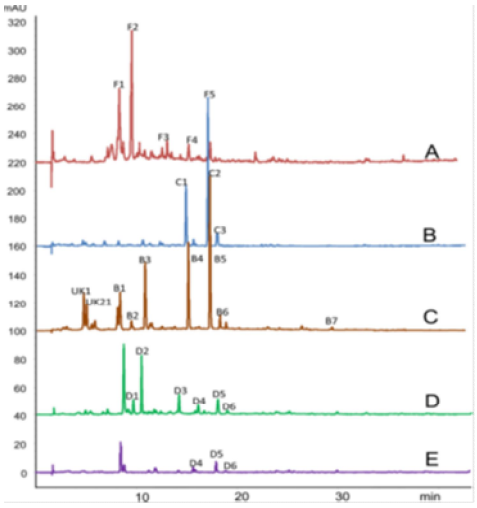

Figure 2. Representative HPLC-DAD chromatograms monitored at 280 nm of free (A, FP), conjugated (B, BHP), and bound (C, BPB) phenolic fractions from pressure-soaked and -cooked regular Red Rider (RR) cranberry bean cultivar. Chromatograms D and E are in vitro gastrointestinal digested phenolics from cooked RR and cooked nondarkening (CND) cranberry beans, respectively.

GENETIC PARAMETERS IN COMMON BEAN UNDER DROUGHT STRESS

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INTRODUCTION

One of the major causes that limits yield instability is drought stress (Ozturk et al. 2002) and consequently, great efforts have been made to breed and obtain drought-tolerant cultivars. Modern agriculture is often challenged to keep crop yields under adverse stress environmental conditions (Lizana et al. 2006). In this context, the objective of this study was to evaluate the performance of 160 common bean recombinant inbred lines derived from the cross IPR-Iapar 81 (drought tolerant) × LP 97-28 (low drought tolerance), under drought stress conditions.

MATERIALS AND METHODS

The populations consisted of 160 $F_{2:7}$ lines from IPR-Iapar 81 × LP 97-28 cross, the parents, and the commercial cultivars Guará, IPR-Tangará, Talismã, IPR-Juriti, Pérola, Flor Diniz and BAT 93, totaling 168 treatments, that were assessed under field conditions at Centro de Treinamento de Irrigação (CTI-UEM), Universidade Estadual de Maringá (UEM), Maringá, Paraná, Brazil, from August to November 2014. The experimental design was conducted as incomplete blocks 13 x 13 triple lattice with three replications. Each experimental plot consisted of two rows of 1.5 m length, spaced 0.5m between rows. The water stress was applied at the beginning of flowering (R_4) to the middle of grain filling stage (R_8). A total of 12 tensiometer, randomly installed in the experimental area, were used to quantify water availability in the soil (Figure 1).

Figure 1. Tensiometers in the experimental area.

The following characters were evaluated: grain yield (YLD; g), number of pods per plant (PPP), number of seeds per plant (SPP) and seed weight (SW; 100 seed wt). Statistical analyses were performed using the SELEGEN-REML/BLUP Model 17 (Resende, 2002).The genotypic model used in this study was $y = X + Zg + Wb + e$, where *y, r, g, b* and *e* refer respectively to: traits

evaluated vector; replication effects vector (fixed) added to the general average; genotypic effects vector (random); blocks effects vector (random); and error vector (random). *X, Z* and *W* correspond to the matrices of incidence of the effects *r, g* and *b*, respectively.

RESULTS AND DISCUSSION

The estimates for genetic and phenotypic parameters for yield and yield production components from IPR-Iapar 81 × LP 97-28 cross are presented in Table 1. The heritability exhibited values that ranged from 5 to 64%, respectively for SPP and PPP characters. These heritability coefficients could be associated with higher additive genetic variances and reduced genotype x environment interaction (Fehr, 1987).

Table 1- Genetic parameters of grain yield (YLD), number of pods per plant (PPP), number of seeds per plant (SPP) and weight of 100 seeds (SW) evaluated in 160 $F_{2:7}$ lines derived from IPR-Iapar 81 × LP 97-28 cross

Random Factors	Estimatives							
	YLD	(%)	PPP	(%)	SPP	(%)	SW	(%)
Genotypic variance - $\hat{\sigma}_g^2$	513.48	25.96	1.28	64.00	0.02	4.54	1.78	52.35
Block - $\hat{\sigma}_b^2$	437.45	22.12	0.09	4.50	0.03	6.81	0.32	9.41
Error - $\hat{\sigma}_e^2$	1026.86	51.92	0.63	31.50	0.39	88.65	1.31	38.24
Phenotypic variance - $\hat{\sigma}_f^2$	1977.80	100	2.00	100	0.44	100	3.40	100
Broad sense heritability - $\hat{h}_g^2$	0.26		0.64		0.05		0.52	
Heritability line - $\hat{h}_{mc}^2$	0.60		0.86		0.14		0.80	
Coefficient determination block	0.22		0.04		0.07		0.09	
Coefficient of genetic variation - CV_g	13.82		16.07		2.93		7.97	
Coefficient of residual variation - CV_e	19.55		11.23		12.33		6.81	
Accuracy of selection of lines - AC_{lin}	0.77		0.92		0.38		0.90	
Prediction of genotypic values PEV	205.38		0.18		0.02		0.45	
Standard deviation of predicted genotypic value SEP	14.33		0.42		0.13		0.59	
Average $\hat{\mu}_m$	163.90		7.05		5.09		16.74	

Thus, the lines derived from IPR-Iapar 81 × LP 97-28 cross showed satisfactory tolerance response to drought. The highest coefficient of genetic variance was observed for PPP (16.07%) whereas the lowest was for NSP (2.93%). This fact suggests the presence of genetic variability with the possibility to select lines with high yield potential when submitted to conditions of hydric stress. The experimental precision based on the efficiency of lineage selection, which varied from 38 to 92%. ????

CONCLUSION

The applied method (REML/BLUP Model 17) was effective in assessing the response to water deficit of 160 evaluated lines. The number of pods per plant and weight of 100 seeds were significantly related to higher water stress tolerance.

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CONSERVATION AND CHARACTERIZATION OF NATIVE *PHASEOLUS VULGARIS* GERMPLASM FROM NORTHWESTERN ARGENTINA

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INTRODUCTION

The Active Germplasm Bank from Northwestern Argentina (BANOA), situated at the Salta Experimental Station of the National Agricultural Technology Institute (INTA-EEA-Salta), conserves an important common bean (*Phaseolus vulgaris* L.) collection of native wild populations and landraces. The BANOA bean collection, which was started in the 80s, consists of 700 accessions, including 400 landraces and 300 wild populations, collected in different regions of Northwestern Argentina. Germplasm collections must provide to breeders genetic variants, genes or genotypes, in order to respond to the challenges demanded by the new production systems. This requires the study and characterization of the preserved germplasm (Abadie and Berreta, 2001; Singh, 2001; De Ron et al, 2015). The aim of this work is to present the preliminary results of the evaluation of part of the BANOA bean collection based on morpho-agronomic characters, microsatellite markers and DNA sequences associated with domestication genes.

MATERIALS AND METHODS

A total of 128 wild populations from the BANOA bean collection were characterized based on 10 morpho-agronomical characters. Twenty pods and 30 seeds per population were measured and a principal components analysis (PCA) was performed. Also, six wild bean populations, selected based on their proximity to cultivated bean fields (< 1 km, > 2 km, and > 5 km), were analyzed with microsatellite markers. The DNA was extracted from seedlings of 10 individuals per population and amplified by PCR using four SSR markers. The amplified fragments were separated by 10% polyacrylamide gel electrophoresis. Allele frequencies for each marker were analyzed using the PowerMarker v3.25 program. On the other hand, two wild populations, two landraces and two commercial bean varieties were studied to analyze the partial sequences of the gene *PvTFL1y*. The amplified regions corresponded to exon I and part of exons II and III of the *PvTFL1y* gene. Sequences were analyzed with BioEdit and compared with the haplotypes described by Kwak et al. (2012).

RESULTS AND DISCUSSION

A Principal Component Analysis (PCA) was generated with 10 morpho-agronomical data: number of pods/plant (P/PL); 100 seeds weight (100 SW), days to 50% maturity (MAT), fruit length (FRL), fruit width (FRW), fruit thickness (FRT), number of grains/pod (GR/P), seed length (SL), seed width (SW) and seed thickness (ST). The first two components (PC1 and PC2) explained 61% of the total variability (figure 1). The variables that most contributed to the differentiation of the wild accessions were: seed length and width, and 100 seeds weight at PC1 and pods/plant, fruit length and grains/pod at PC2. Significant variability was observed for these morphological characters in the accessions analyzed. The spatial analysis performed based on the morphological data showed that the greatest diversity corresponds to accessions collected in

Rosario de Lerma and Chicoana departments in Salta province. The wild populations analyzed based on their proximity to domesticated beans showed differences in allele frequencies for some microsatellite markers. An average of 4.5 alleles per locus was observed over all the loci analyzed. Moreover, for some markers a correlation between the allele frequency and the proximity to crops was observed. After partial amplification of the *PvTFL1y* gene, sequences of approximately 1000 bp length were obtained. The landraces and wild populations analyzed corresponded to the A1 haplotype described previously by Kwak *et al*. (2012) for indeterminate phenotypes. Moreover, haplotypes were obtained for landraces and wild populations of *P. lunatus*, that were not previously described.

CONCLUSIONS

The morphological and molecular characterization confirmed the existence of a significant variability in the accessions conserved in the BANOA (De Ron et al., 2004; Santalla et al., 2005, Galván et al., 2006) and a high degree of introgression in wild populations from domesticated germplasm. This alerts about the importance of conservation of the integer genetic variation of the wild populations in genebanks and also in their natural environments. On the other hand, the knowledge of the variability present in sequences associated with domestication genes it is useful to understand the origin and domestication of the common bean, especially considering the Andean domestication center, which still needs further studies. (Santalla et al., 2004; Bittocci et al. 2013).

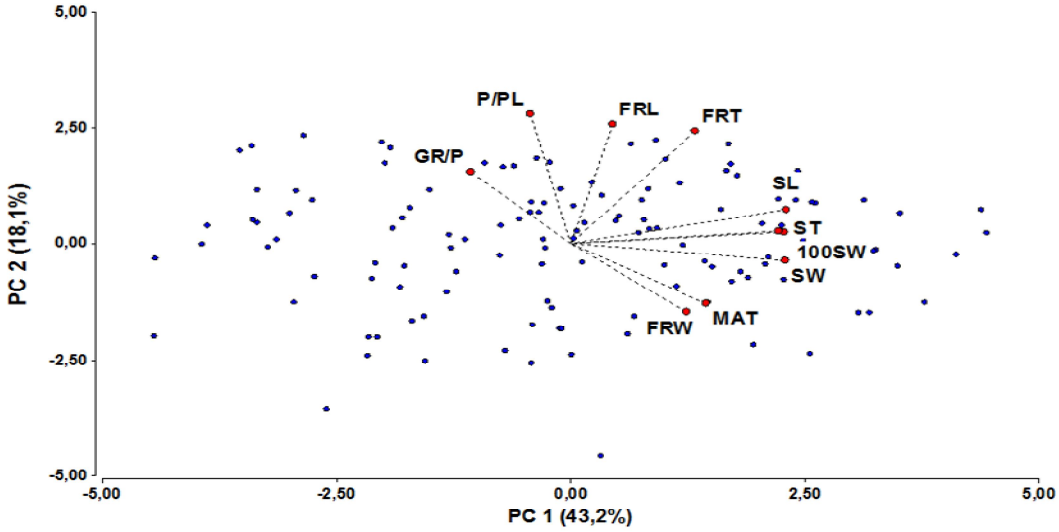

Fig.1. Principal Component Analysis plot using 10 morphological characters.

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GENOTYPE X ENVIRONMENT INTERACTION ANALYSIS BY MIXED MODELS IN BRAZILIAN COMMON BEAN INBRED LINES

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is the world's most important grain legume for direct human consumption. The Americas are the largest bean-producing region and Brazil the world's largest producer and consumer (Singh 1999). However, this crop is affected by several biological, edaphic, and climatic factors that decrease yield (Schwartz and Pastor-Corrales 1989). Genotypic selection methods for recommendation of new cultivars have been widely used in breeding programs to identify inbred lines that have wide yield adaptability and stability. The use of mixed models (REML/BLUP) allows a detailed study of genotype × environment interaction (G × E), and reduce the errors from the effects of this interaction. The objective of this study was to evaluate the yield adaptability and stability of 18 common bean cultivar/inbred lines in four distinct environments of Parana state, Brazil.

MATERIALS AND METHODS

Fourteen elite inbred lines and four commercial cultivars (Perola, IPR Campos Gerais, IPR Uirapuru and CNFP 10104) of common bean were evaluated in four regions of Parana state, Brazil. The experiments were performed in Maringa, Northwest (2012 and 2013), Campo Mourão (Central) and Ponta Grossa (South) regions of Parana state (2012).

The experimental design utilized was complete randomized blocks with three replications. Each experimental unit was constituted of four rows of 5.0 m length, spaced with 0.5 m, and the useful area for analysis was formed by 4.0 m^2 from the two central rows. The genotypic values of grain production were first estimated by REML/BLUP, subsequently, the lines were selected by the method of Harmonic Mean of Relative Performance of Genotypic Value (HMRPGV). The yield adaptability and stability were analyzed using the Model 54 of SELEGEN-REML/BLUP Software (Resende, 2002).

Table 1- Genotypic values obtained by the REML/BLUP methodology of common bean grain yield (Kg ha^{-1}) in four environments in Parana, Brazil

Order	Cultivar/Inbred lines	u + g[1]	New average	Order	Cultivar/Inbred lines	u + g	New average
1	CNFP 10794	3,272.5	3,272.4	10	FT 08-75	3,099.0	3,163.7
2	CHP 01-238	3,220.2	3,246.3	11	IPR Uirapuru	3,085.1	3,156.5
3	LP 09-40	3,205.3	3,232.6	12	FT 08-47	3,080.1	3,150.2
4	IPR Campos Gerais	3,176.0	3,218.5	13	Perola	3,068.4	3,143.9
5	C4-7-8-1-2	3,161.9	3,207.2	14	LEC 01-11	3,054.4	3,137.5
6	CHC 98-42	3,155.0	3,198.5	15	LEP 02-11	3,022.5	3,129.8
7	CNFP 10104	3,132.4	3,189.0	16	C 4-7-7-2-2	3,012.7	3,122.5
8	CNFC 10762	3,107.4	3,178.8	17	TB02-23	3,007.7	3,115.7
9	LP 09-192	3,107.0	3,170.8	18	TB 03-13	2,985.8	3,108.5

[1]predicted genotypic value.

RESULTS AND DISCUSSION

The results obtained for grain yield showed a general mean equivalent to 3,108.6 kg ha^{-1}, being three times higher than the average Brazilian productivity of common bean, showing the high yield potential of genotypes evaluated in the southern region of Brazil.

In the selection based on the best genotypic values (u+g) the genotypes that stood out were: CNFP 10794, CHP 01-238, LP 09-40, C 4-7-8-1-2 and CHC 98-42 with news averages higher than the general mean of 5.27, 4.43, 3.99, 3.17, 2.89%, respectively (Table 1). The results also showed that lines CNFP 10794, CHP 01-238, CHC 98-42, IPR Campos Gerais, C 4-7-8-1-2, LP 09-40 and CNFP 10104 were classified as more stable and adaptable (HMRPGV), with grain yield values that were 1.0 to 1.14 times greater than the general mean (Table 2).

Table 2- Stability of genotypic values (HMGV), adaptability of genotypic values (RPGV), average genotypic values capitalized by the interaction (RPGV*GM), stability and adaptability of genotypic values (HMRPGV) and mean genotypic values in the environments (HMRPGV*GM) for grain yield of common bean cultivars and elite inbred lines evaluated in four environments in Paraná during the years 2012 and 2013

Cultivar/Inbred lines	HMGV	Cultivar/Inbred lines	RPGV	RPGV*GM[1]	Cultivar/Inbred lines	HMRPGV	HMRPGV *GM
CNFP 10794	3,423.0	CNFP 10794	1.14	3,538.0	CNFP 10794	1.14	3,534.0
CHP 01-238	3,305.0	CHP 01-238	1.10	3,405.0	CHP 01-238	1.10	3,405.0
CHC 98-42	3,200.0	LP 09-40	1.06	3,299.0	CHC 98-42	1.05	3,253.0
C4-7-8-1-2	3,178.0	C4-7-8-1-2	1.05	3,281.0	IPR Campos Gerais	1.04	3,248.0
IPR Campos Gerais	3,130.0	CHC 98-42	1.05	3,273.0	C 4-7-8-1-2	1.04	3,243.0
LP 09-40	3,068.0	IPR Campos Gerais	1.05	3,273.0	LP 09-40	1.04	3,237.0
CNFC 10762	3,042.0	CNFP 10104	1.01	3,153.0	CNFP 10104	1.00	3,118.0
FT 08-75	3,024.0	CNFC 10762	1.01	3,129.0	CNFC 10762	0.99	3,106.0
CNFP 10104	3,018.0	FT 08-75	0.99	3,101.0	FT 08-75	0.99	3,096.0
IPR Uirapuru	2,940.0	LP 09-192	0.98	3,068.0	LP 09-192	0.98	3,046.0
FT 08-47	2,939.0	IPR Uirapuru	0.97	3,040.0	IPR Uirapuru	0.97	3,037.0
LP 09-192	2,919.0	FT 08-47	0.97	3,036.0	FT 08-47	0.97	3,021.0
Pérola	2,890.0	Pérola	0.96	2,993.0	Pérola	0.96	2,988.0
LEC 01-11	2,855.0	LEC 01-11	0.95	2,955.0	LEC 01-11	0.94	2,948.0
TB02-23	2,769.0	LEP 02-11	0.92	2,867.0	LEP 02-11	0.92	2,857.0
LEP 02-11	2,757.0	C 4-7-7-2-2	0.92	2,863.0	TB 02-23	0.91	2,842.0
C 4-7-7-2-2	2,747.0	TB02-23	0.91	2,852.0	C 4-7-7-2-2	0.90	2,820.0
TB 03-13	2,702.0	TB 03-13	0.90	2,819.0	TB 03-13	0.88	2,740.0

[1]General Mean of trials.

CONCLUSION

The results showed that genotypic values were higher in overall environments for CNFP 10794, CHP 01-238, CHC 98-42, IPR CAMPOS GERAIS, C 4-7-8-1-2, LP 09-40, and CNFP 10104, which showed superior grain yield when they were selected by the Harmonic Mean of the Relative Performance of Genotypic Values (HMRPGV). These cultivar/inbred lines may be recommended for commercial purposes, since it achieved satisfactory outcomes in the environments assessed.

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NEW ALLELE IN THE AND277, MICHIGAN DARK RED KIDNEY AND JALO EEP558 ANDEAN CULTIVARS LINKED TO THE G2303 MOLECULAR MARKER

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INTRODUCTION

Anthracnose caused by *Colletotrichum lindemuthianum* is one of the most devastating disease of common bean. Currently, the resistance to *C. lindemuthianum* is conferred by 20 resistance *loci*, among them, nine are Andean (Kelly and Vallejo 2004, Trabanco et al. 2015). The *Co-1* and *Co-1*^{4} alleles of the cultivars Michigan Dark Red Kidney (MDRK) and AND 277, respectively, are mapped on Pv01. Although, the Andean cultivar Jalo EEP558 was reported to carry *Co-x* on Pv01, while the *Co-y* and *Co-z* genes are mapped on Pv04. The *Co-y* gene co-segregated with the *Co-3*^{3} allele in BAT 93, also there is evidence that *Co-y*, *Co-z* and *Co-3*^{3} are closely linked on Pv04 (Geffroy et al. 1999). Compared to other chromosomes, the Pv04 is the most important because it contains six R specificities against *C. lindemuthianum*: *Co-3, Co-3*^{2}*, Co-3*^{3}*, Co-3*^{4}*, Co-y* and *Co-z* (Geffroy et al. 2008; David et al. 2008). Three other R specificities against bean rust, caused by *Uromyces appendiculatus*, *Ur-5, Ur-14 and Ur-Dorado* (Souza et al. 2011), and two angular leaf spot, caused by *Pseudocercospora griseola, Phg-1* and *Phg-3* (Gonçalves-Vidigal et al. 2011, Gonçalves-Vidigal et al. 2013) were also mapped in this region of Pv04. Therefore, the objective of this work was to genotype the Andean and Mesoamerican cultivars with the g2303 molecular marker.

MATERIALS AND METHODS

This research was conducted at the Núcleo de Pesquisa Aplicada a Agricultura (Nupagri) at the Universidade Estadual de Maringá, Paraná, Brazil. The common bean cultivars AND 277, Michigan Dark Red Kidney (MDRK), Jalo EEP558, Corinthiano (Andean cultivars) and Ouro Negro, BAT 93, Mexico 222 and Rudá (Mesoamerican cultivars) were genotyped with the g2303 molecular marker previously mapped on Pv04. The amplification products corresponding to the marker g2303 present in the cultivars AND 277, MDRK, Jalo EEP558 and Ouro Negro were purified using PCR Purification Kit PureLink (Invitrogen) for subsequent sequencing. After purification, the DNA fragments were fractionated in agarose gel of 2%. The purified products were sequenced using ABI 3730 DNA Analyser (Life Technologies – Applied Biosystems). Each sequence was edited in BioEdit Program Sequence Alignment Editor Version 7.2.5 (Hall, 1999). After reversing the complement sequence this was aligned generating sequence file FASTA for identifying the differences between the nucleotides and to obtain the genetic positions of AND 277, MDRK, Jalo EEP558 and Ouro Negro, with the genome of the common bean G19833 genotype available in Phytozome.

RESULTS AND DISCUSSION

Two polymorphic DNA fragments were observed at the *Co-3 locus* demonstrating that the Andean cultivars AND 277, MDRK and Jalo EEP558 possess a new allele at *Co-3 locus* different from that present in Mexico 222 (*Co-3*), BAT 93 (*Co-3*^{3}) and Ouro Negro (*Co-3*^{4}) Mesoamerican cultivars (Figure 1). The sequencing of polymorphic DNA fragments from the AND 277, MDRK, Jalo EEP558 and Ouro Negro genotyped with the g2303 molecular marker are showed in the Figure 2.

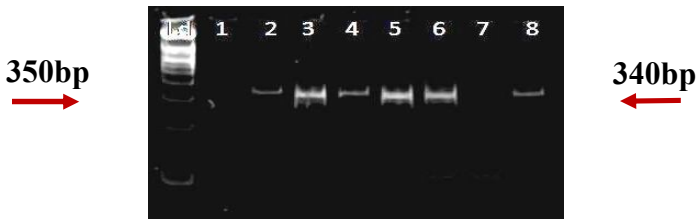

Figure 1- Electrophoretic analysis of the amplification products obtained for the g2303 marker. Lanes: Ld, 100bp ladder; 1, Corinthiano; 2, AND 277; 3, BAT 93; 4, Michigan Dark Red Kidney; 5, Ouro Negro; 6, Mexico 222; 7, Rudá; 8, Jalo EEP558. The arrow left indicates the 350 bp and the right 340 bp DNA bands present in Andean and Mesoamerican cultivars, respectively.

Molecular marker g2303 is mapped at position 3,356,300 bp on chromosome Pv04 (out of a total chromosome length of 45,960,019 bp; PhaseolusGenes). By comparing the sequences of the fragment from the AND 277 cultivar with the genome of the common bean, it was observed its the genetic position at 3,356,178 to 3,356,485 bp on the Pv04. Likewise, the fragment from the MDRK cultivar is positioned at 3,356,179 to 3,356,483 bp, and Jalo EEP558 at the position of 3,356,147 to 3,356,372 bp on chromosome Pv04. It is noteworthy that the sequences of the DNA fragments obtained from AND 277 and MDRK were different in only three nucleotides. However, the allele of Jalo EEP558 is apart by 82 and 79 nucleotides from the alleles of the AND 277 and MDRK cultivars, respectively. In addition, the *Co-3*^{4} allele of the Ouro Negro

cultivar is located at the position of 3,356,225 to 3,356,481 bp on chromosome Pv04.

Figure 2 - Schematic representation of nucleotide sequences of DNA fragments from AND 277, MDRK, Jalo EEP558 and Ouro Negro common bean cultivars genotyped with g2303 molecular marker. Identical and similar nucleotide are indicated by dots.

The STS marker g2303 previously mapped on Pv04 linked to the new alleles present in the AND 277, MDRK, Jalo EEP558 and to the *Co-3*, *Co-3*^{3}, *Co-3*^{4}*/Phg-3 loci*, respectively, present in Mexico 222, BAT 93 and Ouro Negro Mesoamerican cultivars, emphasized the potential usefulness of this marker for marker-assisted selection in Bean Breeding Programs for Andean and Mesoamerican Gene Pools.

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GENETIC MAPPING OF THE ANTHRACNOSE RESISTANCE GENE *CO-14* IN THE COMMON BEAN CULTIVAR PITANGA

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INTRODUCTION

Pitanga is an important source of resistance to anthracnose. It confers resistance to races 23, 64, 65, 73 and 2047 of *Colletotrichum lindemuthianum*. In this study, we conducted molecular analysis on the F_2 population derived from the Pitanga (resistant) × AB 136 (susceptible) cross, inoculated with race 2047 of *C. lindemuthianum*. A total of 35 molecular markers were tested with contrasting amplification patterns in the parental materials and the resistant vs. susceptible bulks. Results showed, that the molecular markers CV 542014^{450} and TGA1.1^{570}, previously mapped on LG Pv01, are linked in coupling phase to the *Co-14* resistance gene at 4.3 cM and 6.3 cM, respectively. In addition, the genetic location of the *Co-14* gene was found to be on chromosome 1 gene cluster.

MATERIAL AND METHODS

This research was conducted under greenhouse conditions and at the Common Bean Breeding and Molecular Biology Laboratory of Núcleo de Pesquisa Aplicada à Agricultura (Nupagri). Total DNA was obtained from 137 F_2 individuals from the cross between Pitanga (resistant) and AB 136 (susceptible) cultivars, by using race 2047 of *C. lindemuthianum.* These individuals were analyzed to construct two homozygous contrasting resistant and susceptible DNA bulks based on virulence data obtained (Michelmore et al. 1991). Equal volumes of DNA from five homozygous resistant and five susceptible F_2 plants were used. A total of 35 molecular markers were tested with contrasting amplification patterns in parental materials and in the resistant vs. susceptible bulks and individuals from the bulks. A goodness of-fit test for a 1:1 segregation ratio was performed for the segregation of the CV208414 and TAG1.1 markers in the BAT 93/Jalo EEP 558 bean consensus mapping population. Linkage analyses were performed using the computer software Mapmaker/EXP 3.0 (Lincoln and Lander 1993). Linkage group nomenclature follows Pedrosa-Harand et al. (2008) and the map was drawn using the computer software MapChart (Voorrips 2002).

RESULTS AND DISCUSSION

A segregation of 177 resistant and 60 susceptible individuals in the F_2 population fitted to the expected 3R:1S ratio (p = 0. 91). This segregation data confirmed the monogenic resistance in the Pitanga cultivar to race 2047 of *C. lindemuthianum* carried out by Gonçalves-Vidigal et al. (2012). The molecular analyses revealed that the molecular markers CV542014^{390} and TGA1.1^{570}, previously mapped on LG Pv01 (McConnell et al. 2010, available from the PhaseolusGenes database: http://phaseolusgenes.bioinformatics.ucdavis.edu/markers/CV542014&format=html) are linked in coupling phase to the *Co-14* resistance gene at 4.3 cM and 6.3 cM, respectively. These molecular markers was tested in the BAT 93/Jalo EEP 558 (BJ) RI population, resulted in segregation of 37 (+):34 (-) (χ^2 = 0.13; p = 0.72) for a good fit to a 1:1 ratio. Pitanga has been shown to be an important source of resistance to anthracnose, and the fact that *Co-14* gene is an

Andean gene reinforces its use in common bean breeding programs where there is a lack of effective Andean resistance genes for anthracnose.

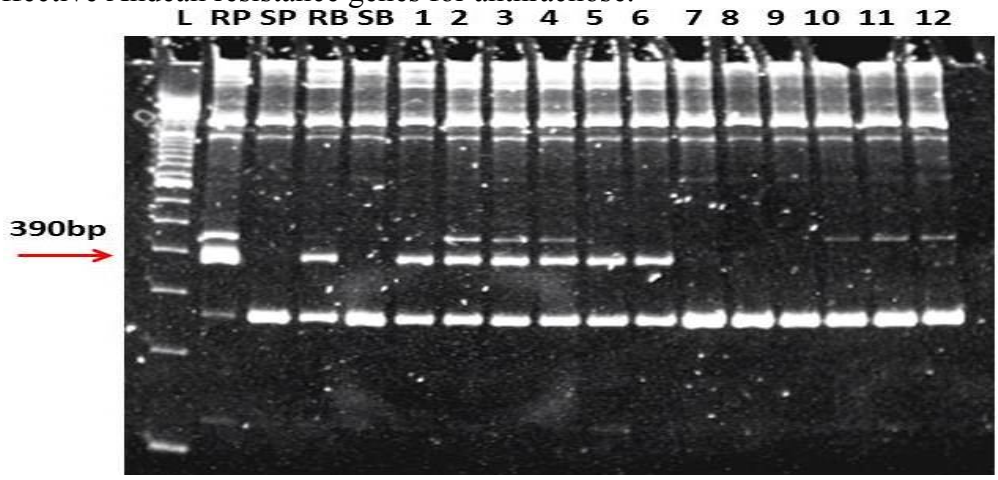
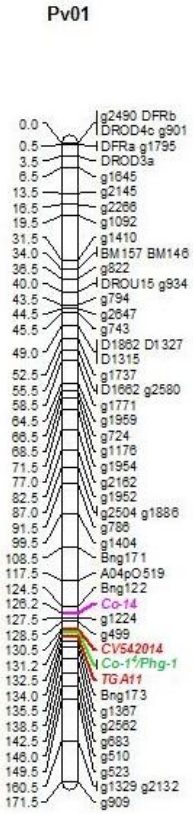


Figura 1-. Electrophoretic analysis of the amplification products obtained for the $CV542014_{390}$ marker. Lines: L, 100bp ladder; RP, Pitanga; SP, AB 136; RB, resistant bulk; SB, susceptible bulk; 1-6, individuals resistant to *C. lindemuthianum;* 7-12, individuals susceptible to *C. lindemuthianum.* The arrow indicates the 390bp DNA band linked to the *Co-14* resistance genes.

Figure 2- Location of *Co-14* for resistance to common bean anthracnose, and $CV542014_{390}$ and TGA 1.1_{570} molecular markers on linkage group Pv01 of *Phaseolus vulgaris* L. using the population from Pitanga × AB 136 cross. The map was obtained from the MapChart program (Voorrips, 2002).



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THE VALIDATION OF QTLS FOR POD QUALITY TRAITS IN SNAP BEANS (*PHASEOLUS VULGARIS* L.) THROUGH ASSOCIATION MAPPING IN THE BEAN CAP POPULATION

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INTRODUCTION

The most important aspect in determining the quality of snap bean relies on pod traits that include pod length, pod shape, and the presence or absence of pod fiber and suture string. These traits are important in order to fit the standard in processing industry and meet consumer preferences. Little is known about the inheritance of pod quality traits in snap bean. Association mapping using a germplasm collection enables the identification of marker-QTL association as well as reveals new QTL in a genetically diverse population. This analysis is a complementary to the QTL mapping, which can provide some insight in understanding the inheritance of pod quality traits in snap beans. The objective of this research is to identify markers associated with pod quality traits through association mapping in a non-biparental population of snap beans.

MATERIALS AND METHODS

75 snap bean accessions from the Bean Coordinated Agricultural Project (Bean CAP) association mapping panel were evaluated in a randomized complete block design in 2014 and 2015 near West Madison, WI. Five pods of sieve 4 were measured for pod length, pod shape, and the presence or absence of fiber and suture string. Pod length was measured from below the pedicel to the tip of pod using a ruler. Pod shape is a ratio between pod diameter 90^{o} off suture over pod diameter on suture and the diameter was measured using a caliper. Pod fiber and suture string were observed by snapping the pod and were rated from 0 to 3. A rate of 0 for pod fiber and suture string when there is no fiber on the break surface and no suture string attached to the pod, respectively. A rate of 3 is for high fiber and thick suture string. A total of 10607 SNP marker that were used in this analysis were genotyped using Illumina Infinium II BeadChip BARCBean6K_1 and BARCBean6K_2 (Song et al., 2015). These SNP markers were provided by the Bean CAP. Association mapping were performed in TASSEL 5.0 software (Bradbury et al., 2007) using mixed linear model that includes principal component analysis and kinship matrix to account for population structure and familial relatedness, respectively (Zhang et al. 2010). Markers with p-value < 0.001 are considered significantly associated with the QTL related to the trait.

RESULTS AND DISCUSSIONS

Pod shape (ratio), pod fiber and suture string showed significant markers at p-value <0.001 in the same chromosome for both years but these are different markers (Table 1). Most markers in all traits show significant association in one year but have lower significant value or not significant in another year (Table 1). Some markers in chromosome 4 showed significant association in either 2014 or 2015 in all traits. Further analysis could possibly reveal interesting findings regarding QTL in this region. For all traits, significant markers at p-value <0.001 explain phenotypic variation ranged from 14 to 50% in 2014 and from 14 to 42% in 2015 as indicated by R^2 value (Table 1). Inconsistent significant markers detected in all traits for both years could

possibly due to genetic by environment interaction effect or weak marker-QTL association. Also, this could be due to some experimental error during data collection.

Table 1. List of markers associated with pod quality traits and the proportion of phenotypic variation explained by markers (R^2) in experiments evaluated in 2014 and 2015 at West Madison, WI.

Trait	Marker§	Chr	Position (bp)	2014			2015		
				p-value		R^2 (%)	p-value		R^2 (%)
Pod fiber	M8046	4	31600660	1.71×10^{-04}	***	19.81	9.60×10^{-01}	ns	0
	M10460	4	17322511	7.15×10^{-03}	**	9.63	1.28×10^{-06}	***	36.40
	M7512	2	12115239	2.15×10^{-01}	ns	1.965	9.80×10^{-04}	***	15.31
Pod length	M632	1	49270510	8.74×10^{-04}	***	15.94	1.72×10^{-03}	**	14.11
	M10460	4	17322511	4.73×10^{-02}	*	5.367	3.85×10^{-04}	***	18.50
Pod shape (Ratio)	M4599	4	42289579	8.98×10^{-04}	***	14.02	2.21×10^{-01}	ns	1.75
	M3700	2	25246500	4.68×10^{-05}	***	21.98	5.45×10^{-02}	ns	4.38
	M1838	2	23710638	9.27×10^{-02}	*	3.38	4.18×10^{-05}	***	21.97
	M4968	10	2792251	3.01×10^{-02}	*	5.70	1.20×10^{-04}	***	19.07
	M6780	11	13000616	3.08×10^{-01}	ns	1.23	5.41×10^{-04}	***	15.10
	M22	7	51233220	1.58×10^{-01}	ns	2.36	7.38×10^{-04}	***	14.31
Suture string	M7838	1	19971327	1.91×10^{-07}	***	47.46	3.72×10^{-03}	**	12.24
	M4700	10	32823435	1.91×10^{-07}	***	47.46	3.72×10^{-03}	**	12.24
	M342	3	50026414	6.77×10^{-05}	***	25.45	1.57×10^{-01}	ns	2.78
	M6287	6	22214805	9.89×10^{-05}	***	24.17	1.87×10^{-01}	ns	2.41
	M3296	4	7945244	3.05×10^{-04}	***	20.46	1.33×10^{-01}	ns	3.13
	M5677	2	43852765	5.01×10^{-04}	***	18.87	4.71×10^{-02}	*	5.54
	M9447	4	22549311	3.37×10^{-01}	ns	1.32	4.95×10^{-07}	***	41.87
	M7512	2	12115239	2.52×10^{-01}	ns	1.89	8.57×10^{-04}	***	16.50

§ Markers are significant at p-value < 0.001 in either in 2014 or 2015

Chr Chromosome

*,**,*** Statistically significant at p-value<0.05, 0.01 and 0.001, respectively

ns Not statistically significant at p-value<0.05

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EFFECT OF IRRIGATION AND PLANT CANOPY ARCHITECTURE ON WHITE MOLD DEVELOPMENT IN DRY BEAN

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INTRODUCTION

Dry bean (*Phaseolus vulgaris* L.) is the most profitable pulse crop in southern Alberta. White mold (WM) caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary can be a major constraint to dry bean production. Registered dry bean cultivars do not have resistance to WM. In favorable conditions, sclerotia present in the soil germinate and produce apothecia which release ascospores that initiate WM. Soil moisture within the top 5-cm is very critical for sclerotial germination (Wu and Subbarao, 2008), and an average temperature of 15^{o}C for several weeks is required for apothecia development (Hall, 1994). Plant architectural traits, such as, upright growth and lodging resistance can influence microclimate within the plant canopy, and thus, can be useful to avoid WM in dry bean (Miklas et al., 2013). In the semi-arid conditions of southern Alberta, dry bean is grown under irrigation which results in favorable conditions for WM development. Our objective was to evaluate the effect of irrigation and plant architecture on microclimate and WM development in dry bean genotypes.

MATERIALS AND METHODS

Studies were conducted in sclerotia-inoculated fields at the Lethbridge Research and Development Centre, Alberta in 2015. Three levels of irrigation (high or 3.8 cm, medium or 1.9 cm and low or 25% less than medium per week) and five genotypes with different canopy architecture (indeterminate prostrate I9365-31 and Othello, indeterminate semi-upright AAC Burdett and AC Island, and determinate upright CDC Pintium) were arranged in a split-plot design. I9365-31 is a black bean line with partial genetic resistance to WM (Miklas et al. 1998), AAC Burdett is a pinto bean cultivar with partial field resistance to WM; all others are pinto bean cultivars with susceptibility to WM. Dry bean plots were evaluated for WM incidence and severity, flower infection, yield and thousand seed weight. Microclimate variables, including leaf wetness, soil moisture and temperature were monitored using data loggers and sensors. Flower infection was assessed by plating flowers onto sclerotinia-specific media. WM incidence and severity were assessed on a 1-4 scale according to Balasubramanian et al. (2014) where 1= healthy and 4= dead plant. Data were analyzed for ANOVA and mean separations were performed using PROC MIXED procedure of SAS 9.2.

RESULTS AND DISCUSSION: Moisture within the top 5-cm of soil and leaf wetness were significantly higher in high irrigation plots as compared to medium and low irrigation. Soil temperatures conducive for apothecia development persisted for several weeks in high irrigation plots (Figure 1). WM severity, incidence and flower infection were greatest in high irrigation plots when compared to medium and low irrigated plots (Figure 2). Susceptible cultivars, Othello and CDC Pintium, exhibited highest WM severity, incidence and flower infection in high irrigation plots. WM development in I9365-31 and AAC Burdett was not affected by irrigation. Plots grown under medium and low irrigation had similar WM levels, however, highest yield and TSW were observed in medium irrigation plots (Figure 3). Thus, medium irrigation was effective

in managing WM while maintaining high yields and TSW. This experiment will be repeated for 2 more years.

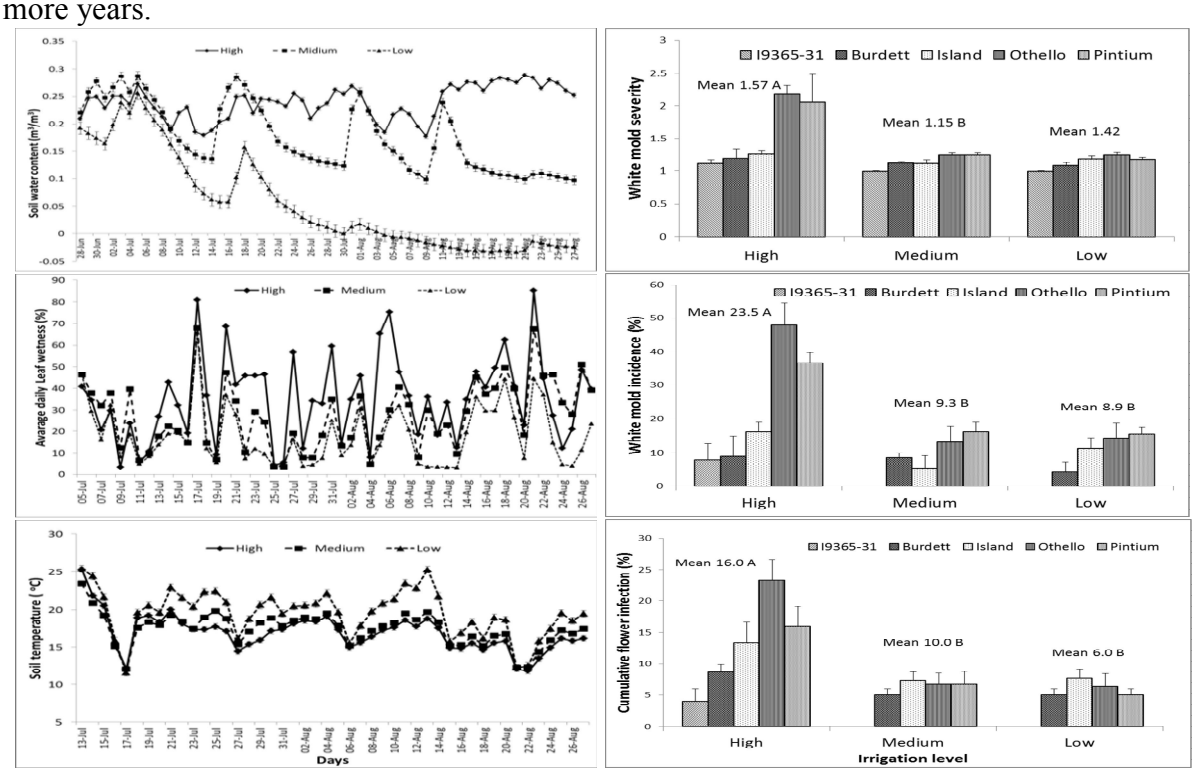

Figure 1. (Left) water content in top 5-cm of soil (above), leaf wetness (middle) and soil temperature (bottom); **Figure 2.** (Right) White mold severity (above), incidence (middle) and flower infection (bottom) in dry bean genotypes under high, medium and low irrigation.

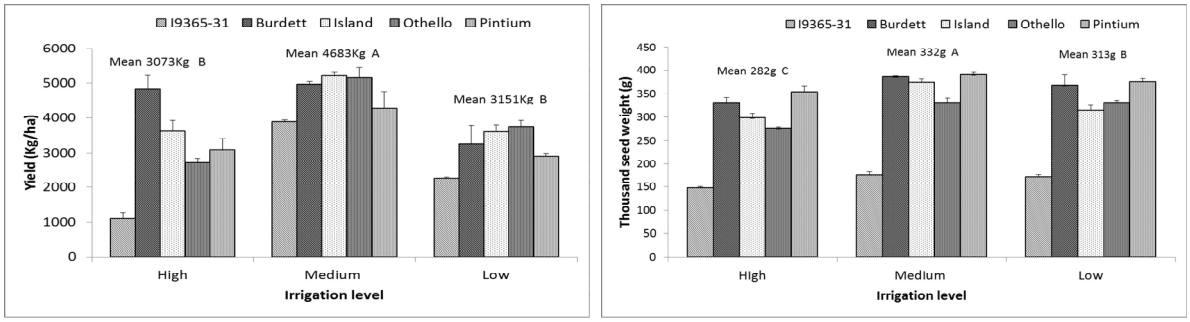

Figure 3. Mean yield (left) and thousand seed weight (right) in five dry bean genotypes cultivars grown under high, medium and low irrigation conditions.

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INVESTIGATING PHENOTYPIC VARIABILITY AND SENSITIVITY *IN VITRO* TO THE FUNGICIDES OF *PSEUDOCERCOSPORA GRISEOLA* STRAINS

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INTRODUCTION

Angular leaf spot (ALS) of common bean, caused by fungus *Pseudocercospora griseola*, is widespread and typical of tropical regions. ALS is responsible for significant crop damage and can result yield losses depending on cultivars susceptibility and environmental conditions. The use of fungicides is the main control method of disease and obtaining of resistant lines is difficult due to wide pathogen variability and presence of major and quantitative resistance genes (Pereira et al. 2015). There are insufficient information about traits of the pathogen involved in spread, penetration, infection and colonization in common bean. Furthermore, studies about *P. griseola* strains sensibility to fungicides have not been found in literature. Thus, this study has evaluated cytological and physiological variability and also sensibility to fungicides of a population of *P. griseola* strains from Brazil.

MATERIAL AND METHODS

We collected 125 *P. griseola* strains from ALS lesions on common bean leaves naturally infected, in Minas Gerais state, Brazil. These strains were evaluated to sporulation capacity, germination percentage, conidia size and sensibility to fungicides. Sporulation capacity was analyzed using a completely randomized design (CRD) with three replicates. Each plot consisted of a Petri dish, containing medium leaf-dextose-agar (LDA) and 1 ml of mycelial suspensions of *P. griseola* strains. Petri dishes were incubated at 24°C and nine days after incubation was determined conidia number of each plot. For evaluation germination percentage, 200 µl of conidial suspensions (2×10^4 conidia.ml^{-1}) of each *P. griseola* strains were incubated at 24ºC for six hours. Conidia with germ tubes length equal or greater than the smallest diameter of the conidia were considered germinated. This assays were performed using a CRD with two replication, using 50 conidia for each replicates. For cytological measurements, length and width of 22 conidia of each *P. griseola* strains were analyzed using CRD. Conidia size were performed using the AxioVision SE64 Rel.4.8 software. For sensibility evaluation of 34 *P. griseola* strains to five fungicides were used Petri dishes containing LDA medium with each fungicide. It was used as control, *P. griseola* strains growth in only LDA medium, totaling 204 treatment. After nine days of incubation was determined conidia number of each *P. griseola* strains. Data were submitted to analysis of variance and treatment means were compared by Scott Knott test using R® software.

RESULTS AND DISCUSSION

Numbers of conidia varied from 0.87×10^4 conidia.ml^{-1} to 27.67×10^4 conidia.ml^{-1} among *P. griseola* strains that were classified in nine groups by Scott-Knott mean test. However, only 2% of *P. griosela* strains presented number of conidia higher 17.79×10^4 conidia.ml^{-1} (Figure 1). Sporulation capacity associated with conidia germination ability are important traits for conidia spread and establishment of disease (Monda et al., 2001). Germination percentages of strains varied from 39 to 74% and two different groups of strains were formed. Conidia length and width varied from 33.6µm to 43.43 µm and 5.2 µm to 10.8 µm, respectively. All fungicides

present efficiency in controlling the mycelial growth and sporulation for all *P. griseola* strains evaluated *in vitro* test. Therefore, this information should be verify *in vivo* test using common bean plants. These results are important because information about these traits evaluated in *P. griseola* are limited and this knowledgement can aid in strategies of management and control of ALS.

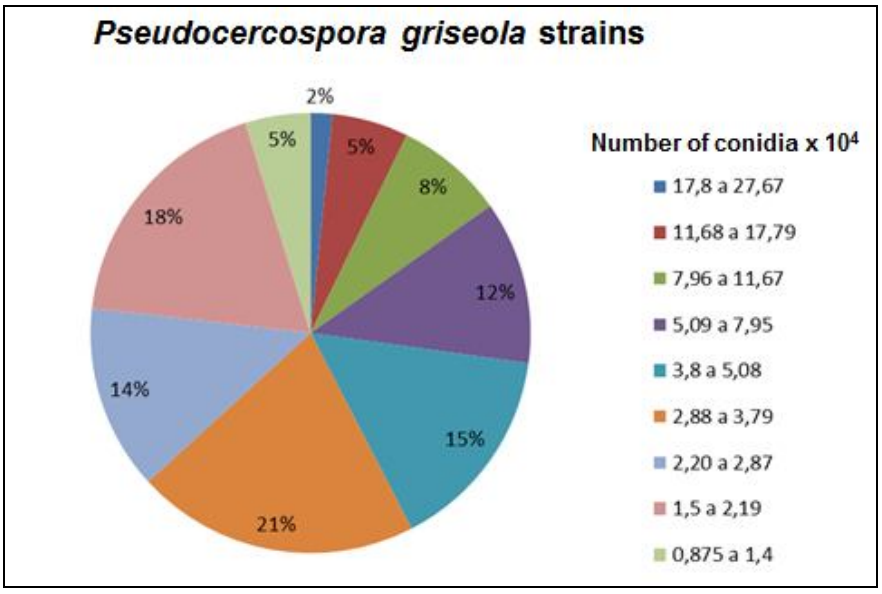

Figure 1- Groups generated by Scott Knott test according to sporulation capacity of *P. griseola* strains.

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PATHOGENIC VARIABILITY OF *COLLETOTRICHUM LINDEMUTHIANUM* IN THE STATE OF PERNAMBUCO, BRAZIL

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INTRODUCTION

Anthracnose, caused by *Colletotrichum lindemuthianum,* is one of the most important diseases of the common bean (*Phaseolus vulgaris* L.) in Brazil and in other regions of the world. High genetic variability for *C. lindemuthianum* has been described worldwide, and more than 247 different races of the pathogen have been identified, which 35 occur exclusively in Brazil (Nunes et al. 2013). The monitoring of *C. lindemuthianum* races present in regions of cultivation is necessary as a way to facilitate the effective control of this disease. The objective of this work was to characterize isolates of *C. lindemuthianum* collected in common bean from Pernambuco state, Brazil.

MATERIAL AND METHODS

In 2014 it was observed that several common bean cultivars were infected by *C. lindemuthianum* pathogen in the common bean fields in Pernambuco state, Brazil (Figure 1).

Leaves and pods infected provided a total of eighteen isolates, which were inoculated on a set of 12 common bean differentials cultivars of *C. lindemuthianum* to characterize the virulence spectra of the pathogen. Monosporic cultures of each isolate were prepared in young green common bean pod medium and incubated at 20 ± 2°C for 14 days (Cárdenas et al. 1964) and adjusted to a final concentration of 1.2×10^6 spores mL^{-1}. Seedlings were grown under natural light in greenhouse for 7 to 10 days until they reached the first trifoliate leaf stage. Ten seedlings of each differential cultivar were inoculated with the spore suspension of each isolate by using an atomizer (De Vilbiss, number 15) powered by an electric air compressor. After inoculation, the seedlings were kept in a mist chamber for 72 h at 20°C with controlled light. After misting, plants were transferred to benches in a greenhouse with suitable environment at 22°C with artificial light (12-h day length at 22°C) for 10 days. The anthracnose disease reactions were scored visually using a scale from 1 to 9 (Pastor-Corrales et al., 1995). Plants with disease reaction scores between 1 and 3 were considered resistant (R), whereas plants that scored 4-9 were considered susceptible (S).

Figure 1 - Regions of Pernambuco state, Brazil, where leaves or pods infected by *C. lindemuthianum* were collected. In the Figure the micro-regions are highlighted: 1- Alto Capibaribe; 2- Vale do Ipojuca; 3- Garanhuns; 4- Sertão do Moxotó.

RESULTS AND DISCUSSION

From 18 isolates evaluated were identified fourteen races: 2, 8, 9, 10, 64, 65, 72, 73, 81, 85, 89, 117, 139 and 331 (Table 1) showing the genetic variability of the pathogen in Pernambuco State. Among these races, 13

were recognized for the first occurrence in this state. As displayed Table 1, the races 2, 9 and 81 were more frequent in Pernambuco state when compared with the other identified races. It is also relevant to emphasize that the cultivars TU, AB 136 and G 2333 were resistant to all isolates evaluated.

Table 1 - Reaction of differential cultivars of common bean to isolates of *C. lindemuthianum* collected in Pernambuco State, Brazil

	Geographic coordinates	Masl[3]	Counties	Isolates	Differential Cultivars [1/2]												Race
					A	B	C	D	E	F	G	H	I	J	K	L	
Micro-region Alto Capibaribe	07° 54' 10" S 35° 59' 16" W	401	Vertente	CLPE 28	S	R	R	R	S	R	S	R	R	R	R	R	81
	07° 49' 55" S 35° 45' 21" W	394	Surubim	CLPE 34	S	S	R	S	R	R	R	S	R	R	R	R	139
	07° 50' 24" S 35° 54' 07" W	494	Sta Maria do Cambucá	CLPE 41	S	S	R	S	R	R	S	R	S	R	R	R	331
Micro-region Vale do Ipojuca	08° 53' 24" S 36° 29' 34" W	470	Bezerros	CLPE 82	R	S	R	R	R	R	R	R	R	R	R	R	2
Micro-region Garanhuns	08° 42' 21" S 36° 29' 20" W	820	Jucati	CLPE 37	S	R	R	S	R	R	S	R	R	R	R	R	81
	08° 42' 43" S 36° 24' 54" W	782	Jupi	CLPE 43	S	S	R	R	S	R	S	R	R	R	R	R	85
	08° 52' 33" S 36° 22' 01" W	716	São João	CLPE 63	R	S	R	S	R	R	R	R	R	R	R	R	9
				CLPE 26	R	R	R	R	R	R	S	R	R	R	R	R	64
				CLPE 06	S	R	R	R	R	R	S	R	R	R	R	R	65
				CLPE 02	S	R	R	S	R	R	S	R	R	R	R	R	73
				CLPE 53	S	R	S	R	S	S	S	R	R	R	R	R	117
Micro-region Sertão do Moxotó	08° 25' 15" S 37° 03' 41" W	663	Arcoverde	CLPE 31	R	S	R	R	R	R	R	R	R	R	R	R	2
				CLPE 46	R	R	R	S	R	R	R	R	R	R	R	R	8
				CLPE 35	R	S	R	S	R	R	R	R	R	R	R	R	9
				CLPE 49	R	S	R	S	R	R	R	R	R	R	R	R	10
				CLPE 29	S	R	R	S	S	R	S	R	R	R	R	R	89

[1]: A- Michelite (1); B- Michigan Dark Red Kidney (2); C- Perry Marrow (4); D- Cornell 49-242 (8); E- Widusa (16); F- Kaboon (32); G- Mexico 222 (64); H- PI 207262 (128); I- TO (256); J- TU (512); K- AB 136 (1024); L- G 2333 (2048). [2]: R- Resistant; S- Susceptible; [3]Masl = meter above sea level.

ACKNOWLEDGEMENTS

This research was financially supported by the CNPq and Capes. Martiniano-Souza MC, Dartibale GB and Lacanallo GF were supported by a scholarship from CNPq. Paulino PPS was supported by a scholarship from Capes. M.C. Gonçalves-Vidigal is grateful for Grants from CNPq. The authors would like to thank IPA for giving the samples of *C. lindemuthianum*.

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SOURCES OF RESISTANCE TO ANTHRACNOSE IN TRADITIONAL ACCESSIONS OF COMMON BEAN FROM PERNAMBUCO STATE, BRAZIL

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INTRODUCTION

Anthracnose caused by *Colletotrichum lindemuthianum* (Sacc & Magnus) Briosi & Cavara is one of the most devastating diseases of common bean (Pastor-Corrales et al. 1995). Bean anthracnose is intensified by the extensive virulence diversity of *C. lindemuthianum* and the seedborne nature of the pathogen (Pastor-Corrales and Tu 1989, Damasceno e Silva et al. 2007). In the Northeastern region of the Brazil Pernambuco state (PE) is considered the third common bean producer. Previous studies of isolates characterization from PE allowed to identify the 7, 23, 81, 87 and 119 races of *C. lindemuthianum* (Alzate-Marin and Sartorato 2004). In recent studies 14 new races were identified: 2, 8, 9, 10, 64, 65, 72, 73, 81, 85, 89, 117, 139 and 331 (unpublished data). To overcome the vast virulence diversity of *C. lindemuthianum* it is required that bean scientists continually broaden the genetic base of the bean crop by identifying new resistance sources and to incorporate new anthracnose resistance genes (Schwartz et al. 1982). The objective of this study was to identify common bean landraces from Pernambuco State for their resistance to different races of *C. lindemuthianum* present in Brazil.

MATERIAL AND METHODS

Twenty common bean landraces collected in the Pernambuco state and kept in the Germplasm Bank of the Instituto Agronômico de Pernambuco (Figure 1), were evaluated with races 8, 73, 89, 139 and 331 of *C. lindemuthianum*. These landraces belong to the following brazilian market class: Branco, Carioca, Jalo, Mulatinho, Preto, Rosinha, Rosa and others (Jalinho, Vermelho, Rajado, Pintado, Enxofre and Pardo). The virulence phenotype of each monosporic isolate was confirmed by inoculating the standard set of 12 differential cultivars. Fourteen-days-old bean seedlings were sprayed with a concentration of 1.2×10^6 conidia mL^{-1}. The plants were incubated and maintained in a mist chamber for 7 to 10 days, 20 ± 2°C and 90-100% relative humidity. Ten days after inoculation, the plants were scored as resistant (R) or susceptible (S) as described by (Pastor-Corrales et al., 1995), where 1 to 3 = resistant and 4 to 9 = susceptible. A pathogenicity index (PI), previously developed by Balardin et al. (1997) for each *C. lindemuthianum* race was computed by dividing the number of bean landrace genotypes with a susceptible reaction by 20 (the total number of landrace genotypes of bean in this study). While, the resistance index (RI), was calculated by dividing the number of landrace bean cultivars that revealed a resistant reaction by 5, the total number of races of *C. lindemuthianum* used for inoculation in this study (Balardin et al. 1997.

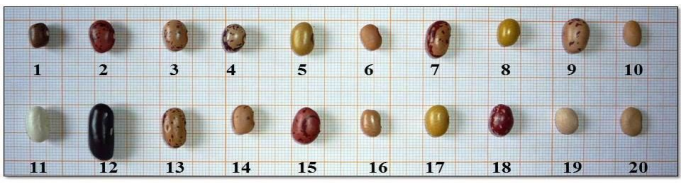

Figure 1. Landraces of common bean from the Germplasm Bank of the Instituto Agronômico de Pernambuco. 1- Brígida; 2- Cocão; 3- Bagajó; 4- Favita; 5- Canarinho; 6- Rosinha Claro; 7- Chita Fina Verdadeira; 8- Jaula; 9- Pintado; 10- Balinha; 11- Praia; 12- Camarão; 13- BSF-1; 14- BSF-2 (Pingo de Ouro); 15- BSF-3 (Fogo na Serra); 16- Brilhoso; 17- Enxofre; 18- Africano 4; 19- IPA 1; 20- IPA 7.

RESULTS AND DISCUSSION

The pathogenicity index (PI) of the *C. lindemuthianum* races utilized in this study ranged from 20 to 75% (Table 1). Race 89 was the most pathogenic with PI 75%, while race 8, 73 and 331 were least pathogenic, with PI values of values of 20, 20 and 25%, respectively (Table 1). The resistance index (RI) of the common bean landraces ranged from 0.0 to 100%, and the RI of the Mesoamerican bean genotypes ranged from 17 to 83% (Table 1). The most resistant landraces were Balinha, Praia and Africano 4, while the more susceptible were IPA 7 and BSF-2 (Table 1).

Table 1 Reaction of 20 common bean landraces from Pernambuco State, Brazil, and the pathogenicity index to the Mesoamerican races of *C. lindemuthianum*

	Landraces	Brazilian Market Class	Reaction to Races[a]					Resistance index(%)
			8	73	89	139	331	
1	Brígida	Carioca	S	S	S	R	S	20
2	Cocão	Diversos	R	R	S	R	R	80
3	Bagajó	Diversos	R	R	S	R	R	80
4	Favita	Diversos	R	R	S	S	R	60
5	Canarinho	Diversos	R	R	R	S	R	80
6	Rosinha Claro	Rosinha	R	R	S	S	R	60
7	Chita Fina verdadeira	Diversos	R	S	S	R	S	40
8	Jaula	Diversos	R	R	S	R	R	80
9	Pintado	Diversos	R	R	S	R	R	80
10	Balinha	Mulatinho	R	R	R	R	R	100
11	Praia	Branco	R	R	R	R	R	100
12	Camarão	Preto	R	R	S	S	R	60
13	BSF-1[b]	Diversos	R	R	S	S	R	60
14	BSF-2 (Pingo de ouro)	Carioca	S	S	S	S	S	0.0
15	BSF-3 (Fogo na serra)	Diversos	R	R	S	R	R	80
16	Brilhoso	Mulatinho	R	R	S	S	R	60
17	Enxofre	Diversos	R	R	R	S	R	80
18	Africano 4	Diversos	R	R	R	R	R	100
19	IPA 1	Mulatinho	S	R	S	S	S	20
20	IPA 7	Mulatinho	S	S	S	S	S	0.0
		Pathogenicity index (%)	20	20	75	50	25	

[a]Resistant (R); Susceptible (S); [b]BSF – Belém de São Francisco.

The results show that landraces of common bean from Pernambuco state evaluated are genetically highly variable in response to different races of *C. lindemuthianum*. Some of this genetic material would be valuable in future common bean breeding programs as new sources of resistance to anthracnose.

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GENETIC ANALYSIS OF YIELD COMPONENTS IN *PHASEOLUS VULGARIS L.* UNDER DROUGHT STRESS

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INTRODUCTION

The drought stress due to occurrence and irregular distribution of rainfall and/or inadequate supply of irrigation, has become one of the main reduction the common bean productivity (Acosta-Gallegos and Kelly, 2012; Asfaw et al., 2012). In this context, the common bean crop requires basic research with low investment and time in order to contribute to the solution of this problem (Beebe et al., 2013). Thus, strategies such as the selection of lines and the knowledge of the genetic control of yield components and the production of common bean subjected to drought are necessary, since they increase the chance of success in breeding programs. Therefore, this work had as objective to evaluate the genetic control of grain yield and its primary components in F_1, F_2, BC_1 and BC_2 and parental plants from IAPAR 81 (drought tolerant) × LP 97-28 (low drought tolerance) under water deficit conditions.

MATERIAL AND METHODS

The experiment was conducted under greenhouse conditions at Núcleo de Pesquisa Aplicada à Agricultura (Nupagri), Universidade Estadual de Maringá. Water deficit was induced over the course of the experiment by withholding irrigation at three different stages of plant growth and development: V_3, R_6 and R_8. Each period of water deficit lasted 96 hours (Figure 1). After each period of induced water deficit, plants were then irrigated normally for a period of 20 days.

Figure 1- Morphological response to water deficit in common bean (*Phaseolus vulgaris* L.).

In stage R_9, the six populations (P_1, P_2, F_1, F_2, BC_1 and BC_2) were evaluated for the following characteristics: number of pods per plant (NPP), number of seeds per pod (NSP), average weight 100 seeds (M100s, g plant^{-1}) and grain yield production (PROD, g plant^{-1}). The genetic statistical analyses were performed to estimate the average and variance for each population and the additive, dominance and environment variance components using the software Genes (Cruz, 2013). The broad and narrow sense heritability, number of genes and selection gain prediction were estimated.

RESULTS AND DISCUSSION

Variance components estimates revealed a high contribution of additive genetic effects in all evaluated traits indicating the occurrence of additive allelic interaction (Table 1). Thus, heritability in broad and narrow sense provided evidence of efficient transmission of drought tolerance character. Usually, breeding methods that benefit from high additive variance to obtain genetic gains are more important in the improvement of autogamous species, among which stands out *P. vulgaris* (Gravina et al., 2004).

Table 1 displays the data of genetic gains, which magnitudes were of 25.2, 11.1, 32.7 and 42.7% for PROD, M100s, NPP and NSP, respectively. These results revealed satisfactory genetic gains conferred by additive genetic effects. Furthermore, selections of superior segregant in early generations have shown to be very efficient.

Table 1- Estimates of variances phenotypic, genotypic, additive, dominance and environmental; heritability broad and narrow sense; number of genes and prediction of selection gain in segregant populations of common bean

IAPAR 81 × LP 97-28	PROD	M100s	NPP	NSP
Phenotypic F_2 variance (σ_f^2)	8.3	8.4	11.5	0.6
Environmental variance (σ_e^2)	1.0	0.9	0.8	0.1
Genotypic variance (σ_g^2)	7.3	7.4	10.7	0.51
Additive variance (σ_a^2)	5.9	5.1	10.3	0.6
Dominance variance (σ_d^2)	1.5	2.3	0.33	0.0
Heritability broad ($H^2_{\%}$)	87.8	88.3	92.7	89.1
Heritability narrow ($h^2_{\%}$)	69.8	60.4	89.8	98.9
Number of genes (n)	5	5	2	2
Selection gain prediction				
Gain from selection (gs)	25.2	11.1	32.7	42.7
Average cycle 1 (μ_{c1})	20.2	29.2	9.42	4.9

CONCLUSION

The results obtained in this study are of high importance, since the genetic gains to grain yield and its primary components in the selection cycles were satisfactory due to the fact of the addictive nature component is one of the most important. These results also reveal that the choice of contrasting parenting IAPAR 81 (drought tolerant) and LP 97-28 (low drought tolerance) were appropriate, showing the transmission of the character tolerance to drought.

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INTERACTION OF COMMON BEAN WITH *RHIZOCTONIA SOLANI* AND *TRICHODERMA* SPP.

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INTRODUCTION

Spain together with Italy and Greece are the main common bean producers. León, a province located at the northwest of Spain, is the main producer province by quantity and quality, with almost 45% of Spanish production in 2014. In the last years, however dry bean production has gone through difficulties due to relatively low yields (mainly caused by fungus, virus, and bacteria) and insufficient income for growers.

The cultivation of the bean is affected by numerous fungal infections, one of the most frequent "Rhizoctonia disease" caused by *Rhizoctonia solani* JG Kühn. (Teleomorph: *Thanatephorus cucumeris*). *Trichoderma* spp. is a secondary opportunistic invasive, fast-growing, which it produces large numbers of spores, enzymes capable of degrading cell wall and antibiotic substances. Many *Trichoderma* species are well-known for their ability to promote plant growth and defence. The aims were study how the interaction of bean plants with *R. solani* and/or *Trichoderma* affect bean plants.

MATERIALS AND METHODS

The present study was conducted with twenty-three isolates of *Trichoderma* and one isolate of *Rhizoctonia solani* collected from the production area of the Protected Geographical Indication (PGI), called "Alubia La Bañeza - León", without any genetic manipulation and three isolates from other collections.

***In vitro* antifungal assays.** *Trichoderma* isolates were evaluated for their *in vitro* potential to antagonize the plant pathogenic fungus *R. solani* using two different tests: direct confrontation and assay on membranes. The procedure was performed as previously described (Mayo et al., 2015).

***In vivo* assay of the antifungal activity.** The bioassays were performed in climatic chambers with 15 *in vitro* selected *Trichoderma* isolates. The procedure was performed as previously described (Mayo et al., 2015).

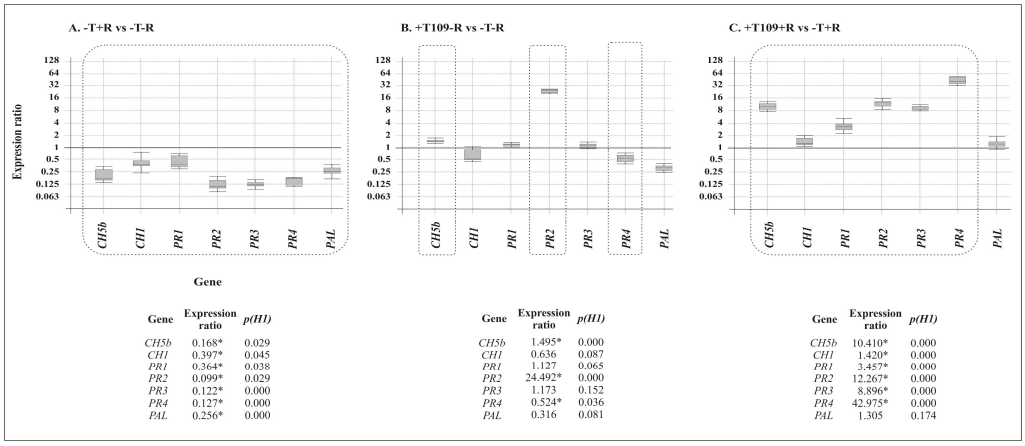
Analysis of expression of bean defence-related genes. Three bean leaves from 45 day-old plants of each treatment were randomly collected and they were detached from plants inoculated with *Trichoderma* isolate showing positive phenotypic results in the *in vivo* test. The procedure was performed as previously described (Mayo et al., 2015).

Quantification of ergosterol and squalene. Total intracellular sterols of the *Trichoderma* selected strain were extracted and ergosterol and squalene content were quantified as previously described (Mayo et al., 2015).

RESULTS AND DISCUSSION

Trichoderma isolates inhibited *R. solani* growth by more than 75%, were T003, T004, T006, T020, T022, T012, T013, T025, T016, T007, T024, T005 and T010 and T019 in membrane

assays. T021 was the *Trichoderma* isolate showing the highest percentage of inhibition (72.77%) in the direct confrontation assays, whereas T009 showed the lowest inhibition values (14.63%).
T. harzianum T019 shows a positive effect on the level of resistance of bean plants to *R. solani*. This strain induces the expression of plant defence-related genes and produces a higher level of ergosterol, indicating its ability to grow at a higher rate in the soil, which would explain its positive effects on plant growth and defence in the presence of the pathogen.
An increased production of ergosterol and squalene by *Trichoderma* resulted in the induction of defence genes in the bean plants. In this way, the plant would grow better under a pathogen presence in the soil.



Gene	Expression ratio	p(H1)
CH5b	0.168*	0.029
CH1	0.397*	0.045
PR1	0.364*	0.038
PR2	0.099*	0.029
PR3	0.122*	0.000
PR4	0.127*	0.000
PAL	0.256*	0.000

Gene	Expression ratio	p(H1)
CH5b	1.495*	0.000
CH1	0.636	0.087
PR1	1.127	0.065
PR2	24.492*	0.000
PR3	1.173	0.152
PR4	0.524*	0.036
PAL	0.316	0.081

Gene	Expression ratio	p(H1)
CH5b	10.410*	0.000
CH1	1.420*	0.000
PR1	3.457*	0.000
PR2	12.267*	0.000
PR3	8.896*	0.000
PR4	42.975*	0.000
PAL	1.305	0.174

Fig. 4: Expression of *CH5b, CH1, PR1, PR2*, *PR3, PR4* and *PAL* genes in comparison with *α-actin* and *EF1α* reference genes. Comparison of the gene expression of the bean defence-related genes A) in plants infected with *R. solani* R43 *versus* control plants. B) In plants treated with *Trichoderma* T019 *versus* control plants C) In plants infected with *R. solani* R43 and treated with *Trichoderma* T019 *versus* plants infected with *R. solani* R43. (Mayo et al., 2015)

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GENOTYPIC VARIATION FOR TOLERANCE TO LOW SOIL PHOSPHOROUS IN COMMON BEAN UNDER CONTROLLED GREENHOUSE CONDITIONS

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INTRODUCTION

Phosphorus deficiency limits crop yield. The two major components of P efficiency are P acquisition efficiency and P use efficiency. Phosphorus acquisition efficiency refers to the plant's ability to acquire greater amounts of P per unit root length, whereas P use efficiency refers to the plant's ability to produce yield per unit of acquired P from soil (Beebe, et al., 2006). With a given P supply in soil, P acquisition per plant might be improved with a root system that provides greater contact with P and with greater uptake per unit of root due to enhanced uptake mechanisms, and with an ability to use insoluble organic or inorganic P forms that are relatively unavailable or poorly available to plants (Clark and Duncan, 1991). There is a need to identify varieties tolerant to low P mineral in order to reduce cost of production to farmers. Therefore this project aims: to determine the effect of phosphorus levels on vegetative plant growth and to evaluate genotypic variability in terms of phosphorus uptake under limiting soil phosphorus.

MATERIALS AND METHODS

The experiment was conducted in the glasshouse at USDA-Prosser WA, following a randomized completely block design. Eight Andean genotypes, representing parents of RIL populations, were grown in three mono-ammonium phosphate (MAP) fertilizer levels applied at planting time at a rate of 0kg P ha^{-1} (0ppm), 50kg P ha^{-1} (22.5ppm) and 100kg P ha^{-1} (45ppm). The first set of experiment with 4 replications, 8 bean genotypes and 3 fertilizer levels was harvested at flowering maturity. The second set of experiment with the same number of treatments was harvested at seed maturity. In total 192 plants were planted for this experiment. The experiment was repeated three times to confirm the results. Shoot and root dry weights were collected at flowering maturity, tissues were dried for 48hrs at 75 °C. Seed yield were collected at harvest maturity. To determine internal P concentration (ppm) for shoot, root and seed all samples were ground to pass 1 mm sieve using a large Wiley mill and analyzed by ICP nitric acid digest method (Isaac and Johnson, 1975). Calculations for P susceptibility index (PSI) and P use efficiency (PUE) were done. Statistical analysis of phenotypic traits measured was conducted with SAS 9.4 software. Analysis of variance and correlation among variables was performed.

RESULTS

For all varieties, shoot biomass was lowest when no P was added. G122, Cardinal and Rojo were consistently more susceptible to P deficiency as indicated by significantly lower shoot dry weight in no P treatment. The PSI calculations for each variety using shoot and root dry weight are shown in Table 2.

The results confirm Tukey comparisons of mean (Fig. 1) that G122, Cardinal and Rojo have higher PSI values above 1.1 suggesting that they might also be susceptible to P deficiency. All other varieties have PSI values between 0 – < 1.0 indicating moderate tolerance to P deficiency.

There is significant variation ($P \leq 0.05$) between Montcalm and G122 means for PUE (Table 3), but all other genotypes showed variations which were not statistically significant. Montcalm, Bukoba, and Kijivu exhibited high internal P use efficiency in all phosphorus treatments. A high

PUE score reflects more shoot biomass obtained by remobilizing a higher portion of internal P. When no P is added, G122, CAL143 and Cardinal have the lowest PUE scores. Shoot: root ratio was expected to be lower in no P treatment and increase with higher P levels. Bukoba and Rojo have higher shoot: root ratio at no P treatment (data not presented) suggesting that they were more efficient in P utilization. The significant correlations ($P \leq 0.05$) for shoot and root biomass production ($r = 0.80$), and shoot internal P and root internal P ($r = 0.83$) suggest that shoot and root biomass production are related for beans grown in P deficient soils. Significant ($P \leq 0.05$) but negative correlations were observed for PSI shoot and shoot dry weight and PSI root and root dry weight. This was expected because lower PSI value indicates a higher tolerance to P-deficiency.

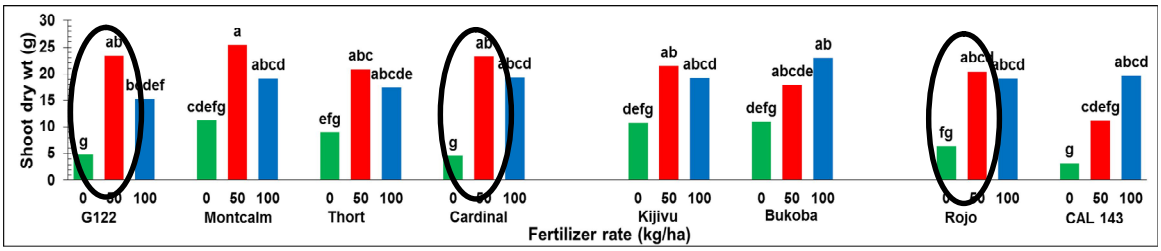

Fig. 1. Shoot biomass in the first experiment for eight bean varieties grown in sunshine media with three different phosphorus (P) treatments.

Table 2: PSI calculations using lsmeans from three experiments

Variety	Shoot dry weight	Root dry weight
G122	1.63	1.83
Cardinal	1.19	0.60
Rojo	0.96	1.46
Thort	0.94	0.91
CAL143	0.92	0.94
Montcalm	0.89	0.75
Kijivu	0.85	0.75
Bukoba	0.49	0.71

Table 3: PUE for all varieties with different P treatments using lsmeans from three experiments

Genotype	P 0 kg/ha	P 50 kg/ha	P 100kg/ha
Montcalm	0.12	0.10	0.06
Bukoba	0.12	0.07	0.06
Kijivu	0.10	0.08	0.05
Rojo	0.08	0.07	0.05
Thort	0.07	0.06	0.04
Cardinal	0.06	0.06	0.05
CAL143	0.05	0.05	0.05
G122	0.04	0.08	0.05

DISCUSSION AND CONCLUSIONS

When no P is added, susceptible varieties like G122 have the lowest PUE scores while Bukoba and Montcalm have high PUE indicating they may be displaying tolerance via high use of their internal P. Low PSI values for root and shoot biomass (Table 2) were a good indicator that Bukoba and Kijivu were less susceptible to P deficiency. Higher shoot:root ratio is an indication that some varieties responded to the low P treatment by increasing root growth.

Bukoba exhibits good Phosphorus use efficiency with high root:shoot ratio and low PSI values for both shoot and root dry weight. Such characteristics suggest Bukoba could be useful for breeding elite bean varieties tolerant to soil P deficiency in the future.

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RECURRENT SELECTION TO DEVELOPMENT OF CARIOCA TYPE COMMON BEANS CULTIVARS AT IAPAR

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INTRODUCTION

Brazil is the main world producer and consumer of common beans (*Phaseolus vulgaris* L.). The annual area cultivated is around 3,366 million hectares with an approximated production of 3,454 million tons and the estimated consumption of 17kg per capita per year (Conab, 2015). Among the various types of common beans consumed in Brazil, carioca beans represent about 67% of the total production, corresponding to about 2,314 million tons per year. In the development of new autogamous cultivars pedigree, bulk or SSD method and their variants are usually is used in a population often obtained by biparental crosses. The major limitations of these methodologies are low genetic variability and low probability of genetic recombination occurrence, reducing the genetic gain. The recurrent selection (RS) has been used as a way to overcome these but also to broaden the genetic base of cultivars. This work aimed to develop new carioca type common beans cultivars with high yield potential, production stability and higher technological and nutritional grain characteristics.

MATERIAL AND METHODS

Eight parents were selected, based on the yield potential, resistance to main diseases, tolerance to edaphoclimatic stress, plant architecture, technological and nutritional quality of grains and genetic divergence. They were intercrossed in a partial diallel scheme, to generate the base population (C_0S_0). This population was conducted by single pod descent method (SPD) until to generation (C_0S_3) in which individual plants were selected and their progeny (C_0S_4) conducted by the pedigree method. In this generation, 300 progenies (C_0S_5) were selected and evaluated in augmented Federer's blocks design, and the 20 promising progenies were selected and coded with the LP abbreviation followed by the year of acquisition and the sequence number. These progenies were recombined using circulating crosses, in which the population (C_1S_0) was used to initiate the second cycle in which the previous steps were repeated.

RESULTS AND DISCUSSION

A Recurrent selection program was carried out at IAPAR in 1998 and until now has been completed five cycles of RS. During these cycles was obtained two new cultivars named IPR Tangará and IPR Quero-quero, five superior lines, LP09-33, LP09-34, LP09-40, LP09-41 and LP09-47, which are in the genetic seed production phase and twelve lines coded as LP13-, that are being evaluated in the test VCU. The cultivars IPR Tangará and IPR Quero-quero were registered by the Ministry of Agriculture, Livestock and Supply for cultivation in five Brazilian states. IPR Tangará (breeding line LP02-02) was obtained in the first cycle of RS, presenting resistance to rust (*Uromyces appendiculatus*), moderate resistance to angular leaf spot (*Phaeoisariopsis griseola*), curtobacterium (*Curtobacterium flaccumfaciens* pv. *Flaccumfaciens)* and powdery mildew (*Erysiphe polygoni*) and susceptibility to anthracnose (*Colletotrichum lindemuthianum)* and common bacterial blight (*Xanthomonas axonopodis* pv. *Phaseoli).* It has 3,326 kg/ha of yield potential, excellent grain quality and intermediate drought and heat tolerance (Table 1). IPR Quero-quero (breeding line LP07-80), from the second cycle, has shown

better plant health, it presents resistance or moderate resistance to the important pathogens diseases, reduced cooking time and higher iron content in the grain (9mg.100g^{-1}). It presents a yield potential of 4,425kg.ha^{-1}, exceeding in 14% the cultivar Perola, (Table 1). SR uses provided gain in yield, plant health, technological and nutritional quality of grains and increased genetic variability.

Table 1. Average grain yield (kg.ha^{-1}) of common beans IPR Tangará and IPR Quero-quero cultivars compared to the control cultivars and Relative Yield (RY%) in trials of Value for Cultivation and Used (VCU) conducted in the states of Paraná (PR), Santa Catarina (SC), São Paulo (SP) and Rio Grande do Sul (RS), in three growing seasons.

Trial	Cultivars	Seasons			Overall	RY (%)
		Rainy	**Dry**	**Autumn-Winter**	**Average (kg.ha^{-1})**	
VCU – PR	IPR Tangará	1,906	2,105	-	2,005	104.8
	Carioca	1,863	1,968	-	1,916	100.2
	Iapar 81	1,731	1,843	-	1,787	93.4
	IPR Juriti	2,035	2,034	-	2,035	106.4
VCU – SC	IPR Tangará	3,468	2,500	-	2,984	112.4
	Perola	3,289	2,275	-	2,782	104.7
	SCS 202 Guará	3,370	2,119	-	2,744	103.4
	IAC Alvorada	3,036	1,840	-	2,438	91.8
VCU – SP	IPR Tangará	2,705	2,355	3,074	2,712	109.8
	IAC Alvorada	2,315	1,885	3,041	2,414	97.7
	Pérola	2,670	2,092	2,811	2,524	102.2
VCU – PR	IPR Quero-quero	2,378	2,300	-	2,339	111.8
	IPR Siriri	2,100	2,191	-	2,146	102.6
	IAPAR 81	2,101	2,033	-	2,067	98.6
	Carioca	2,166	1,959	-	2,062	98.6
VCU – SC	IPR Quero-quero	3,621	2,703	-	3,162	115.5
	SCS 202 Guará	3,317	2,177	-	2,747	100.4
	Pérola	3,247	2,205	-	2,726	99.6
VCU – RS	IPR Quero-quero	2,477	1,891	-	2,184	108.3
	Carioca	2,494	1,699	-	2,097	104.0
	Pérola	2,357	1,514	-	1,936	96.0
VCU – SP	IPR Quero-quero	3,554	3,983	1,655	3,064	115.4
	IPR Juriti	3,104	3,326	1,428	2,619	98.6
	IAC Alvorada	3,296	3,103	1,676	2,692	101.4

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ESTABLISHING IN VITRO REGENERATION TECHNOLOGY FOR COMMON BEAN (*PHASEOLUS VULGARIS*) VARIETIES

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INTRODUCTION

Herbicides are widely used in common bean (*Phaseolus vulgaris*) fields to control weeds. However, herbicide damage to common bean crop is also a concern. This research aims to develop common beans that are genetically resistant to herbicide Pursuit via genome editing approach. The first phase of the research is to develop *in vitro* regeneration system. A large number of common bean varieties representing different backgrounds were evaluated for regeneration using cotyledons and embryonic axes as explants. The regeneration system established will be used for genome editing to develop herbicide resistance in common bean.

MATERIALS AND METHODS

To date, 27 varieties belonging to ten market classes have been tested in tissue culture experiments (Table 1) following a modified protocol [1]. The seeds were germinated for 5-7 days instead of 3 days [1]. The tissues used in our experiments were different from the published protocol. Following germination, the seeds were dissected into several different tissues: cotyledon and embryonic axis (Figure 1). The cotyledons were placed on callus induction medium (CIM) either in the abaxial or the adaxial orientation. The embryonic axis or hypocotyl was cut into three 2 to 3-mm transverse sections which were placed onto another CIM plate. All explants were kept on CIM in the dark for the first seven days and later were placed under light, following Collado *et al.* [1] protocol. After three weeks on CIM, calli were removed from the explants and, if green, were placed on callus proliferation medium (CPM) for another three weeks [1]. Shoots were allowed to regenerate on

Table 1. Common bean varieties used in the experiments to date, their respective market classes, and abbreviations (used in this report).

Market class	Variety	Abbreviation
Black	Carman Black	CB
	ACC Black Diamond 2	BD2
	Zorro	ZRO
	Ica Pijao	IPJ
Cranberry	Cardinal	CRL
Great Northern	AAC Tundra	ATN
	AAC Whitehorse	AWH
	Matterhorn	MTH
Navy	Portage	PR
	OAC Rex	ORX
	BAT93	BAT
	Mist	MST
	PI-440795	PI
Pink	Sedona	SDN
Pinto	AC Pinta	APN
	AAC Burdett	ABR
	Island	ISL
	Olathe	OLT
Red Kidney	Montcalm	MTM
	Red Hawk	RHK
Small red	AC Earlired	AER
	Merlot	MRL
	JALO-EEP558	JAL
Yellow bean	L11YL012	L12
	L11YL015	L15
Mixture	XAN-159	XAN
Andean	G19833	G19

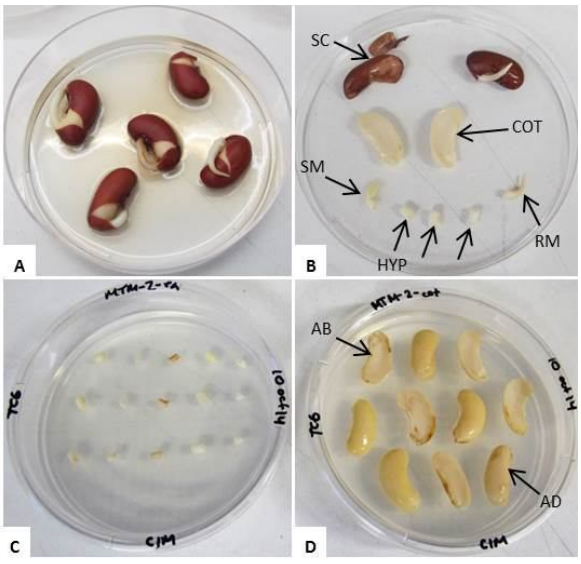

Figure 1. Dissection strategy for the tissue culture protocol. A Seeds were germinated on GM. B The seed coat (SC) and cotyledons (COT) were removed. The shoot (SM) and root meristems (RM) were excised. The remaining embryonic axis/hypocotyl (HYP) was cut into three transverse sections. C The hypocotyl sections were placed onto CIM. D The cotyledons were placed onto CIM either in the adaxial (AD) or the abaxial (AB) orientation.

shoot regeneration medium (SRM) for at least three weeks, longer if necessary, and then were transferred to shoot elongation and rooting media (SERM) [1]. When shoots were more than 3 cm in length, they were transferred to pots with ProMix BX potting mix and kept in a growth chamber until they set seed.

RESULTS

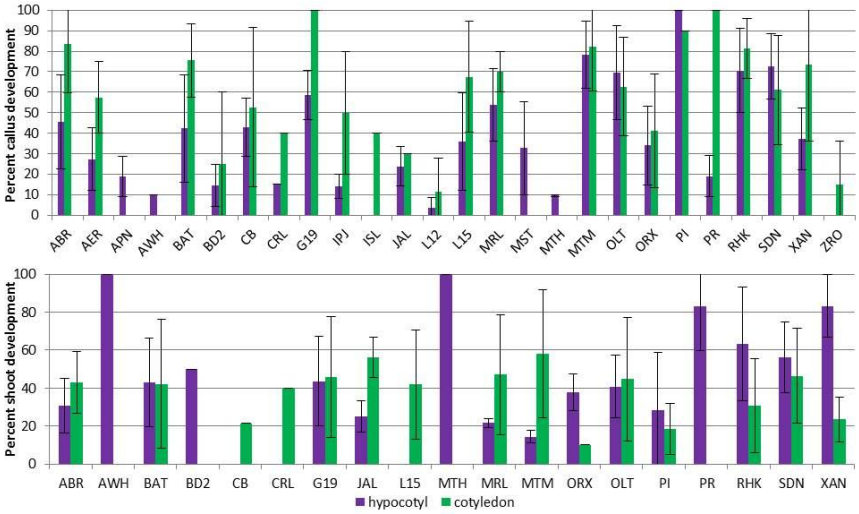

Figure 2. Percent of callus development (top) and percent of shoot development (bottom) in each variety for each of the tissues tested: embryonic axis/hypocotyl (purple) and cotyledon (green).

Twenty-seven bean varieties were investigated for callus and shoot development. Twenty-six out of the 27 varieties showed various degrees of callus development (Figure 2). From these, 19 varieties showed some degree of shoot development (Figure 2) and four varieties further produced growing shoots (Table 2). Twenty-two or 81% of the varieties tested developed calli from cotyledon tissues, while twenty-four or 89% produced calli from embryonic axis or hypocotyl tissues. Further, fifteen (56%) or sixteen (59%) of the varieties tested produced shoots from either cotyledon or embryonic axis tissues, respectively. While many varieties developed shoots, roots developed in smaller number of varieties. Only four out of the 27 varieties regenerated into plants. These included AAC Burdett, Olathe, Montcalm, and Red Hawk. Plants from the latter variety were regenerated from hypocotyl explants and did not survive the transfer to soil. Plants were successfully regenerated from cotyledon explants for the other three varieties and produced mature plants from which seeds were collected.

SUMMARY

The regeneration ability of common bean varieties appears to be largely genotype-dependent. From the 27 varieties tested, AAC Burdett and Olathe (Pinto) and Montcalm and Red Hawk (Red Kidney) show the best potential for shoot regeneration using the current protocol. Further plant growth will be evaluated to determine whether the produced seeds will prove to be viable. After further optimizing the regeneration technology developed, we will conduct genome editing to mutate the targeted plant endogenous gene(s) to develop herbicide resistant common beans.

Variety	Number of shoots per callus	Explant type
AAC Burdett	3.29 ± 2.33	Cotyledon
Olathe	2.06 ± 0.70	Cotyledon
Montcalm	1.62 ± 0.54	Cotyledon
Red Hawk	2.0 ± 1.0	Hypocotyl

Table 2. Shoot regeneration from calli in two Pinto (AAC Burdett and Olathe) and two Red Kidney (Montcalm and Red Hawk) bean varieties.

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AN *IN SILICO* ANALYSIS OF PHENYLPROPANOID PATHWAY GENE FAMILIES IN COMMON BEAN: CYTOCHROME P450

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INTRODUCTION

Cytochrome P450 (CYPs) are ubiquitous monooxygenase enzymes involved in the oxidation of various substrates using oxygen and NADPH. The focus of this study was P450 gene families encoding enzymes of the phenylpropanoid pathway in common bean.

MATERIALS AND METHODS

The availability of the complete genome sequences enabled a thorough inventory of putative P450 genes encoding key enzymes of this metabolic pathway. The P450 gene sequences from common bean were compared to homologs from Arabidopsis and soybean (which is highly syntenic to bean) and confirmed with the information published for both soybean and common bean genomes. The expression of these genes in different tissues and syntenic relationships between common bean and soybean were examined. Finally, we compared genomes of G19833 (Andean) and OAC Rex (Mesoamerican) by aligning their sequences and searching for polymorphisms in phenylpropanoid pathway P450s – focusing on CYP73A family.

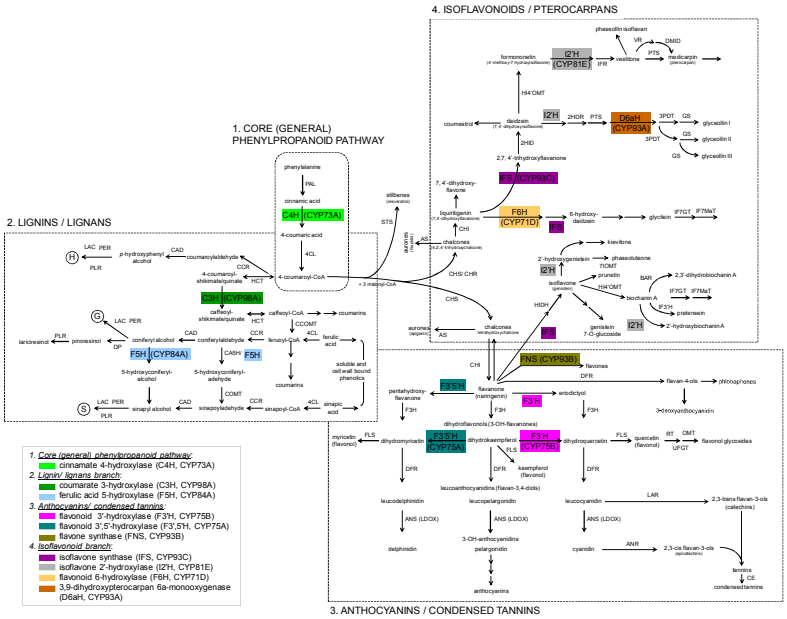

Figure 1. The positions of ten P450s in the phenylpropanoid pathway.

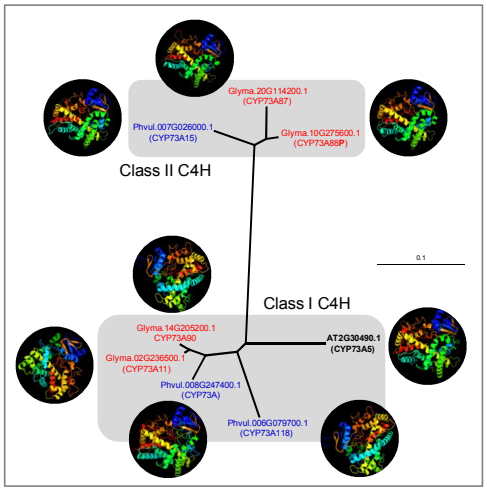

Figure 2. Comparison of C4H proteins from common bean, soybean and Arabidopsis.

RESULTS

Cytochrome P450, clan CYP71 gene families encode enzymes that catalyze various reactions in different branches of the phenylpropanoid pathway (Fig. 1).

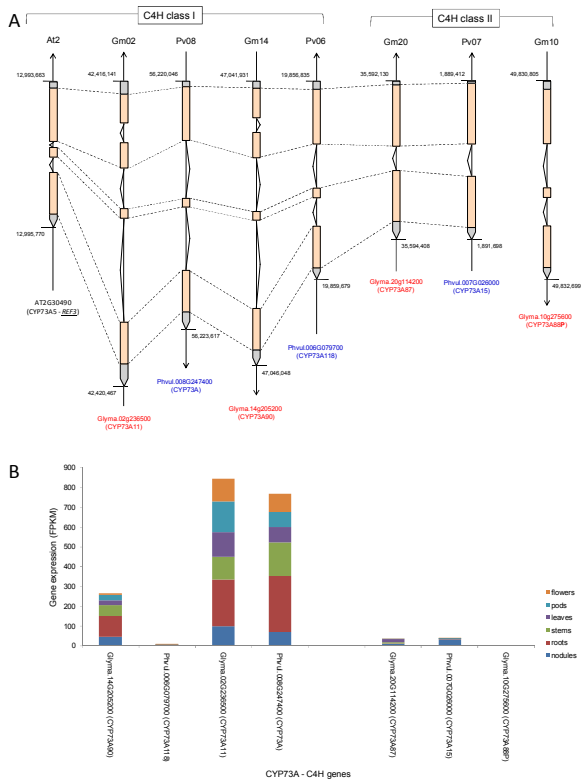

Figure 3. C4H gene structure (A) and FPKM (Fragments Per Kilobase of transcript per Million fragments mapped)-based relative expression levels (B)

Cinnamate 4-hydroxylase (C4H, EC:1.14.13.11, CYP73A) is the first P450 enzyme in the phenylpropanoid pathway. It is an ER membrane-bound P450, and belongs to the family of oxidoreductases that act on paired donors with incorporation of molecular oxygen. The enzyme catalyzes region-specific hydroxylation of the aromatic ring of *trans*-cinnamic acid to produce *p*-coumaric acid, a precursor for many phenylpropanoids including: flavonoids, phytoallexins and monolignols. A phylogenetic tree separated the sequences into class I and Class II C4H proteins (Fig. 2). C4H proteins are highly similar (85% - 98% within five C4H class I proteins and 90% between two class II C4H proteins). All proteins contain identical (PPGP) proline-rich regions and conserved motifs including: a heme-binding region (FxxGxRxCxG), a PERF motif (PERF/W), K helix (KETRL) and a I helix region (AGxDT). In addition, all proteins have secondary structures (Phyre2 available at http://www.sbg.bio.ic.ac.uk/phyre2/).) similar to the previously published P450s. No clear differences between class I and class II enzymes have been reported.

C4H is encoded by the relatively small *CYP73A* gene family consisting of three genes in common bean (Phvul.006g079700, Phvul.007g026000 and Phvul.008g247400), four genes (including one pseudogene, Glyma.10g275600) in soybean and a single gene in Arabidopsis (At2g30490; CYP73A5; *REF3*). Figure 3 (A) shows C4H gene structure. The gene is well conserved in plants, including soybean and common bean, with well conserved exons and more variable introns. Class I and class II C4H genes have different exon/intron structure. Based on RNA data (https://phytozome.jgi.doe.gov/pz/portal.html), C4H genes were differentially expressed in six common bean and soybean tissues (Figure 3 (B). In general, the expression of the genes encoding class I C4H enzymes were higher in all tissues, compared to those encoding class II enzymes. Sixty nine *cis*-regulatory elements were predicted in the 5'UTR regions of the C4H genes using PlantCARE database (http://bioinformatics.psb.ugent.be/ webtools/plantcare/html/); 26 (38%) were unique to class I C4H and 11 (16%) were specific to class II C4H (not shown). The identification of the *cis*-acting sequences regulating differential expression of C4H genes and transcription factors that interact with these sequences will lead to the understanding of the mechanism(s) of differential regulation of these highly similar genes.

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GENETIC ANALYSIS OF SEED HARDNESS TRAIT IN A BLACK BEAN RECOMBINANT INBRED LINE (RIL) POPULATION

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INTRODUCTION

Seed hardness in dry beans (*Phaseolous vulgaris*) refers to the inability of the seeds to imbibe sufficiently when soaked in water for a reasonable period of time. Differences in seed hardness in bean varieties can affect their cooking time, nutritional quality and hydration process during canning. Plant breeders often target this trait for improvement during variety development, even though the genetic factors controlling seed hardness are not yet fully understood (Konzen and Tsai 2014; Kinyanjui et al. 2015; Sánchez-Arteaga et al. 2015). The purpose of the current research is to identify the quantitative trait loci (QTL) that are associated with seed hardness in dry bean. For this purpose, we have developed a recombinant inbred line (RIL) population from a cross between H68-4 (black bean, very high percentage of stone seeds) with BK04-001 (black bean, no or very low percentage of stone seeds).

MATERIAL AND METHODS

The recombinant inbred line ($F_{2:8}$) population was derived through single seed decent approach from a cross between hard and soft seeded black bean parents (H68-4 and BK04-001, respectively). In year 2014, 131 RILs and parents were planted in 5 m single rows at two locations in southern Manitoba. Three replications of each line were grown in a randomized block design. Seed hardness trait was phenotyped through overnight soaking and a lab cracking assay. For hydration assay, 100 seeds were soaked in water for 16 h. Seeds were weighed before and after the soaking. Hydration capacity was calculated as percentage weight change based on the hydrated- and dry-seed weights (change in seed wt. after soaking/dry seed wt. x 100). At the end of 16 h soaking, un-hydrated seeds were counted and weighed to calculate the stone seed percentage. For seed cracking assay, 20 seeds per replication and six replications per RIL were used. Intact seeds (no visible cracks) were selected and shaken in Qiagen TissueLyserII (Valencia, CA) for a pre-determined speed and time. The rate of seed coat cracking was calculated based on the number of cracked seeds count.

RESULTS AND DISCUSSION

Seeds of 134 lines (parents, check and RILs) from Morden location were used for the hydration test. The distribution of lines is shown in Fig. 1a and 1b. In 2014, hydration capacity ranged from 51.2% to 171.9% in the RIL population. Also significantly, the rate of stone seed ranged from 0% to 63.3%, and the rate of seed coat cracking in the lab assay ranged from 5% to 62.5% (Fig. 2). As expected, the Pearson Correlation Coefficient between wt. change after hydration and seed coat cracking rate is moderately positive (r = 0.69). The Pearson Correlation Coefficient between wt. change after hydration and stone seed percentage is also moderately negative (r = -0.62) as expected.

We have observed that there is a loss of whole-bean-seed yield due to cracking during combine harvesting in the field. In order to determine that if careful variety selection can reduce this loss, we calculated the proportion of cracked seeds in combine harvested samples of all bean lines. The seed cracking due to combine showed positive but weak correlation (r = 0.45) with the

hydration values and lab cracking rates (r = 0.59). In future, RIL population will be further phenotyped and genotyped to identify genomic regions and markers associated with the seed hardness trait.

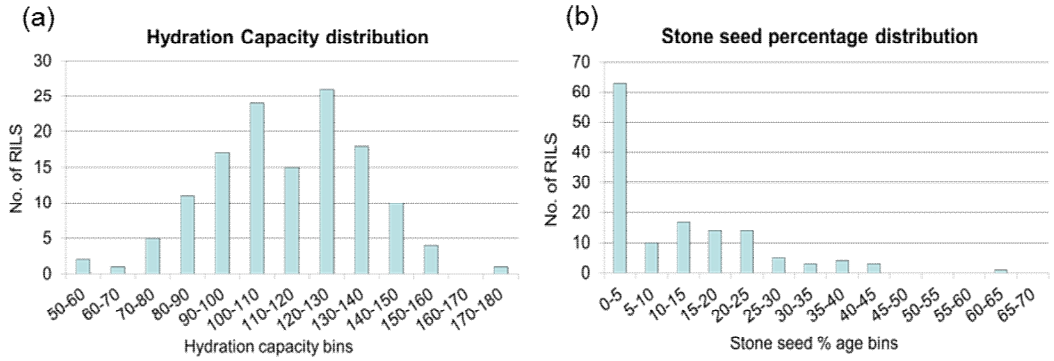

Fig. 1 (a) Distribution of 134 black bean lines for percent wt. change after hydration. The wt. change percentage values in the population ranged from 51.2% to 171.9%. The wt. change percentage values for parents H68-4 and BK04-001 were 92.9% and 124.2% respectively. (b) Distribution of 134 black bean lines for stone seed percentage. The range for stone percentage values in the population was from 0% to 63.3%. The stone seed percentage values for parents H68-4 and BK04-001 were 25.6% and 0.3% respectively.

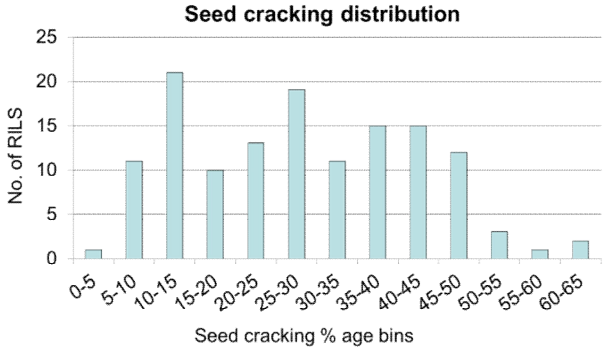

Fig. 2 Distribution of 134 black bean lines for rate of seed coat cracking. The range for cracking rate in the population was from 5% to 62.5%. The cracking rate values for parents H68-4 and BK04-001 were 17.5% and 45% respectively.

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MODIFIED SCREENING METHOD REVEALS PARTIAL ANTHRACNOSE RESISTANCE TO RACE 73 IN 16 GENOTYPES OF THE MESOAMERICAN DIVERSITY PANEL OF DRY BEANS

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INTRODUCTION: Anthracnose, caused by the fungal pathogen *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib., is one of the most devastating diseases in dry beans (*Phaseolus vulgaris* L.) with yield losses up to 100% under favorable conditions (Junaid *et al.* 2014). Plant disease resistance and tolerance mechanisms can be correlated to plant maturity and have been reported in several crops (Beebe and Pastor-Corrales 1991; Kolmer et al., 2015). Resistance to anthracnose is typically evaluated at the unifoliate stage and rated 7 days post-inoculation. The objective of this study was to evaluate resistance to *C. lindemuthianum* in the Mesoamerican Diversity Panel (MDP) at the trifoliate stage under greenhouse conditions.

METHODOLOGY: A total of 296 lines from the MDP, with three replicates of five samples per replication were screened in a randomized complete block design. Plants at the trifoliate leaf stage were inoculated with race 73 ($1.2x10^6$ spores/ml) and incubated in humidity chambers for five days (180sec/h humidity, 12h day/night cycle). Disease severity was evaluated on the trifoliate leaves and new plant growth 12 days post inoculation (Figure 1). Disease severity was rated as 1 = no symptoms, 2 = small lesions, and 3 = numerous enlarged lesions or sunken cankers on the lower sides of leaves or hypocotyls.
After filtering for minor allele frequency (MAF) of $\geq$ 5%, 15,000 SNP markers were obtained from a combination of two Illumina iSelect 6K Gene Chips (BARCBEAN6K_1 and BARCBEAN6K_2) (Song *et al.*, 2015) and genotyping by sequencing (GBS) data. The genotypic and phenotypic data was used to perform a Genome Wide Association Study (GWAS).

Figure 1: Anthracnose susceptible genotypes (left), resistant genotypes with known resistance genes (middle), and resistant genotypes with unknown resistance and CDC-Nordic (Co-1^5) (right), 12 dpi.

RESULTS AND CONCLUSION: Based on the rating of the trifoliate leaves and new plant growth, 30 lines were categorized as resistant, another 11 lines had small lesions and the remainder were susceptible. Interestingly, 16 of the 30 resistant lines do not contain reported anthracnose resistance genes (Table 1). These genotypes have been rated as susceptible in previous studies based on the evaluation of unifoliate leaves.

The 14 genotypes with known anthracnose resistance genes were excluded from GWAS analysis. The GWAS results indicated that SNP-markers associated with anthracnose resistance were found on Pv04 and Pv11 (Figure 2). Stepwise regression for all SNP markers below a 0.1% cut off revealed that the combination of four markers accounts for 25% of the variation in the best model (5PC-EMMA), whereas the marker with the highest -log10(p) contributes 13% of the variation.

Validation experiments are currently underway.

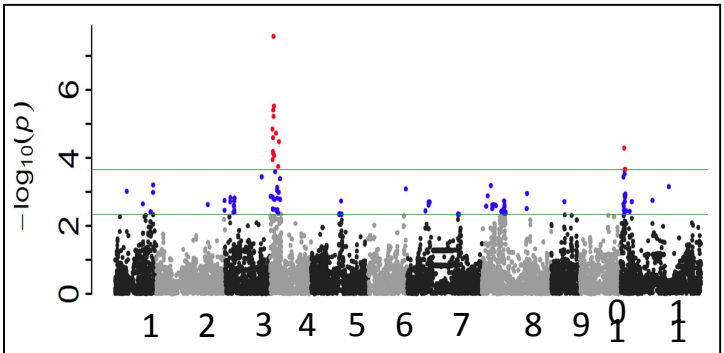

Figure 2: Manhattan plot of anthracnose resistance associated with Pv04 and Pv11 (lower line = 1% cut off, upper line = 0.1% cut off).

Genotype	Market Class	Resistance Gene
Grand Mesa	Pinto	NR
Agassiz	Pinto	NR
CDC Nordic	Great Northern	**Co-1^5**
CDC Pintium	Pinto	NR
CDCWM-2	Pinto	NR
Envoy	Navy	**Co-1^2 and Co-2**
GTS-900	Pinto	NR
Loreto	Black	**Co-1**
Mackinac	Navy	**Co-1**
Merlot	Red	NR
N05324	Navy	**Co-1**
Newport	Navy	**Co-1 and Co-2**
Raven	Black	**Co-1**
Sanilac	Navy	**Co-1**
UI-911	Black	NR
Maverick	Pinto	NR
Phantom	Black	**Co-1**
USPT-ANT-1	Pinto	**Co-4^2**
Jaguar	Black	**Co-1**
Dehoro	Small Red	NR
Condor	Black	**Co-1**
I9365-25	Pink	NR
Morden 003	Navy	**Co-4^2?/Co-3?**
Win Mor	Pinto	**Co-1?**
Remington	Pinto	NR
F07-014-22-2	Red	NR
F07-449-9-3	Red	NR
Huron	Navy	NR
CENTA Pupil	Small Red	NR
Topaz	Pinto	NR

Table 1: List of resistant genotypes and their corresponding Co-genes. NR = resistant genotypes with no resistant genes reported.

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GENETIC MAPPING OF THE COMMON BEAN RECOMBINANT INBRED LINES CALIFORNIA DARK RED KIDNEY × YOLANO USING SSR AND STS MOLECULAR MARKERS

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.), is a legume of tropical origin grown worldwide. This species is a self-pollinating diploid (2n=22); its main genome sequence assembly encompasses approximately 587 Mb (Schmutz et al. 2014). In common bean breeding programs, molecular markers have been used in various ways, such as genetic mapping. The genetic mapping is carried out using segregating populations and molecular markers, which enable a more complete coverage. Molecular markers also enable the identification of genomic regions that control traits of economic importance. Thus, the objective of this work was to add microsatellite and STS markers to a pre-existing molecular map of the CY recombinant inbred mapping population (Johnson and Gepts 2002).

MATERIALS AND METHODS

A F_9 recombinant inbred line (RIL) population of 111 individuals was derived from the cross between cultivars California Dark Red Kidney (Andean gene pool) × Yolano (Mesoamerican gene pool) (CY). The RIL population was used to study the relationship between patterns of inheritance and performance of inter-gene pool hybrid populations (Johnson and Gepts 1999, 2002). Seed samples of the RIL population were provided by P. Gepts. This study was conducted under greenhouse conditions at the Laboratório de Biotecnologia do Núcleo de Pesquisa Aplicada á Agricultura (Nupagri) at the Universidade Estadual de Maringá, Paraná, Brazil. Three hundred fifty-one molecular markers were used in this study, including 140 microsatellites (43 BM, 44 BMd, 27 IAC, 10 PVBR, 4 PVM, 11 Pv, and one CV) and 211 STS (McConnell et al., 2010); some of these were located already on the consensus map of common bean BAT93 / Jalo EEP558 covering all linkage groups. All primer pairs (forward and reverse) were adjusted for PCR amplification conditions and tested for polymorphism between the parental and among the CY lines. Each marker was tested for goodness-of-fit to the expected 1:1 ratio with a chi-square (χ^2) test to identify markers (p-value > 0.05). Linkage analysis was conducted using the software MapDisto (Lorieux, 2012) and genetic distances reported in centiMorgans (cM) after conversion by the the Kosambi mapping function (Kosambi, 1944). The linkage groups were determined using a LOD of 3.0 and a maximum recombination fraction of 0.3 to place new markers in the CY population genetic map.

RESULTS AND DISCUSSION

Of the 351 markers evaluated after optimizing the PCR conditions, 113 (32%) were polymorphic between the two parents. In the CY RIL segregating population, 82 marekrs were polymorphic. Of the 82 markers tested in the CY population, 25 could not be located in any of the linkage groups (LGs). The linkage map reported in the present study spanned 289 cM with an average LG of 26 cM (Table 1). The average distance markers was 6 cM, with an average of 5 markers in

each group. A total of 57 (70%) markers were mapped and distributed along the 11 LGs. The number of markers varied from 2 to 10 per LG (Table 1).
The molecular markers were not well distributed across linkage groups and did not sufficiently cover the genome, when compared with other available maps, such as Galeano et al. (2012) with a total length of 2,041 cM, distributed among 11 linkage groups, and an average length per linkage group of 185 cM.

Table 1. Number of polymorphic molecular markers, length in centimorgans (cM), maximum and minimum distance (cM) of the 11 Linkage Groups (LG) in the genetic map of *Phaseolus vulgaris* L. of the CY RILs population

LG	Map length (cM)	Polymorphic markers	Maximum distance (cM)	Minimum distance (cM)	Average distance (cM)
01	32.41	7	12.0	1.2	5.4
02	26.85	7	12.9	0.8	4.5
03	76.67	10	19.5	2.1	8.5
04	15.18	5	8.3	1.4	3.8
05	79.25	7	29.7	4.7	13.2
06	6.25	3	3.2	3.1	3.1
07	13.12	4	6.8	1.9	4.4
08	18.42	5	6.1	1.7	4.6
09	0.75	2	0.7	0.7	0.7
10	3.19	2	3.2	3.2	3.2
11	16.58	5	7.1	1.9	4.1
Total	288.67	57	-	-	6.2

The increase of the number of markers resulting from this study will improve the resolution of the linkage map of the CY RIL population. Originally, this map included 392 markers, mostly AFLPs (Johnson and Gepts 2002). This population is now being genotyped further with new SSR and SNP (Single Nucleotide Polymorphism) markers, which will be included to obtain an even higher saturation of this map.

ACKNOWLEDGEMENTS
We would like to thank Dr. Paul Gepts for sharing CY mapping population. Financial support from Capes and CNPq.

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CHARACTERIZATION OF THE ANTHRACNOSE RESISTANCE GENE PRESENT IN THE ANDEAN CULTIVAR PERLA

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INTRODUCTION

Biotic stress is a major cause of yield losses in common bean production worldwide. Anthracnose (ANT), caused by the fungus *Colletotrichum lindemuthianum* (Sacc & Magnus) Briosi & Cavara is among the diseases that affect beans and can cause yield losses of up to 100%, especially when environmental conditions are favorable for the development of the disease (Singh and Schwartz, 2010). The use of disease resistance genes is the most economical and ecological strategy for disease control. Finding and characterization of ANT resistance genes in different materials and available cultivars is important (Mahuku and Riascos, 2004). To date different anthracnose resistance genes were identified in common bean, most of them in Mesoamerican germplasm. Thus, characterization of new Andean sources of resistance is important for bean breeding. In the present work the resistance gene present in the Andean cultivar Perla was studied.

MATERIALS AND METHODS

Inheritance test was carried out to characterize the resistance of Perla cultivar to races 65 and 73 of *C. lindemuthianum*. F_2 populations from the crosses between Perla (resistant) × Mexico 222 (susceptible) and Perla × Cornell 49-242 (susceptible) were inoculated with races 65 and 73, respectively. Allelism tests were conducted in F_2 populations derived from independent crosses between Perla and other resistant cultivars that have genes previously characterized (Table 1). After the emergence of the first trifoliate leaf, seedlings of the parents, F_1 and F_2 populations from each cross were inoculated with races 65, 73 and 2047 independently. Inoculum was prepared adjusting the spore concentration to 1.2×10^6 spores.mL^{-1} for each race. After inoculation, plants were placed in a 20 ± 2°C growth chamber with high relative humidity (>95%). Ten days after inoculation, the disease severity index (DSI) was rated using the 1-9 scale proposed by Pastor-Corrales *et al*. (1995) and plants with scores of 1-3 were considered as resistant. Statistical analyses for the inheritance and allelism tests were performed through Chi-square test.

RESULTS AND DISCUSSION

Inheritance and allelism tests results are shown in Table 2. Inheritance test performed with the F_2 population derived from the cross Perla × Mexico 222 showed a 3R:1S ratio ($p = 0.67$), indicating the presence of a single dominant resistance gene in Perla cultivar to race 65. Also, a 3R:1S ratio was observed in the F_2 population from the cross Perla × Cornell 49-242 ($p = 0.86$), suggesting the presence of a single dominant resistance gene in Perla cultivar to race 73. Allelism tests showed 15R: 1S ratios for the F_2 populations from each cross (R × R). These results indicate the action of two dominant independent resistance genes, one of them present in Perla cultivar and the other in the remaining cultivars. From these results we conclude that the

Andean cultivar Perla possess a new gene that confers resistance to races 65, 73 and 2047 of *C. lindemuthianum* and it is an important source of resistance to be use in bean breeding programs.

Table 1. Disease reaction in F2 populations from Inheritance tests (R × S) and allelism tests (R × R) for the genetic characterization anthracnose resistance in Perla cultivar

Crosses with Perla	Resistance Gene[a]	Race	Expected ratio[b]		Observed ratio		χ^2	*p* value
			R	S	R	S		
Inheritance tests								
Mexico 222 (M)	*Co-3*	65	3	1	92	28	0.1777	0.6733
Cornell 49-242 (M)	*Co-2*	73	3	1	81	28	0.0275	0.8682
Allelism tests								
Paloma (A)	NI	65	15	1	111	9	0,3200	0.5716
Kaboon (A)	*Co-1*[2]	65	15	1	117	9	0,1714	0.6788
MDRK[c] (A)	*Co-1*	65	15	1	110	8	0,0564	0.8121
TU (M)	*Co-5*	65	15	1	82	6	0,0484	0.8257
Pitanga (A)	*Co-14*	65	15	1	78	5	0,0071	0.9322
AB-136 (M)	*Co-6, Co-8*	65	15	1	91	6	0,0006	0.9791
PI 207262 (M)	*Co-4*[3]	73	15	1	105	7	0.0000	0.1000
Ouro Negro (M)	*Co-3*[4]	73	15	1	97	6	0.0006	0.9804
AC[d] (A)	NI	2047	15	1	73	6	0.2440	0.6210
Jalo Pintado 2 (A)	NI	2047	15	1	97	6	0.0310	0.8580

[a] NI: not identified gene; [b] R: resistant; S: susceptible; [c]MDRK: Michigan Dark Red Kidney; [d]AC: Amendoim Cavalo; (A): Andean gene pool, (M): Mesoamerican gene pool.

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RELATIONSHIP BETWEEN PHYSIOLOGICAL AND PHENOLOGICAL TRAITS WITH ROOT PULL-OFF FORCE OF COMMON BEAN

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INTRODUCTION

Cultivated over all Brazilian territory, common beans are subjected to many different environmental conditions, including drought and high temperature, requiring genetic improvement and development of more rustic cultivars with lush roots (CIAT, 2015). Plants with well-developed roots are more efficient in nutrient and water absorption, present high germination potential and more tolerance to soil amendments resulted from machine traffic. The full development of the root system is the result of physiological, morphological and genetic factors. The use of genetic diversity for physiological factors such as photosynthesis rate, stomatal conductance, carboxylation and water use efficiency is a relevant strategy to obtain genotypes able to survive in different environmental conditions, as well improves crop yield, turning it a sustainable alternative to increase production. The aim of this study was evaluate physiological and phenological traits of *Phaseolus vulgaris* L. and their relationship with root pull-off force.

MATERIAL AND METHODS

Twenty genotypes of Brazilian Carioca group were grown in 0.8m x 0.3m tubes, under greenhouse conditions. The experiment was conducted in randomized blocks design, with six replications. The variables collected were temperature (ΔT), stomatal conductance (*gs*), photosynthesis rate (*A*), internal carbon (*Ci*) and transpiration (*E* – data not shown). All variables were measured in V4, R6 and R8 stages. In the physiological maturation, plant's lap diameter (PLD) and root pull-off force (RPOF) also were assessed. Results were submitted to Anova analysis and grouped by Scott-Knott test ($p<0.05$) and by Principal Component Analysis.

RESULTS

Genotypes presented physiological variation at R6 and R8 stages but not in V4 stage, suggesting that in early stages, genetic differences between genotypes do not influence on plant homeostasis in terms of gas exchange (Figure 1). Notably, gas exchange rates decrease according to the progress of plant development (Figure 1B and C), as differences between atmospheric and leaf temperatures increase (Figure 1A). The mean value for PLD and RPOF were 7.2 mm and 10.6 KgF, respectively, similar to those presented by Stoffella et al., (1979). PLD and *A* in R8, *gs* and *Ci* in R6 and RPOF were grouped together by PCA (Figure 2), demonstrating that those variables are correlated to RPOF in common beans, what indicates that plants with better gas exchange in late stages of development presented roots better developed. In this contest, IAC Alvorada, BRS Estilo, IPR Bem-Te-Vi and IPR Quero-Quero cultivars and LP 08-157 lineage were clustered together, presenting high RPOF related with high values of PLD, *A*, *gs* and *Ci*.

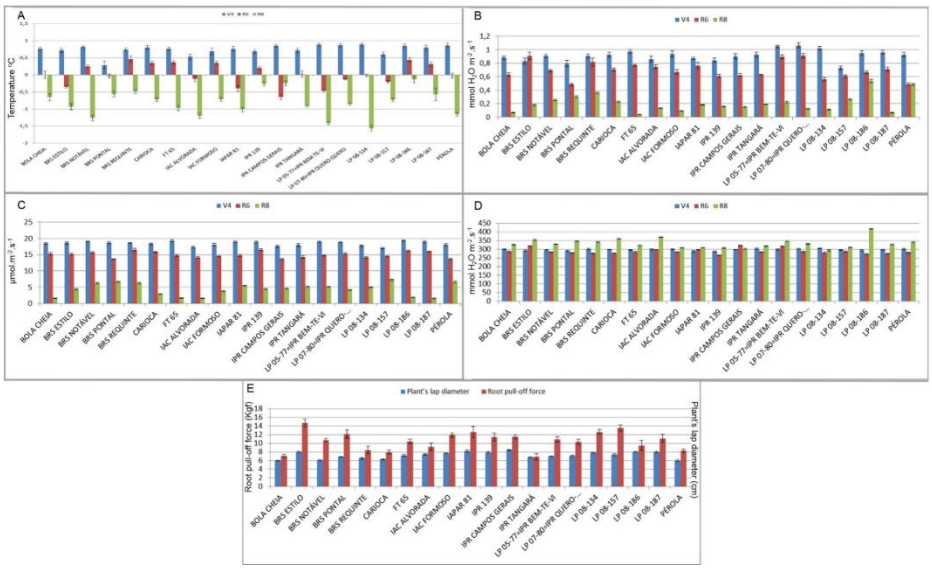

Figure 1. Monitoring of gas exchange of twenty common bean cultivars during V4, R6 and R8 stages. **A.** ΔT = leaf temperature – chamber temperature. **B.** *gs*. **C.** *A*. **D.** *Ci*. **E.** RPFO and PLD.

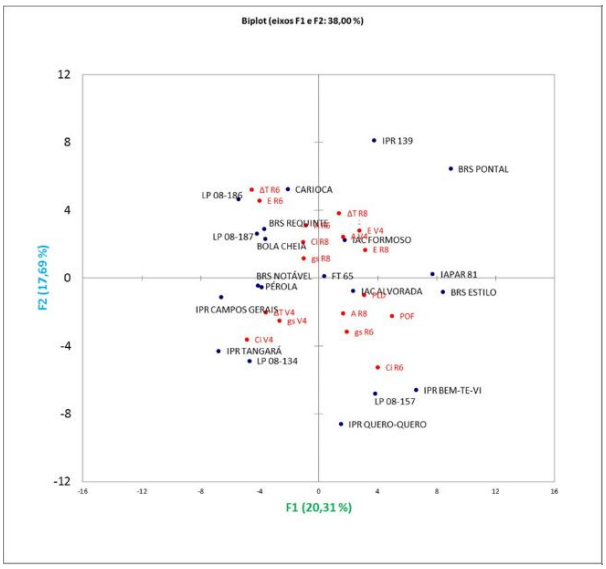

Figure 2. Grouping of twenty common bean cultivars in function of phenological and physiological traits by Principal Component Analysis (PCA) using phenological (PLD and RPOF) and physiological (*E*, *gs*, *A*, ΔT and *Ci*) traits in V4, R6 and R8 stages.

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VERDIN, OPAQUE DRY BEAN CULTIVAR TOLERANT TO TROPICAL TERMINAL DROUGHT OF SOUTHEAST PRODUCTION AREAS OF MEXICO

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INTRODUCTION

Terminal drought is the most limiting abiotic factor for dry bean production under residual soil moisture cropping system in Veracruz and Chiapas, Mexico (López *et al*., 2011). In 2015, the INIFAP Bean Program for Southeastern Mexico released Verdín, an improved small, opaque-black bean cultivar which originated from SXB114/DOR605//SXB123, a cross made in CIAT. As a result the breeding line SEN-70 was introduced in to Mexico in 2009 and its field adaptation evaluated. The aim of this study is to show the results of Verdín´s field productivity during the evaluation process in Veracruz and Chiapas, Mexico.

MATERIALS AND METHODS

Verdín was evaluated from year 2011 to 2013 in an elite regional yield trial across 11 environments of Veracruz and Chiapas, Mexico under both residual soil moisture and rainfed conditions. During the winter-spring (February-May) season of 2013 in Cotaxtla, Veracruz, Verdín was evaluated under irrigation and terminal drought conditions along with other 22 breeding lines and the black bean; Negro Tacaná, Negro Jamapa and Negro INIFAP cultivars used as checks. Drought susceptibility index DSI = 1- (Yii / Yci) / IIS (Fisher and Maurer, 1978) and the relative yield efficiency index RYEI = (Yii / Yi) (Yci / Yc) (Graham, 1984) were determined. A uniform yield trial composed of 10 breeding lines that were selected based on their response to terminal drought was conducted in both bean seasons, autumn -winter (October-January), 2013-14 and winter-spring (February-May), 2014. Same check cultivars were used to assess the field performance under residual soil moisture in Veracruz and Chiapas, Mexico. Verdín was characterized in year 2014 according to UPOV international guidelines for dry beans. Verdín was registered in the National Service of Seed Inspection and Certification (SNICS) and released for commercial used in year 2015.

RESULTS AND DISCUSSION

Verdín was the most productive cultivar in either residual soil moisture or rainfed conditions with an average yield (1446 kg ha^{-1}) higher than check cultivars Comapa (12.8%) and Papaloapan (10.2%). In the terminal drought field trial, Verdín, was the earliest season among all cultivars, with 67 days to physiological maturity, while the check cultivars Negro Jamapa, Negro Tacaná and Negro INIFAP reached maturity on average 8 days later. Verdín was selected for its tolerance to terminal drought (DSI=0.8) and high productivity (RYEI=1.45) under both irrigated and dry conditions (Table 1). Verdín exhibits the type-II upright short vine (indeterminate) growth habit. Plants average 59 cm in height, are more upright than Negro Jamapa, and exhibit an overall upright appearance similar to Negro Tacaná. Verdín produces purple blossoms, matured pods are yellow-cream in color and seeds are small (24.7 g/100 seeds) opaque black. One of the traits that has a high agronomic acceptance is that Verdín is an early maturing cultivar with 68 d after planting with a range of 67 to 70 d, depending on season and location of the tropical and subtropical conditions of southern Mexico, compared with a range of 73 to 75 and

mean of 74 d for Negro Jamapa. The earliness allows Verdín reduce the risks of yield losses due to the occurrence of terminal drought. Verdín is resistant to anthracnose and to BGYMV, it possesses the single dominant hypersensitive *I* gene which conditions resistance to seed-borne BCMV, but is sensitive to the temperature-insensitive-necrosis inducing strains of BCMNV like NL 3, viral diseases already present in bean farmer´s fields of Veracruz and Chiapas.

Table 1. Seed yield (kg ha^{-1}) under drought and non-drought stress of Verdín and check cultivars.

Cultivar	NonDrought stress	Drought stress	DSI†	RYEI††
Verdín	2118.67 *	1420.33 *	0.80	1.45
Negro INIFAP	1710.33	961.00	1.06	0.79
Negro Tacaná	1984.33 *	1192.33	0.96	1.14
Negro Jamapa	1672.00	866.67	1.16	0.70
Mean	1879.57 *	1101.65	1.0	1.0
LSD (0.05)	383.69	242.27		

*Cultivars statistically superior according to LSD (0.05). † Drought Susceptibility Index. †† Relative Yield Efficiency Index.

According to the soil moisture balance there was drought stress in four out six environments where uniform yield trial were evaluated, under these conditions Verdín exceeded 49.9 and 41.8% seed yield of Negro Tacaná and Negro Jamapa checks; even without drought stress Verdín was 17.4 and 39.9% higher than same checks (Table 2).

Table 2. Seed yield (kg ha^{-1}) of Verdín and cultivar checks with and without drought stress.

Site/State	Verdín	Negro Tacaná	Negro Jamapa
La Candelaria, Veracruz (DS)†	1253	747	854
Ocozocoautla, Chiapas. (DS)†	687	468	549
Cintalapa, Chiapas (DS)‡	1407	1065	738
La Candelaria, Veracruz (DS)‡	1140	713	1024
Martinez de la Torre, Veracruz. (NS)†	1427	1200	1318
Martinez de la Torre, Veracruz (NS) ‡	1709	1471	924
Average	**1270**	**944**	**901**

DS=Drought stress, NS=Non stress †Fall (October-January) and ‡Winter (February-May) seasons.

CONCLUSIONS

Verdín was registered as a new improved cultivar for commercial use in Veracruz and Chiapas due to its earliness, drought tolerance, disease resistance and high productivity. Verdín is the first early season tropical black bean cultivar tolerant to terminal drought with high yield potential adapted to tropical areas of southeastern Mexico.

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IDENTIFICATION OF QTL FOR RESISTANCE TO WHITE MOLD IN DRY BEAN I9365-25

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INTRODUCTION

White mold, caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, is a disease of major impact on dry bean (*Phaseolus vulgaris* L.), as it limits the production potential and reduces the quality of seeds and pods. The disease regularly causes losses of up to 30%, and is favored by high humidity and moderate temperatures. The interaction of the pathogen with its host is complex, and host tolerance is inherited as a quantitative trait with low to moderate heritability. Higher levels of white mold resistance are found in the secondary gene pool such as *Phaseolus coccineus* L. The goal was to identify QTLs conditioning partial resistance from the I9365-25 pink bean which derives from a *Pv* x *Pc* interspecific population.

MATERIAL AND METHODS

Two RIL populations were generated: 130 RILs (F5:7) were derived from the cross between 'Montrose', a white mold susceptible pinto cultivar, and I9365-25, a resistant pink line; a second F5:7 derived RIL population (n=127) was originated from the cross between 'UI-537', a white mold susceptible pink cultivar, and I9365-25. The plants were evaluated for white mold resistance with the straw test method with six reps and the reaction rated 1 (resistant) to 9 (susceptible) under greenhouse conditions. The plants were also evaluated in the field at Paterson, WA. The RILs were genotyped with the 5398 SNP BARCBean6K_3 Bead Chip. The genetic map was made using JoinMap 4.1® and QGene was used for QTL analysis using composite interval mapping.

RESULTS AND DISCUSSION

Soule et al. 2011 also found QTL on Pv05 (WM 5.4^{R31}), and on Pv07 (WM 7.3^{R31}) in the Raven/I9365-31 RIL population. Both I9365-31 and I9365-25 derive from an interspecific population between *P. vulgaris* L. and *P. coccineus* L. suggesting these two QTL may derive from *P. coccineus*. WM6.2^{U25} and WM7.6^{M25} have distinct physical positions from previously identified QTL and pending validation may represent new minor effect QTL.

A major QTL detected on chromosome Pv05 (WM $5.3^{R31,M25,UI25}$) for the greenhouse evaluation for both populations shows a promising region for physiological resistance in dry bean I9365-25. Future work should be done to validate candidate genes and markers flanking this region and the QTL should be considered for MAS for white mold disease resistance in common bean.

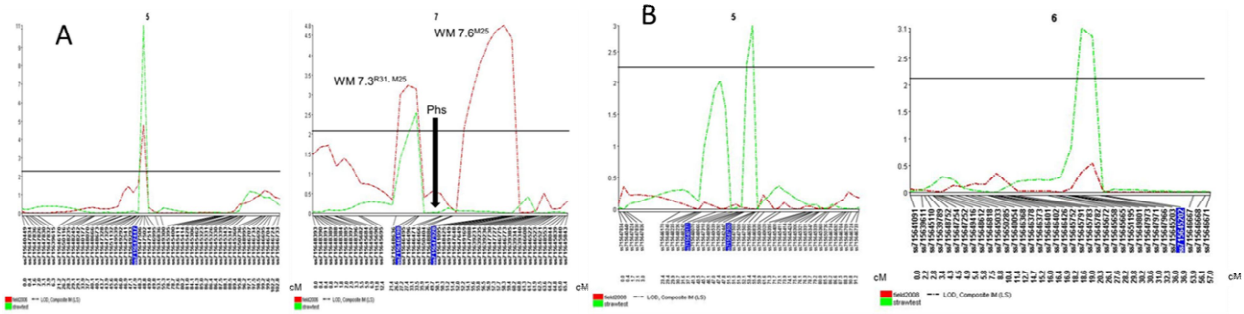

Figure 1: (A) QTL for white mold resistance on Pv05 (WM $5.3^{R31,M25,UI25}$) and Pv07 (WM $7.3^{R31,\ M25}$ and WM 7.6^{M25}) for Montrose/I9365-25 population. (B) QTL for white mold resistance on Pv05 (WM $5.3^{R31,M25,UI25}$) and Pv06 (WM $6.1^{B60,R31,XC,UI25}$) in the UI-537/I9365-25 population. [field - red line and GH - green line]

Table 1: Genomic position and R^2 (phenotypic variation explained by significant QTL-linked markers) for the Montrose/I9365-25 population.

QTL	Position (cM)	Position (Mb)	R^2 (%)
WM 5.4	46.92 – 49.84	29.45 – 35.49	0.16 (Field) 0.31 (GH)
WM 7.3	21.43 – 27.49	1.81 – 2.67	0.11 (Field) 0.09 (GH)
WM 7.6	49.76 – 50.38	11.93 – 15.77	0.16 (Field)

Table 2: Genomic position and R^2 (phenotypic variation explained by significant QTL-linked markers) for the UI-537/I9365-25 population.

QTL	Position (cM)	Position (Mb)	R^2 (%)
WM 5.4	47.86 – 52.16	30.98 – 34.91	0.19 (GH)
WM 6.2	30.23 – 36.91	28.85 – 29.75	0.20 (GH)

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IDENTIFYING WATERLOGGING TOLERANT DRY BEAN (*PHASEOLUS VULGARIS* L.) GENOTYPES USING CHLOROPHYLL CONTENT

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INTRODUCTION

Waterlogging occurs when the soil is completely saturated with water, causing hypoxia or anoxia conditions (Striker, 2012). Waterlogging can severely impact crop production and dry bean (*Phaseolus vulgaris* L.) is one of the most susceptible crops to waterlogging. A reduction in greenness of the leaves is one of the first responses to waterlogging stress (Yordanova and Popova, 2007). The resulting reduction in greenness can influence the health and production of the plant, especially during crop emergence and establishment (Caudle and Maricle, 2012). The objective of this study was to determine which MDP genotypes are able to maintain leaf greenness in waterlogged conditions.

MATERIALS AND METHODS

A total of 279 genotypes from a Middle American Panel (MDP) were screened in greenhouse conditions using Royalty as a check. A randomized complete block design (RCBD) with a split-plot arrangement was used with the treatments (control vs. waterlogged) being the main plots and genotypes being the sub-plots. Four replications were performed and two samples per experimental unit were considered. All plants were germinated in 4 inch square plastic pots filled with a sandy clay loam, well-drained soil. Waterlogging stress was introduced to the waterlogged main-plot after the primary (cotyledonary) leaves were unfolded (V2 stage of development). The waterlogging stress was applied for 10 days, then chlorophyll content was measured using SPAD-502. Flooding effect was calculated using the following equation

$$flooding\ effect = \frac{(SPAD\ value\ in\ waterlogged\ condition - SPAD\ value\ in\ control\ condition)}{(SPAD\ value\ in\ control\ condition)}$$

. Data were analyzed using SAS 9.3 (SAS Institute, Cary NC).

RESULTS

Plants grown in the control conditions have a significantly higher mean SPAD value than those grown in the waterlogged condition with means of 47 and 34 SPAD units, respectively. For the flooding effect, pinto and great northern market classes (Durango race) have the least reduction in greenness (~17%), whereas black and navy (Mesoamerica race) have the most (34% and 49%, respectively) (Figure 1). There is a significant genotype by treatment interaction which explains the genotypic variation in greenness. The flooding effect for each genotype ranged from -89.0% to 1.6% with an average of -27% (Tables 1 and 2).

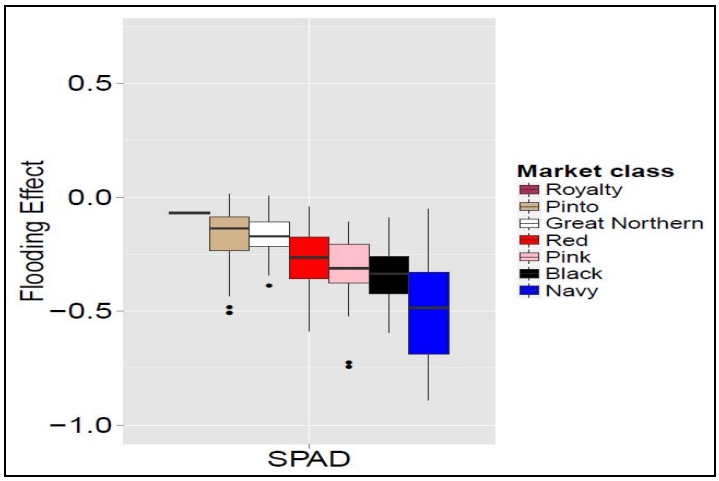

Figure 1. Flooding effect for each market class.

Table 1. Most susceptible genotypes in terms of flooding effect.

Genotype	Flooding Effect (%)	Market Class
Midland	-89.0	Navy
Huron	-79.2	Navy
HY 4181	-78.5	Navy
Albion	-77.4	Navy
CDC Rosalee	-74.5	Pink
Newport	-74.3	Navy
NW 395	-74.2	Navy
Arthur	-72.8	Navy

Table 2. Most tolerant genotypes in terms of flooding effect.

Genotype	Flooding Effect (%)	Market Class
Apache	1.6	Pinto
Sawtooth	0.5	Great Northern
CDC Camino	-2.3	Pinto
Arapaho	-2.3	Pinto
Chase	-2.5	Pinto
Win Mor	-2.9	Pinto
Sonora	-3.1	Pinto
Royalty	-3.4	Cream

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DIVERSITY FOR SYMBIOTIC NITROGEN FIXATION AND RELATED TRAITS IN COMMON BEAN (*PHASEOLUS VULGARIS* L.)

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INTRODUCTION

Symbiotic nitrogen fixation (SNF) and related agronomic traits were examined in the Mesoamerican Diversity Panel (MDP) for an on-going genome-wide association study (GWAS).

MATERIALS AND METHODS

The 300 genotypes of the MDP, 19 additional modern AAFC-UofG Mesoamerican cultivars, and a non-nodulating dry bean mutant were grown at low-nitrogen field sites in Elora, Ontario (2014). Measurements recorded in the field include days to flowering, days to maturity, leaf chlorophyll content readings at 20 and 48 days after planting, 100 seed weight, yield, and harvest index. Percent nitrogen derived from the atmosphere (%Ndfa), a measure of nitrogen fixing capacity, was obtained through gas-chromatography mass-spectrometry analysis of seed samples.

RESULTS AND DISCUSSION

Analysis of data from the 2014 field season indicated that leaf chlorophyll content was significantly different among the 320 genotypes at first trifoliate stage ($P<0.0001$) and at mid-flowering stage ($P<0.0001$). The panel exhibited significant differences among genotypes for days to 50% flowering ($P<0.0001$; Fig1A), yield ($P<0.0001$; Fig1B) and %Ndfa ($P<0.0014$; Fig1C). Average %Ndfa was 54.7, and OAC Mist which has been previously reported as a high nitrogen-fixing genotype had below average performance (51%) in this study. Some genotypes fixed as much as 86% of their total nitrogen.

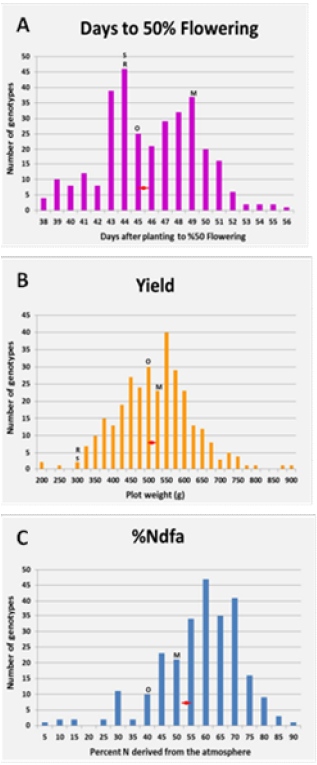

Figure 1. A) Days to 50% flowering for 320 genotypes. **B**) Yield (g) for 314 genotypes. Six genotypes did not mature and were not harvested. **C**) Percent nitrogen derived from the atmosphere (%Ndfa) for 260 genotypes (54 genotypes yet to be analyzed). Where available, bean genotypes Mist (M), Sanilac (S), OAC Rico (O) and R99 (R) are indicated. Mean +/- 95% confidence limits are shown (circle and line).

Genome-wide Association (GWA) analysis was carried out in TASSEL for %Ndfa using genotypic data (271556 SNPs). The Manhattan plot (Fig2) generated from the output of the mixed linear model (MLM; $Y = X\alpha + P\beta + K\mu + \varepsilon$) revealed peaks associated with %Ndfa: Pv01 (37.7Mbp), Pv02 (9.2Mbp), Pv04 (8.1 and 29.0Mbp), Pv05 (0.8Mbp), Pv07 (17.7Mbp), and Pv08 (37.3Mbp). Genomic regions associated with %Ndfa under dry field conditions (Pv07, 19.2Mbp) and under optimum field conditions (Pv08, 35.7Mbp) we previously reported by Farid (2015).

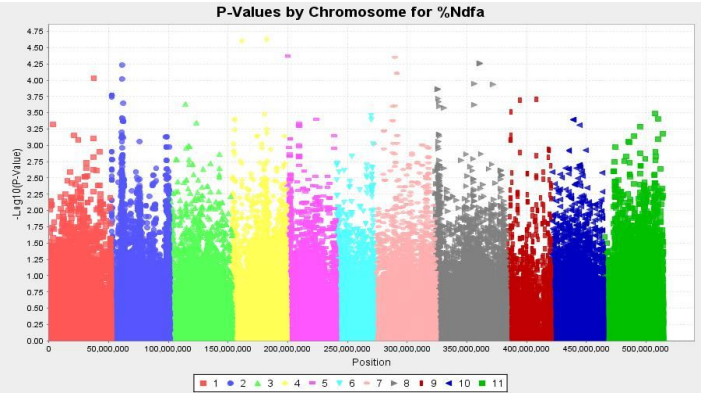

Figure 2. Manhattan plot for %Ndfa. After removing monomorphic and low frequency SNPs, 159 884 markers and %Ndfa values for 226 MDP genotypes were used for MLM analysis.

CONCLUSIONS AND FUTURE DIRECTIONS

Diversity for nitrogen fixation capacity as measured by %Ndfa and other agronomic traits was found in the MDP. Genetic diversity is prerequisite to breeding for trait improvement. High SNF varieties with good yield potential identified in this study could be used in a breeding programme.

Unique genomic regions associated with %Ndfa on Pv01, Pv02, Pv04, and Pv05 were observed, as indicated by peaks in the Manhattan plot. Further trials will be carried out to determine if these peaks are repeatable.

Genomic regions on Pv07 and Pv08 are near those reported under dry and optimal field conditions in a bi-parental navy bean population (Farid, 2015).

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REGULAR REPORTS

IDENTIFICATION OF TROPICAL BLACK BEAN BREEDING LINES RESISTANT TO BCMV AND BCMNV USING MOLECULAR MARKERS

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INTRODUCTION: In Mexico, the small, opaque black bean is a seed class under high demand by consumers, particularly in the center and southeast regions of the country, with an annual requirement of 400,000 T. Tropical small black beans are the predominant cultivars grown under rainfed and residual soil moisture cropping systems of Veracruz and Chiapas. BCMV (Bean common mosaic virus) can reduce bean yield by up to 100% when the virus occurs before flowering, and 50% when the virus is transmitted through seed harvested from infected plants (Morales, 1979). In this region, the BCNMV (Bean common necrotic mosaic virus) has appeared sporadically, its incidence has caused death of plants by systemic necrosis and significant grain yield reduction. A strategy for the control of these viral diseases is to plant varieties that carry resistance genes. The objectives of this study was to identify tropical black bean germplasm with BCMV and BCNMV resistance genes, by artificially inoculating bean seedlings and verifying the presence of molecular markers associated with such resistance genes.

MATERIALS AND METHODS: A collection of 70 Mesoamerican black bean germplasm was evaluated, which included breeding lines derived from three crosses (Papaloapan/SEN 46, Negro Citlali/RAV 187 y Jamapa plus/RAV) and commercial cultivars, most developed by INIFAP. The BCMV and BCNMV strains have been stored at 4°C in bean seeds for long-term storage. The BCNMV NL3 strain is kept in Michelite 62 seeds. Phenotypic and genotypic evaluations were undertaken. Single inoculations were made using the NL3 strain. Inoculation was performed early morning (7:00 to 9:00 am), for which 1.0 g of primary leaf tissue was macerated in 0.01M phosphate buffer (pH 7.5) in a ratio of 1:10 (w/v). Sterile cotton gauze was moistened and steeped in well-spread solution and primary leaves of bean seedlings (7-10 days after sowing) were rubbed. Incidence of symptoms was visually assessed one week after inoculation according to Drijfhout (1978). To confirm that absence of disease symptoms were not due to an escape, same seedlings were re-inoculated in both, primary and first trifoliate leaves, then incidence was determined 16 days after inoculation. Bean seedlings not showing any symptoms after re-inoculation were considered resistant. Then, molecular markers (MM) were used to identify the presence of resistance genes: SW13 for the *I* gene (Haley et al, 1994; Melotto et al., 1996.), CAPS marker for *PveIF4E* gene: $eIF4E^2$ allele linked to *bc*-3 gene (Naderpour et al., 2010); and SBD5 for the *bc*-1^2 gene (Miklas et al., 2000). Mixed and individual samples were used to run three PCRs per sample, one for each MM. PCR reactions with different types of labels were made separately as reported by Haley et al. (1994), Melotto et al. (1996) and Miklas et al. (2000). CAPS was made according to Naderpour et al. (2010) using *Rsa* I and *Fau* I to discriminate $eIF4E^2$ from $eIF4E^3$ allele.

RESULTS AND DISCUSSION: As a first approximation, a population scrutiny was undertaken comprising the total DNA mixture of 12 individual plants per bean genotype, which

gave rise to 70 composite samples. With such information together with phenotypic response then single plants were selected to run an individual scrutiny. A total of 840 individuals were assessed. Data from the population scrutiny indicated that all genotypes had the *I* gene in different proportions except Jamapa Plus/RAV-3-4-4 breeding line, some of which also carry the *bc*-3 gene. Inoculation with BCNMV NL3 strain, coupled with confirmation of presence of the *I* gene by molecular marker SW13 is sufficient to consider the resistance to no necrosis-inducing strains of BCMV. Molecular marker analyses were undertaken in 192 single plant samples in the individual scrutiny study. The *bc*-3 gene (Fig 1) without the presence of *I* or *bc*-1^2 genes was found in a very low proportion, only one individual seedling of breeding lines Papaloapan/SEN 46-7-6, Papaloapan/SEN 46-7-13 and Jamapa Plus/RAV-3-4-4 carrying that gene were found. However, 15 breeding lines showed high proportion of individuals with the *I* + *bc*-3 genetic combination such as Papaloapan/SEN 46-7-10 (92%), Jampa Plus/RAV-3-4-4 (88%), Papaloapan/SEN 46-5-5 (82%), Papaloapan /SEN 46-7-7 (75%), Negro Citlali/RAV-187-3-1-9 (75%) and the elite line SCN-2 (75%), the rest of the genotypes had a variable proportion (8 to 67%) individuals with both genes. This group of breeding lines might represent sources of BCMV and BCNMV resistance genes in the tropical black bean gene pool.

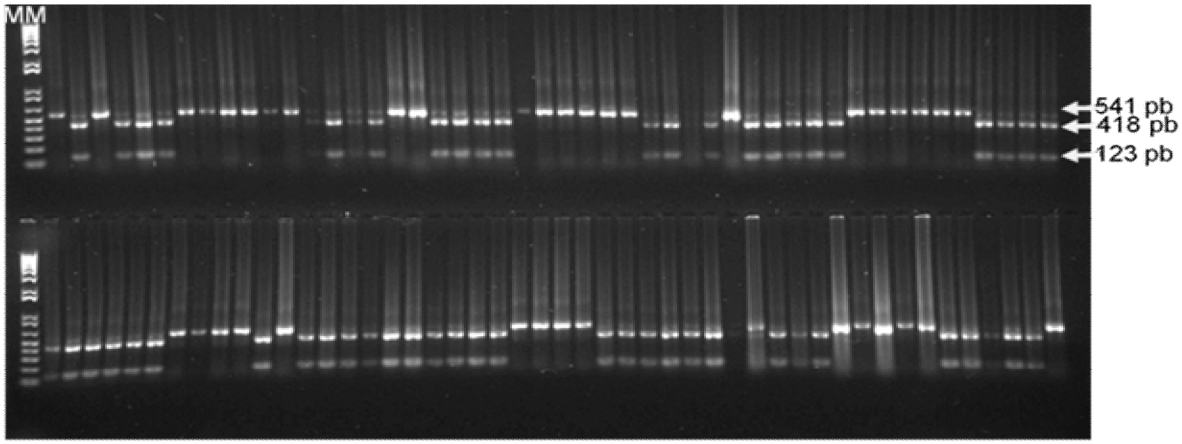

Fig. 1. Molecular marker product CAPS type linked to *bc*-3 gene after digestion of the 541 bp fragment with *Fau* I enzyme. Each lane corresponds to one seedling. The presence of two fragments (418 bp and 123) indicates the homozygous state of the allele *eIF4E2/eIF4E2* (*bc*-3/*bc*-3), the single 541 bp fragment indicates the absence of *bc*-3 gene. MM: size marker 1Kb DNA ladder (Invitrogen).

CONCLUSIONS: A group of tropical black breeding lines were identified carrying *I*, *bc*-3 and *bc*-1^2 genes. Such breeding lines might be used as a source to develop black bean cultivars with high levels of resistance to BCMV and BCNMV.

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BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF *BEAN COMMON MOSAIC VIRUS* ISOLATES FROM PATHOGENICITY GROUPS I AND V

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INTRODUCTION: *Bean common mosaic virus* (BCMV) exists as a complex of strains classified into eight pathotypes according to the interactions with six recessive resistance alleles in common bean (1,2). These strains, thus, represent pathogroups (PGs) numbered I through VIII and defined by the ability of a BCMV isolate to replicate in a set of 12-14 bean differential lines carrying different combinations of recessive resistance alleles. A substantial genetic diversity was found between BCMV isolates from pathogroups I, and VI to VIII, with whole genome sequence differences ranging up to 10-15% (2,3). The genetic determinants involved in interactions of BCMV with different resistance alleles have not yet been identified, and in order to reveal these genetic determinants of pathogenicity for BCMV, we performed a complete biological and molecular study of two isolates of BCMV belonging to PG-I and V. Inspection of complete genome sequences for BCMV isolates US1, NY15P, and NL1 revealed that recombination between BCMV strains may be quite common and may result in changes in virus pathology. The data obtained suggest that the search for the genetic determinants responsible for BCMV pathogenicity should continue through comparative genomics of BCMV isolates with known pathogenicities.

MATERIALS AND METHODS: The biological characterization of US1 (PG-I) and NY15P (PG-V) was conducted as described previously (3). The cloning strategy for all BCMV isolates was based on the initial amplification of three genome fragments using degenerate primers developed for conserved areas of potyvirus genomes, as described by Ha et al. (4). Briefly, three pairs of degenerate primers were used to amplify conserved areas in the HC-Pro, CI, and NIb-3'-end regions. Once these initial fragments were cloned and sequenced, the remaining gaps were filled through RT-PCR using specific primers based on the sequences determined. The sequences were initially analyzed using BLAST tool available at the National Center for Biotechnology Information (NCBI). Complete viral genomes were assembled using SeqMan (DNASTAR,Madison,WI). ORFs were identified using the ORF Finder program available at the NCBI. Complete sequences of BCMV isolates were aligned using ClustalX. Further analysis was conducted with the RDP4.

RESULTS: The whole genomes of BCMV isolates US1 and NY15P were cloned and sequenced, using the same approach (3). Upon sequence assembly, the US1 genome was found to be 10,052-nt long, excluding the poly (A) and, based on conceptual translation, encoded a single polyprotein of 3,221 aa. NY15P genome was found to be 10,053-nt long, excluding the poly (A) and encoded a single polyprotein of 3,222 aa. The sequences of both isolates were compared to the known BCMV and BCMNV genomes, and the US1 and NY15P sequences were found the closest to the isolate NL1 [PG-I, accession number AY112735; 94% and 97% nucleotide identity to US1 (PG-I) and NY15P (PG-V), respectively]. The whole genomes for US1 (accession number KT175569), and NL1 (accession number AY112735) both from PG-I,

together with NY15P (accession number KT175568, PG-V), were aligned using CLUSTALX and further analysis was conducted with the RDP4 program package. Figure 1 shows the comparison of all three sequences using the manual distance plot analysis, with the full-length NY15P sequence as the reference (Fig. 1). Based on the RDP4 analysis, the 5'- terminal sequences of isolates US1 and NL1, between nt 1-2,124, shared more similarities to each other (98% identity) than to NY15P isolate (96% identity). The sequences downstream between nt 2,125-6,717 (position in alignment) in the NL1 and NY15P genomes were quite similar (97% identity), while US1 had 89% similarity to the NL1 and NY15P sequences in this segment. The 3'-terminal genome segments between nt 6,718-9,381 (position in alignment), were very similar among all three isolates (97-98% identity). In the 3'-terminal region, between nt 9,382-10,055 (position in alignment), sequences of US1 and NL1 were similar to each other (98% identity) while NY15P shared only 91-92% identities with the two other sequences. It appears that genomes of isolates NL1 (PG-I) and NY15P (PG-V) on one hand, and isolate US1 (PG-I) on the other hand, carry long sequences between nt 2,125 to 6,717 that are quite different, while most of the other areas of the genomes for all three are much closer. This indicates a possible recombination event leading to these similarity patterns (Fig. 1).

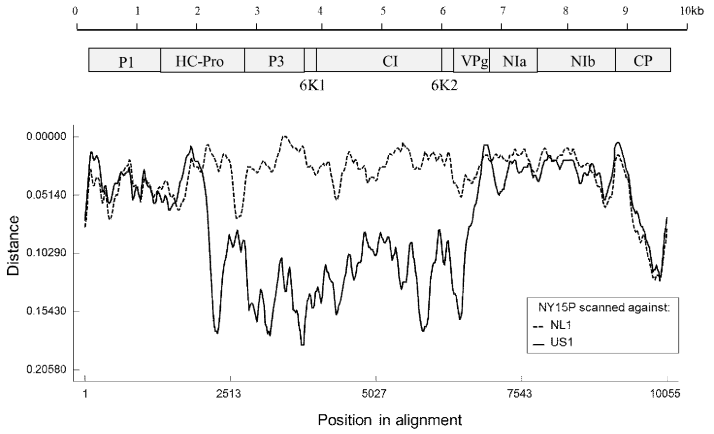

Fig. 1. Recombination analysis of US1 and NY15P isolates of BCMV. Manual distance plot based on the aligned full-length nucleotide sequences of BCMV isolates US1, NL1 and NY15P; NY15P was used as the reference strain. X axis represents nucleotide position in the alignment, Y axis represents relative distance from the reference sequence which is calculated using Kimura model.

CONCLUSIONS: Based on the sequence analysis of NY15P, and two other complete genomes of BCMV isolates with PG-I pathogenicity (Fig. 1), the most likely genome areas involved in interactions with genes *bc-1* and *bc-2* may be located between positions 1 to 2,124 or positions 9,382 to 10,055. The data obtained suggest that the search for the genetic determinants responsible for BCMV pathogenicity should continue through comparative genomics of BCMV isolates with known pathogenicities.

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UNRAVELING THE BROAD AND COMPLEX RESISTANCE IN COMMON BEAN CULTIVAR MEXICO 235 TO *UROMYCES APPENDICULATUS*

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INTRODUCTION

The Mesoamerican common bean cultivar Mexico 235 (M235) is known to have a broad spectrum of resistance to the hypervirulent bean rust pathogen (*Uromyces appendiculatus*). This cultivar is resistant to 83 of 94 races of the rust pathogen maintained in Beltsville, MD. These 83 races overcome nine of the 10 named and mapped *Ur* genes (*Ur-3*, *Ur-4*, *Ur-5*, *Ur-6*, *Ur-7*, *Ur-9*, *Ur-11*, *Ur-12*, *Ur-13*, and *Ur-14*) as well as other very important sources of resistance including PI 260418, PI 310762, and Compuesto Negro Chimaltenango. The rust resistance in M235 is conferred by the *Ur-3* gene and another or possibly two additional rust resistance genes. The *Ur-3* gene was originally described in dry bean Aurora (1) which is resistant to only 55 of the same 94 races. Hence, the additional gene(s) in M235 provides resistance to 28 races to which Aurora is susceptible. Although M235 was used as source of rust resistance of many released cultivars, no genetic study has been conducted describing the gene(s) conditioning this resistance. The objective of this study was to conduct an in-depth analysis to elucidate the complex rust resistance of M235 using differential races of *U. appendiculatus* on a F_2 segregating population.

MATERIALS AND METHODS

All of the studies reported here were conducted under greenhouse conditions using published methodologies. To determine the inheritance of rust resistance in M235, 253 F_2 plants from the cross Pinto 114 (S) x Mexico 235 (R) were inoculated with four different races (41, 53, 49, and 67) of *U. appendiculatus***.** Races 41 and 53 in interaction with M235 result in a hypersensitive reaction (HR) which is the characteristic resistant phenotype of *Ur-3* in Aurora. Races 49 and 67 in interaction with M235 produce typical tiny pustule reaction. Aurora (*Ur-3*) is susceptible to races 49 ad 67. These two races were used to study the inheritance of the additional resistance present in M235 and absent in Aurora. Controls in this study included the Pinto 114 and Mexico 235 parents, Aurora (*Ur-3*), NEP 2 (*Ur-3*$^+$), Early Gallatin (*Ur-4*), Mexico 309 (*Ur-5*), Golden Gate Wax (*Ur-6*), Great Northern 1140 (*Ur-7*), Pompadour Checa 50 (*Ur-9-Ur-12*), PI 181996 (*Ur-11*), Redlands Pioneer (*Ur-13*), PI 260418 and PI 310762. Evaluations were conducted 12 days after inoculations. Statistical analyses were performed using the Chi-square test.

RESULTS AND DISCUSSION

The rust resistant conferred by the natural combination of resistant genes in M235, known as the "*Ur-3*$^+$", is much broader than the resistance conferred by *Ur-3*, the rust resistance gene present in Aurora (Table 1). Moreover, the broad resistance in M235 is more closely comparable to that found in PI 181996 and PI 260418 (Table 1).

The resistant rust phenotype observed on the F_2 population from the Pinto 114 (S) x Mexico 235 (R) cross, inoculated with races 41 and 53 was an HR response typical of *Ur-3*. The resistant and susceptible phenotypes fitted a 3R:1S ratio (Table 2). The resistant phenotype from the interaction between races 49 and 67 with the F_2 resistant plants were tiny uredinia and faint or tiny chlorotic spots. The resistant and susceptible phenotypes best fitted a 13R:3S ratio (Table 2). These results suggest that the additional resistance in M235 is controlled by two genes in an epistatic dominant II interaction (Table 2).

Thus, the broad and complex rust resistance in M235 is conditioned by three genes. The first resistant gene in M235 is dominant and independent which produces an HR, like that produced by the *Ur-3* gene in Aurora. The resistant phenotype of the other two genes, which interact in epistasis with each other, is a tiny pustule. To our knowledge, this is the first report of the complex epistatic interaction between the two additional rust resistant genes in M235. The elucidation of the genetic mechanisms of these two genes will enhance our understanding of the complex gene interactions in Mexico 235. Additional crosses, bulk segregant analysis, and a large set of SNP DNA markers are being used to identify the two newly discovered genes and to develop SSR DNA markers linked to them.

Table 1. Reactions of common beans Aurora and Mexico 235 to four races of *Uromyces appendiculatus*. Other cultivars with rust resistance genes are included for comparison.

		Races of *U. appendiculatus*			
Bean Genotypes	Ur-Genes	41	53	49	67
Aurora	*Ur-3*	2, 2+	2	4, 5	4, 5
Mexico 235	*Ur-3*+	2	2	3, f2	3, f2
NEP 2	*Ur-3*+	2	2	4, 5	4, 5
Early Gallatin	*Ur-4*	4	4, 5	2	4, 5
Mexico 309	*Ur-5*	3, f2	f2	4, 5	4, 5
Golden Gate Wax	*Ur-6*	4, 5	4, 5	4, 5	4, 5
Great Northern 1140	*Ur-7*	4	5, 4	3, f2	3, f2
Pompadour Checa 50	*Ur-9, Ur-12*	4, 5	4, 5	2, 2+	4
PI 181996	*Ur-11*	f2	3, f2	3, f2	f2
Redlands Pioneer	*Ur-13*	4, 5	4	4, 5	4, 5
PI 260418	Unnamed	3	3, f2	3, f2	3
PI 310762	Unnamed	f2,3	f2,3	3	f2, 3

Standard bean rust grading scale: 2, 2+ = Necrotic spots without sporulation; 3 = Tiny uredinia (sporulating pustules) less than 0.3mm in diameter; f2 = faint and tiny chlorotic spots; 4 = Medium uredinia, 0.3-0.5mm in diameter; 5 = Large uredinia, 0.5-0.8 mm in diameter. 1, 2, 3, f2 = Resistant; 4, 5, 6 = Susceptible (in gray).

Table 2. Inheritance of rust resistance in an F_2 population from the cross Pinto 114 (S) x Mexico 235 (R) inoculated with races 41, 53, 49, and 67 of *Uromyces appendiculatus*.

	Total	Observed		Expected		Ratio	χ^2	P-	Putative
Races	F_2 plants	R	S	R	S			value	R gene
41	247	178	69	185.3	61.8	3:1	1.135	0.2867	*Ur-3*
53	243	176	67	182.3	60.8	3:1	0.857	0.3545	*Ur-3*
49	249	199	50	200.1	46.7	13:3	0.289	0.5907	M235
67	253	203	50	205.6	47.4	13:3	0.170	0.6798	M235

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RESISTANCE TO FUSARIUM WILT IN COMMON BEAN CULTIVARS AND LINES IN PRE-COMMERCIAL STAGE

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Among the diseases that affect common bean (*Phaseolus vulgaris* L.), Fusarium wilt is one of the most deleterious. It is caused by the fungus *Fusarium oxysporum* f.sp. *phaseoli* and is present in the soil in many common bean-producing regions, mainly in areas with successive crops or under center pivot irrigation. Integrated management is the best strategy to control this disease and the use of resistant cultivars is decisive (Costa et al., 2007). The objective of this study was to identify common bean cultivars with resistance to Fusarium wilt.

Thirty common bean cultivars with different grain types were evaluated from 2009 to 2014 in experiments installed in an area infested with the pathogen in Santo Antônio de Goiás, under center pivot irrigation, in the winter growing season, in a randomized block design with two replications. From 2009 to 2012, only one of the replications was assessed and in 2013 and 2014, two replications. The plots were evaluated on a 1-9 scale (1 - completely resistant to 9 -fully susceptible). Analysis of variance was performed in a randomized block design, considering each replication as a block, regardless of the year, totaling eight blocks. Means were compared by the Scott Knott test, at 10% probability.

There were differences among cultivars, indicating variability for resistance to Fusarium wilt, which was confirmed by the variation between cultivars (mean scores 1.9 - 7.1). The experimental precision was satisfactory, as indicated by the coefficient of variation of 23.5%. The most resistant cultivars were BRS Esplendor and CNFP 10794, both with black grain. The cultivars BRS Radiante, BRS Embaixador, BRS Executivo, Jalo Precoce, BRSMG União, BRS Campeiro, BRS Notável, and BRSMG Realce also had a good resistance level and were grouped in the second cluster. A noteworthy cultivar is BRS Notável, the most resistant to Fusarium wilt, with carioca grain. The other cultivars of this group have large grains (over 38 g/100 seeds), which are jalo (yellow), DRK (dark red kidney), cranberry, or striped, native to the Andean origin, except for BRS Campeiro, with black beans. The third group consisted of BRS Ametista, BRS Requinte, BRSMG Majestoso, BRS Utopia, and Pérola, all with carioca beans and intermediate resistance to Fusarium wilt. Pérola is not only the cultivar with highest acreage in the country, but also a reference in terms of field resistance to Fusarium wilt. In addition to these, BRS Agreste with mulatinho (cream) and BRS Pitanga with purple grain also have an intermediate resistance level; a fourth group consisting of cultivars with low resistance: CNFC 10762 and BRS Pontal, carioca; BRS Ártico, white; and BRS Vereda, with pink grain. A fifth group was formed by highly susceptible cultivars: CNFC 10467, BRS Sublime, BRSMG Madrepérola, CNFC 10729, BRS Cometa, and BRS Estilo, all with carioca grain; BRS Esteio, VP-22 and BRS Supremo, with black grain. Based on these results, it was concluded that there are common bean cultivars of almost all grain types tested with good levels of Fusarium wilt resistance, which should be preferred for cultivation in areas infested with the disease.

Table 1. Summary of analysis of variance for reaction to Fusarium wilt in 30 common bean cultivars.

SV	DF	MS	F	P-value
Replications	7	6.41	-	-
Cultivars	29	21.97	17.98	0.000
Residue	203	1.22	-	-
Mean (1-9)	4.7			
CV (%)	23.5			

Table 2. Types of grains (TG) and means of 30 common bean cultivars for reaction to Fusarium wilt (FUS).

Cultivar	TG	FUS		Cultivar	TG	FUS	
BRS Esplendor	black	1.9	a	BRSMG Utopia	carioca	4.5	c
CNFP 10794	black	2.0	a	Pérola	carioca	4.6	c
BRS Radiante	striped	2.8	b	CNFC10762	carioca	5.0	d
BRS Embaixador	drk	3.1	b	WAF 75	white	5.3	d
BRS Executivo	sugar bean	3.1	b	BRS Pontal	carioca	5.3	d
Jalo Precoce	jalo	3.1	b	BRS Vereda	pink	5.6	d
BRSMG União	jalo	3.3	b	CNFC 10467	carioca	6.5	e
BRS Campeiro	black	3.3	b	BRS Esteio	black	6.5	e
BRS Notável	carioca	3.3	b	BRS Sublime	carioca	6.6	e
BRSMG Realce	striped	3.3	b	BRSMG Madrepérola	carioca	6.8	e
BRS Ametista	carioca	3.8	c	CNFC 10729	carioca	6.9	e
BRS Agreste	mulatinho	3.9	c	VP-22	black	6.9	e
BRS Pitanga	purple	3.9	c	BRS Cometa	carioca	7.0	e
BRS Requinte	carioca	4.0	c	BRS Supremo	black	7.1	e
BRSMG Majestoso	carioca	4.5	c	BRS Estilo	carioca	7.1	e

Means followed by the same letter do not differ from each other (Scott Knott, 10 %)

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SCREENING ANDEAN DRY BEAN GERMPLASM FOR ROOT ROT RESISTANCE

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INTRODUCTION: In 2014 and 2015, a field trial was conducted in East Lansing, Michigan to assess 50 Andean dry bean germplasm lines for resistance to common root rot pathogens.

MATERIALS AND METHODS: Three meter single-row plots were inoculated in furrow at planting with either *Rhizoctonia solani* (AG2-2IIIB and AG4), *Fusarium phaseoli*, or *Pythium* (*P. torulosum* and *P. dissoticum*), and compared to a non-inoculated control. Pathogen inoculum was planted with dry bean seed at 23 ml per m except *R. solani* which was inoculated at 3 ml per m. Stand counts were conducted three times during the season, and plant heights were collected once. In 2015, dry bean germplasm was inoculated with *R. solani* AG2-2IIIB or AG4 at 1.5 g per m and compared to a non-inoculated check, stand counts were collected twice.

RESULTS AND DISCUSSION: In 2014, the *Rhizoctonia* treatment significantly reduced plant stands in all 50 dry bean lines assessed, often killing nearly 100% of the plants (Fig 1). The *Pythium* and the *Fusarium* treatments significantly reduced plant stands compared to the control for 37 and 9 of the 50 dry bean lines, respectively (Fig 2). In 2015, non-inoculated control stand counts were significantly reduced due to heavy rainfall and root rot pathogens in the soil. We observed differential responses among germplasm screened against *R. solani* anastomosis groups AG2-2IIIB and AG4 (Fig 3). The screens were very effective at identifying material with resistance to the pathogens tested. In 2015, the screening was refined for assessment of *Rhizoctonia* resistance, by screening *R. solani* anastomosis groups AG2-2IIIB and AG4 individually and with less inoculum input.

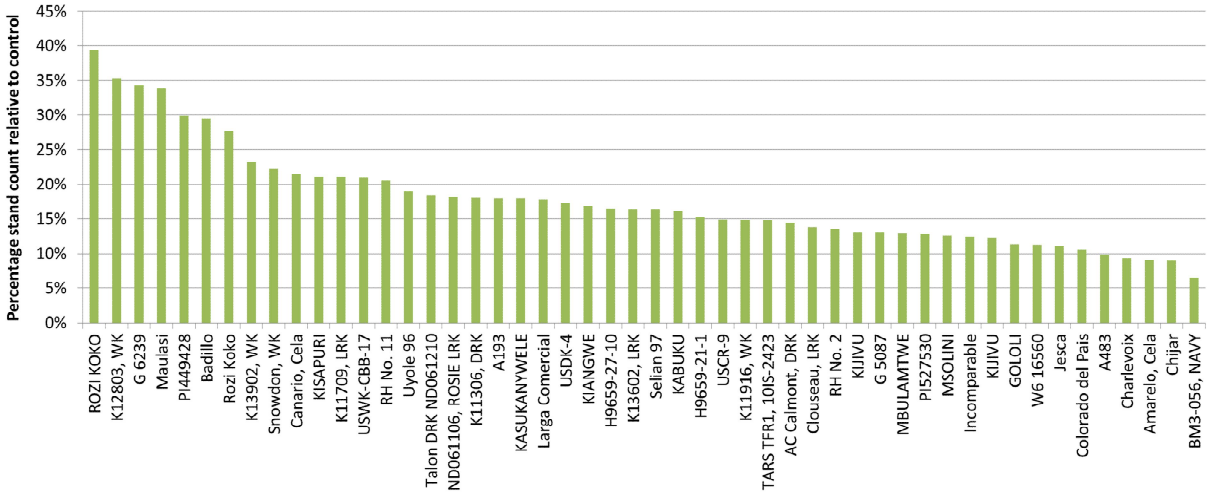

Figure 1. 2014 dry bean plant stand count relative to control as affected by *Rhizoctonia solani*

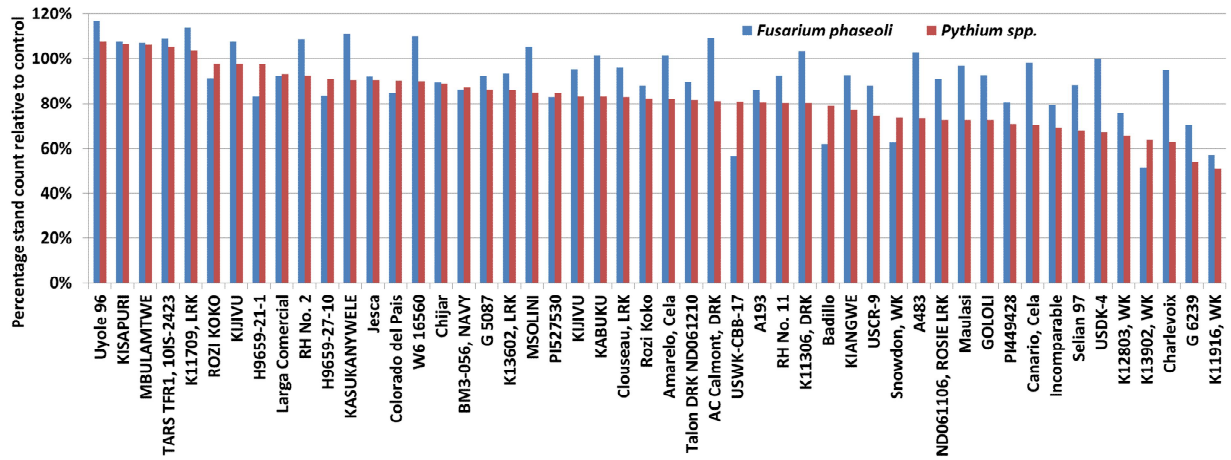

Figure 2. 2014 dry bean plant stand count relative to control as affected by *Pythium* spp. and *Fusarium phaseoli* inoculum

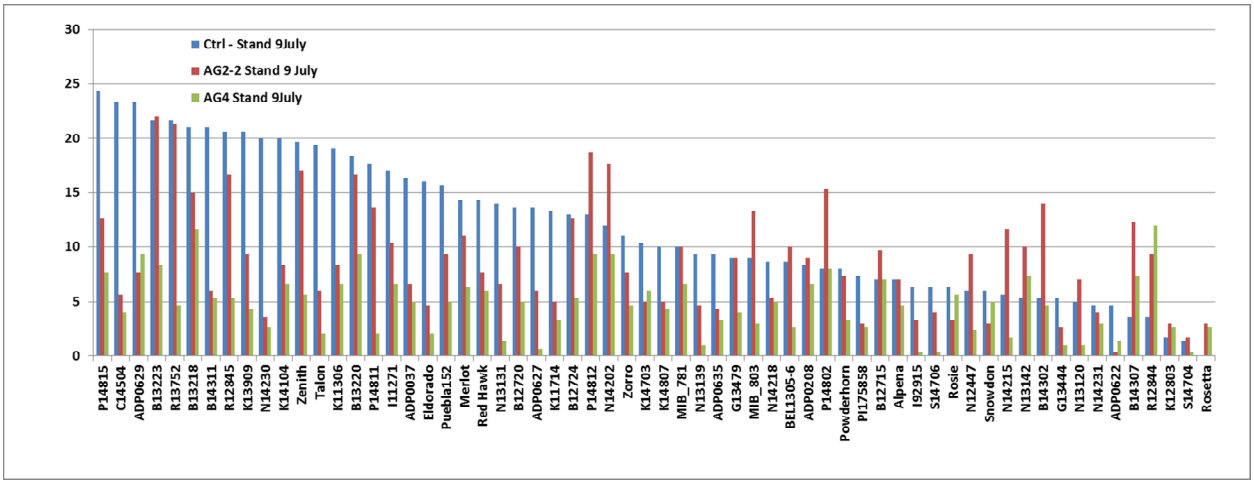

Figure 3. 2015 dry bean plant stand count for non-inoculated control and *Rhizoctonia solani* AG2-2IIIB or AG4 inoculated plots

PATHOGENICITY AND VIRULENCE OF RHIZOCTONIA SOLANI AND PYTHIUM SPP. ON DRY BEAN

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INTRODUCTION: Root rot, seedling damping-off, and seed rot are chronic problems associated with dry bean plant stand establishment in Michigan. Significant losses can occur, especially when cool temperatures, wet soil conditions, and soil compaction occur after seeding. It is not uncommon for growers to replant sections or whole fields due to poor stand establishment as a result of these damping-off and root rot pathogens. In addition to increased costs due to replanting, these diseases can lead to yield reductions as a result of a weakened root system and delayed plant development. In 2015, it was estimated that 10% of the dry bean acreage in Michigan was replanted due to plant stand establishment issues (Greg Varner, personal communication). These diseases are primarily caused by the soil borne pathogens, *Fusarium* spp., *Rhizoctonia solani*, and *Pythium* spp.

Therefore, the objectives of this study were to first identify which pathogens are associated with poor stand establishment due to seed rot, damping-off and root rot of Andean and Mesoamerican dry bean production in Michigan; and to determine which organisms are pathogenic and identify the stage of plant development infected.

MATERIALS AND METHODS: A survey was conducted in 2014 (9 fields) and 2015 (14 fields) in Andean and Mesoamerican dry bean fields in Michigan that had stand establishment issues or plants with root rot symptoms. Plant roots and stems were washed and cultured on corn meal agar amended with pimaricin, ampicillin, rifampicin, PCNB, and benomyl (CMA-PARPB) for recovery of oomycetes and on water agar amended with metalaxyl and streptomycin (WMS) for recovery of fungi. In addition, *Fusarium solani* species complex (FSSC) clade 2 isolates were recovered by the transfer of conidia with an insect needle from sporodochia that developed directly on diseased root tissue in culture on WMS medium. Cultures were purified by single spore technique onto PDA.

Pathogenicity and aggressiveness were evaluated with a representative oomycete panel on red kidney and black bean in a seedling assay. *Rhizoctonia solani* isolates representing anastomosis groups, AG2-2, AG2-3, AG4, AG5, and AG11 were screened against dry bean (cv. Zorro), soybean (cv. Sloan), and corn (cv. DK52-61) in seedling and seed rot assays. Inoculum for pathogenicity testing on seedlings was prepared by culturing all oomycetes on sterilized rice grains and *R. solani* on sterilized un-hulled barley grains for 2-3 weeks, depending on the growth rate of organisms.

Results and Discussion. A total of 169 *Pythium* spp., 260 *Fusarium* spp., and 179 *R. solani* isolates were recovered and identified based on isolate morphology in pure culture. Isolates of *P. ultimum*, *P. myriotylum*, and *Phytopythium* aff. *vexans* significantly reduced emergence of red kidney bean (cv. Red Hawk) and black bean (cv. Zorro), with *P. ultimum* having the greatest impact at nearly 80% reduction. In addition, *P.* aff. *diclinum*, *P. irregulare*, *P. lutarium*, and *P. sylvaticum* significantly reduced dry root weight, root length, and root area in both Andean and Mesoamerican germplasm. *Rhizoctonia solani* isolates in the seed root assay were mostly consistent in their virulence across hosts, isolates within AG2-2 had the highest seed rot index in

dry bean (Fig. 1). AG11 isolates were the least virulent. However, in the seedling pathogenicity assay, all of the isolates were less virulent on corn (Fig. 2). In addition, isolates within AG2-2 and AG4 (with one exception) were the most aggressive on dry bean and soybean resulting in decreased root dry weight.

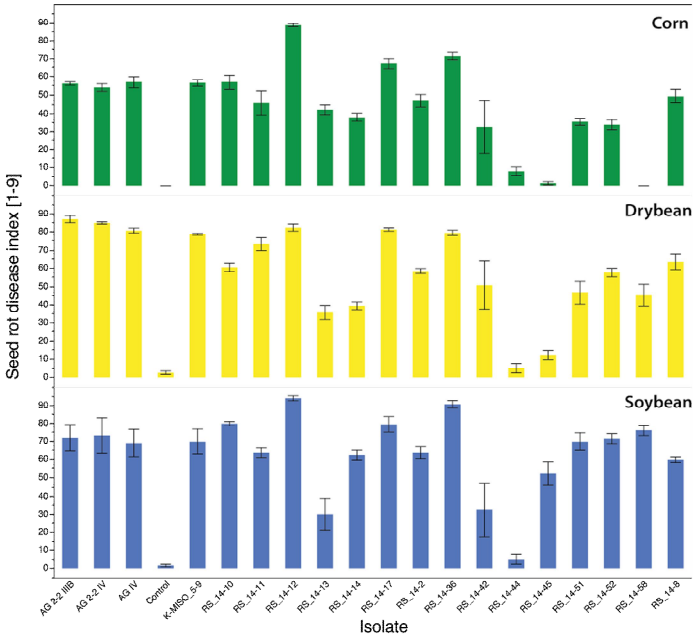

Figure 1. Seed rot disease index of corn, dry bean, and soybean as affected by *Rhizoctonia solani* isolates.

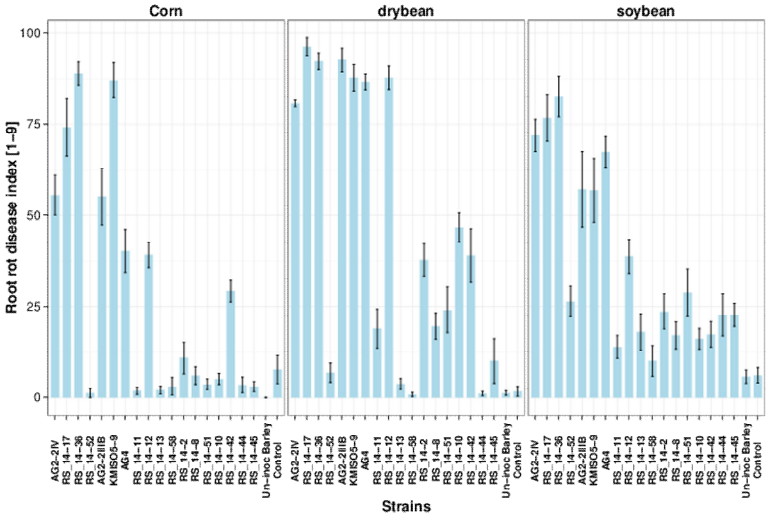

Figure 2. Seedling root rot disease index as compared to the control in a pathogenicity assay on corn, dry bean, and soybean inoculated with *Rhizoctonia solani*

SNP MAPPING OF QTL ASSOCIATED WITH RESISTANCE TO APHANOMYCES ROOT ROT IN SNAP BEAN

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Root rot caused by the fungus *Aphanomyces euteiches* f.sp. *phaseoli* is a major disease of snap beans (*Phaseolus vulgaris* L.) in irrigated central sands of Wisconsin. The disease infects hypocotyls of seedlings resulting in decreased nodulation and stunted growth of plants. Traditional methods for disease control such as fungicides, crop rotations, cover crops, seedbed preparations have been proven ineffective against root rot.

In previous research a recombinant inbred line (RIL) population derived from crossing 'Eagle' a bush snap bean cultivar and 'Puebla-152', a black-seeded Mexican bean landrace cultivar was evaluated for resistance to *Aphanomyces euteiches* by root rot severity and plant height in green house and a major QTL (Quantitative Trait Locus) was identified and mapped using RAPD markers in a low density linkage map (Navarro et. al. 2005 a and b). Two closely linked flanking RAPD markers, S18.1500 and AD9.950, were identified on Linkage group 6 with an R^2 of 0.49 in greenhouse experiments, and an R^2 of 0.28 in field experiments (Navarro et. al. 2005 a and b).

The objective of this research was to remap the QTL for resistance using SNP markers in a high density linkage map in the same Eagle x Puebla 152 RIL population. Single Nucleotide Polymorphism (SNP) data was used to assemble a high density linkage map containing 2488 markers across 1782cM. The map was populated with 1 SNP for every 0.7cM, spanning across 11 chromosomes.

Table 1. Analysis of variance and heritiability of resistance to *A. euteiches* root rot (1=resistant, 9=suseptable) in an Eagle x Puebla RIL population. Walnut Street Greenhouse 2005

Source of variance	d.	Mean square	P>F
Among RIL	68	17.83	<0.001
Within RIL	131	1.78	
Heritability (h^2)			0.75
Mean Puebla 152		1.6	
Mean Eagle		7.1	

Phenotypic data were combined with SNP map to identify quantitative trait loci (QTL) associated with resistance using composite interval mapping. The putative QTL for root rot severity were found on chromosomes 4 and 6 explaining 7.2% and 61.2 % variation, respectively (Table 2).

The greenhouse evaluation of resistance to root rot in the RIL population was performed using four plants per 9 cm plastic pot in a three replicate randomized complete block design. The week old plants were watered and inoculated on the hypocotyl surface is 0/5 ml of lake water containing 2000 zoospores of *A. Euteiches* and then flooded for five days using distilled water and scored for disease reaction (Fig. 1).

Fig. 1. Histogram of response Eagle x Puebla 153 RIL population to inoculation by A. euteiches

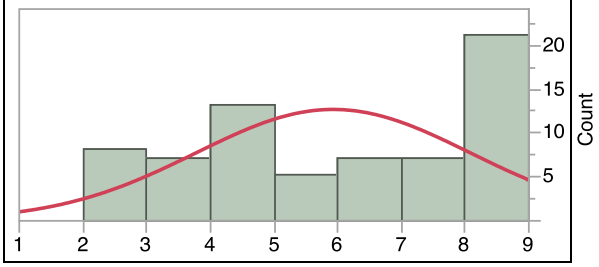

Table 2. Single Nucleotide Polymorphism (SNP) markers associated with putative quantitative traits loci (QTL) in 'Eagle' x 'Puebla 152' recombinant inbred line (EP-RIL).

	QTL discovery in EP-RIL population				
Trait	Marker	LOD score	Chromosome	Position (cM)	R^2
Root rot score	ss715647804	4.3	4	1.41	7.24
	ss715639366	16.8	6	52.26	61.22

LOD score= logarithm to the base 10 of the likelihood odds ratio

The magnitude of the variation, R^2 = 49%, explained by flanking RAPD markers S18.1500 and AD9.950, previously identified by Navarro et. al., 2008 and 2009 and the amount of variation, R^2 = 61% identified in the present study associated with SNP marker ss715639366, combined with their shared location on linkage group 6 suggest that the RADP and SNP markers may be associated the same major QTL for resistance to *A. euteiches*. Additional minor RAPD markers accounting for from 11 to 14 % of the variation were also observed on linkage groups 1, 2 and 9 (Navarro et. al., 2008 and 2009. In contrast, only one minor SNP marker which accounted for 7 % of the variation located on linkage group 4 was identified associated with SNP marker ss715647804.

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IDENTIFYING GENOMIC REGIONS ASSOCIATED WITH HALO BLIGHT RESISTANCE WITHIN THE USDA CORE COLLECTION OF COMMON BEAN

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INTRODUCTION: Halo blight, an important common bean (*Phaseolus vulgaris* L.) bacterial disease, is caused by a seed-borne bacterium *Pseudomonas syringae* pv. *phaseolicola* (*Psp*). Out of the nine races identified, race 6 of *Psp* is the most prevalent worldwide including North Dakota and Minnesota, and it has been reported to cause yield loss up to 45%. To date, five putative major R genes conferring resistance to all *Psp* races except for race 6 have been identified (Taylor et al., 1996). The development of resistant cultivars to race 6 has had limited success, with US14HBR6 being among the few resistant genotypes identified to date (Duncan et al., 2014). Therefore, this study was aimed at evaluating the USDA-NPGS common bean core collection to identify novel sources of resistance to race 6 of *Psp*, and the genomic regions linked to the resistance using Genome-wide Association Study (GWAS) for potential use in Marker-Assisted Selection (MAS).

MATERIALS AND METHODS: Due to issues with photoperiod sensitivity and germination, only 383 accessions out of 422 were included in the study. One plant of each accession and 10 standard commercial cultivars (checks) were arranged in a randomized complete block design (RCBD) with four replications under greenhouse conditions. The second trifoliate from each accession was inoculated 21 d after planting with a 48 h old *Psp* race 6 culture (at a density of $1x10^8$ cfu/mL) using the multiple-needled florist pin frog method (Mills and Silbernagel, 1992). The inoculated plants were maintained at 100% RH and 19°C ± 1°C for 48 h in a humidity chamber. Inoculated trifoliates were then rated 10 days post inoculation using a disease severity scale of 1 – 5 [1 = resistant (R), 2-5 = susceptible (S)] (Taylor et al., 1996). A DNA sample from each genotype was extracted at the Univ. of California-Davis (Dr. Paul Gepts) and genotyped in USDA-ARS at Beltsville-MD using a 6K SNP chip (Song et al., 2015). Marker-trait associations were identified using GAPIT (Genome Association and Prediction Integrated Tool) in the R software (Zhang et al., 2010).

RESULTS AND DISCUSSION: Of the 383 accessions evaluated, 10 accessions showed high levels of resistance with an average disease score of ≤ 1 (Table 1), suggesting these lines are potential sources of resistance to race 6 of *Psp*. A total of 5398 SNPs were obtained from 383 accessions. For data imputation FastPHASE v. 1.2 software was used (Scheet and Stephens, 2006). Markers with minor allele frequency (MAF) >5% were removed to obtain a total of 4988 polymorphic markers that were used for GWAS. Of the four models tested for controlling population structure, naïve, Principal Component Analysis (PCA), Efficient Mixed Model Association (EMMA), and PCA + EMMA, EMMA was selected as the best model based on the QQ-plot (Quantile-Quantile). Stepwise regression was performed to select the markers that showed more significant marker-trait association. GWAS analysis identified potential chromosome regions on Pv 04 (1.2 Mbp) and Pv05 (39.4 Mbp) associated with resistance to race 6 of *Psp* (Fig. 1), where the region on Pv05 was also consistent across other models tested. Nucleotide-binding (NB-ARC domain-containing) disease resistance proteins in Pv04 suggests activation of innate immune

responses under disease pressure. Step-wise regression revealed three markers on both Pv05 and Pv04 that explained 18% of the genetic variation. However, among these, the most significant marker accounted for 8% of the genetic variation. These markers will be useful for MAS in the common bean breeding program to develop cultivars resistant to race 6 of *Psp*.

Table. 1. Least square means and standard error of 10 resistant PIs and 10 standard checks.

Accession/entry	Mean ± S.E.‡	Country of Origin	Accession/entry	Mean ± S.E‡	Market Class
PI 313531	1.0 ± 1.4	Mexico	VAX3¶	1.0 ± 0.46	Dark Red Kidney
PI 313727	1.0 ± 1.4	Mexico	US14HBR6¶	1.5 ± 0.41	Pinto
PI 325684	1.0 ± 1.4	Peru	Montcalm†	2.6 ± 0.41	Dark red kidney
PI 311940	1.0 ± 2.6	Mexico	Red Hawk†	2.6 ± 0.46	Dark red kidney
PI 319677	1.0 ± 2.6	Ecuador	CELRK†	2.7 ± 0.41	Light red kidney
PI 355419	1.0 ± 2.6	Peru	Eclipse†	2.8 ± 0.41	Black
PI 511767	1.0 ± 2.6	Colombia	Long's Peak†	3.2 ± 0.46	Pinto
PI 531862	1.0 ± 2.2	Peru	Croissant†	3.3 ± 0.46	Pinto
PI 207193	1.0 ± 3.1	Colombia	Pink Panther§	3.5 ± 0.41	Light red kidney
PI 290995	1.0 ± 3.1	Mexico	Red Rover†	3.5 ± 0.41	Dark red kidney

‡ *Pdiff* was used to compare Lsmeans at $\alpha = 0.05$.
¶ Resistant check. § Susceptible check. † Commercial checks.

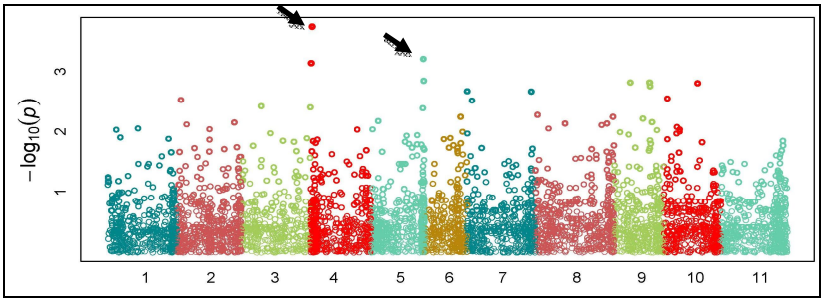

Figure 1. Manhattan plot representing markers associated with halo blight resistance using EMMA. Arrows point to the most significant markers after stepwise regression.

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RACES OF *PSEUDOMONAS SYRINGAE* PV. *PHASEOLICOLA* FROM YELLOW BEANS GROWN IN SINALOA, MÉXICO

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In 2013 the dry bean production at the state of Sinaloa was 114,993 t in 74, 451 ha, that is 10.5% of the national production. From 2013 to 2015, 95% of the land sown with beans in Sinaloa was with cv. Azufrado Higuera (yellow seeded) with a mean yield of 2.0 t/ha. Azufrado Higuera is resistant to rust and tolerant to virus. In the past three years the incidence of halo blight (*Pseudomonas syringae* pv. *phaseolicola* (*Psp*)) was 100%, and estimated losses during the fall-winter growing season were of 40-60%. *Psp* has been reported inducing the disease in other common bean growing areas of Mexico during the summer-fall season[1,5]. *Psp* strains isolated from seeds of several yellow bean cultivars growth in Sinaloa showed different severity levels during the inoculation in yellow bean cultivars, under greenhouse conditions[2]. In Mexico 38% of the farmers use the grain they produce as seed. The aim of this study was to determine the physiological races of twelve *Pseudomonas syringae* pv. *phaseolicola* (*Psp*) strains isolated from cv. Azufrado Higuera, an Andean cultivar, grown at different locations in Sinaloa in the fall-winter of 2013/2014.

MATERIALS AND METHODS

Azufrado Higuera leaves and pods were collected in commercial fields in the municipality of Ahome, Sin. Twelve isolates were obtained and characterized (morphological, cultural and biochemical) as *Psp.* The pathogenicity of the strains was tested on Azufrado Higuera. Fully expanded leaves were taken from plants of the differential set of Taylor *et al*.[6], which were grown in a greenhouse. Suspension of each of 12 strain of *Psp* (3 $X10^8$ ufc/ml), was inoculated with an insulin needle, with nine replicates for isolate; different leaves were used with each isolate. In the control leaves we used distilled water. Plants were kept at 100% RH, 23ºC/10 days. Infection scored with 1-9 scale[4]. The assay was replicated four times.

RESULTS

It is well known that the cultivars from the Nueva Granada race, like Azufrado Higuera, are susceptible to *Psp*. The extensive use of a single susceptible cultivar and the use of grain as seed, are factors that had increased the inoculum present in Sinaloa. Also, the temperate weather during the fall-winter growing season is favorable for *Psp* growth. Our working hypothesis was that all the strains isolated belonged to a single race, since all isolates were collected from same cultivar, Azufrado Higuera. Six of the strains fitted the reaction of race six[6] and the rest did not fit any of the known races (Table 1). Nevertheless, race six can be 'any strain' that infects all differentials. In 1996 Taylor *et al*.[6] used isolates from different places, 18 from America and only 4 from Mexico to establish the differential set for halo blight. In a recent work Miklas *et al*.[2] showed slight variation in the reaction of some cultivars from the differential set compared with Taylor's reaction. It might be that the differential set needs to be improved? It seems necessary to increase our knowledge on the interaction between *Pseudomonas syringae* pv. *phaseolicola* and *Phaseolus vulgaris.*

Table 1. Races of Taylor *et al*.[6], reactions observed by Miklas *et al*.[2], and of inoculated strains from Sinaloa.

#	Cultivar	Resistance genes	Races* 1	2	3	4	5	6	7	8	9
1	Canadian Wonder	-	+	+	+	+	+	+	+	+	+
2	A52 (ZAA 54)	4	+	+	+	+	-	+	+	+	+
3	Tendergreen	3	+	+	-	-	+	+	+	+	+
	Tendergreen[&]	3	+	+	-hr	-hr	+	+	+	+	+
4	Red Mexican UI3	1, 4	-	+	+	+	-	+	-	+	-
5	1072	2	+	-	+	-	-	+	-	+	+
6	A53 (ZAA 55)	3, 4	+	+	-	-	-	+	+	+	+
	A53 (ZAA 55)[&]		+	+	-	-hr	+	+	+	+	+
	Minuette[&], I9365-31[&]		+	+	-hr	-hr	-	+	+	+	+
7	A43 (ZAA 12)	2, 3 , 4, 5	+	-	-	-	-	+	-	-	-
8	Guatemala 196-B	3, 4	-	+	-	-	-	+	-	+	-
	Inoculated strains							2, 4, 7, 16, 18, 20			

* Taylor *et al*.[6]; [&] Miklas *et al*.[2]; + compatible (susceptible); - incompatible (resistant), -hr incompatible reaction with severe hypersensitive response.

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IDENTIFICATION OF COMMON BEAN RESISTANT SOURCES TO ANGULAR LEAF SPOT DISEASE IN A BRAZILIAN GERMPLASM COLLECTION

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INTRODUCTION

Angular leaf spot disease caused by *Pseudocercospora griseola* is one of the most important diseases of the canopy of common bean, occurring in most places where the crop is cultivated (MODA-CIRINO et al., 2012). Although the frequent use of fungicides common bean crop, the most economically viable alternative to control disease is the use of resistant cultivars. The germplasm collection of the Universidade Federal de Lavras has about 888 entries and its origins are genotypes developed by different breeding programs, collected in productions areas and other countries. The common bean breeding program from the University conduces a recurrent selection program to grain yield and angular leaf spot resistance since 1980. The aim of this study was to identify sources of resistance in the germplasm collection providing information of potential sources of resistance to introduce in breeding programs.

MATERIAL AND METHODS

A group of 209 common bean genotypes from the germplasm collection of the Universidade Federal de Lavras (UFLA), Minas Gerais State, Brazil where evaluated. The race 63-63 of *P. griseola* is one of the most frequent races in Brazil (SILVA et al.2008; PEREIRA et al. 2015) and was used in all inoculations. Pathogenicity tests were conducted using the inoculation in early plant stages as proposed by Pereira et al. (2011). Mycelial plugs were inoculated in leaf-dextrose-agar medium and incubated at 24ºC for seven days in the dark to producing spores. Spore solutions were made by harvesting in sterile water and the concentration adjusted to 2 x 10^4 spores/mL. Nine seeds of each cultivar were sown in a polystyrene tray of 162 cells with Multiplant® substrate. Two replicate trays were used totalizing 18 seeds of each cultivar. The cultivars Rosinha and the inbred line MAI-18-13 were used as susceptible and resistant controls, respectively. When seedlings had fully expanded primary leaves the spore suspension was sprayed and trays were maintained in the greenhouse at 24ºC and 85% humidity for 14 days when evaluations were performed. Scale from 1 to 9 developed by Librelon et al. (2015) was used to evaluate plant symptoms. Average scores were estimated and scores below 3 were considered as resistant, whereas plants scoring more than 3 were susceptible

RESULTS AND DISCUSSION

The germplasm collection from the breeding program at UFLA has about 888 entries. In this work we evaluated the reaction of 209 inbred lines using the 63-63 race of *P. griseola*. The reaction of the evaluated lines showed different levels of resistance to race 63-63. Figure 1 shows that 54(26%) lines were identified as resistant and 155(74%) as susceptible.

This result demonstrates the severity of the disease and the importance of obtaining resistant cultivars. Recurrent selection breeding method allows the introduction of new genotypes and among the lines evaluated have been identified potential sources of resistance that can be employed directly in the breeding program.

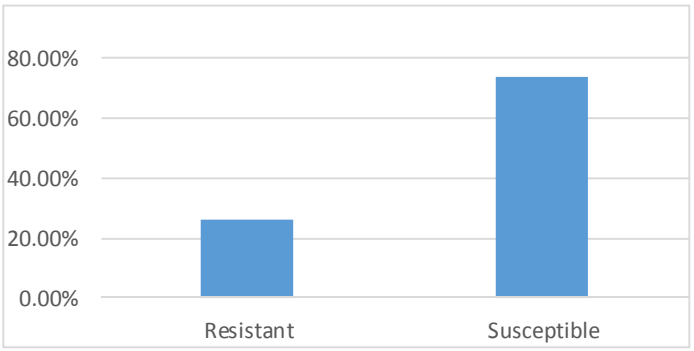

Figure 1. Percentages of resistant and susceptible common bean inbred lines to 63-63 race of *P. griseola*.

The evaluation of resistance to angular leaf spot has been carried out in germplasm collections in of different research institutions in Brazil (MODA-CIRINO et al., 2012) identifying about 14% of resistantant lines. Most of the evaluations were conducted under field condictions depending of the natural occurrence of the pathogen. Evaluations performed at the Universidade Federal de Viçosa found three lines (MAI-18-13, VC16 and Vermelhão) resistant in both field and greenhouse evaluations, therefore considered as sources of resistance to breeding programs (MODA-CIRINO et al., 2012). Interestingly, the resistant line MAI-18-13 has origins in the recurrent selection program from UFLA. Moreover, in the present work were identified 26% of resistant lines and seven were obtained from the recurrent selection program showing its efficiency to improve resistance to angular leaf spot disease.

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EFFECT OF CHEMICAL CONTROL OF ALS (*PSEUDOCERCOSPORA GRISEOLA*) ON SEED YIELD OF TROPICAL BLACK BEAN CULTIVARS IN VERACRUZ, MEXICO

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INTRODUCTION: In Veracruz, Mexico, angular leaf spot (ALS) caused by the fungus *Pseudocercospora griseola* (Sacc.) Ferraris, is a disease that affects bean yields in both cropping systems, rainfed and residual soil moisture (Lopez *et al*., 2006; Tosquy *et al*., 2012). This situation becomes more critical, as most local farmers grow landraces and black bean cultivars introduced from different places such as the USA; both of these type of cultivars have shown susceptibility to bean diseases such as ALS. In addition, producers do not apply fungicides for disease control. The aim of the study was to determine the effect of ALS chemical control on seed yield performance of tropical black bean cultivars by applying dithiocarbamate, a non-systemic agricultural fungicide.

MATERIALS AND METHODS: The research was conducted in the of summer season (July-October), 2011, under rainfed environmental conditions, in Rincon Grande, Orizaba, a central region of the state of Veracruz. A field experiment was planted as a RCBD with four replications with a 2x3 factorial arrangement of treatments. Two levels of dithiocarbamate, a non-systemic agricultural fungicide were assessed: 0.0 kg ha^{-1} (regional treatment) and 1.5 kg ha^{-1} of the fungicide commercial product, which was applied only once during the course of the field experiment, particularly when the first symptoms of the disease occurred. Three bean cultivars were evaluated, Negro Jamapa, Negro Comapa and T-39 (control check). The variables assessed were disease reaction and grain yield. To evaluate the effect of fungicide treatments and genotype reaction to ALS, the 1 to 9 scale (CIAT, 1987) was used and seed yield was measured as well. ANOVA was performed for each variable and statistical test for separation of means was based on the Least Significant Difference (LSD, 0.05). To determine the effect of angular leaf spot on seed yield a correlation analysis was performed as well.

RESULTS AND DISCUSSION: With the application of the dithiocarbamate, a non-systemic agricultural fungicide, bean plants showed lower damage caused by ALS. An average of 100% seed yield increased was obtained with the chemical control treatment compared to non-fungicide application. In general, Negro Comapa and Negro Jamapa cultivars had less ALS symptoms and higher average seed yield than the check T-39 (Table 1). These results indicate that both, Negro Comapa and Negro Jamapa, are best cultivar alternative for growing beans on central region in Veracruz, and that even with the presence of the disease had higher seed yield than the check cultivar T-39. Correlation analysis (r = -0765 **) indicated that no chemical control of ALS caused a seed yield reduction, that ranged from 47.1 (Negro Comapa) to 70.9% (T-39). According to the data about reaction to ALS, Negro Comapa and Negro Jamapa were moderately tolerant 4.5 and 5.0 respectively, while T-39 was susceptible (6.5). In addition, both varieties with no chemical control of ALS showed lower grain yield reduction than the check T-39 (Table 2). These results suggest that, if farmers do not have the resources to apply fungicide and thus chemically controlling ALS, then farmers can still grow beans using Negro Comapa and/or Negro Jamapa cultivars and still obtain higher seed yields than the check cultivar T-39.

Table 1. Effect of fungicide application on the chemical control of ALS and seed yield in black bean cultivars. Rincon Grande, Orizaba, Veracruz, Mexico. Summer season 2011.

Factor	Angular leaf spot (1 – 9) ††	Seed yield (kg ha^{-1})
Fungicide treatment		
No application (control)	5.3 a	743.3 b
With application	3.0 b	1,599.2 a
LSD (0.05) †	0.4	153.7
ANOVA	**	**
Cultivar		
Negro Comapa	3.5 b	1,356.2 a
Negro Jamapa	4.0 b	1,345.0 a
T-39 (Check)	5.0 a	812.5 b
LSD (0.05)†	0.5	188.3
ANOVA	**	**
CV (%)	11.3	15.1

** = Significant (0.01). † Means with same letter in each variable within each factor are statistically similar.
†† CIAT scale (1= resistant to 9= susceptible).

Table 2. Reaction of common bean cultivars to ALS and effect of chemical control on seed yield.

Cultivar	ALS control type	ALS (1 a 9) †	Seed yield (kg ha^{-1})	Seed yield reduction (%)
Negro Comapa	+A	2.5	1,773.7	
Negro Comapa	-A	4.5	938.7	47.1
Negro Jamapa	+A	3.0	1,765.0	
Negro Jamapa	-A	5.0	925.0	47.6
T-39	+A	3.5	1,258.7	
T-39	-A	6.5	366.2	70.9
ANOVA		n.s.	n.s.	
CV (%)		11.3	15.1	

+A, with fungicide application and –A, no fungicide application. n.s. = non-significant.
† CIAT scale (1= resistant, 9= susceptible).

CONCLUSIONS: Disease control of angular leaf spot was obtained by applying dithiocarbamate, a non-systemic agricultural fungicide, and higher seed yields were obtained across all bean cultivars assessed as well. Without chemical protection Negro Comapa and Negro Jamapa bean cultivars were tolerant to angular leaf spot and showed higher productivity than the check cultivar T-39, which showed some level of susceptibility to this disease.

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CHARACTERIZATION OF THE RESISTANCE LOCUS TO ANGULAR LEAF SPOT IN THE COMMON BEAN ACCESS 'JAPONÊS'

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INTRODUCTION - The angular leaf spot caused by the fungus *Pseudocercospora griseola* (Sacc.) Crous & U. Braun, is a major diseases that causes large losses to common bean producers (Pastor-Corrales et al., 1998). The allelism test is commonly used to determine whether various phenotypes of a given character, observed in a population of individuals, by participation of a number of alleles or genetic interaction. This test consists of crossing the individuals with the many phenotypes, two by two, in all possible combinations and study the phenotypic segregation in offspring. If a different monogenic inheritance is found, there is a case of gene interaction and not multiple allelic. Thus, this study aimed to characterize the R loci for resistance to angular leaf spot, present in 'Japanese' creole acess, in relation to the five other related sources.

MATERIAL AND METHODS - For allelic tests, genotypes 'Azulão', 'Carioca Precoce', 'Casquinha', 'Criangu', 'Pardo' and 'Madrepérola' were crossed with 'Japonês'. In all cases, 'Japonês' genotype was used as pollen donor. F_2 populations from each cross were used to test the allelic. To ensure that the F_1 seeds were actually the result of hybridization, we analyzed the color, shape, size and brightness of the F_2 seeds. To support the understanding of allelic relations was executed also heritage studies. The genotypes 'Azulão', 'Carioca Precoce', 'Casquinha', 'Criangu' and 'Pardo' were crossed with 'Madrepérola'. In all cases, 'Madrepérola' was the female parent. Main populations were used in inoculations with races 63.23 and 63.39, which are known to be aggressive and virulent. The suspension of conidia of each race was obtained by scraping the surface of the fungus colonies grown in Petri dishes containing a mixture of distilled water, tomato sauce, agar and calcium carbonate ($CaCO_3$), for 12 days at 24°C. This suspension was filtered through cheesecloth and then adjusted to a final concentration of 2.0×10^4 conidia/mL (Sanglard et al., 2009). The chi-square test (χ^2) were analyzed using the GENES program (Cruz, 2006).

RESULTS AND DISCUSSION - The race 63.63 was inoculated in F_2 populations (Japonês x Azulão), F_2 (Japonês x Casquinha), F_2 (Japonês x Criangu) and F_2 (Japonês x Pardo), yielding the pattern of segregation of two genes typically 15:1 (two independent dominant genes) and genes involved three (63:1, 61:3 and 60:4, respectively). The F_2 population (Japonês x Casquinha) did not indicate gene interaction by presenting a segregation of 63:1 (three independent dominant genes), indicating the simultaneous participation of two genes present in 'Casquinha'. The inheritance study of this population resulted in a segregation of 15:1, which corroborates with this idea. Similar results were observed in inoculation with race 63.39, when the populations F_2 (Japonês x Casquinha) and F_2 (Japonês x Criangu) also showed segregation of 63:1 and 15:1, respectively. The F_2 population (Japonês x Carioca Precoce) segregated in 13:3 for this breed, a typical behavior of digenic inheritance where two epistatic genes, one dominant and one recessive, respectively. From the reviews in this work, it is suggested that the locus genotype present in the 'Japonês', distinct from other sources studied. Allelic tests are particularly important for breeding programs aimed at the pyramiding of different genes that confer resistance to different races of a pathogen. The pyramiding of genes has been suggested as a strategy of obtaining genotypes with

durable resistance. The accumulation of resistance genes with major effects on a genotype delays the appearance of new races of the pathogen (Servin et al., 2004). The basis for the stability of resistance is the reduction of the pathogen avirulence adaptation when multiple genes must be inactivated to "break" host resistance (Van der Plank, 1984). Therefore, the intercrossing of these materials analyzed lines derived from 'Japonês' is justified and possibly would increase the spectrum of resistance to *P. griseola* races.

Table 1. Allelism tests and inheritance studies for genetic characterization of resistance to *P. griseola* present in the acess 'Japonês'

Cross	Race (isolate)	F_2 plants		[a]Expected ratio		Value of χ^2	P (%)
		R	S	R	S		
Japonês x Azulão	63.63 (158-1)	421	27	15	1	0.0140	97.0072
Japonês x Carioca Precoce	63.39 (29-3)	389	74	13	3	0.0449	83.2113
Japonês x Casquinha	63.63 (158-1)	512	14	63	1	0.2984	58.4875
Japonês x Casquinha	63.39 (29-3)	334	4	63	1	0.0786	77.9149
Japonês x Criangu	63.63 (158-1)	356	16	61	3	0.0232	87.8891
Japonês x Criangu	63.39 (29-3)	345	24	15	1	0.0016	96.7127
Japonês x Pardo	63.63 (158-1)	339	22	60	4	0.0027	95.8442
Japonês x Madrepérola	63.63 (158-1)	226	71	3	1	0.1698	62.8720
Japonês x Madrepérola	63.39 (29-3)	367	123	3	1	0.2661	57.7021
Azulão x Madrepérola	63.63 (158-1)	140	43	3	1	0.0014	65.4243
Carioca Precoce x Madrepérola	63.39 (29-3)	323	102	3	1	0.0712	62.2926
Casquinha x Madrepérola	63.63 (158-1)	376	28	15	1	0.2194	91.4625
Casquinha x Madrepérola	63.39 (29-3)	375	27	15	1	0.0805	94.0584
Criangu x Madrepérola	63.63 (158-1)	367	119	3	1	0.0764	59.3658
Criangu x Madrepérola	63.39 (29-3)	141	43	3	1	0.1802	66.2363
Pardo x Madrepérola	63.63 (158-1)	315	103	3	1	0.0798	58.4486

R: resistance; S: susceptibility; [a]Nine proportions were tested (Table 1 shows the best explain of observed data, i.e., that provided the lower chi-square values - χ^2); P (%): Probability of estimated value.

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REACTION OF COMMON BEAN LINES TO *PSEUDOCERCOSPORA GRISEOLA* IN DIFFERENT ENVIROMENTAL CONDITIONS

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INTRODUCTION

Angular leaf spot (ALS) in common bean (*Phaseolus vulgaris* L.) caused by fungus *Pseudocercospora griseola* of is one of the main diseases that affects the crop. This disease mainly infects leaves and pods, inducing premature leaf dropping and consequently reduction in grain quality (Mahuku et al., 2009). Losses in grain yield caused by ALS can reach 80% (Singh and Schwartz, 2010). The use of resistant cultivars is one of the most efficient method to control this disease. Evaluation of common bean lines reaction to *P. griseola* is important to identify newresistance sources that can be used in common bean breeding program. Furthermore, to determine the best environment condition for this evalution is a challenge. Therefore, this study aimed to compare the reaction of common bean lines to *P. griseola* in different enviromental conditions.

MATERIAL AND METHODS

Two experiments were conducted, one in field and other one in greenhouse to evaluate the ALS severity in 144 common bean genotypes, cultivars and lines, using artificial inoculation of *P. griseola*. Experimental design was a triple lattice 12x12 and the plot size was two rows of one-meter and sowing on February in dry season. Randomized block design with three replications was used to evaluate the same common bean genotypes in greenhouse. Plot consisted of a pot which four seeds were sown. Mixture *P. griseola* strains, races 63.23 and 63.63, was used for artificial inoculation in both environment conditions. Each strain was cultivated in leaf dextrose agar medium and incubated at 24°C for 14 days with 12 hours of photoperiod. Concentration of conidia was adjusted to 2×10^4 conidia mL^{-1}. Plants in V3 stage were inoculated and remained in greenhouse with relative humidity of 80% and temperature of 24°C. After 14 days of inoculation was evaluated ALS severity of plants according to the descriptive scale 1-9, developed by Pastor-Corrales and Jara (1995). Artificial inoculation on plants in field was carried three times from the V3 stage and ALS severity was evaluated at 33 days after flowering using the same scale (Rezende et al. 2015). Plants scoring between 1 and 3 were considered resistant and higher 3, susceptible. The severity data were submitted to analysis of variance using the GENES software.

RESULTS AND DISCUSSION

Of the 144 genotypes evaluated, only 9 and 12 were resistant to *P. griseola* in greenhouse and field, respectively (Table 1) showing the difficulty of identifying common bean genotypes with high resistance to ALS. Furthermore, it was observed that in general, the genotypes behavior was not consistent in the two environment conditions evaluated. Low coincidence should have occurred due to temperature and humidity conditions that are not controlled in field. Another point is the natural pathogen occurrence in field that is responsible for the presence of other pathogen races. It is expected that common bean lines identified as resistant in greenhouse present different reaction to ALS under field conditions. Interestingly, the most of resistant

common bean lines (acronym MA) in both environments are derived from a recurrent selection program for ALS. These data corroborate with results commonly found in the literature (Pereira et al., 2015). This recurrent selection program used 17 genotypes for the establishment of the population base and, among those, ten are recognizable resistance sources to ALS.(AN512561, AND-277, Ouro Negro, Campuesto Negro Chimaltenango, CAL143, MAR-2, MAR-1, G5686, MA4.137, and Jalo) (Amaro et al., 2007). In general, all common bean lines derived from different cycles of this program exhibited a good level of resistance to the *P. griseola,* proving that recurrent selection has been efficient in obtaining resistant lines to ALS. The common bean line MAIV-15.524 exhibited the lowest scores for the severity of ALS in both environments. Thus, this line is a possible source of resistance to be used in breeding programs, with a view toward resistance to ALS.

Table 1 Mean scores (1 = minimum, 9 = maximum) of ALS severity of common bean lines more resistant in field and greenhouse.

Elite line/cultivar	Scores (Greenhouse)	Elite line/cultivar	Scores (Field)
MAII-8	2.9	MAIII-16.155	2.3
PT 65	2.8	MAI-18.13	2.0
VC 28	2.8	MAVIII-128	2.7
MAIV-8.102	2.8	MAII-10	2.7
MAIV-15.204	2.8	MAVII-244	1.3
MAIX-4	2.7	MAIII-9.91	2.6
RC2 RAD 155	2.6	CNFC 10432	2.8
MAIV-15.524	2.4	BRS NOTÁVEL	2.6
BRS RADIANTE	1.7	VC 17	2.3
-		MAV-1.7	3.0
-		MAIV-15.524	2.6
-		RPCVIII 7	2.4
Check-1 BRSMG Talismã	4.8	Check-1 BRSMG Talismã	5.2
Check-2 BRSMG Majestoso	4.7	Check-2 BRSMG Majestoso	4.3
Check-3 BRSMG Madrepérola	4.6	Check-3 BRSMG Madrepérola	4.7

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PRODUCTION OF ANTHRACNOSE INFECTED DRY BEAN SEED UNDER GREENHOUSE CONDITIONS

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INTRODUCTION

Anthracnose of dry edible bean (*Phaseolus vulgaris* L.), caused by the fungal pathogen *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib., results in reduced yield and seed quality. Anthracnose is most often introduced into a field by infected seeds. The transmission of seed-borne anthracnose to dry bean plants has been previously evaluated using field-infected seeds, or seeds coated with spore suspensions (Tu, 1983; Mohammed and Sangchote, 2005; 2007; Conner et al., 2009). The use of either of these seed sources can introduce variability into evaluations; therefore, methods were developed to produce seed with varying levels of anthracnose under controlled conditions. The objective of this study was to develop a method to produce of seeds infected with *C. lindemuthianum* under controlled conditions, for experimental use.

MATERIALS AND METHODS

The pinto bean cultivar AC Pintoba was grown in the greenhouse to the R3 and R4 growth stage, approximately 6 to 8 weeks. At R3, the pods were wounded with medium grit sand paper and sprayed with a 1×10^6 conidia/ml suspension of *C. lindemuthianum*, race 73. To generate severely infected seeds, the pods were stabbed with a 3cc syringe with a 21 gage needle containing a 1×10^6 conidia/ml suspension at the R4 growth stage. The inoculated plants were placed in a walk-in growth chamber with a 14 hr photoperiod, 22˚C day and 20˚C night temperatures, and 95% relative humidity (Fig.1). The plants were kept in the chamber until the pods started striping (R7 growth stage) and moved to the greenhouse until maturity (Fig. 2). At harvest, the seeds were separated into 5 categories; symptomless, slightly discolored, discolored, lesions <50%, and severely infected (Fig. 3).

Figure 1. Wounding with sandpaper and inoculation of dry bean pods with *C. lindemuthianum*.

RESULTS AND DISCUSSION

The stabbing method was the most labor intensive; however, it produced the highest frequency of seeds of the more severely infected seed types (Fig. 4). The wounding method did not produce as many seeds in the more severely diseased categories because severely infected pods did not produce seeds. When pods were inoculated prior to R3, no seeds were produced. If pods were inoculated after R4, seeds with lesions were not commonly produced. The development of this method has enabled the evaluation of seed-to-seedling transmission of *C. lindemuthianum* while controlling for pathogen race and limiting the likelihood of seed discoloration resulting from environmental factors or infection by other pathogens.

Figure 2. Dry bean pods wounded with sandpaper and inoculated with *C. lindemuthianum*.

Figure 3. Dry bean pods stabbed with a needle and syringe filled with a *C. lindemuthianum* conidial suspension.

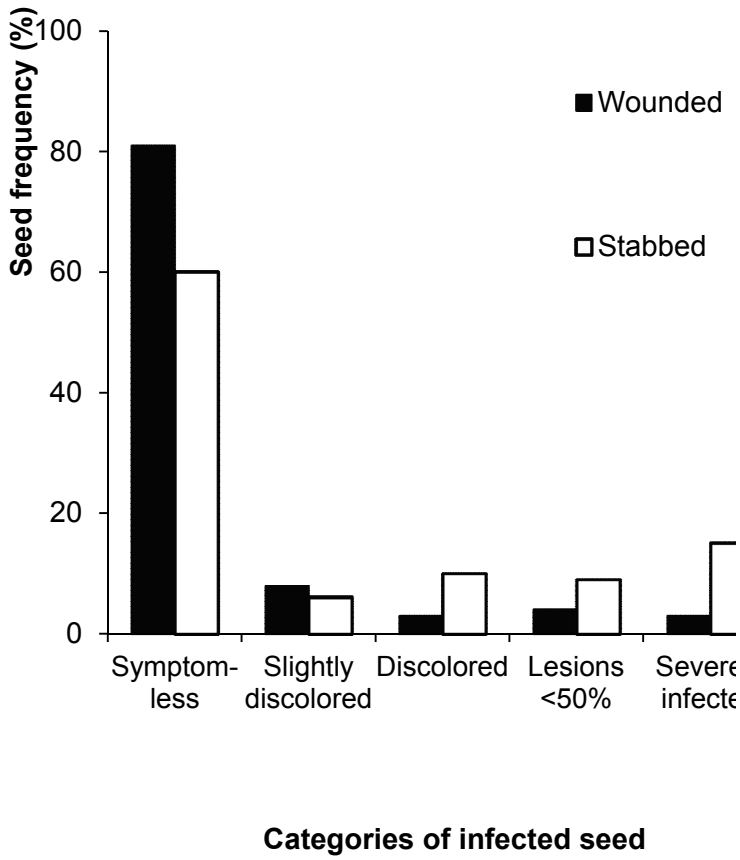

Figure 4. Frequency of seeds in each category produced by wounding with sand-paper and inoculating or stabbing with a needle and injecting a suspension of *C. lindemuthianum* conidia.

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PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF COMMON BEAN GENOTYPES FOR RESISTANCE TO ANTHRACNOSE

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Anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Scrib., is one of the most important diseases that occur in the common bean (*Phaseolus vulgaris* L.) in the main producing regions of the world. This disease can cause losses of up to 100% in the production of common bean (Méndez-Vigo, 2005). This study aimed to perform phenotypic and molecular characterization of cultivars and breeding lines of common bean for resistance to anthracnose.

The experiment was conducted at the National Research Center for Rice and Beans of the Brazilian Agricultural Research Corporation (EMBRAPA-CNPAF), located in the county of Santo Antônio de Goiás, GO, Brazil. Fifty-five common bean genotypes (cultivars and elites breeding lines) were evaluated. The field experiment was carried out during the winter period of 2014 (June to August) in a randomized block design (RBD) with three replications. Each plot had a line of 3 m and spaced 0.5 m each other. In the field, the genotypes were inoculated simultaneously with the races 65, 73, 81, 91, 475 and 1609 (Melo, 2009). Leaf samples of each genotype were collected from bulks (ten genotypes in each access) for DNA extractions according to Doyle & Doyle (1987). The DNA samples for each genotype were amplified using six types SCAR markers.

The results of phenotypic and molecular evaluations are summarized in Table 1. In the field experiments we found 26 resistant genotypes (score < 3.0) and ten were considered immune (score = 1.0). All the controls (Ouro Negro, TO, SEL 1308, TU and AB 136) were resistant and Rosinha G2 cultivar was susceptible (score = 9.0). In the molecular characterization of cultivars and common bean lines, the SH18 markers (*Co-4*^{2}), SAS 13 (*Co-4*^{2}), SAB3 (*Co-5*) and SAZ20 (*Co-6*) were specific for their respective loci, but they did not discriminate alleles. Only the SH18 marker proved to be allele-specific, discriminating *Co-4*, *Co-4*^{2} and *Co-4*^{3}. Of the 55 genotypes studied, 31 had the SF10 brand including cultivars, lines and controls.

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Table 1. Phenotypic reaction and molecular amplification of common bean genotypes to anthracnose.

Genotypes	Score	Molecular markers					
		$SF10_{1072}$ *Co-3*[4]	$SY20_{830}$ *Co-4*	$SH18_{1100}$ *Co-4*[2]	$SAS13_{950}$ *Co-4*[2]	$SAB3_{400}$ *Co-5*	$SAZ20_{845}$ *Co-6*
BRS Agreste	6.67	+	-	-	-	-	-
BRS Campeiro	7.33	+	-	-	-	+	-
BRS Embaixador	1.33	-	-	-	-	-	-
BRS Esplendor	4.67	+	-	-	-	+	-
BRS Esteio	1.00	+	-	-	-	-	-
BRS Estilo	1.33	+	-	-	-	-	-
BRS Executivo	2.33	-	-	-	-	-	-
BRS Grafite	6.33	+	-	-	-	-	-
BRS Notável	2.33	-	-	-	-	-	-
BRS Radiante	1.67	+	-	-	-	-	-
BRS Realce	1.00	+	-	-	-	-	-
BRS Requinte	6.00	-	-	-	-	-	-
BRS Sublime	1.00	+	-	-	-	-	-
BRS Supremo	2.00	+	-	-	-	+	-
BRSMG Madrepérola	8.00	-	-	-	-	-	-
BRSMG Majestoso	6.00	+	-	-	-	-	-
BRSMG Talismã	7.00	+	-	-	-	-	-
CNFC 10729	1.00	+	-	-	-	-	-
CNFC 15873	7.33	-	-	-	-	-	-
CNFC 15874	7.00	-	-	-	-	-	-
CNFC 15875	8.00	-	-	-	-	+	-
CNFP 10120	4.67	+	-	-	-	-	-
CNFP 10794	5.67	-	-	-	-	-	-
CNFP 15330	5.33	-	-	-	-	-	-
IAC Alvorada	4.67	+	-	-	-	-	-
IPR Uirapuru	6.33	+	-	-	-	-	-
Jalo Precoce	5.33	-	-	-	-	-	-
Pérola	5.00	-	-	-	-	-	-
Rudá	5.67	-	-	-	-	-	-
BRS Valente	7.00	+	-	-	-	-	-
MDRK	2.00	-	-	-	-	-	-
Kaboon	1.33	+	-	-	-	-	-
Perry Marrow	2.00	+	-	-	-	-	-
AND 277	2.00	-	-	-	-	-	-
Widusa	2.00	-	-	-	-	-	-
Cornell 49-242	7.33	+	-	-	-	-	-
Mexico 222	3.00	+	-	-	-	-	-
BAT 93	7.67	+	-	-	-	-	-
PI 207262	1.67	+	+	-	+	-	-
G 2333	1.33	-	+	-	-	+	-
K10	1.00	+	+	-	+	+	+
K13	1.00	-	+	-	+	-	-
K23	3.33	+	-	-	-	+	-
SEL 1360	5.67	+	-	-	-	+	-
H1	5.67	+	-	-	-	-	-
Michelite	7.33	-	-	-	-	-	-
Jalo Vermelh	1.33	-	-	-	-	-	-
Jalo Listras Pretas	1.67	-	-	-	-	-	-
BRS Pitanga	6.67	+	-	-	-	-	-
Rosinha G2[a]	9.00	-	-	-	-	-	-
Ouro Negro[b]	1.00	+	-	-	-	-	-
TO[b]	1.00	+	+	-	-	-	-
SEL 1308[b]	1.00	-	+	+	+	-	-
TU[b]	2.00	+	-	-	-	+	-
AB 136[b]	1.00	+	-	-	-	-	+

[a]Susceptible control; [b]Resistant control; [+]Band presence; [-]No band.

FURTHER CHARACTHERIZATION OF THE BROAD-SPECTRUM ANTHRACNOSE RESISTANCE IN ANDEAN COMMON BEAN AMENDOIM CAVALO

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INTRODUCTION: Anthracnose is one of the most significant diseases of common bean (*Phaseolus vulgaris*) in temperate and subtropical bean production areas of the world. This disease is caused by *Colletotrichum lindemuthianum*, known for its extensive virulence diversity. Dozens of races of this pathogen have been characterized and reported in the literature. Having genes with broad spectrum resistance to the races of *C. lindemuthianum* is a very important step for the development of common bean cultivars with effective resistance to this highly variable pathogen. The Andean dry bean Amendoim Cavalo (AC) has broad resistance to *C. lindemuthianum* (Nanani et al. 2014). We report here a study of the inheritance of anthracnose resistance in AC. In addition we used bulk segregant analysis and of a large set of single nucleotide polymorphism (SNP) DNA markers to establish the location of the anthracnose resistance gene of AC in the linkage group of common bean.

MATERIALS AND METHODS: Two separate crosses between AC (resistant) and PI 207262 (suscetpible), used to conduct these studies, were made at the Núcleo de Pesquisa Aplicada à Agricultura (Nupagri) at the Universidade Estadual de Maringá, Paraná, Brazil. The inheritance of anthracnose resistance study in AC was conducted in the Soybean Genomics and Improvement Laboratory, ARS-USDA, Beltsville, Maryland. To accomplish this study we used 112 F_2 seedlings from the AC x PI 207262 which were inoculated with race 3481 of *C. lindemuthianum*. The anthracnose differential cultivars Cornell 49242, Kaboon, TO, TU, and G2333 were included as checks (Table 1). Primary leaves of the F_2 seedlings were inoculated with a small hair brush soaked in a spore solution of 2.0×10^6 spores/ml. Plants were evaluated seven and ten days after inoculation using a 1-9 scale. Plants with disease reaction scores between 1 and 3 were considered resistant and plants with scores from 4 to 9 were considered susceptible. Another F_2 population from the AC x PI 207262 cross was used to conduct the molecular studies to determine the location of the anthracnose resistance gene of AC in a linkage group of the common bean. A total of 63 $F_{2:3}$ families derived from the F_2 population were inoculated in Maringá with race 3481 of *C. lindemuthianum*. Based on the anthracnose phenotype of the 63 $F_{2:3}$ families, resistant and susceptible bulks, for bulk segregant analysis (BSA), were prepared from the F_2 population. Only F_2 plants that were homozygous for resistance and susceptibility were used to prepare the resistant and susceptible bulks. Each bulk consisted of equal amounts of DNA from eight F_2 plants. DNA of the AC (R) and PI 207262 (S) parents were also obtained for BSA. The DNA of the two parents and of the resistant and susceptible bulks was screened with the 5,399 SNPs on an Illumina BeadChip following the Infinium HD Assay Ultra Protocol (Illumina, Inc. San Diego, CA). BeadChips were imaged using the Illumina BeadArray Reader to measure fluorescence intensity. The SNP alleles were called using the GenomeStudio Genotyping Module v1.8.4 (Illumina, Inc. San Diego, CA). Each allele call was manually checked. Positive hits for BSA were recorded when a SNP was polymorphic between PI 207262 and AC and the resistant bulk clustered tightly with AC and the susceptible bulk clustered with PI 207262.

RESULTS AND DISCUSSION: The Andean bean AC has broad resistance to *C. lindemuthianum*. It is resistant to races 2, 7, 64, 65, 73, 89, 2047 and 3481 of this pathogen. This broad anthracnose resistance is also revealed in the inheritance of resistance study of AC reported here. This study was conducted using the Mesoamerican race 3481 of *C. lindemuthianum*. AC is resistant to race 3481; however, this race overcomes the broad resistance present in several anthracnose differential cultivars, such as G 2333 (*Co-3*^{5}, *Co-4*^{2}, *Co-5*^{2}), AB 136 (*Co-6*, *co-8*), TO (*Co-4*), PI 207262 (*Co-3*^{3}, *Co-4*^{3}). Race 3481 also overcomes the resistance of Widusa (*Co-1*^{5}), Cornell 49242 (*Co-2*), Michelite (Co-*11*), Jalo Vermelho (*Co-12*), Pitanga (*Co-14*), and Corinthiano (*Co-15*). The Andean differential cultivars Michigan Dark Kidney (*Co-1*), kaboon (*Co-1*^{2}), and Perry Marrow (*Co-1*^{3}) and the Mesoamerican TU (*Co-5*) are resistant to race 3481. The inheritance of resistance study in AC was based on the reaction to race 3481 of 112 F_2 plants from the AC x PI 207262 cross. A total of 80 plants were resistant and 32 were susceptible. This segregation fits a 3R:1S ratio, indicating monogenic dominant inheritance in AC (Table 2). Nanami et al. (2014) had similar results using an F_2 population from the cross AC x Mexico 222. These authors also conducted many allelism tests between AC and most of the anthracnose resistance genes and concluded that AC has an independent gene that is different from most of the reported Andean and Mesoamericans anthracnose resistance genes. We also determined the location of the resistance gene of AC in a linkage group of common bean. A total of 21 SNPs resulted positive in the BSA. The SNP analysis indicated that these 21 SNPs were located in three scaffolds of the reference genome of *Phaseolus vulgaris* spanning a 1,77 Mb on the lower arm of the Linkage PV01. Thus, the resistance locus of AC resides on PV01.

Table 1. Reaction to race 3481 of selected genes for resistance to the anthracnose pathogen.

Cultivars	Genes	Reaction	Note
Cornell 49242	*Co-2*	S	7.9
Kaboon	*Co-1*^{2}	R	1
PI 207262	*Co-4*^{3},*Co-3*^{3}	S	8.0
TO	*Co-4*	S	7.4
TU	*Co-5*	R	1.5
G 2333	*Co-4*^{2}/*Co-5*^{2}/*Co-7*	S	7

* Plants with disease reaction scores between 1 and 3 were considered resistant and plants with scores from 4 to 9 were onsidered susceptible.

Table 2. Inheritence of resistance in F_2 population from the cross Amendoim Cavalo x PI 207262 inoculated with race 3481 of *Colletotrichum lindemuthianum*.

	Observed		Expected Ratio(3:1)				
	*R	*S	R	S	Total	Chi squared	P value
Amendoim Cavalo	16	0	-	-	16	-	-
PI 207262	0	14	-	-	14	-	-
F_2 Population	80	32	84	28	112	0.762	0.3827

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SCREENING PHASEOLUS LUNATUS FOR RESISTANCE TO *SCLEROTINIA SCLEROTIORUM*

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INTRODUCTION: Delaware is the number one producer of fresh market lima beans (*Phaseolus lunatus*) for commercial freezing and canning in the United States. Substantial yield loss occurs due to *Sclerotinia sclerotiorum* annually. No currently grown lima bean cultivars have resistance to *S. sclerotiorum*. To evaluate the level of resistance within *P. lunatus*, thirty-eight cultivars and accessions were grown and inoculated using the cut stem method. Thirty lines of *P. lunatus* were selected from USDA Plant Introductions (PI) of Mesoamerican and Andean lineages, six lines were chosen from cultivars and PI lines from the American Southwest, and two additional lines, coastal and mountainous, were selected from India.

The following plant introduction lines were tested in the fall and winter of 2014-2015: 256804 (Frijol Rojo), 257365, 355841 (Haba Pallara), 256885, 355839 (Torta Pallara), 256861, 260411 (Arvita), 256816 (Frijol Tierno), 256811, 256841, 257363, 257381, 200915, 256417, 310624 (Ixtapacal), 347779 (Hopi 13), 451782 (L 122A), 224713 (Pacle), 257548, 202830, 310627 (Chaparota), 195342, 535343 (DGD 453), 311795 (Frijol Curona), 549469 (Sieva), 535341 (NI 675), 257409 (Mariguita), 433928, 197025, 256389 (Chilipuca Negra Redonda), 451925, 189403 (Piloy), 549521 (Maffei 15), 438911 (Bacalar claro), 164155 (Bins), 180324 (Val), 549465 (Fordhook Bush), and the commercial line 184-85.

METHODS AND MATERIALS: Sclerotia as well as infected stems and pods were collected from a commercial lima bean field in Lewes, Delaware, and a single-hyphal tip isolation was obtained and used for inoculation. Seeds of each line were scarified and planted in LC1 growing mix. Replicate pots (one seed per pot) were randomly placed in four blocks within a greenhouse maintained at 20-24° C, with 12-hour light. Osmocote slow release fertilizer, and Imidacloprid, a pesticide to manage thrips, were applied to the soil. Avid 0.15% was also sprayed to manage thrips. Six-week-old plants were inoculated using the cut stem method. The main stem was severed 5 cm above the 5th node using a sterilized blade, and a plug of agar from a 72 hour old subculture was placed on the cut stem. Plants were evaluated on sclerotial development, length of infection progression on the main stem, and the node to which the infection reached. Plant collapse was defined as infection which had spread to the cotyledon node and/or infection of all side shoots in addition to the main stem

RESULTS: Variation in *P. lunatus* plant structure resulted in difficulty in disease resistance evaluation. Overall height of plants at the 5th node ranged from 25 cm to more than 1 m, and distance between nodes ranged from 1 or 2 cm to 20 cm. On taller plants the pipette caps had difficulty remaining in place, which may have reduced infection.

Mycelial agar plugs varied in thickness, and adhered differently based on strength of the main stem. Despite inoculation challenges, variation in disease response was found in *P. lunatus* cultivars and landraces. Infection in five of the thirty-eight lines screened did not move beyond the severed stem [Table 1]. Infection progressed to the main stem in the other lines. However in

eight lines where infection advanced, the plants continued to produce strong side shoots, vigorous growth, and even set pods [Table 2].

Table 1. *Phaseolus lunatus* lines in which infection did not spread beyond the tip of the severed stem.

Variety	Origin	Infection level
L122A; PI 451782	California, US	No infection
PI 256417	Alajuela, Costa Rica	3 out of 4 no infection
Mariguita; PI 257409	San Jose, Costa Rica	3 out of 4 no infection
Sieva; PI 549469	cultivar, US	3 out of 4 no infection
PI 256861	La Libertad, Peru	3 out of 4 no infection

Table 2. *Phaseolus lunatus* lines in which vigorous plant growth continued after infection of the main stem by *Sclerotinia sclerotiorum*.

Variety	Origin	Post-infection
PI 257363	Sucre, Colombia	4 of 4 maintained strong side shoots, vigor
PI 197025	San Salvador, El Salvador	4 of 4 maintained strong side shoots, vigor
Haba Pallara; PI 355841	Esmeraldas, Ecuador	4 of 4 maintained strong side shoots, vigor
Piloy; PI 189403	Suchitepéquez, Guatemala	2 of 4 maintained strong side shoots, vigor, set flowers
PI 256885	Ica, Peru	2 of 4 maintained strong side shoots, vigor, set pods
PI 256841	Piura, Peru	2 of 4 maintained strong side shoots, vigor, set pods
Hopi 13; PI 347779	Arizona, US	3 of 4 maintained strong side shoots, vigor, set pods

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POPULATION STRUCTURE AND FUNGICIDE SENSITIVITY OF 366 *SCLEROTINIA SCLEROTIORUM* ISOLATES FROM DRY COMMON BEAN

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Isolates of *Sclerotinia sclerotiorum* were obtained in 2003 to 2012, from white mold screening nurseries and bean grower fields. These were locations throughout most of the dry and snap bean production areas of the U.S., and included additional locations in France, Mexico, and Australia. Nearly half of all isolates (48.4%) were from cultivars Beryl, Bunsi, and G122. Isolates were phenotyped for aggressiveness using the straw test, which was reported previously (Jhala *et al.* 2014). The goals of the present study were to characterize the population structure of *S. sclerotiorum* populations obtained and report results of fungicide sensitivity determined for a selection of isolates.

***Population Structure Analysis* –** Isolates were genotyped at 16 microsatellite loci described previously (Sirjusingh and Kohn 2001). Eliminating loci that were either compound or dinucleotide repeat motifs resulted in a set of 11 loci with alleles following a stepwise model. Genotyping 366 isolates identified a total of 165 multilocus genotypes (MLG). Among these, 70 were unique MLG, represented by a single isolate, and 95 were clonal genotypes, which were represented by 80.9% of all isolates genotyped (**Fig. 1**). This genetic structure is typical of primarily clonal pathogens, with a small degree of out-crossing, as evidenced by a relatively high and significant amount of linkage detected among pooled populations clone-corrected by region/year (N=275, I_A=0.711; P=0.001).

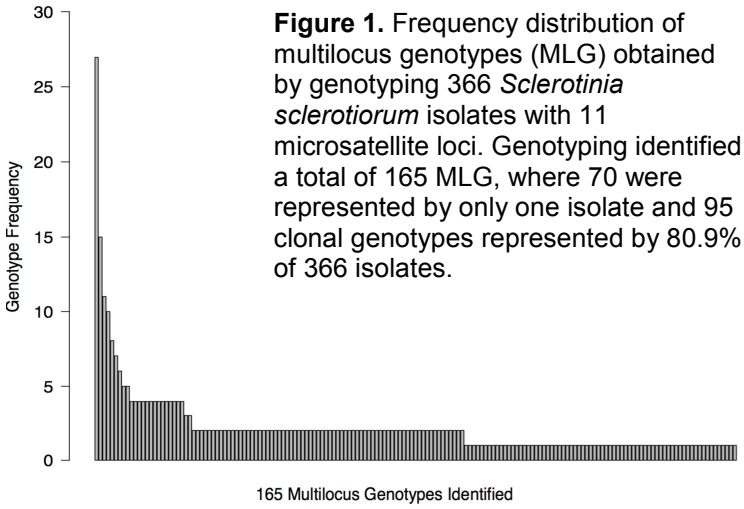

Figure 1. Frequency distribution of multilocus genotypes (MLG) obtained by genotyping 366 *Sclerotinia sclerotiorum* isolates with 11 microsatellite loci. Genotyping identified a total of 165 MLG, where 70 were represented by only one isolate and 95 clonal genotypes represented by 80.9% of 366 isolates.

Analysis of molecular variance (AMOVA) showed some populations were significantly different when defined by region/year, but the most distant populations showed little to no significant population differentiation. For example, populations from France and Australia were not genetically distinct from most U.S. populations. This is a surprising result considering the soil-borne nature and infrequent sporulation of this pathogen, which would lead us to expect differentiation of populations

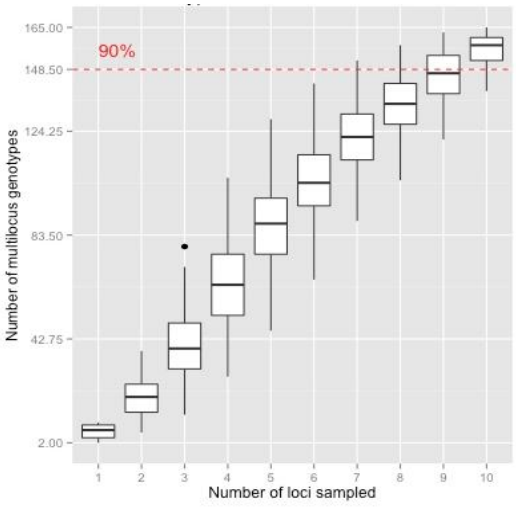

Figure 2. Despite evidence of sexual out-crossing in approximately ¼ of populations by region and year, rarefaction analysis of genotype resolution with increasing number of loci sampled showed lack of plateau, indicating existing SSR loci do not resolve genotypes sufficiently.

between fields. Although our results are congruent with previous studies that indicated populations of *S. sclerotiorum* are mostly clonal (Carbone et al. 1999), lack of differentiation may be due to existing microsatellite markers not providing sufficient resolution.

Geotype resolution was tested using an accumulation curve estimated from the entire data set of 366 isolates, where genotypes resolved was estimated for an increasing number of microsatellite loci (**Fig. 2**). Lacking an observed plateau in the number of genotypes resolved suggests existing SSR loci are not sufficient for genotype resolution for *S. sclerotiorum* and may explain why the most geographically and temporally distant populations show little to no differentiation.

Fungicide Sensitivity **–** Fungicide sensitivity was determined for a selection of 64 *S. sclerotiorum* isolates (**Table 1**). These isolates were representative of all geographic locations (U.S., Mexico, France, and Australia) and fungicides tested were selected because they are registered for white mold control and represent different modes of action (inhibition of electron transport chain, inhibition of nucleic acid metabolism/protein synthesis, inhibition of sterol synthesis). Mycelial growth inhibition was measured using a conventional plate-dilution method that was calibrated for each fungicide and effective concentration of 50% growth inhibition (EC_{50}) estimated by log-logistic regression in R. Results showed average concentrations for control were highest for Thiophanate methyl and lowest for Pyraclostrobin (amended with SHAM to inhibit the alternative oxidative pathway).

TABLE 1. Effective concentration of 50% inhibition (EC_{50}) estimated for 64 *S. sclerotiorum* isolates to fungicides using a plate-dilution method

Fungicide	Concentrations	Summary of EC_{50} Estimates			
		Mean	Min	Max	Std Dev
Thiophanate methyl	0, 0.1, 0.5, 1, 10, 100, 500	4.44	2.89	6.78	1.13
Iprodione	0, 0.1, 0.5, 1, 5, 10, 50	0.465	0.326	0.627	0.112
Proline	0, 0.05, 0.1, 0.5, 1, 5, 50	1.42	0.312	3.50	0.642
Metconazole	0, 0.1, 0.5, 1, 5, 10, 50	0.766	0.28	1.429	0.472
Pyraclostrobin (70ppm SHAM)	0, 0.001, 0.005, 0.01, 0.1	0.0156	0.00362	0.03266	0.0078

Future Direction – Our future direction is to explore the *S. sclerotiorum* published genome (Amselem *et al.* 2011) to identify additional SSR loci suitable for new marker development. Additional fungicide sensitivity data will be obtained for isolates using a spiral plating method. This high-throughput method will be validated against the plate-dilution method and supplant the technique for subsequent sensitivity estimates.

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USE OF MUTI SITE SCREENING TO IDENTIFY AND VERIFY PARTIAL RESISTANCE TO WHITE MOLD IN COMMON BEAN IN 2015

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

Breeders sent seed of 9 bean lines for field testing and 11 bean lines for greenhouse testing with putative sources of resistance to our laboratory. The seeds were divided in equal amounts for field (400g/line) and/or greenhouse (25 seeds/ line) tests and then sent to eight locations to be evaluated by standardized greenhouse and/or field screening methods. Three bean lines were included in both tests as controls: a good source of partial resistance G122, Bunsi with mostly field avoidance and susceptible GN Beryl.

The field tests consisted of two rows of each of the 9 entries and one row of a local semi-vine WM susceptible genotype, resulting in a three-row plot 4.6 m (15 ft.) long replicated three times in a randomized complete block design. There were seven field tests conducted in seven locations; two locations were not recorded due to wind damage or low WM. Field nurseries were evaluated using a CIAT 1 to 9 scale (1 = no visible symptoms to 9 = death) (Van Schoonhoven *et al*., 1987). Data (Table 1) at most locations supported USPT-WM-12 and 039-A-5 as the most resistant with Mist and Lighthouse similar to Bunsi in resistance/avoidance. Lines N13140 and R12844 were more variable, but they had some lower ratings. Although the field tests were somewhat inconsistent, the value of multiple location testing to assist in selecting for improved WM resistance is evident. More than one location lacked useful data.

The greenhouse trials tested 11 entries, plus 3 controls, using the straw test to inoculate 21- to 28-day-old plants. The plants were inoculated 2.5 cm above the fourth node with a plug of PDA media containing young *S. sclerotiorum* mycelia pressed into a 2.5 cm clear drinking straw sealed at one end or microfuge tube and8 fitted over the cut internode. The length of infection was evaluated 8 days later using the modified Petzoldt and Dickson scale (Teran et al, 2006). The greenhouse results (Table 2) indicate that USPT-WM-12 and 031-A-11 are similar to the more resistant G122. R13752, Lighthouse, Mist, 039-A-5 and R12844 rank as similar to Bunsi showing little resistance. The other four lines were similar to Beryl and evaluated as susceptible.

Progress in incorporating WM resistance into dry bean lines with commercial potential validates use of multisite screening and National Sclerotinia Initiative support over the last 10 years. The slow, but steady progress may be invigorated by the release of Dr. Shree Singh's bean lines from wide crosses that survived multiple inoculations in his greenhouse trials.

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

Line	Seed Class	MI	NE	OR	WA	WI	Mean	t Grouping
BERYL	GN	3.0	6	6.3	6.4	9.0	6.1	A
P14815	PINTO	5.0	4.3	8.0	5.1	8.2	6.1	A
B12724	BLACK	5.0	1.7	6.0	4.6	6.5	4.8	A B
R13752	SM RED	4.3	1.7	6.0	4.3	7.0	4.7	A B
N 13140	NAVY	5.7	3	4.0	4.6	5.0	4.5	A B
R12844	SM RED	4.7	1.7	5.7	4.3	5.3	4.3	A B
BUNSI	NAVY	3.7	2	4.0	4.9	6.5	4.2	A B
Lighthouse	NAVY	4.7	1.3	6.3	4.6	3.5	4.1	A B
Mist	NAVY	4.7	3.7	4.0	4.4	3.3	4.0	A B
039-A-5	PINTO	4.3	1	3.3	3.9	6.2	3.7	B
USPT-WM-12	PINTO	4.0	2.7	1.7	2.8	6.3	3.5	B
G122	CRAN	3.0	1	4.0	4.2	3.0	3.0	B

*CIAT Scale: 1 = no disease, 9 = plants dead **Alpha = 0.05, LSD = 2.1

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

Line	Seed class	BEL	CO	NE	OR	WA	WI	Mean	t Grouping
B12724	BLACK	9.0	8.0	8.8	8.7	7.9	9.0	8.6	A
P14815	PINTO	7.8	8.6	8.9	7.1	6.8	9.0	8.0	A
N13140	NAVY	5.8	8.3	8.5	8.1	8.2	9.0	8.0	A B
ASR 1003	SNAP	4.6	7.7	8.7	7.4	8.0	8.9	7.6	A B C
Beryl	GN	8.0	7.6	7.4	6.3	7.5	7.9	7.5	A B C
R13752	SM RED	4.0	6.7	8.0	7.6	5.8	8.1	6.7	B C D
Bunsi	NAVY	6.2	4.8	7.7	6.6	6.3	7.8	6.6	C D
Lighthouse	NAVY	5.5	6.2	7.8	5.8	6.8	7.1	6.5	C D
Mist	NAVY	5.2	5.6	8.2	6.1	6.6	7.3	6.5	C D
039-A-5	PINTO	4.9	5.7	7.8	7.2	7.0	6.2	6.5	C D
R12844	SM RED	4.3	5.3	6.3	5.7	6.2	8.0	6.0	D E
USPT-WM-12	PINTO	3.2	3.8	5.5	6.4	5.8	4.8	4.9	E F
031-A-11	GN	3.7	3.5	5.3	7.9	4.7	3.5	4.8	E F
G122	CRAN	3.0	4.9	5.1	3.9	4.1	4.0	4.2	F

*Straw test rating scale based on modified Petzoldt and Dickson scale (Teran *et al.,* 2006)
(1-3 = resistant, 4-6 = intermediate, 7-9 = susceptible) **Alpha = 0.05, LSD = 1.3

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EFFICIENCY OF PROGENY SELECTION IN COMMON BEAN FOR OXALIC ACID REACTION

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INTRODUCTION

The common bean is affected by several diseases like white mold (*Sclerotinia sclerotiorum*), that reduces the grain quality and grain yield in many countries, mainly in culture under mild temperature and high humidity. Genetic resistance is one of the measure that contribute for diminish these problems, although higher level of resistance are not still available in most of the cultivars. Some resistance sources are promising although in general they are not adapted especially in the growing conditions of Brazil. There are several procedures for evaluating white mold resistance in common bean, and an indirect one is through measuring the ability of young plants to support the oxalic acid solution for some hours, expressed by the level of wilting of the plants (Kolkman & Kelly, 2000). This acid is exuded by the fungus during the plant infection process, and those that tolerate this acid are considered more resistant.This indirect method of measuring the resistance is fast, easy, many plants can be evaluated at a time, and also it is free of the error, which is caused by the variability of the pathogen in the direct evaluation of the resistance. However, due to the quantitative nature of the white mold resistance, it is important to use an efficient procedure of selection like evaluate progeny instead of individual plant. Thus the objective was to check the efficiency of evaluating common bean progenies reaction to oxalic acid compared to individual plants.

MATERIALS AND METHODS

The experiments were set up at Biology Department of Universidade Federal de Lavras (UFLA), MG State, Brazil. Two lines were crossed, one is the source of resistance to white mold G122, which is not adapted to Brazilian conditions, and the other is the adapted line M20 that has the accepted carioca grain type, upright plant and anthracnose resistance due to *Co.5* and *Co.4*^{2} alleles. There were obtained the F_1, the F_2 and 170 $F_{2:4}$ progenies that were evaluated in 14 experiments with two common testers, the parents G122 and M20. The completely randomized design was used in all experiments with three replications of plots with ten plants.The parents, the F_1 and each progeny were represented by one plot per replication, and the F_2 were represented in 4 plots per replication, totaling 30 F_1 and 120 F_2 plants.

The plants without roots were evaluated individually in a container with 4 liters of oxalic acid solution 20mM, pH 4 adjusted with NaOH. The exposition time of the plants to the solution of oxalic acid was 20 hours under 22°C to 24°C temperatures. The reaction of each plant was evaluated using a grade scale proposed by Kolkman & Kelly (2000).

The analysis of variance was set up using the software SAS 8.0 (SAS Institute). The experimental precision was evaluated through the coefficient of variation (CV) and the selective accuracy ($\hat{r}_{gg}$)(Resende & Duarte, 2007). The components of variance, the heritability (h^2) and the gain with selection (GS) were estimated based on individual plant data for selection in the F_2 population, and on plot mean data for selection among $F_{2:4}$ progenies.

RESULTS AND DISCUSSION

The experimental precision can be considered high based on the values of the CV and the $\hat{r}_{\hat{g}g}$ (Table 1). Wide genetic variability for the reaction to oxalic acid was expressed by the progenies and between the parents ($P < 0.01$), indicating the chance of success with the selection of the more resistant progenies.

Table 1. Estimates of heritability (h^2), gain with selection (GS), mean reaction to oxalic acid, selective accuracy ($\hat{r}_{\hat{g}g}$) and coefficient of variation (CV) for the reaction of common bean to oxalic acid.

	F_2	$F_{2:4}$	Mean reaction to oxalic acid	
h^2	54%	79%	G122	3,78
GS	0.25	0.86	M20	4,11
$\hat{r}_{\hat{g}g}$	89%		F1	3.57
CV	10.5%		F2	3.56

The mean reactions of the parents, F_1 and F_2 were similar indicating mainly additive allelic interaction, which facilitate the selection because the individual or the group of the resistant genotypes will generate also resistant descent. Antonio et al. (2008) using similar evaluations, also observed the genetic control of the reaction of common bean to oxalic acid is due to the predominance of the additive effect but with the presence of partial dominance.

According to the wide genetic variability observed among progenies, high heritability was also estimated, although the h^2 under individual level, in the F_2 population, was lower similar to the values of Kolkman & Kelly (2002).The estimates of gain with selection considering the five more resistant plants of the F_2, and of the five more resistant $F_{2:4}$ progenies also indicated a three times higher success with progenies selection (Table 1), indicating the higher efficiency of using progenies. It is important to mention that it is impossible to do mass selection in the F_2 population through the oxalic acid procedure, because the plants are killed after the evaluation. This is not the case with progenies when one sample of each is used for evaluating the reaction to oxalic acid. Therefore the use of the F_2 evaluation was done only for having an idea of the efficiency of the progeny selection based on its mean reaction in replicated experiments, and also having an idea of the genetic control of the trait.

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EFFECT OF OXALIC ACID ON COMMON BEAN PROGENIES DERIVED FROM RECURRENT SELECTION FOR WHITE MOLD RESISTANCE

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INTRODUCTION

White mold is an important disease of common bean mainly under mild temperatures and high humidity, like in the irrigated cultures in the winter of Brazil.The main measure for controlling the disease is chemical, although it is not fully efficient and resistant cultivars would be the ideal for the farmers. However, the level of resistance of the available cultivars is low and it needs to be improved for being efficient for controlling the disease. The genetic control of the white mold resistance in common bean is quantitative with a considerable influence of the environment. Therefore, we have used recurrent selection for obtaining progenies with physiological resistance using the straw test procedure for evaluation. Another way of accessing the white mold resistance is through the evaluation of genotypes in oxalic acid solution (Kolkman & Kelly, 2000), and according to Carvalho et al. (2013) the evaluations obtained by straw test and by oxalic acid presented low correlation, and different genes of resistance can be acting. Therefore, aiming to improve the level of resistance, the selected progenies were also evaluated in a solution of oxalic acid, considering that plants more tolerant to this acid may have additional resistance.

MATERIALS AND METHODS

The experiments were set up in greenhouse and in chamber with temperature of 22^0C, in the Department of Biology of the "Universidade Federal de Lavras", Lavras, MG State, Brazil. Sixty-seven progenies from the cycles 7, 8 and 9 of recurrent selection for white mold resistance, and selected through the "straw test", were evaluate for the reaction to oxalic acid, plus two common testers, the susceptible lines "Corujinha" and CNFC9506. Thirty more uniform seedlings per progeny, with the second trifoliate leaf, of around 21 days old were selected and had their root cutoff. In the experiments we used the completely randomized design with three replications and plot with ten plants. The seedlings had their stem partially immersed in a solution of oxalic acid 20mM, pH 4, for 20 hours. The intensity of wilting of the progenies was evaluated through a grade scale (1=no wilting to 6=highest wilting) (Kolkman & Kelly 2000).

The analysis of variance was done by the software SAS 8.0 (SAS Institute). The experimental precision was estimated by the coefficient of variation (CV) and the selective accuracy ($\hat{r}_{\hat{g}g}$) (Resende & Duarte, 2007). The genetic heterogeneity of the mean reaction of the progenies was ranked according to Scott & Knott (1974) at 5% level of probability.

RESULTS AND DISCUSSION

The experimental precision was high as revealed by the CV and $\hat{r}_{\hat{g}g}$ (Table 1). The progenies of all cycles were genetically different for the oxalic acid reaction ($P < 0.01$) indicating the possibility of selection of some resistant ones, which may have higher resistance to the white mold as long as they are also resistant based on the inoculation through the straw test. Comparing the mean of the progenies of all cycles we noted two groups in cycle 7 (the group A with mean 2.58 and B with mean 3.02), three groups in cycle 8 (the group A with mean 2.19, the group B with mean 2.73 and C with mean 3.15), and three groups in cycle 9 (the mean of the

group A is 2.23, group B is 2.79 and group C is 3.31). So we could see some progeny reactions with higher resistance to oxalic acid. Therefore, they can exhibit higher resistance to white mold in the field. When we compared the mean of cycles we didn't see improvement in resistance by oxalic acid procedure, however, the selection for obtaining the progenies was by straw test method and according to Carvalho et al. (2013), there is low correlation between these methods. Considering the high speed and the easiness of evaluating many progenies by the "straw test", and the complexity of the resistance to the disease, both evaluations straw test and oxalic acid should be used for selecting progenies with higher resistance.

Table1- Mean squares, coefficient of variation (CV), selected accuracy ($\hat{r}_{\hat{g}g}$) and mean reaction of common bean progenies for oxalic acid reaction.

Sources of variation	Cycle 7	Cycle 8	Cycle 9
Progenies	0.2575**	0.3092**	0.3284**
Error	0.0632	0.0813	0.0622
CV	8.71%	9.77%	7.98%
$\hat{r}_{\hat{g}g}$	86.85%	85.84%	90.03%
Mean reaction	2.81 (2.27-3.22)[a]	2.91(2.06-3.46)[a]	3.12(2.23-3.7)[a]

** Significant by the F test at the level of 1% of probability.

[a] Lower and upper limit of mean reaction in each cycle of recurrent selection.

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WITHIN-ROW PLANT DENSITY FOR TYPE III COMMON BEAN GENOTYPE WITH PARTIAL RESISTANCE TO WHITE MOLD

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INTRODUCTION. White mold (WM) is the most serious common bean disease during the fall-winter season in Brazil, where farmers prefer to sow seeds of the "carioca" (beige with brown stripes) market class, especially those of Type III growth habit. For Type III cultivars susceptible to WM, many farmers have used the within-row density of 5-6 plants/m (Vieira et al., 2010). Recently, the carioca line VC 17 (Type III) has exhibited high levels of WM resistance in the field (Lima et al., 2015) and no study has been made to determine the optimal plant density that should be used for WM-resistant cultivars. Our objective was to determine, under WM pressure, the optimal range of plants per meter for genotypes with partial resistance to WM, keeping constant the between-row spacing of 0.5 m.

MATERIAL AND METHODS. A sprinkler irrigated trial was conducted during the fall-winter season in a field naturally infested with sclerotia of *Sclerotinia sclerotiorum* in Oratórios, Zona da Mata region, Minas Gerais State, Brazil. Treatments were arranged as 4 x 2 x 2 factorial: plants per meter (4, 7, 10 or 13), genotypes (Madrepérola or VC 17), with or without fungicide applications. The statistic design was a randomized complete block with four replications. The cultivar Madrepérola is susceptible to WM and the elite line VC 17 exhibits partial resistance to this disease, both under field conditions. The fungicide fluazinam (0.62 L/ha) was applied at flower onset followed by one application 10 d later. Each plot had four 4 m-long rows, spaced 0.5 m apart. WM severity index (WMSI) was calculated for each plot according to methodology employed by Vieira et al. (2010). After harvest, sclerotia on the soil surface were collected in five randomly positioned quadrats (400 cm^2) in each plot and weighted. Sclerotia were also removed from each seed lot harvested from the two central rows and weighted.

RESULTS AND DISCUSSION. WM mold pressure was moderate in the trial. Genotype affected lodging and sclerotia left on the plot; whereas fungicide affected lodging, WMI, WMSI, and sclerotia left on the plot (Table 1). Lodging was greater for Madrepérola than for VC 17 and for the plots where fluazianam was not applied compared with plots where fungicide was applied. Fungicide decreased WMI by 42%, WMSI by 59%, and sclerotia left on the plot by 70%. G x F interaction was significant for sclerotia mixed with seeds. Without fungicide application, VC 17 had lower amount of sclerotina mixed with seeds than Madrepérola; with fungicide applications, difference between genotype for this variable was nonsignificant (Fig. 1). On average, fungicide increase yield by 57%. P x F interaction was significant for seed yield. With fungicide applications, yields were not affected significantly by the within-row densities; without fungicide, yield obtained with 13 plants per meter was lower compared with the other plant densities (Fig. 2). Our preliminary results suggest that the range between 4 and 10 plants per meter might be the optimal plant density when the WM pressure is moderate, regardless the level of resistance of the host to WM. Further studies are needed under different WM pressure to amplify our conclusion.

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Table 1. Effects of genotype, within-row density and fungicide levels on lodging, WM incidence (WMI), WM severity index (WMSI), mass of sclerotia and yield in Oratórios, 2015.

Factor	Lodging[1]	WMI (%)	WMSI (%)	Sclerotia left on the plot (mg/ 2000 cm^2)	Sclerotia with seeds (mg/plot)	Yield (kg/ha)
Genotype(G)						
VC 17	4.9***	68	37	148***	1572	3073
Madrepérola	5.7	66	39	440	2839	2985
Plants/m (P)[2]						
4	5.2	70	35 B	199	2079	2984
7	5.2	62	34 B	271	1894	3091
10	5.2	67	40 AB	341	2265	3103
13	5.4	69	43 A	366	2583	2939
Fungicide (F)						
with	5.0***	49***	22***	137***	771	3697
without	5.6	85	54	451	3639	2361
				P values		
G	<0.001	0.515	0.580	<0.001	<0.001	0.376
P	0.495	0.484	0.055	0.266	0.506	0.575
F	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
G x P	0.861	0.557	0.593	0.207	0.399	0.320
G x F	0.303	0.863	0.920	0.075	0.014	0.115
P x F	0.612	0.677	0.298	0.364	0.469	0.014
G x P x F	0.197	0.923	0.218	0.698	0.390	0.712

[1] 1 = no lodging ; 9 = > 90% lodging. [2] Duncan's test, 5%. *** = significant at < 0.01%.

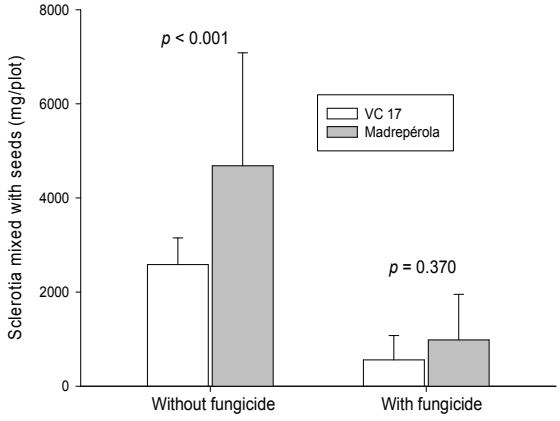

Figure 1. Interaction between genotype and fungicide levels on mass of sclerotium mixed with seeds harvested. Mean ± SD.

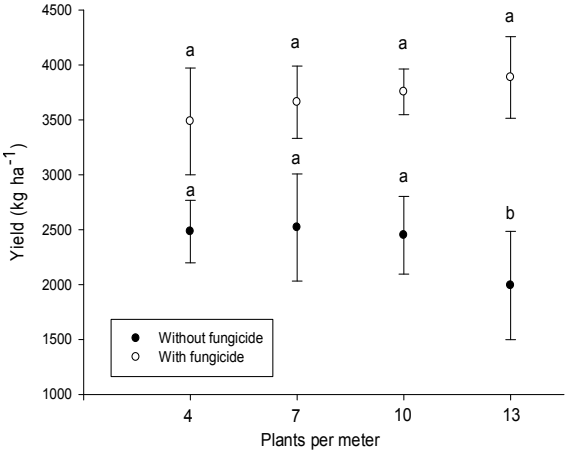

Figure 2. Interaction between plant density and fungicide levels on yield. Duncan's test (5%) was use to compare means of plant densities within each fungicide level. Mean ± SD.

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PERFORMANCE OF LINES/CULTIVARS SELECTED FOR PARTIAL RESISTANCE TO WHITE MOLD IN THE FIELD

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INTRODUCTION: White mold (WM), caused by the fungus *Sclerotinia sclerotiorum*, is a serious disease affecting common bean during the fall-winter season in the State of Minas Gerais, Brazil. The most commonly used control measure is fungicide application at flowering. However, the high cost and the potentially deleterious effects on human health and environment have motivated the search for new options for WM management. Genetic resistance is a key component of the WM management, because it is easier for farmers to adopt and is environmentally safe. Common bean elite lines developed by Federal University of Lavras (UFLA), Federal University of Viçosa (UFV), and Embrapa Rice and Beans are evaluated each year at several locations in Minas Gerais through the "Value for Cultivation and Use" (VCU) experiments. From 2008 to 2014 we have screened lines/cultivars from the VCU experiments for their relatively less symptoms of WM and high yield. Our objective was to evaluate under field conditions the lines/cultivars selected from the VCU experiments in comparison with popular cultivars and with international genotypes with partial resistance to WM.

MATERIAL AND METHODS: Twelve genotypes selected from the VCU experiments for partial resistance to WM and high yield were evaluated in the field. These genotypes were compared to five popular cultivars used in Brazil and to the international WM resistant controls A 195, G 122, and Cornell 605, which are adapted to the Brazilian conditions (Lehner et al., 2015). To evaluate these 20 genotypes, we used two sites with history of WM located in the districts of Viçosa and Oratórios, Zona da Mata region, Minas Gerais State, Brazil. The experiments were conducted during the fall-winter season with sprinkler irrigation. A randomized complete block design with four replications was used. Plots were two 3 m-long rows, spaced 0.50 m apart. Preventive control of anthracnose and angular leaf spot were made during the vegetative phase of the plants. Data were subjected to individual and combined ANOVA for the two sites.

RESULTS AND DISCUSSION: WM intensity in the experiments was low/moderate (Viçosa) or very low (Oratórios) (Table). In Viçosa, WM scores ranged from 1.8 to 5.8; in Oratórios, from 1.0 to 2.3. The two cultivars (Ouro Vermelho and Ouro Negro) with higher lodging scores exhibited the highest WM scores. Site x genotype interaction was significant for yield. Thus, genotypes performance was presented separately by experiment. Besides WM disease, three genotypes exhibited symptoms of anthracnose, but only VC 17 and Pérola had moderate or moderate/high severity of this disease in the experiments. Probably, anthracnose was the main reason for the relatively low yield of these two genotypes. VC 17, in particular, has shown high yield potential in previous experiments (Lima et al., 2015), especially under high WM pressure. BRS Estilo, which belongs to carioca market class, is widely used in Brazil. The elite lines CNFP 11990, CNFC 10720, VC 27, and CNFC MG11-08 showed yield potential similar to BRS Estilo under very low to low/moderate pressure of WM. The international genotypes with partial resistance to WM (A 195, G 122, and Cornell 605) exhibited lower yield potential than the elite lines, especially G 122 and Cornell 605.

Table. White mold intensity, lodging, and seed yield of genotypes selected from previous trials for partial resistance to WM compared to five popular cultivars (in bold) and to three international genotypes with partial resistance to WM (A 195, G122, and Cornell 605) in two districts located in Zona da Mata region, Minas Gerais State, Brazil, 2015.

Line/cultivar (market class)[1]	WM scores[2]		Lodging[3]		Yield (kg/ha)	
	Viçosa	Oratórios	Viçosa	Oratórios	Viçosa	Oratórios
CNFP 11990 (P)	2.3 B[4]	1.5	4.9 C	4.6 C	4179 A	2904 A
CNFC 10720 (C)	1.8 B	1.0	4.5 C	5.0 B	4117 A	3525 A
VC 27 (C)	2.0 B	1.5	4.6 C	5.0 B	4021 A	3208 A
CNFC MG11-08 (C)	2.0 B	1.5	5.1 C	5.4 B	3829 A	3383 A
BRS Estilo (C)	2.0 B	1.3	4.9 C	4.8 C	3767 A	3738 A
CNFC 10432 (C)	1.8 B	1.5	3.9 D	4.0 C	3613 B	3858 A
VC 26 (C)	1.8 B	1.8	5.1 C	5.3 B	3554 B	3263 A
CNFP 10798 (B)	2.0 B	1.8	4.6 C	4.6 C	3517 B	3333 A
CNFC 10722 (C)	2.0 B	1.0	3.9 D	3.8 C	3467 B	3004 A
Ouro Vermelho (R)	5.5 A	1.8	8.0 A	6.5 A	3213 B	3638 A
BRS Vereda (R)	1.8 B	1.0	4.5 C	4.4 C	3258 B	2667 B
CNFC 11946 (C)	2.3 B	1.5	4.9 C	4.8 C	3113 B	2683 B
Ouro Negro (B)	5.8 A	2.3	7.1 B	5.3 B	3046 B	2742 B
A 195 (A)	1.8 B	1.3	4.8 C	4.8 C	2613 C	3058 A
Majestoso (C)	2.3 B	1.8	5.6 C	5.4 B	3042 C	2625 B
VC 17 (C)	2.0 B	1.5	5.0 C	4.8 C	2971 C	1942 C
Pérola (C)	2.0 B	1.0	5.3 C	5.0 B	2633 C	2013 C
Ouro Branco (A)	2.3 B	1.3	5.0 C	4.6 C	2396 D	2721 B
G 122 (A)	1.8 B	1.5	3.1 D	4.1 C	2229 D	2367 B
Cornell 605 (A)	2.0 B	1.5	5.0 C	5.1 B	1842 D	1717 C
Mean	2.4	1.5	5.0	4.9	3221	2919
CV(%)	34	34	12	13	13	16

[1] C = carioca, B = black, A = Andean gene pool, R = red. [2] 1 = no diseased plants; 9 = 80 to 100% diseased plants and/or 60 to 100% infected tissue (Miklas et al., 2001). [3] 1 = no lodging; 9 = > 90% lodging. [4] Means followed by the same letters belong to the same group (Scott-Knott test, $p = 0.05$).

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PARTIAL RESISTANCE TO WHITE MOLD AMONG COMMON BEAN ELITE LINES

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INTRODUCTION: Genetic resistance is a key component of the white mold (WM) management in common bean, because it is easier for farmers to adopt. 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) experiments. In these experiments, traditional cultivars are used for comparison. The objective of this study is to screen lines/cultivars of common bean with relatively less WM symptoms associated with high seed yield.

MATERIAL AND METHODS: Fourteen VCU irrigated trials were conducted from 2008 to 2011 during the fall-winter season in a field naturally infested with sclerotia of *Sclerotinia sclerotiorum* in Oratórios, Zona da Mata region, Minas Gerais State, Brazil. These trials embrace four common bean market classes: carioca (beige with light brown stripes), black, colored (especially red, pink, cranberry, and cream), and especial (seeds with different colors of Andean gene pool for export purpose). Some trials were repeated with the same set of genotypes in different years. In total, we evaluated 106 genotypes (elite lines and cultivars): 47 carioca, 29 blacks, and 30 that belong to other market classes. Among the 30 genotypes of other market classes, 15 belong to Andean gene pool and 15 to the Mesoamerican gene pool. The trials were arranged in a randomized complete block design with three replications. Each plot had two 4 m-long rows, spaced 0.50 m apart. Fungicide was not applied. Genotypes were screened for their relative less symptoms of WM associated with high seed yield. We evaluated WM incidence (in 2008) or WM intensity (from 2009 to 2011). WM incidence represents the percentage of plants with WM symptoms. WM intensity (incidence + severity) was evaluated visually, using a 1-to-9 scale (Miklas et al., 2001).

RESULTS AND DISCUSSION: Based on the maximum score of WM incidence or WM intensity, WM pressure was considered moderate in one trial (maximum score of 5.3), moderate/high in four trials (5.8 or 6.3), and high in nine trials (>6.7 or more than 70% of plants diseased) (Table 1). Besides WM disease, angular leaf spot, anthracnose, and rust also occurred in the trials, and genotypes varied in susceptibility to these foliar diseases. Maximum yield varied from 1946 to 3420 kg ha^{-1} and minimum yield, from 433 to 2097 kg ha^{-1}. Genotype affected WM incidence (or WM intensity) significantly in half of the trials. In two trials, genotype did not affect yield significantly. On the other hand, genotype affected yield in a very highly significant way in seven trials. WM incidence (or WM intensity) correlated negatively with seed yield, but this correlation was not significant in three trials. In the 2009 carioca trial, in addition to the significant WM score-seed yield correlation (r = -0.56***), angular leaf spot score also correlated significantly with seed yield (r = -0.46***). In 2011, in the black bean trial, WM score-seed yield correlation was nonsignificant (Table 1), but anthracnose score-seed yield correlation was significant (r = -0.55***). Five carioca lines were selected from the VCU trials: CNFC 10720, CNFC 10722, CNFC 10432, VC 17, and CNFC 11965. Except for the line VC 17, which has indeterminate semiprostrate Type III growth habit, the other carioca lines have indeterminate upright Type II growth habit. The black lines with

Type II growth habit VP 21, CNFP 10798, CNFP 11990 and CNFP 11980 were also selected. From the colored and especial trials we selected BRS Vereda (Type III, Mesoamerican origin) and the Andean genotypes BRS Executivo, Ouro Branco and CAL96. CAL96 and Ouro Branco are early maturity genotypes with determinate Type I growth habit. BRS Executivo has indeterminate upright Type IIb growth habit. The cultivars Pérola, BRS Cometa, BRS Estilo (carioca), BRS Valente, BRS Supremo, BRS Esplendor (black), Jalo EEP 558, BRS Timbó (colored), BRS Embaixador (especial), and the carioca line RP-1 exhibited intermediate resistance to WM. Among the most susceptible cultivars to WM in the VCU trials were BRSMG Majestoso, BRSMG Madrepérola, BRSMG Talismã, BRSMG Pioneiro (carioca), BRS Campeiro, Ouro Negro (black), Ouro Vermelho, and BRS Radiante (colored).

Table 1. Number of common bean genotypes evaluated each month/year, market class, maximum and minimum yield, maximum and minimum white mold (WM) incidence (or intensity), F value for genotype, and correlations (r) between WM incidence (or intensity) and seed yield in 14 VCU experiments conducted in Oratórios from 2008 to 2011

Month/ Year[a]	Bean market class[b]	Genotype screened	WM incidence or score			Seed yield (kg ha^{-1})			r
			Max[c]	Min[c]	F genotype	Max	Min	F genotype	
Apr. 2008	car	26	91	31	1.3ns	2558	1035	2.0*	-0.84***
Apr. 2008	bla	16	93	36	1.9ns	1968	666	2.6*	-0.63**
Jul. 2008	car	26	86	45	1.7ns	3391	1826	3.3***	-0.30*
Jul. 2008	bla	16	70	29	2.3*	3051	2097	0.8ns	-0.70***
Jun. 2009	car	26	5.8	2.3	2.6**	2255	871	3.2***	-0.56***
Jun. 2009	bla	16	6.7	2.3	3.5**	2231	630	11.1***	-0.57***
Jul. 2010	car	25	6.3	3.3	2.3**	3305	1464	2.1*	-0.28**
Jul. 2010	bla	16	6.3	3.5	2.8**	2587	1281	2.8**	-0.69***
Jul. 2010	esp	16	5.3	2.3	1.1ns	3276	1910	1.3ns	-0.60***
Jul. 2010	col	16	6.7	2.0	1.8ns	3420	861	4.5***	-0,36**
Apr. 2011	car	25	8.0	5.2	4.4***	1996	470	3.5***	-0.68***
Apr. 2011	bla	16	7.8	4.5	4.9***	2021	433	10.0***	-0.11 ns
Apr. 2011	col	16	6.7	4.8	0.5ns	1946	1181	2.4*	-0.24 ns
Apr. 2011	esp	16	6.3	3.7	1.2ns	2087	629	11.8***	-0.16 ns

[a] Month and year of the experiment installation. [b] car = carioca, bla = black, col = colored, esp = especial. [c] In 2008, percentage of plants with WM symptoms are presented; from 2009 to 2011, WM intensity are presented: 1 = no diseased plants, 9 = 80-100% diseased plants and/or 60-100% infected tissues. *, **, and *** indicate $P < 0.05$, 0.01, and 0.001, respectively; ns = nonsignificant.

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IDENTIFICATION OF GENOMIC REGIONS INTROGRESSED IN NEAR-ISOGENIC LINES OF THE MARKET CLASS FABADA USING MASSIVE GENOTYPING

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Resistance to anthracnose and bean common mosaic viruses, conferring by the genes *Co-2, Co-3/Co-9, I* and *bc-3,* were introgressed into the A25 line, a bean genotype belonging to the fabada market class. Resistant plants were selected using resistance tests or combining resistance tests and marker-assisted selection. A set of near-isogenic lines (NIL) with fabada seed phenotype but different combinations of resistance genes were obtained. In this study, we physically mapped the introgressed genomic regions into the A25 line using genotyping-by-sequencing (Elshire et al. 2011) in order to approach to genes involved in the genetic control of the introgressed resistances.

MATERIAL AND METHODS: Sixteen NILs derived from the A25 line, carrying different genes combination and all them belong to the market class fabada (very large-seeded white bean cultivar with 100 g/100 seeds), were analyzed in this study (Ferreira et al. 2012). NILs were obtained in two steps; backcrossing and pyramiding. In the first step, different genes were introgressed into line A25 using a backcrossing method; lines A1231, A1258, A1220, A1183 A1878 A2418 and A2648. Line Xana, having determinate growth habit controlled by the *Fin/fin* gene, was simultaneously obtained through pedigree method from the cross A25xV203. In a second step, pyramiding of different genes were developed using the pedigree method from a single cross between lines obtained in the first step: lines A1699, A2438, A2806, A3308, X1612, X1358, X1319 and X2776.

Genotyping-by-sequencing as described by Elshire et al. (2011) was performed at the Institute of Genomic Diversity, Cornell University. DNAs were digested with *ApeKI* restriction enzyme. SNPs were filtered and extracted using the TASSEL 5.0 software (Bradbury et al. 2007). Data analysis was carried out as follows. First, polymorphisms between line A25 and the seven donor lines were analyzed along the eleven chromosomes. Second, introgressed SNPs (SNP showing genotype of donors) were investigated in the obtained lines derived from backcrossing programs and in line Xana. This analysis allowed the identification of introgressed genomic regions (blocks of adjacent SNPs showing donor genotype) in the obtained lines. Third, the common introgressed genomic regions among NILs derived from the same donor genotype were analyzed in specific chromosomes in order to delimit the region carrying the resistance genes introgressed in line A25.

RESULTS AND DISCUSSION: A total of 50758 SNPs were detected of which 50503 were assigned to physical positions in one of the eleven bean chromosomes (www.phytozome.net). The proportion of unknown data in the plate was 0.048 while the proportion of heterozygous sites was 0.036. The number of SNPs varied across chromosomes, ranging from 3344 on chromosome 10 to 5907 on chromosome 2. The SNP coverage (distribution and polymorphisms) was not homogeneous along the eleven chromosomes. The highest level of polymorphism was detected between the genotypes A25 & BRB130, while the genotypes A25 & V203 exhibited the lower. Average distance between SNPs ranged between 0.037 Mb in chromosome 9 (A25 & BRB130) and 1.90 Mb in chromosome 9 (A25 & V203).

Number and physical positions of introgressed SNPs after the backcross process were investigated comparing the A25 line, the donor lines, and the resulting NILs. Results revealed that backcrossing programs resulted in introgressions of specific genomic regions. The number, position and size of introgressed regions varied among the seven near isogenic lines directly obtained from backcrossing programs. For example, the line A1258 showed 21 introgressed blocks in the chromosome 11 corresponding with 69% of chromosome or 6.78% of bean genome. In contrast, line A1231 exhibited a unique introgressed fragment in chromosome 4 corresponding with 0.24% of chromosome or 2.8% of bean genome.

Analyses of specific chromosomes among the fabada near-isogenic lines derived from the same donor, allowed a more precise delimitation of the introgressed regions containing the resistance gene (Table 1). Results of physical positions agree with the results of genetic mapping which mapped the genes *I, Co-3/Co-9, bc-3* and *Co-2* in the linkage groups Pv02, Pv04, Pv06 and Pv11, respectively (Table 1). Bounded physical regions for genes *I* and *bc-3* also agree with the previously reported in other genotypes (Bello et al 2014; Naderpour et al 2010). Interestingly, were identified different physical regions in the end of chromosome 11 involved in anthracnose resistance (Table 1). In this position, a cluster of race-specific resistance genes to anthracnose was reported (Campa et al. 2014). Finally, this study revealed the usefulness of massive genotyping in delimitation of physical positions carrying specific genes using near isogenic lines.

Table 1. Bounded physical regions from analysis of introgressed segments in near isogenic lines derived from the same donor resistance source. Number of annotated genes in these positions is also indicated.

Donor	Analyzed near isogenic lines	Gene	Chrom.	Physical position (Mb)	% Chrom.	N. genes
BRB130	A2418, A2806, A3308, X2776	I^{BRB130}	**2**	47.93 - 48.72	1.61	123
SANILAC	A1878, A2806, A3308, X1612, X2776	$I^{SANILAC}$	**2**	47.93 - 48.04	0.22	31
A493	A1220, A2438, A1699, A3308, X1319	$Co\text{-}9^{A493}$	**4**	0.37 - 1.30	2.09	74
A321	A1231	$Co\text{-}9^{A321}$	**4**	0.37 - 1.72	3.03	115
BRB130	A2418, A2806, A3308, X2776	$bc\text{-}3^{BRB130}$	**6**	28.0 - 29.08	3.38	127
IVT7214	A2648, A3308	$bc\text{-}3^{IVT}$	**6**	28.0 - 29.08	3.38	127
A252	A1258, A1699, A3308	$Co\text{-}2^{A252}$	**11**	45.18 - 47.07	3.75	134
SANILAC	A1183, A1878, A2806, A2438, X1358, X1612, X2776	$Co\text{-}2_{SANILAC}$	**11**	46.72 - 48.85	4.23	155

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DEVELOPMENT AND RELEASE OF NEW BIOFORTIFIED BEAN VARIETIES IN EASTERN AFRICA

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INTRODUCTION: Development and utilization of biofortified varieties is regarded as probably the most effective and sustainable and potentially long-lasting strategy for reducing micronutrient deficiencies in Africa. Micronutrient malnutrition is now recognized as one of the most serious health challenges facing vast sectors of Africa's population particularly resource-poor women and children (Kimani et al, 2001). Major deficiencies include iron, zinc, vitamin and protein. Micronutrient deficiency is often referred as 'hidden hunger' because the problem does not show any easily recognisable symptoms in the early stages, until considerable, and often irreversible damage has occurred. Prevalence of iron deficiency anaemia (IDA) varies from 8% in Ethiopia, 67% in Tanzania, to 69% in Burundi. The main cause of these deficiencies is diet rich in energy but poor in proteins, minerals and vitamins. In 2004, a regional breeding program supported by ASARECA and led by the University of Nairobi was initiated to develop and disseminate micronutrient dense bean varieties. The objectives of this program were to (i) characterize the variation of grain iron and zinc concentration in east, central and southern Africa, ii) identify lines which could be fast-tracked as mineral dense lines for cultivation by farmers in regions with severe Fe and Zn malnutrition; iii) determine whether there regions with high diversity for this trait, and iv) identify potential parents for further breeding work.

MATERIALS AND METHODS: Bean germplasm was collected in nine countries in east and central Africa. Collections included landraces, varieties, introductions, germplasm accessions and breeding lines held by national bean programs and gene banks. Samples were analyzed at the University of Nairobi (Kenya), and subsequently at CIAT (Colombia), Cornell University (USA), University of Copenhagen (Denmark) and Sokoine University (Tanzania) to facilitate cross-lab comparison (Kimani et al 2001,2006). Thirty-eight fast track lines were identified and distributed for regional evaluation across agro-ecological zones in more than 15 countries in east, central and southern Africa, and later in west Africa (Kimani et al, 2004, 2007). Fast track lines were also evaluated for anti-nutritional factors (tannins and phytates), cooking time, mineral retention and other organoleptic characteristics (Kimani et al 2005, 2006, 2007). Genotypes with high micronutrient concentration but lacking in preferred agronomic traits and susceptible to major diseases were entered in hybridization program at Kabete to generate segregating populations and select for lines combining mineral density with resistance to biotic and abiotic stress factors, marketable grain types and high yield potential. Mineral analyses were performed used ashing and wet digestion techniques (Zarcinas et al, 1983). In Kenya, 19 selected bush and climbing bean lines were validated in national performance trials in 2008 and 2009. Normal agronomic practices were followed in field trials. Data was analyzed using Genstat and/or SAS statistical software.

RESULTS AND DISCUSSION: More than 2853 germplasm accessions collected from nine countries in east and central Africa were analyzed for Fe and Zn. Fe concentration varied from 40 to 105 ppm. Sixty-six lines had more than 90 ppm indicating that potential of increasing iron concentration in the grain by more than 90%. Bean showed higher iron concentration compared to whole maize (19 ppm), dehulled maize (7ppm), cassava (5ppm), cassava flour (5 ppm), cocoyam (4 ppm), potato (3ppm), sweet potato (5 ppm) and cooking bananas (5 ppm), which are widely

consumed in the region. Iron concentration in grey and yellow beans was higher than beef (98 ppm) and comparable to fish (124 ppm). This implied that beans are among the most important sources of iron in local diets, and is critical to the vast majority who have limited access to animal sources. Zinc concentration varied from 20 to 52 ppm. The highest diversity of mineral concentration was found in Great Lakes Region. Fast track biofortied bean nursery was distributed to more 25 countries in east, central, west and southern Africa. Most of the countries have identified and/or released new biofortified varieties from this nursery. In Kenya, three new climbing biofortified bean varieties were formally released in 2012 (Table1). Four new bush varieties were recommended for release in 2013 after validation in multi-location trials by KEPHIS (Table 2).

Table 1. Grain yield across environments, iron and zinc concentration of new micronutrient rich climbing bean varieties released in Kenya.

Line	Status	Mean yield across environments (kg ha-^{1})	Yield over best check (%)	Fe (ppm)	Zn (ppm)
MV 19	**Released**	2230	45.8	86	32
MV17	**Released**	2200	44.1	104	44
MV14	**Released**	2150	40.1	75	34
MAC 34	**Check**	1530		<70	<25
MAC 13	**Check**	1190		<70	<25
MAC 64	**Check**	1180		<70	<25
Vunikingi	**Check**	1110		<70	<25

Source: KEPHIS, 2008, 2012.

Table 2. Performance and grain iron and zinc concentration of micronutrient dense lines in Kenya.

Line	Lowland sites			Highland sites			Fe	Zn
	Mean yield (kg ha-^{1})	Yield over best check (%)	Yield over mean of checks (%)	Mean yield (kg ha-^{1})	Yield over best check (%)	Yield over mean of checks (%)	ppm	ppm
MN 1	1150	20.9	33.8	2030	32.6	45.1	147	38
MN 3	1130	18.8	31.5	1960	28.7	45.1	73	41
MN 6	1130	18.0	30.6	2800	80.6	97.7	106	30
MN 9	1010	6.1	17.5	1890	23.3	35.0	75	45
Checks								
GLP 92 (Mwitemania)	950			1380			<70	16
GLP 1127 (Mwezi Moja)	800			1530			<70	<25
GLP 2 (Rosecoco)	830			1280			<70	<25

Source: KEPHIS, 2008, 2012

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CONTENT OF POLYPHENOLS AND ANTIOXIDANT CAPACITY OF DIFFERENT BEAN CULTIVARS AFTER AN ENZYMATIC EXTRACTION

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INTRODUCTION: Phenolic compounds are metabolites synthesized by plants which have the potential to be used by humans for a variety of applications (Puri et al., 2011), in this sense, common bean has been wildly researched for its polyphenols content and antioxidant capacity; however, quantification of these compounds is often realized in organic extracts, meaning that the extraction methods are not carried out under physiological conditions, and it is difficult to determine to what extent these results could be transferred to the human organism. The objective of the present study was to evaluate the effects of digestive enzymes in the polyphenols extraction an antioxidant capacity of different common bean cultivars.

MATERIAL AND METHODS: Bean cultivars Pinto Saltillo (PS), Pinto Centauro (PC), Bayo Zacatecas (BZ), Flor de Mayo Dolores (FMD) and Flor de Junio Dalia (FJD) were ground, and polyphenols were extracted from samples by two methods: an organic extraction using 1 g of sample and 10 mL of acidic acetone [acetone/water/acetic acid (70:29.5:0.5, v/v/v)] as described by Xu et al. (2007), and an enzymatic extraction using pepsin, pancreatin and α-amylase under physiological *in vitro* conditions. In brief, 300 mg of sample were incubated with pepsin solution containing 300 mg/mL of HCl-KCl buffer during 1h, then a solution of 5 mg/mL of pancreatin in a physiological buffer were added and incubated during 6h; finally, an α-amylase solution (120 m/mL of Tris-maleate buffer) was added and incubated during 16h. Samples were centrifuged and supernatants were removed. Content of total phenols, total flavonoids and condensed tannins were determined from both extracts as well as the antioxidant capacity by scavenge of DPPH and ABTS free radicals, expressed as IC_{50}.

RESULTS AND DISCUSSION: There was an interaction between the extraction method and the cultivars, resulting in differences in the phenolic compounds (Table 1). Concentration of total phenols was higher in the enzymatic extracts of all bean cultivars tasted, however FJD had the highest concentration, with an upsurge of 70% compared with its organic extract. This may suggest that the enzymatic treatments hydrolyze starch and protein, which may favor the release of phenolic compounds. Although flavonoids and condensed tannins were higher in the enzymatic extracts of cultivars PS and PC in comparison to their organic extract, BZ, FMD and FJD showed a higher content of these compounds when extraction was obtained by organic solvents. Enzymatic digestion showed marked favorable variations in the DPPH radical scavenging activity of bean cultivars, while organic extracts showed better antioxidant capacity against ABTS radical (Table 2). The enzymatic extract of PC and the organic extract of FMD had the greater capacity to scavenge the DPPH and ABTS radicals respectively, this suggests that both types of extracts possess free radical-scavenging activity but in different levels, depending on the existing radical.

Table 1. Phenolic compounds of organic and enzymatic extracts of common bean cultivars.

Sample	Extraction	Total phenols[1]	Total flavonoids[2]	Condensed Tannins[2]
Pinto Saltillo	Organic	3.5 ± 0.3^{f}	4.1 ± 1.3^{bcde}	1.2 ± 0.3^{e}
	Enzymatic	11.6 ± 0.2^{bc}	3.1 ± 0.1^{def}	2.2 ± 0.1^{de}
Pinto Centauro	Organic	2.8 ± 0.0^{f}	5.1 ± 2.3^{abcd}	1.6 ± 0.4^{e}
	Enzymatic	12.7 ± 0.2^{ab}	0.7 ± 0.3^{ef}	3.4 ± 0.0^{d}
Bayo Zacatecas	Organic	10.3 ± 0.2^{d}	8.0 ± 0.8^{a}	8.5 ± 0.1^{b}
	Enzymatic	10.9 ± 0.4^{cd}	3.2 ± 0.2^{cdef}	5.3 ± 0.7^{c}
FM Dolores	Organic	7.7 ± 0.6^{e}	7.0 ± 0.5^{abc}	11.6 ± 0.0^{a}
	Enzymatic	11.6 ± 0.4^{bc}	2.4 ± 0.5^{def}	1.7 ± 0.1^{e}
FJ Dalia	Organic	8.1 ± 0.3^{e}	7.8 ± 1.1^{ab}	11.7 ± 0.5^{a}
	Enzymatic	13.6 ± 0.0^{a}	0.2 ± 0.1^{f}	3.4 ± 0.1^{d}

Values are presented as mean ± SD. Means in the same column with a common letter are not significantly different ($p<0.05$, Tukey). FM, Flor de Mayo; FJ, Flor de Junio. Results are expressed as [1]mg equivalent of gallic acid/g and [2]mg equivalent of (+) catechin/g.

Table 2. Antioxidant capacity of organic and enzymatic extracts of common bean cultivars.

Sample	Extraction	DPPH	ABTS
Pinto Saltillo	Organic	861.7 ± 5.0^{a}	153.1 ± 3.8^{ab}
	Enzymatic	885.1 ± 17.5^{a}	186.2 ± 20.0^{a}
Pinto Centauro	Organic	632.2 ± 11.6^{c}	103.0 ± 4.8^{de}
	Enzymatic	61.2 ± 2.3^{e}	133.0 ± 3.5^{bcd}
Bayo Zacatecas	Organic	138.6 ± 7.7^{de}	109.2 ± 5.8^{cde}
	Enzymatic	81.1 ± 1.5^{de}	110.0 ± 0.9^{cde}
FM Dolores	Organic	123.4 ± 21.1^{de}	82.9 ± 4.4^{e}
	Enzymatic	736.4 ± 58.4^{b}	97.6 ± 16.1^{de}
FJ Dalia	Organic	152.7 ± 11.5^{d}	99.5 ± 0.1^{e}
	Enzymatic	56.7 ± 5.5^{e}	144.6 ± 6.2^{de}

Values are presented as mean ± SD. Means in the same column with a common letter are not significantly different ($p<0.05$, Tukey). FM, Flor de Mayo; FJ, Flor de Junio. Results are expressed as IC_{50}.

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USE OF COMMON BEAN FLOURS TO ENHANCE PHENOLIC CONTENT AND ANTIOXIDANT CAPACITY OF SOME BAKERY PRODUCTS

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INTRODUCTION: Common bean is the most important grain legume for direct human consumption. Being rich in protein and an often supply of an important proportion of carbohydrates, bean plays a significant role in the human diet, and is also a key source of some minerals, especially iron (Beebe et al., 2013). However, in recent years, bean producers have faced problems in marketing, leading to economic losses; in this context, it is important to carry out strategies to achieve agro-industrial processing and to improve the nutritional quality of food products. The objective of the present study was to enhance the content of phenolic compound and antioxidant capacity of two bakery products by using common bean flour in their processing.

MATERIAL AND METHODS: Bean cultivars Pinto Saltillo and Flor de Mayo Dolores were ground and used to produce cookies and biscuits, respectively. Cookies were made with the addition of whole wheat and oat flour, and a commercial cookie was used as the control. On the other hand, biscuits were produced with whole wheat flour and the incorporation of different proportions of common bean Flor de Mayo Dolores flour (90/10, 70/30 and 50/50 of bean/wheat flours) and a 100 % wheat flour biscuit was used as the control. Samples were freeze dried and processed in a domestic grinder, phenolic compounds were extracted as described by Xu et al. (2007). Determination of total phenols, total flavonoids, condensed tannins and total anthocyanins was carried out on the extracts of the baked products and antioxidant capacity was evaluated by scavenge of DPPH and ABTS free radicals, expressed as IC_{50}.

RESULTS AND DISCUSSION: Table 1 shows the phenolic compounds and antioxidant capacity of commercial and common bean cookies. Almost all variables evaluated in this study demonstrate that the addition of Pinto Saltillo common bean in the cookie mixture improves not only the phenolic compounds content, but also increases the antioxidant capacity by scavenging of free radicals. An up surge of 253%, 382% and 186% was observed for total phenols, flavonoids and anthocyanins respectively, while scavenging of DPPH and ABTS was greater by 83% and 52% compared with the commercial cookie. This suggest that incorporating this legume improves some bakery products, however, increases in antioxidant capacity may be given by a synergistic effect between the phenolic profile and some other phytochemicals with free radicals-scavenging capacity (e.g. non-digestible carbohydrates, proteins and same minerals). Content of condensed tannins was higher in the commercial cookie, which may indicate that some ingredients of this product provided a higher amount of those compounds.

Table 1. Phenolic compounds and antioxidant capacity of commercial and common bean cookies.

Compound	Commercial cookies	Common bean cookies
Total phenols[1]	1.7 ± 0.1	4.3 ± 0.1**
Total flavonoids[2]	5.0 ± 0.2	19.1 ± 2.0**
Condensed Tannins[2]	0.5 ± 0.0	2.0 ± 0.0***
Anthocyanins[3]	4.3 ± 0.2	8.0 ± 1.0***
Antioxidant capacity		
DPPH[4]	2863.8 ± 33.0	480.3 ± 17.6***
ABTS[4]	325.0 ± 31.0	156.0 ± 11.5*

Values are presented as mean ± SD. *** $p \leq 0.001$, ** $p \leq 0.01$ y * $p \leq 0.05$ vs the control (commercial cookies) indicates significant differences ($p<0.05$, Dunnett). Results are expressed as [1]mg equivalent of gallic acid/g; [2]mg equivalent of (+) catechin/g; [3]mg equivalent of cyanidin 3-glucoside/g and [4]IC_{50}.

Differences where observed in the phenolic profile and antioxidant capacity of biscuits (Table 2). Although the control sample had the highest capacity to scavenge the DPPH radical, content of phenolic compounds was lower in comparison to the biscuits that had a proportion of common bean flour. The biscuit with a proportion of 50/50 bean/wheat flours exhibited the highest concentration of total flavonoid and anthocyanins, while total phenols and condensed tannins were higher for the 70/30 and 90/10 proportions respectively. Additionally, the 90/10 biscuit showed the highest antioxidant capacity by scavenging the ABTS free radical. Recently, we determined the content of phytochemicals of different common bean cultivars, including Flor de Mayo Dolores which had outstanding results in the majority of the compounds evaluated. In this context, results clearly indicates that using cultivar Flor de Mayo Dolores as a source of phenolic compounds is a good option in order to improve the nutraceutical quality of some bakery products.

Table 2. Phenolic compounds and antioxidant capacity of wheat and common bean biscuits.

Compound	Control biscuit	Biscuit 90/10	Biscuit 70/30	Biscuit 50/50
Total phenols[1]	1.3 ± 0.2	4.0 ± 0.0**	4.2 ± 0.5**	3.6 ± 0.2**
Total flavonoids[2]	2.9 ± 0.1	2.8 ± 0.4	2.5 ± 0.2	10.4 ± 0.6***
Condensed Tannins[2]	1.5 ± 0.1	5.7 ± 0.1***	2.5 ± 0.1***	3.5 ± 0.1***
Anthocyanins[3]	39.3 ± 0.7	19.5 ± 0.2***	36.1 ± 1.2**	41.0 ± 0.2
Antioxidant capacity				
DPPH[4]	387.1 ± 65.5	615.3 ± 22.6	1116.6 ± 38.4**	910.8 ± 173.3*
ABTS[4]	316.0 ± 22.2	159.3 ± 8.1**	205.1 ± 22.0**	220.0 ± 3.3**

Values are presented as mean ± SD. *** $p \leq 0.001$, ** $p \leq 0.01$ y * $p \leq 0.05$ vs the control (100% wheat flour biscuit) indicates significant differences ($p<0.05$, Dunnett). Results are expressed as [1]mg equivalent of gallic acid/g; [2]mg equivalent of (+) catechin/g; [3]mg equivalent of cyanidin 3-glucoside/g and [4]IC_{50}.

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CENTESIMAL COMPOSITION OF CREOLE ACCESSIONS OF COMMON BEANS CULTIVATED IN NORTH OF MINAS GERAIS STATE, BRAZIL

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The bean (*Phaseolus vulgaris* L.) is an important food because of its nutritional composition and has high mineral content, vitamins, carbohydrates, fiber and protein (Broughton et al., 2003). Creole or traditional accessions can be defined as genotypes in use by farmers, obtained from natural crossings and that have not undergone any genetic breeding (Elias et al., 2007). The cultivation of these genotypes by small and medium farmers provides the conservation of genetic resources. Furthermore, there is the possibility of this diversity is exploited by the bean crop breeding programs, since they are well characterized according to the agronomic interest in technological and nutritional qualities (Pereira et al., 2009). The aim of this study was to analyze the centesimal composition of Creole accessions of common beans cultivated in mesoregion of North of Minas Gerais State, Brazil.

Ten Creole bean accessions commonly grown by small farmers in the mesoregion of Northern of Minas Gerais State were analyzed: 'Curiango', 'Penquinha', 'Meia Corda', 'Roxo', 'Olho de Pombo', 'Cores', 'Branco', 'Mulatinho', 'Fava Branca' and 'Fava Cores'. Samples were collected in August 2015 in the county of Montes Claros-MG, in geographical coordinates of 15º96'69" South Latitude, 08º50'59" West Longitude and 596 meters. Samples were collected at random from different points of crops. The analysis of the centesimal composition involved the moisture content, ash, lipids, carbohydrates and proteins, which were performed according to the methodology described by AOAC (1995). Statistical analyzes were carried out from 30 repetitions of each completely randomized design (CRD) access. The means (μ), variances (σ^2) and analysis of variance (ANOVA) were calculated using the statistical program GENES (Cruz, 2006).

The variance analysis of centesimal composition was significant for all contents (Table 1). When considering the wide genetic base of a Creole genotype it is known that the response to environmental conditions can also be changed. Thus, there is both effect on climate variations, such as genotype and location on the differential accumulation in the centesimal composition (Rangel et al., 2007). For example, the protein content varies from 18.1173% to 26.1524% (Table 2), demonstrating broad-spectrum with respect to the materials traditionally improved (Lemos et al. 2004). The use of Creole genetic resources in research can contribute to increasing the technological and nutritional quality of beans.

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Table 1. Summary of analysis of variance on the centesimal composition of Creole accessions of common beans cultivated in mesoregion of the North of Minas Gerais State, Brazil.

ANOVA	DF	SS	MS	F	P value	F critical
Proteins	9	153.0576	17.0064	35.1268	2.5041E-10	2.3928
Lipids	9	3.2431	0.3603	24.3369	1.9572E-11	2.2106
Carbohydrates	9	79.4180	8.8242	16.5496	1.9861E-07	2.3928
Ash	9	4.0827	0.4536	237.9143	2.2239E-25	2.2106
Moisture	9	20.5644	2.2849	13.8546	8.7122E-07	2.3928

ANOVA: Analysis of variance; DF: Degree of freedom; SS: Sum of Square; MS: Mean Square.

Table 2. Means (μ) and variances (σ^2) of centesimal composition of Creole accessions of common beans cultivated in mesoregion of the North of Minas Gerais State, Brazil.

Creole common bean accessions	Contents (%)									
	Proteins		Lipids		Carbohydrates		Ash		Moisture	
	μ	σ^2	μ	σ^2	μ	σ^2	μ	σ^2	μ	σ^2
Curiango	23.9874	0.9668	1.0804	0.0143	62.0888	1.5587	3.9231	0.0016	8.9200	0.1236
Penquinha	24.9945	0.7370	1.1841	0.0069	61.0519	0.5820	3.8389	0.0045	8.9304	0.0468
Meia Corda	23.2322	1.9234	1.5572	0.0194	62.5735	1.5184	3.3852	0.0010	9.2517	0.0236
Roxo	22.5752	0.0819	1.6444	0.0087	63.7764	0.2569	3.3429	0.0003	8.6609	0.1467
Olho de Pombo	23.5970	0.1801	0.8161	0.0049	62.7126	0.2630	3.3083	0.0052	9.5658	0.0029
Cores	26.1524	0.0449	0.7172	0.0667	62.3220	0.0121	3.4569	0.0001	8.3513	0.3775
Branco	19.7293	0.1652	1.2437	0.0220	65.9643	0.0340	3.7575	0.0016	9.3050	0.0297
Mulatinho	20.4943	0.4568	0.9533	0.4920	64.8341	0.5636	3.4965	0.0003	10.2214	0.0104
Fava Branca	19.4345	0.2197	1.2111	0.0014	65.4877	0.1724	3.2670	0.0014	10.5994	0.1783
Fava Cores	18.1173	0.0651	1.0720	0.0033	65.4768	0.3705	4.3040	0.0025	11.0297	0.7093

GENETIC VARIABILITY OF BEAN GRAINS CREOLE FOR WATER ABSORPTION

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The bean cooking time is important in the definition of farming to be made available for research institutions due to the cooking time that can prevent consumer acceptance. Thus, breeding programs should prioritize the identification of genotypes that have reduced cooking time, with coats without damage during cooking and high volumetric expansion after cooking. Furthermore, it is also important to the productivity and disease resistance as well as technological product quality (Costa et al., 2001). The faster the higher water absorption is also the capacity of the cooking grains (Perez-Ibarra et al., 1996). During cooking, the grain structure is modified, the gelled starch and denatured proteins. In this work, we aimed to perform the analysis of the water absorption of Creole beans accessions cultivated in the mesoregion of North of Minas Gerais State, Brazil.

Ten Creole bean accessions commonly grown by small family farmers in the middle region north of the Minas Gerais state were analyzed: 'Curiango', 'Penquinha', 'Meia Corda', 'Roxo', 'Olho de Pombo', 'Cores', 'Branco', 'Mulatinho', 'Fava Branca' and 'Fava Cores'. In August 2015, samples were collected in the county of Montes Claros, Minas Gerais State (Geographical coordinates of 15º96'69" South Latitude, 08º50'59" West Longitude and 596 meters). Samples were taken at random from different points of crops. The water absorption tests were performed at the Biotechnology Laboratory of the ICA/ UFMG, using a completely randomized design with three replications. The means (μ), variances (σ^2) and analysis of variance (ANOVA) were calculated using the GENES program (Cruz, 2001). Moisture content was estimated by drying the samples at 105°C according to AOAC (1995). The humidity was obtained by the expression H = [(iw-fm) / iw] x100; where H = humidity (%), iw = initial weight of sample (g) and fm = the final mass of sample (g). To measure the capacity of water absorption, the grains were placed in cups becker with 200 ml of distilled water remaining in soaking for a period of 12 hours. Samples of the grain were removed and partially dried with paper towels, proceeding to count the normal grain, hard (hardshell) and total (normal + hard). The number of normal grains relative to the total grains evaluated in samples of 50g of grains, or those which have absorbed water after immersion in distilled water were used to calculate the percentage of water absorption. The number of hard grains (without moisture) in relation to the total number of grains was used to calculate the percentage hardshell grains.

There was a significant effect on moisture and the ability of water absorption by the grains of the studied accessions (Table 1). The average value of the capacity of water absorption by the grains was 57.4605%. However, it was observed that the accessions differ for the evaluated parameters and great amplitude values (21.0692% to 96.6251%) (Table 2). This information is important since accessions that showed high rate of water absorption by the beans are associated with a shorter cooking time (Plhak et al., 1989). Thus, in the germplasm it is possible to identify that access may have lower cooking time, which can be identified by some accessions that had near total absorption of water by the grains. The existence of genetic variability for water absorption by the beans has been reported by other authors (Costa et al., 2001; Ramos Junior &, 2002). This indicates that selection for this character may be useful for early identification of strains with greater facilities for cooking. However, it is important that standardized methodology for fast and efficient identification

of the percentage of water absorption by the grains because this method is questioned (Carbonell et al., 2002). Moreover, Plhak et al. (1989) found that cooking capacity seems to be indeed associated with rapid absorption of water by the beans. In this work, we found strong evidence about the genetic variability of absorption of water by the grains in Creole bean accessions, suggesting that it is possible genetic selection for this genetic character.

Table 1. Summary of variance analyzes of moisture and the ability of water absorption in Creole bean accessions cultivated in mesoregion of North of Minas Gerais State, Brazil.

ANOVA		FD	SS	MS	F	P value	F critical
Moisture	Accessions	9	20.5644	2.2849	13.8546	8.7122E-07	2.3928
	Residue	20	3.2984	0.1649			
	Total	29	23.8628				
Absorption of water	Accessions	9	26.481.0439	2.942.3382	189.7549	2.2769E-17	2.3928
	Residue	20	310.1197	15.5059			
	Total	29	26.791.1637				

ANOVA: Analysis of Variance; FD: Freedom Degree; SS: Sum of Squares; MS: Mean Square.

Table 2. Means (μ) and variances (σ^2) of the units and water absorption capabilities in Creole bean accessions cultivated in mesoregion of North of Minas Gerais State. Brazil.

Creole bean accessions	Contents (%)			
	Moisture		Water Absorption	
	μ	σ^2	μ	σ^2
Curiango	8.9200	0.1236	95.4338	4.6733
Penquinha	8.9304	0.0468	96.6251	1.4721
Meia Corda	9.2517	0.0236	94.6860	64.5622
Roxo	8.6609	0.1467	21.0692	0.9740
Olho de Pombo	9.5658	0.0029	31.2023	0.8023
Cores	8.3513	0.3775	80.9878	8.2009
Branco	9.3050	0.0297	46.9986	26.6431
Mulatinho	10.2214	0.0104	23.8680	2.6848
Fava Branca	10.5994	0.1783	32.5433	26.4680
Fava Cores	11.0297	0.7093	51.1909	18.5788

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PHYSICAL PROPERTIES OF CREOLE BEAN GRAINS

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During storage, the bean drying is the process most commonly used to ensure quality and stability, reducing the biological activity and possible physical and chemical changes. Reducing the water content of the beans decreases the size of the grains and influences directly on its physical properties during the drying process. Thus, the correct determination of the physical properties is of fundamental importance in optimizing industrial processes, aerodynamic studies, design and sizing of equipment used in harvesting and post-harvest operations (Resende et al., 2005). The objective of this study was to measure in Creole beans forms, the grain sizes (sphericity and volume) and their densities, grown in mesoregion of North of Minas Gerais State, Brazil.

This work was developed in the Biotechnology Laboratory of the Universidade Federal de Minas Gerais, Montes Claros-MG, Brazil. Ten Creole bean (*Phaseolus vulgaris* L.) accesses were studied. The bean accesses were collected manually with a water content of about 0.92 (b.s.). Drying was carried out at constant temperature of 40°C. Reducing the water content over drying was followed by gravimetric (weight loss), determining the initial moisture level of the grain until the final water content of 0.13 (b.s.), using analytical balance accurate to 0.01g . The grain water content was determined by the oven-drying method at 105 ± 1°C to constant weight. The shape and size of grains analyzed by means of spherical shape and volume from measurements of 30 grains of each access (Moshsenin, 1986). The data on the characteristics and dimensions orthogonal axes (Figure 1) were obtained using a digital caliper with an accuracy of 0.01mm.

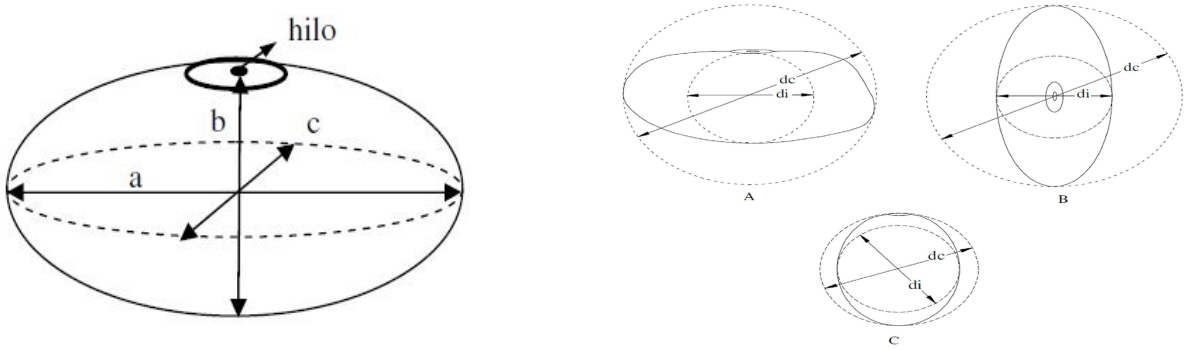

Figure 1. Schematic bean grain design considering the oblate spheroid shape with its characteristic dimensions: (a) major axis of grain (mm); (b) Average grain axis (mm); (c) the grain minor axis (mm); (di) diameter of the largest inscribed circle (mm); (dc) diameter of the smallest circumscribed circle (mm); (A) major axis; (B) median axis; (C) minor axis.

The density (g/cm^3) was determined by dividing the sample weight by volume (Ferreira et al. 2002). Statistical analyzes were performed in a completely randomized design (CRD), and the mean ( ), the variance (σ^2) and the analysis of variance (ANOVA) were determined by the GENES program (Cruz, 2001).

The analysis of variance of the physical properties of sphericity, volume and density were all significant (Table 1). Thus, it is apparent that the analyzed Creole beans exhibit variations of its characteristic dimensions (Table 2). This is observed for most biological products, which, during drying, contract up irregularly in the various directions, as noted by Correa et al. (2002). The volumetric changes

of the products due to dehydration are reported to be the main causes of changes in the physical properties of agricultural grains (Sokhansanj & Lang, 1996). Zogzas et al. (1994) observed that the plant products shrinkage during drying is not solely a function of water content, but also dependent on the process conditions and product geometry. In this work, it can be seen that the characteristic dimensions of the grains decrease with decreasing water content. The grain sphericity also reduced during the drying process, whereas circularity did not show a tendency to set their values to reduce the water content. Moreover, the surface/volume ratio of the grains increased by reducing the water content during the drying process (Table 2). Despite the theoretical basis for the knowledge of the shrinkage process involved complex mechanical and material deformation laws (Towner, 1987). There is an increasing tendency for the bean breeding programs intensify their work in relation to the physical properties of the grains.

Table 1. Summary analysis of variance on the sphericity, volume and density in grain Creole bean accesses cultivated in mesoregion of North of Minas Gerais State, Brazil.

ANOVA		DF	SS	MS	F	P value	F critical
Sphericity	**Accesses**	9	27,785.4616	308.2735	149.7538	1.6181E-103	1.9122
	Residue	29	5,978.5415	20.6156			
	Total	29	33,764.0031				
Volume	**Accesses**	9	41,877.8110	4,654.6457	49.6429	1.1275E-53	1.9123
	Residue	29	27,884.6250	93.3435			
	Total	29	69,762.4360				
Density	**Accesses**	9	0.2740	0.030449289	16.2184	2.9625E-09	2.2106
	Residue	29	0.0563	0.001877451			
	Total	29	0.3303				

ANOVA: Analysis of variance; DF: Degree of freedom; SS: Sum squared; MS: Mean squared.

Table 2. Means (μ) and variances (σ^2) of units and water absorption capabilities in Creole bean accesses cultivated in mesoregion of North of Minas Gerais State, Brazil.

Creole bean accesses	Measurements					
	Sphericity (%)		Volume (mm^3)		Density (g/cm^3)	
	μ	σ^2	μ	σ^2	μ	σ^2
Curiango	68.1401	6.0258	113.7088	62.4484	1.2200	0.0004
Penquinha	64.4329	11.3016	107.4405	42.6366	1.1899	0.0009
Meia Corda	86.4798	62.8349	177.4813	19.4447	1.0861	0.0028
Roxo	82.7521	27.7154	164.9587	51.2726	1.1568	0.0011
Olho de Pombo	74.2842	11.0413	203.8478	22.3590	1.2881	0.0005
Cores	65.8439	19.3919	127.4270	52.0175	1.2412	0.0005
Branco	72.6522	6.6235	222.1668	11.6740	1.3508	0.0011
Mulatinho	89.5283	21.1343	228.8581	13.2107	1.3343	0.0001
Fava Branca	60.6864	9.0797	436.2075	14.8561	1.2843	0.0001
Fava Cores	64.3607	31.0078	455.1603	58.5153	1.3422	0.0110

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GENETIC VARIABILITY ASSOCIATED TO PHYSIOLOGICAL QUALITY IN SEEDS OF COMMON BEAN GENOTYPES OF SPECIAL GRAIN

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INTRODUCTION: The genetic variability among common bean genotypes for traits related to physiological quality, characterized by germination and seed vigor, it has been reported by Maia *et al*. (2011). However, there is a lack of this type of study for Cultivation and Use Value tests (VCU) of beans, in which it was evaluated the performance of common bean elite lines, which can be recommended for planting in different growing regions. Thus, the evaluation of characteristics related to seed physiological quality of bean lines can support and greater security to the recommendation of new cultivars. The objective of this study was to evaluate the genetic variability for traits related to seed physiological quality of common bean genotypes of "special grain" group.

MATERIAL AND METHODS: The experiment was carried out at Laboratory of Seed Analysis at State University of Montes Claros, Campus Janaúba, MG. The seeds were from the Experimental Farm of Federal University of Viçosa, located in Coimbra- MG, harvested in the autumn-winter season of 2012. We used 12 common bean elite-lines and four cultivars ones selected to compose the VCU test of the "special grain" group. The design was in randomized block with three replications. From each plot 50 seeds were collected at random for evaluations. The evaluated characteristics were the percentage of germination (GER), the seed vigor, evaluated from the plantlets emergence test (PE) (BRAZIL, 2009), besides the emergence speed index (IVE), according to Maguire (1962). The data were submitted to analysis of variance and when significant, effects were studied by Scott-Knott test at 5% significance level.

RESULTS AND DISCUSSION: There was a significant effect of genotypes on all traits, indicating that there is genetic variability among genotypes studied for those characteristics. Genotypes ‘Ouro-Vermelho’, ‘BRS Timbó’, ‘BRS Vereda’, ‘CNFJ 15288’, ‘CNFRx15275’, ‘Jalo EEP’, ‘PT-68’, ‘RAD/E550-284’ and ‘VR-17’ showed germination above the minimum standard required for sale of bean seeds (Table 1) which is 80% for certified seed (BRAZIL, 2005). Except for VR-14 line, the other genotypes showed vigorous plantlets, ranging from 90-99% in the emergence. The CNFJ 15288 CNFRx 15275, VR-16 and VR-17 lines besides the ‘Ouro-vermelho’, ‘BRS Timbó’ and ‘BRS Vereda’ cultivars had the highest EVI values (Table 1). High values of EVI are desirable, since the plantlets are less susceptible to adverse environmental conditions to emerge and develop more quickly and in a uniform way, establishing, therefore, plantlets with greater vigor.

Table 1: Average percentage of germination (GER), plantlet emergence (PE) and the emergence velocity index (EVI) of common bean genotypes of the "special grain" commercial group, grown in the autumn-winter crop 2012 in Coimbra-MG.

GENOTYPE	GER (%)	PE (%)	EVI
OURO VERMELHO	85.3 a[1]	97 a	16.0 a
BRS TIMBÓ	90.7 a	96 a	15.7 a
BRS VEREDA	87.3 a	99 a	15.8 a
RADIANTE	78.7 b	98 a	14.6 b
CNFJ 15288	86.7 a	97 a	16.0 a
CNFRx 15275	90.0 a	96 a	15.5 a
JALO EEP	92.0 a	97 a	13.9 b
PT-65	72.0 b	99 a	14.0 b
PT-68	88.0 a	97 a	14.9 b
RAD/E550-284	84.7 a	95 a	14.6 b
RC2RAD-155	80.0 b	98 a	14.0 b
VR-14	40.7 c	51 b	6.6 c
VR-15	74.7 b	90 a	14.1 b
VR-16	70.7 b	95 a	15.6 a
VR-17	84.7 a	94 a	15.4 a
VR-18	81.3 b	93 a	14.7 b
CV (%)	7.85	3.99	3.93

[1]Means followed by the same letter in the column belong to the same group for the Scott-Knott test. a 5 % significance level.

CONCLUSIONS: There is genetic variability among genotypes as for germination, plantlets emergence, and emergence speed index. The CNFJ 15288. CNFRx 15275 and VR-17 lines and the 'Ouro-Vermelho'. 'BRS Timbó' and 'BRS Vereda' cultivars have higher seed physiological quality.

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CHANGES IN TECHNOLOGICAL QUALITY OF CARIOCA BEANS

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INTRODUCTION

As a result of storage conditions and time, common bean grains can harden and darken their seed coat. Studies suggest that intensification of seed coat color and the pH of the broth from soaking the grains may reflect cooking time. Investigation of the qualitative properties of the common bean grains packaged by different companies is desired by consumers who hope to purchase quick-cooking grains. Thus, the aim of this study was to evaluate the technological quality of carioca (beige with brown stripes) common beans of different brands in the municipality of Lavras, MG, Brazil, in the period of August and September 2012.

MATERIALS AND METHODS

The experiment was developed in the Grain, Root, and Tuber Laboratory in the Food Science Department of the Universidade Federal de Lavras, adopting a completely randomized statistical design with six replications and four treatments (predominant brands in local retail sales).

The following determinations were made: the percentage of water imbibition by the grains before cooking (PWIBC) and after cooking (PWIAC), according to the methods of Garcia-Vela and Stanley (1989) and of Plhak, Caldwell and Stanley (1989), modified according to Oliveira et al. (2012); the volumetric expansion rate of the grains after cooking (VER), according to Martin-Cabrejas et al. (1997); and the mean cooking time (MCT), by the Mattson Cooker, according to the method of Sartori (1982), modified by Proctor and Watts (1987). Color was evaluated with the MINOLTA Chroma Meter CR-400 colorimeter, which takes a reading in a three-dimensional system, evaluating color on three axes - a, b, and L, according to the Hunter Coordinate System. The vertical "L" axis evaluates the color of the samples from black to white and reflects the lightness of the grain, which is the most used in evaluations of grain storage. The pH of the broth from soaking the grains was also evaluated, obtained through dilution of 10 g of meal from selected grains in 50 mL of distilled water; the mixture was homogenized in a magnetic stirrer for five minutes and left at rest for ten minutes for sedimentation. After that period, the pH was directly measured in the supernatant.

The data were subjected to analysis of variance through use of the software Sisvar version 4.0 and, in cases of significance for treatments, the mean values were clustered through the Scott-Knott means test.

RESULTS AND DISCUSSION

The commercial brands used were similar in regard to VER, with mean increase in volume of around 53% and almost all the grains remained whole after cooking (Table 1). There were differences in regard to the other characteristics evaluated, with the results of greatest acceptance for consumption in brand C: high hydration capacity and low cooking time. In addition to the economic benefit, the lowest nutritional loss was seen in this brand, inferred as based on the pH from soaking, possibly a consequence of greater integrity of membranes (OLIVEIRA et al., 2012).

In contrast, inferior results were found in brand A in comparison to the other brands. In addition to color opacity, the brand had more acidic tissue, with greater leaching of solutes to the solution, and mean cooking time was 2.31 times greater than the mean value of the other samples.

Although brand A had the lowest retail price (Table 1), which could appear advantageous to the consumer, the results of MCT denote a final cost equivalent to or even greater than that of the other brands studied. In addition, the nutritional value and the sensory properties could be compromised, leading the consumer to question if the reduced price would justify purchase of the brand.

Table 1. Mean values of the percentage of imbibition before cooking (PWIBC) and after cooking (PWIAC), volumetric expansion rate (VER), L index of seed coat coloring, pH from soaking, and mean cooking time (MCT) of carioca common bean grains sold in Lavras, Minas Gerais, Brazil.

Brands	Price* U$/kg	PWIBC ---------(%)---	PWIAC ----------	VER (g mL^{-1})	L Index ----------	pH from soaking ----------	MCT (minutes)
A	0.95	85.21 d	113.39 b	0.49	46.6 d	6.50 c	67 b
B	1.12	96.07 b	120.13 a	0.53	52.7 c	6.60 b	30 a
C	1.18	100.82 a	125.26 a	0.54	56.7 b	6.64 a	24 a
D	1.18	92.31 c	116.67 b	0.55	56.9 a	6.59 b	34 a
Mean	1.11	93.60	118.86	0.53	53.3	6.58	38
CV (%)	-	2.36	4.99	9.28	3.27	0.27	17.82

Mean values followed by the same letters belong to the same group according to the Scott-Knott test at the level of 5% probability. *Values obtained in the local market on 06/08/2012.

In summary, it may be inferred that the technological quality of common bean grains varies according to the brands, whose products may be in different stages of storage. It is also fitting to note that all the characteristics cited may have been initially affected by the genetic composition of the grain and by the environmental conditions of production, and negatively intensified when the grains were exposed to inadequate storage conditions or to a long storage period.

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COOKING TIME OF COMMON BEAN: GENETIC PARAMETERS AND SELECTION OF ELITE LINES

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In common bean breeding programs, the grain cooking time (CT) is an important property, which can restrict the advance of lines in subsequent selection stages. The shorter the CT, the better, in view of the consumer demand for fast food preparation. Furthermore, the longer the CT, the greater is the loss of grain nutrients. Consequently, the development of lines with a shorter cooking time represents a breakthrough in terms of energy savings and food quality improvement. The objectives of this study were to investigate the genetic variability and select elite lines for CT, since these lines aggregate essential agronomic traits.

In preliminary tests, 140 elite lines, grouped according to their grain type were evaluated (68 with carioca grain, 30 with black, 16 with mulatinho (cream), 14 early cycle carioca and 12 with purple grain. The experiments, separated by grain type, were conducted in Santo Antônio de Goiás, in the winter growing season of 2011, in a randomized complete block design with two replications. The grain samples were used to determine the CT by the Matson method, modified by Proctor & Watts (1987). The following genetic and phenotypic parameters were estimated: genetic variance, phenotypic variance, environmental variance, heritability, and expected gain with selection. Based on the results, 17 lines with shorter CT were selected for a validation test in three environments: (Ponta Grossa/PR/dry season/2013; Santo Antônio de Goiás/GO/winter/2013, and Brasília/DF/rainy season/2013) in a randomized complete block design with two replications, to study the genotype-environment interaction and selection of the best lines. Individual and combined analysis of variance were performed and the group means tested (Scott-Knott, at 10%).

Significant differences were detected in the tests between the lines with carioca, black and mulatinho grain. The heritability estimates were 44.71% (Carioca), 65.79% (black) and 81.01% (mulatinho), all nonzero, with moderate to high magnitude, indicating the existence of variability between lines and the possibility of successful selection for CT. The gain expected with selection at a selection intensity of 20% was 8.03% (carioca), 14.54% (black) and 13.11% (mulatinho) (Table 1), confirming the genetic potential for selection of lines with shorter CT.

In the validation phase, significant differences between lines were observed in the three environments. The overall mean of the environments varied widely, with32.6 min (Ponta Grossa/PR/dry/2013), 44.8 min (Santo Antônio de Goiás/GO/winter/2013) and 41.1 min (Brasília/DF/rainy/2013), reflecting the environmental effect on CT. The analysis showed significant differences ($p \leq 0.05$) for lines, environments and line by environment interaction, which is the presence of genetic variability between lines, heterogeneity between environments, as well as the differential response of lines to different environments, as reported elsewhere (Perina et al., 2014).

The grouping test defined three groups of which nine elite lines were selected (CNFP 11976, with black grain; CNFC 15710, 15723, 15861 and 15732, carioca; CNFM 15647 and 15656 mulatinho, and CNFRx 15614 and 15602 with purple grain) with similar cooking time to that of the best controls (BRS Estilo and Jalo Precoce) and shorter CT than Pérola. No line had a shorter CT than the best control (Table 2). The mean CT of the selected lines was 17% lower than that of cultivar Pérola, highlighting the good potential to reduce grain CT.

Table 1. Estimates of genetic variance (σ^2_g), phenotypic variance (σ^2_f), environmental variance (σ^2_e), mean heritability in the narrow sense (h^2), associated error ($S(h^2)$), genetic variation coefficient (CV_g), index b, expected selection gain (SG%) and differential selection (DS) for cooking time.

	σ^2_g	σ^2_f	σ^2_e	h^2	$S(h^2)$	SG(%)
Carioca	10.10	22.59	12.49	44.71	13.31	8.03
Black	37.06	56.32	19.27	65.79	12.29	14.54
Mulatinho	14.37	17.74	3.37	81.01	9.21	13.11

Table 2. Mean cooking time (in minutes) of 17 common bean lines evaluated in three environments.

Lines	Means	Lines	Means
CNFP 11976	35.08 a	BRS Estilo	38.62a
CNFC 15710	35.39 a	CNFRx 15602	39.38a
CNFC 15723	35.45 a	CNFP 15685	41.72b
CNFM 15647	36.18 a	CNFM 15640	42.14b
CNFC 15861	36.19 a	Pérola	42.31b
CNFM 15656	36.40 a	CNFP 15684	43.11b
Jalo Precoce	37.12 a	CNFC 15628	44.84b
CNFC 15732	37.53 a	IAC Centauro	52.11c
CNFRx 15614	38.16 a	Mean	39.51

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THE COMMON BEAN (*Phaseolus vulgaris* L.) DEMONSTRATION UNITY SYSTEM - SUDF - APPLICABILITY POTENTIAL FOR FAMILY FARMING

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INTRODUCTION

Rio Grande do Sul (RS) State, in southern Brazil, is characterized by the important role that family farming occupy in agriculture, being directly responsible for about 70% of common bean production. In the early 90s, the Embrapa Temperate Climate found that many farmers were not taking advantage of the productive potential presented by the new released bean cultivars, about 40% higher in productivity to those under cropping and resistant to major diseases. From this recognition, the Embrapa Temperate Climate common bean breeding team, with the contribution of Emater / RS, the official extension institution, put in place the Common Bean Demonstration Units System - SUDF - having as assumptions, giving producers the knowledge on new cultivars released by research institutions, the possibility of comparison with farmer's cultivars, the possibility of selection of those best suited to farmer's environmental conditions and the subsequent use of their seeds. Simultaneously, the SUDF made possible to avoid the expression of genotype x environment interaction for the testing at farm's level. This paper describes the behavior of SUDF's common bean cultivars in Soledade region, one of the regions with the highest bean production in Rio Grande do Sul, compared to the cultivar used by farmers.

MATERIAL AND METHODS

Demonstration Unities (UD) were composed of seventeen cultivars already recommended by research Institutions located in southern Brazil, as well as by the cultivar in use by the farmer, as check. The plots consisted of four 4m rows, with 0.50m between rows, with a density of 12 seeds per meter. In the region of Soledade, headquarters of one of the twelve Emater / RS administrative regions, composed of 39 municipal offices, were installed 25 UDs in the period 1996/97 - 2014/15, being each UD considered as one block. UD's, for the most part, were installed in properties of farmers selected by Emater / RS employees. At harvest, the two central row plants were threshed, weigh and the seeds sent to Embrapa accompanied by the field notebook, specifying region and county, sowing date, farmer's name, Emater / RS technician's name, harvest date, maintenance and cover fertilization, lime soil improvement, phytosanitary treatment, cultivar's grain yield, assessment of the occurrence of diseases, general grade and Emater / RS and producer's individual technical assessment on the performance of each cultivar, and the diffusion of technology conducted through meeting, field day, visit, meet and / or tour. Statistical analysis involved the analysis of variance for the variable grain yield and the Dunnett's test mean comparison having the farmer's cultivar as term of comparison.

RESULTS AND DISCUSSION: As shown in Table 1 among the seventeen SUDF cultivars tested, only FT Nobre (with productivity 20.2% higher) and Iapar 31 (with productivity 19.8% higher) differ from the cultivar in use by the farmer. These data reveal a wide adaptation of these cultivars to the environments encountered in the target region, since they result from observation in many years,

at varying climatic conditions and farming management conditions conducted by the different farmers. These aspects do not guarantee that these cultivars are to be adopted by farmers because they take into account other aspects, such as cooking characteristics, regional preference for color, and relevant cultural aspects of each family. However, results achieved by Chollet (2005), reveal that 90% of the SUDF farmers have adopted at least one of SUDF cultivars, suggesting that both FT Nobre and Iapar 31 can be adopted by them. At the same time, characteristics other than productivity, presented by SUDF cultivars, such as seed coat color, can induce the farmer to adopt them. The results obtained suggest that SUDF constitutes an effective tool in disseminating knowledge about new bean cultivars, as well as source of information to the common bean breeder, and especially to the extension agent, on the adaptation of cultivars released from research.

Table 1. Mean yield ($kg.ha^{-1}$) of SUDF cultivars in comparison to farmer's cultivar. Soledade region, RS, Brazil.

Cultivar	Grain yield ($kg.ha^{-1}$)
FT Nobre	2352,20*
Iapar 31	2345,01*
Macotaço	2258,21
Carioca	2250,22
Guapo Brilhante	2208,77
Minuano	2196,39
BRS Expedito	2152,42
Soberano	2134,65
Macanudo	2067,89
Guateian 6662	2044,67
Iapar 44	2005,91
Iraí	1992,82
Farmer's cultivar (check)	**1957,43**
FT 120	1900,90
Valente	1892,23
Pérola	1840,56
Rio Tibagi	1817,23
Diamante Negro	1790,20

*: Cultivar differs from the check by Dunnett's test at $\alpha = 0.10$ level

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FLOWERING, DISTRIBUTION OF DRY MATTER AND POD YIELD IN CLIMBING BEAN WITH PRUNING

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INTRODUCTION

The pruning is a farming practice that is applied usually in fruit trees. In beans this handling is not common. Some reports under field conditions and greenhouse indicate that the elimination of the apical bud increased the number of pods and the yield in climbing bean (Escalante *et al*., 2015). On the other hand, the grain yield per pod, is one of the components that has received little attention and which could be the subject of study to increase yield. Reports on the effect of the pruning in the phenology, accumulation and distribution of dry matter in the structures of the plant and the pod yield in climbing bean are limited and this was the objective of the present study.

MATERIALS AND METHOD

The experiment was stablished under glasshouse in Montecillo Méx., Mexico (19°29' N, 98°53'W, 2250 m of altitude) of temperate climate. The climbing bean (*Phaseolus vulgaris* L.) HAV-14 of indeterminate habit was shown in five-liter pots, in June 20. In each plant, the apical bud was removed when stem had 2, 4 and 6 nudes (treatments). The test (reference) was without removal. The beginning of flowering (FB) and at physiological maturity (PM) were evaluated by plant, just like the dry matter (DM) accumulation (DMA) in stem, valves, grain and total, DM distribution (DMD=(dry matter in stem, valves or grain/total DM)*100) and the pod yield (grain DM by pod (g), PY) following the criteria indicated in Escalante and Kohashi (2015). The experimental design was completely randomized with four replicates. An analysis of variance and Tukey test were applied. It is also recorded the maximum (Tmax) and minimum (Tmin) temperature during crop development.

RESULTS AND DISCUSSION

During the development of the experiment the Tmax ranged between 30 to 45ºC and the Tmin between 13 to 16 ºC. In the test the FB was to 64 days after sowing (das), the pruning to 2, 4 and 6 nudes causes the delay the FB in 20, 15 and 8 days, respectively. This indicates that earlier you apply the pruning, the cycle of vegetative parts will be of greater length and the flowering will be later. PM was to 102 das. The pruning caused greater DMA in the structures of the plant and in the total. The highest DMA found with the pruning to 2 nudes; followed by the 4 and 6 nudes; the test presented the lowest values. Similar trend was observed for the PY (Figure 1), where the pruning led to greater DMA in the grain in each pod. On the other hand, with the exception of the stem where the DMB was greater in the test, with the pruning this was highest in valves and grain (Table 1). This indicates that the pruning is necessary to achieve a greater DMA in the pods and consequently greater grain yield.

Table 1. Accumulation and distribution of dry matter in stem, valves and grain of climbing bean (*Phaseolus vulgaris* L.) in function of the pruning. Montecillo, Méx., México.

Pruning to node	Stem	Valves	Grain	Total
2	18 a (32)	9 a (16)	29 a (52)	56 a
4	13 b (28)	8 ab (17)	25 b (55)	46 b
6	15 ab (34)	7 b (16)	22 b (50)	44 b
test	16 ab (40)	6 b (15)	18 c (45)	40 b
Cv %	16	12	9	7
Mean	15 (33)	7 (15)	24 (52)	46
Tukey 0.05	4.7	1.7	4	6
F Prob.	*	**	***	*

***, **,* P < 0.001, 0.01, 0.05, respectively. In the Columns values with similar letter are statistically equal (Tukey 0.05). The value within the parentheses indicates the distribution in percent.

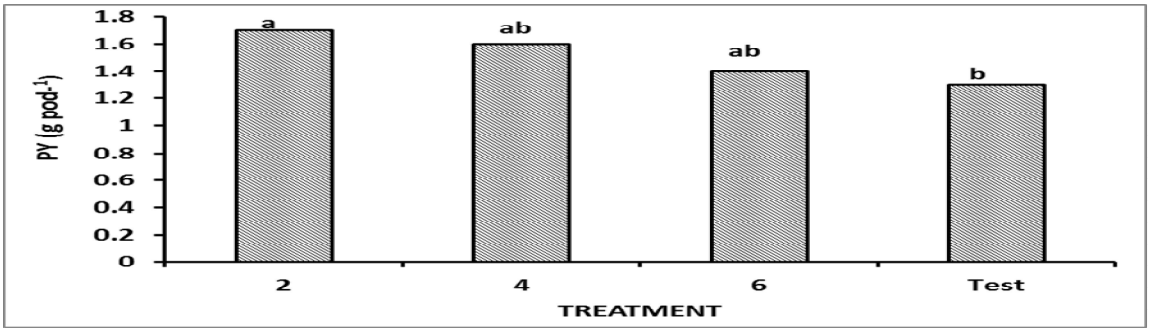

Figure 1. Grain yield per pod (g, PY) in climbing bean in function of the apical pruning. Montecillo, Mexico.Mexico. Summer. Treatment of pruning to 2, 4 and 6 nudes. Test is without pruning. In columns different letter indicates differences according to the Tukey 0.05.

CONCLUSIONS

In climbing bean HAV14 the pruning causes the delay of the flowering, increase in the accumulation and distribution of dry matter, particularly in valves and grain and increases too the yield pod.

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FERTILIZATION OF COMMON BEAN CV. BRSMG MADREPÉROLA WITH NPK ORGANOMINERAL FERTILIZER CONTAINING SOIL CONDITIONER

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INTRODUCTION – The nutritional imbalance of plants hurts agricultural production and may result in low yield. In the case of common bean, a plant with a limited root system and short cycle, adequate fertilization is fundamental.

Organomineral fertilization arises as an alternative for this nutrition. In it, the low concentrations of N, P, and K of the organic fertilizers are complemented by mineral fertilization such that plants may take better advantage of the nutrients through synchronized release throughout their growth. Another strategy available, which is being used in a growing way in Brazil, is the use of bioactivators or soil conditioners, whose function is to lead to improvement in physical or physical-chemical properties or soil biological activity, which may recover degraded soils or soils with nutritional imbalance.

This study aimed to evaluate growth and yield of common bean cv. BRSMG Madrepérola subjected to different application rates of an organomineral NPK formulation containing soil conditioner and compare them with the mineral formulation commonly used for the crop.

MATERIALS AND METHODS – An experiment was carried out in a greenhouse of the Department of Agriculture of the Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil. A completely randomized experimental design was used, with four replications and a (6x2) + 1 factorial arrangement, involving six application rates (0, 100, 200, 400, 600, and 800 kg ha^{-1}) of organomineral fertilizer (OMF) in the presence or absence of a soil bioactivator (SB). An additional treatment was used corresponding to 400 kg ha^{-1} of the commercial mineral formulation 4-14-8.

Each plot was composed of a 5 L pot filled with a *Latossolo Vermelho distrófico* (Oxisol). Two seeds were sown of the cv. BRSMG Madrepérola of type III indeterminate growth habit and low tolerance to lodging. One plant from each pot was harvested in the R6 stage, evaluating the shoot fresh weight (SFW) and shoot dry weight (SDW) of the plant, as well as height (HGT). The second plant was evaluated in the R9 stage, determining number of pods per plant (PP), grains per pod (GP), hundred grain weight (W100), and yield (YLD).

The organomineral fertilizer was composed of 15.15% chicken manure, 7.5% urea, 2% cottonseed meal, 40.6% natural phosphate, 33.4% double sulfate of potassium and magnesium; 1% zinc sulfate, and 0.35% borax. Topdressing fertilization was 20 kg ha^{-1} of N from urea. The soil bioactivator, when present, was used at the proportion of 0.5% in relation to fertilizer weight and contained basically monocarboxylic and tricarboxylic acids.

After transformation of W100 and YLD in $(x+1)^{0.5}$ to meet the requirements of normality and homoscedasticity, all the data were subjected to analysis of variance. In cases of occurrence of significance of the application rate factor, regression analysis was used. Comparisons between the bioactivator levels, as well as the Factorial vs. Additional contrast, were carried out through the F test.

RESULTS AND DISCUSSION – The application of SB at sowing did not affect crop growth. Similar results were found in evaluations of yield and its components. The lack of synchrony between the periods of greatest action of the product and of the greatest development of the common bean plant, and even the low experimental precision with which these characteristics were evaluated, may have led to this result. According to the manufacturer, SB has a period of greatest activity estimated at ten days. As this time coincides only with the period necessary for germination and emergence of common bean, in these stages, the root system of the plants was still not very developed and, therefore, not very responsive to the action of the SB. Split applications at other steps of the crop cycle could favor utilization of the product and more greatly benefit the legume. Another possibility of intensifying the effect of SB would be increasing the proportion of the bioconditioner source, which was only 0.5% in weight.

The SFW and the SDW had a linear increase with the increase in the application rate of OMF. In spite of this effect, however, it was seen that with the application rate of 400 kg ha^{-1}, common bean growth was less than that obtained with the control. In relation to HGT, there was an increase as a result of the increase of the application rate of OMF up to 565 kg ha^{-1} (Table 1). As of this application rate, however, the HGT steadily reduced. Certainly, as of this application rate, the increase in nutrients came to have a greater effect on lateral branching of common bean, which would be favored by the type III growth habit of the cultivar used.

The PP also had a linear increase with the increase in fertilization, showing that the greater availability of nutrients may have reduced abortion of flowers and/or pods. In the present study, application rates greater than 535 kg ha^{-1} of OMF led to greater increases in PP than 400 kg ha^{-1} of the commercial formulation (Table 1). An increase in the number of grains formed and in hundred grain weight was also observed from the increase in the application rates of OMF up to 493 and 429 kg ha^{-1}, respectively. As of those levels, the increase reduced the GP and the W100, but not the YLD, which increased in a linear manner, accompanying the increase seen in PP (Table 1). Thus, it may be seen that even with the reduction in the number of grains per pod, there was an increase in production per plant because the number of pods continued to increase.

The results also indicate that the mineral fertilizer used as a control led to greater initial growth of common bean, which is certainly related to the greater initial solubility and availability of the mineral sources of the product. Upon comparing YLD, however, the performance of the organomineral fertilizer was better. In addition to this effect, the OMF reduces environmental impacts, increases soil fertility, and has a lower cost than the mineral fertilizers (Table 2), providing a series of advantages to the producer.

Table 1. Regression equations for estimate of the characteristics evaluated as a function of application rates of the organomineral formulation and mean values obtained with the commercial fertilizer NPK 4-14-8.

Characteristic[1]	Equation	Coefficient of Determination	CV%	Mean values of the additional*
SFW	$Y = 4.221580 + 0.039619\ x$	$R^2 = 96.06$	19.8	18.5 g
SDW	$Y = 0.692177 + 0.006056\ x$	$R^2 = 96.70$	35.0	2.9 g
HGT	$Y = 0.169589 + 0.002261\ x - 0.000002\ x^2$	$R^2 = 95.03$	32.2	0.63 m
PP	$Y = 2.513596 + 0.017342\ x$	$R^2 = 93.40$	3.30	8.6 units
GP	$Y = 3.642441 + 0.009855\ x - 0.000010\ x^2$	$R^2 = 74.12$	21.8	5.0 units
W100	$Y = 17.636372 + 0.123621\ x - 0.000144\ x^2$	$R^2 = 30.34$	41.9	31.9 g
YLD	$Y = 5.485149 + 0.028418\ x$	$R^2 = 73.55$	34.9	15.4 g

[1]SFW and SDW – shoot fresh weight and shoot dry weight, respectively; HGT – plant height; PP and GP – number of pods per plant and grains per pod, respectively; W100 – mean hundred grain weight; YLD – common bean yield.

Table 2. Cost of organomineral fertilizer (and its components) compared to the commercial formulation NPK 4-14-8*.

Components	**Unit value**	**Percentage in the fertilizer**	**Cost per ton**
	R$	**(%)**	**R$**
Chicken manure	120.00 (ton)	15.15	18.18
Cottonseed meal	950.00 (ton)	7.5	71.25
Urea	750.00 (ton)	2	15.00
Natural phosphate	450.00 (ton)	40.6	182.70
Double sulfate of K and Mg	1100.00 (ton)	33.4	367.40
Zn sulfate	1.70 (kg)	1	17.00
Borax	2.33 (kg)	0.35	8.15
Total			679.68
Commercial fertilizer 4-14-8			1200.00

*Values found on the market on 24/01/2014.

CONCLUSIONS – The increase in the application rates of organomineral fertilizer increases common bean growth, with a linear increase in weight and a quadratic effect on plant height. These effects, however, are less than those provided by the mineral fertilizer used as a control. In regard to plant production, the effect of the organomineral fertilizer surpasses the effect of the mineral fertilizer control, as a result of its greater effect on the yield components.

At the proportion of 0.5% in the formulation of the organomineral fertilizer, there is no effect from the soil bioactivator.

GRAIN YIELD COMPONENTS OF THE CULTIVAR *BRSMG MADREPÉROLA* AS A FUNCTION OF NITROGEN AND MOLYBDENUM FERTILIZATION AND INOCULATION WITH *RHIZOBIUM* SPP.*

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INTRODUCTION – Common bean is demanding in nutrients, and nitrogen (N) is the nutrient most taken up. Due to losses, environmental pollution, and the high cost of nitrogen fertilizers, biological N fixation (BNF) becomes a possible solution. Molybdenum (Mo), present in nitrogenase (from BNF) and nitrate reductase, is decisive in nitrogen metabolism. The aim of this study was to verify the effect of leaf application of Mo, combined with seed inoculation with two strains of rhizobium and nitrogen fertilization at planting.

MATERIALS AND METHODS – Two field experiments were conducted in the 2011/2012 spring-summer crop season in soils of the municipalities of Patos de Minas and Pitangui (Table 1), in Minas Gerais (state), Brazil. The experiments were set up in a conventional tillage system in areas without registry of previous inoculation for the common bean crop. The statistical design used was randomized blocks with three replications and a (3 x 2 x 2) + 1 factorial arrangement, involving types of inoculation (the strains CIAT 899 of *Rhizobium tropici*, UFLA 02–100 of *R. etli*, and absence of inoculation), leaf application rates of Mo (0 and 80 g ha^{-1}), and application rates of N at planting (0 and 20 k g ha^{-1}), plus an additional treatment (80 kg ha^{-1} of N, applying half at planting and half in topdressing). The cultivar was BRSMG Madrepérola, of type III indeterminate growth habit, and low tolerance to lodging. The sources of N and of Mo were urea and sodium molybdate, respectively. Evaluations were made of number of pods per plant (PP), grains per pod (GP), and the mean one hundred grain weight (W100).

All the data were subjected to analysis of variance and, in cases of significant effect of the inoculation treatments, comparison of means was carried out by the Scott-Knott test at the level of 5% probability. Comparisons between the levels of locations, Mo and N were carried out through use of the F test. Analyses of variance and application of the tests were carried out using the Sisvar® statistical analysis software.

Table 1. Results of chemical analysis of samples of soil material, 0-20 cm depth layer, in Patos de Minas and Pitangui, MG, Brazil before sowing.

Locations	Characteristics											
	pH (H_2O)	P avail. mg dm^{-3}	K	Ca	Mg	Al	SB	t	T	m	V	MO dag kg^{-1}
			---------- $cmol_c$ dm^{-3} ----------							-------%-------		
[1]Patos de Minas	5.0	73.79	51.48	0.9	0.3	0.3	1.33	1.36	8.37	18.4	15.91	3.14
[2]Pitangui	5.8	9.4	210.0	5.3	1.5	0.1	7.3	7.4	10.9	1.3	67.1	2.2

[1]*Latossolo Vermelho eutrófico* (Oxisol); [2]*Latossolo Vermelho distrófico* (Oxisol)

RESULTS AND DISCUSSION – There was significance of the effects of Locations (L) on all the variables. The effects of inoculation (I), mineral nitrogen (N), and molybdenum (Mo) and of the contrasts Factorial *vs*. Additional and L *vs*. Factorial *vs*. Additional were not significant. The interactions N x Mo and I x N x Mo (in relation to W100) and L x N (regarding GP) were significant.

A greater mean value of PP was obtained in Pitangui (Table 2), where the low stand of plants (93 thousand plants ha^{-1}) may have had an influence on this result since the lower number of plants leads to better use of the light, water, and nutrient resources by the surviving plants, with production of a greater number of pods on each plant. In contrast with PP, greater mean values of GP, regardless of the nitrogen application rate at sowing, and W100 were found in Patos de Minas, which is related to the lower PP of common bean in this location, where the physiological balance between source and sink may have determined the production of a greater number of grains and larger grains in each pod.

The mean values of PP, GP, and W100 brought about by inoculation were very near those obtained without inoculation, which is an indication that the native bacteria had a level of effectiveness similar to that of the inoculated strains. Molybdenum fertilization likewise did not affect the primary components of yield (Table 2).

In relation to W100, it may be seen that the rate of 20 kg ha^{-1} of N reduced the mean grain weight only in the absence of Mo and of inoculation (Table 3). In Table 4, where the breakdown of the interaction is made in another manner, it may be seen that the inoculated strains had the same effect as the native strains on W100, except in the absence of Mo and presence of 20 kg ha^{-1} of N. In this situation, the UFLA 02-100 strain proved to be superior to the other inoculation treatments.

In any case, the results found in the literature on the effect of leaf Mo on one hundred grain weight are not in agreement. Biscaro et al. (2011) found that W100 was negatively influenced by molybdenum fertilization, regardless of the N application rate. In contrast, Calonego et al. (2010) concluded that this characteristic responded positively to application of Mo together with a lower N application rate in topdressing.

Table 2 Mean values of the number of pods $plant^{-1}$ (PP) and of grains pod^{-1} (GP) and one hundred grain weight (W100) of the BRSMG Madrepérola cultivar as a function of locations, inoculation, and N and Mo application rates.

Treatments	PP	GP	W100
	----(unit)----		(g)
Location			
Patos de Minas	9.0 B	5.5 A	23.26 A
Pitangui	22.0 A	4.5 B	19.00 B
Inoculation			
Absent	15.0	4.9	21.00
CIAT 899	16.0	4.9	20.89
UFLA 02-100	16.0	4.9	21.50
Nitrogen (kg ha^{-1})			
0	16.0	4.9	20.94
20	16.0	5.0	21.31
Molybdenum (g ha^{-1})			
0	15.0	5.0	21.13
80	17.0	4.9	21.13
Factorial[1]	16.0	4.9	21.13
Additional[2]	19.0	5.0	20.88
Overall Mean	16.0	4.9	21.11

Mean values followed by the same uppercase letters in the column do not differ among themselves by the F test at 5% probability. [1]Mean value of the factors of Location, Inoculation, Nitrogen, and Molybdenum. [2]Mean value of the treatment fertilized with 80 kg ha^{-1}, applied half at planting and half in topdressing.

Table 32 Mean one hundred grain weight (g) of common bean as a function of inoculation and N and Mo application rates.

Inoculation	**Nitrogen (kg ha^{-1})**	**Molybdenum (g ha^{-1})**	
		0	80
Absent	0	22.58 Aa	20.23 Ab
	20	19.35 Bb	21.85 Aa
CIAT 899	0	20.51 Aa	20.40 Aa
	20	20.76 Aa	21.87 Aa
UFLA 02-100	0	21.06 Aa	20.89 Aa
	20	22.51 Aa	21.56 Aa
Mean		21.13	21.13

Mean values followed by the same lowercase letter in the line and uppercase letter in the column do not differ among themselves by the F test at 5% probability.

Table 43 Mean one hundred grain weight (g) of common bean as a function of inoculation for each combination of N and Mo application rates.

Molybde-num (g ha^{-1})	**Nitrogen (kg ha^{-1})**	**Inoculation**		
		Absent	CIAT 899	UFLA 02-100
0	0	22.58 a	20.51 a	21.06 a
	20	19.35 b	20.76 b	22.51 a
80	0	20.23 a	20.40 a	20.89 a
	20	21.85 a	21.87 a	21.56 a
Mean		21.00	20.89	21.51

Mean values followed by the same lowercase letter in the line do not differ among themselves by the Scott-Knott test at 5% probability.

CONCLUSIONS – There is no effect of molybdenum on the primary components of common bean grain yield. The native populations at the two locations provide primary yield components equal to those of the inoculated strains, except for the treatment without molybdenum and fertilized with 20 kg ha^{-1} of N-urea, where the UFLA 02-100 strain stood out in one hundred grain weight.

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INFLUENCE OF NITROGEN FERTILITY LEVEL ON GROWTH, GRAIN YIELD, AND YIELD COMPONENTS OF DIFFERENT DRY BEAN CULTIVARS

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INTRODUCTION

The amount of N_2 fixed by dry bean (*Phaseolus vulgaris* L.) is low compared with other legume crops (Hardarson, 1993). Thus, fertilizer N is often applied to dry bean crops throughout the world. Although N applications are currently profitable, identification of new genotypes that use N more efficiently (i.e., that require less N fertilization) have the potential added benefit of reducing environment effects. The objective of this study was to evaluate physiological traits of four greenhouse-grown dry bean cultivars when subjected to three levels of soil nitrogen.

MATERIAL AND METHODS

Seed of (CO-46348, Long's Peak, Rio Rojo, and UI-537) were sown in 11.3 L pots (8 kg of soil) in the greenhouse (nine pots per cultivar) on 2 August 2015 in Laramie WY (2200 m elevation). Seed were inoculated with a commercial inoculant that listed *Rhizobium leguminosarum* biovar. *phaseoli* (presumably now *Rhizobium etli*). The soil mix was 33% sand, 33% soil amendment (i.e., potting soil), and 33% native soil. Seedings were thinned to three per pot and a completely randomized design was used with three replicates. Aqueous fertilizer treatments (NH_4NO_3) were applied on alternate weeks (seasonal equivalents were 0, 42, and 84 kg N ha^{-1}). Leaf chlorophyll was determined (34, 40, 50, 62 dap) and seed yield/yield components were determined at maturity.

RESULTS AND DISCUSSION

They were significant differences between cultivars and N levels for all variables but very few significant interactions between cultivars and N levels. The seed weight (g), pod number, seed number, and pod harvest index were different among cultivars (Table 1) and among N levels (Table 2). Seed yield appeared to be optimized at the 42 kg N ha^{-1} rate. N application at 42 kg N ha^{-1} increased seed size and pod harvest index and there was no additional benefit from the high N rate. Rio Rojo exhibited a differential response for leaf chlorophyll (Fig. 1). Leaf chlorophyll in Rio Rojo was conspicuously lower than the other three cultivars at 40 dap in the Medium and High N treatments and at 50 and 62 dap at Medium N but equaled the other three cultivars by 62 dap in the High N treatment. Since these chlorophyll results represent only a single greenhouse experiment, further investigations are underway to confirm or refute the observations here.

Table 1. Effect of dry bean cultivars on the yield and yield components of dry bean.

Cultivar	Seed weight per pot (g)	Pod number per pot	Seed number per pot	Seed size (mg)	Pod harvest index
UI-537	29.4 a	24.4 a	89.5 a	320 c	0.79 a
Longs Peak	26.1 ab	17.1 b	67.8 b	380 a	0.74 b
CO-46348	23.9 bc	15.7 b	66.2 b	350 b	0.78 a
Rio Rojo	19.5 c	17.4 b	72.4 b	270 d	0.75 b
LSD (0.05)	4.8	3.2	14.3	10	0.01

Table 2. Influence of nitrogen levels on the yield and yield components of dry bean.

Treatment kg N ha^{-1}	Seed weight per pot	Pod number per pot	Seed number per pot	Seed Size mg	Pod harvest index
0	18.2 b	15.3 b	58.5 b	310 b	0.75 b
42	29.5 a	20.8 a	84.9 a	340 a	0.77 a
84	26.5 a	19.9 a	78.5 a	340 a	0.77 a
LSD (0.05)	4.1	2.8	12.3	10	0.01

Maturity (67% of pods brown) of Rio Rojo was 2, 7, and 9 days later than Long's Peak, UI-537, and CO-46348, respectively, but it is unclear if this might be related to the lower chlorophyll in Rio Rojo.

For morphological traits, plant height was greater in the 42 and 84 kg N ha^{-1} treatment (66 and 59 cm, respectively) than the zero N treatment. Stalk weight paralleled height (6.3 g and 5.5 g per pot for 42 and 84 kg N ha^{-1}, respectively) vs. 3.4 g for zero N. Root weight was greater for the 42 and 84 kg N ha^{-1} treatment (2.6 and 2.4 g per pot, respectively) vs. 2.1 g per pot for zero N. Nodule number and nodule weight did not differ among treatments averaging 33 nodules per pot and 360 mg per pot. Stalk weight was greatest in Rio Rojo, Long's Peak, and UI-537 (6.1, 5.9, and 5.0 g per pot, respectively) than in CO-46348 (3.4 g per pot). Long's Peak had higher mature root weight (3.5 g per pot) than Rio Rojo, UI-537, and CO-46348 (2.3, 2.3, and 1.4 g per pot, respectively).

Although this is just one greenhouse experiment, it appears that UI-537 may have a high nitrogen use efficiency (yield/N) for this soil type and at this altitude.

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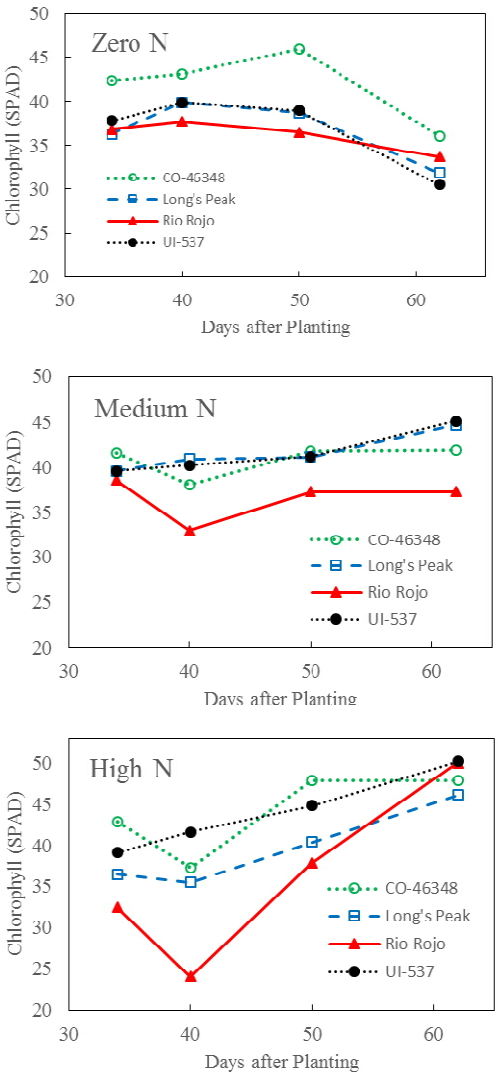

Figure 1. Leaf chlorophyll as a function of time for four dry bean cultivars.

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COMMON BEAN AND ORGANIC FERTILIZER USED AS COMPONENTS OF SUSTAINABLE AGROFORESTRY SYSTEMS IN DURANGO, MÉXICO

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INTRODUCTION: Forest production has been considered one of the most important economic activities in the State of Durango, México, where forest plantations have been recommended for intensive production of wood, pulp and firewood, as well as Christmas trees. Forest plantations cultivated with woody species reached 1,866 ha in 2013 (SEMARNAT, 2013). In 2015, several plots were planted at different municipalities such as: Durango, Canatlán and Nuevo Ideal, for intensive production of forest biomass using different pine species such as: *Pinus greggii*, *P. cembroides* and *P. engelmannii*. High yield potential has been also detected in the same production areas for agricultural crops such as common bean (*Phaseolus vulgaris*), which is considered as a productive option by landowners in order to obtain food and economic benefits during pine timber production. Organic and low cost fertilizer options are also needed in Durango due to the low input agriculture, which is traditionally considered a productive and profitable option used by farmers. The objective of this study was to evaluate the importance of common beans and organic fertilizer as components for sustainable agroforestry systems in Durango, México.

MATERIALS AND METHODS: In 2015, an agroforestry (alley cropping) system was implemented at INIFAP´s experiment station located in Durango, México. The agroforestry system included an 8 year-old *Pinus greggii* plantation and two common bean cultivars (Pinto Saltillo and Pinto Centauro), both showing different maturity. Common bean cultivars were sown when the rainy season started (June 24th) using strips between pine tree lines, planted 3 m apart (strips) and 1.5 m between plants. Pine trees showed average values of 3.3 m for plant height, 11.0 cm for stem basal diameter and 2.0 m for canopy diameter.

Common bean cultivars were planted in alternate strips using two rows, with variable length (20-130 m) and 0.81 m apart. A traditional intercropping system was established in an adjacent plot without pine plantings to be used as a control. An AccuPAR (Decagon LP-80, Decagon Devices, Inc.) ceptometer was used for light interception measurements at two cropping systems. Readings were taken at 10:00 h and 15:00 h on two sampling dates (57 and 64 days after planting; DAP) during the reproductive period in both common bean cultivars.

Fertilizer treatments included compost (Maxicompost®) at the rate of 4 MT/ha and chemical fertilizer at the dose of 25-35-00 (N-P_2O_5 and K_2O). Both treatments were applied by hand and then incorporated during the first (1/4 compost and chemical fertilizer) and second (3/4 compost) mechanical weeding. Weed control included two mechanical weeding complemented with two hand weeding. The experimental plot consisted in one strip with two rows and three replications per treatment. At maturity, three equidistant plant samples were taken from each crop strip for yield determination. Samples consisted of two rows with 5 m in length by 0.81 m in width (8.1 m^2). Analysis of variance was obtained under randomized complete block design with nine replications and mean comparisons were performed by Tukey´s test ($P \leq 0.05$).

RESULTS AND DISCUSSION: Supplemental irrigation was applied in order to avoid crop loss caused by intermittent drought registered during 2015 cropping season, which was associated with low rainfall accumulation (221 mm) compared to historical average (436 mm). Light interception (shade) registered an average value of 29 % and variations from 15 to 43 %. Significant ($P \leq 0.05$) differences were observed for maturity between intercropping systems and cultivars. Pinto Centauro show early maturity (96 DAP) and a delayed response in the agroforestry system (98 DAP) mainly caused by shade effects (Table 1). Significant reduction was observed for seed yield between intercropping systems. The highest seed yield was observed at traditional intercropping system in Pinto Centauro using compost (1,772 kg ha^{-1}) and chemical fertilizer (1,770 kg ha^{-1}). A greater yield reduction was also observed in Pinto Centauro under the agroforestry system (489 kg ha^{-1}) compared to Pinto Saltillo (800 kg ha^{-1}). Differences in results were detected compared to those during 2014 at the same site, where common beans registered similar seed yield under traditional intercropping (1,445 kg ha^{-1}) and agroforestry (1,392 kg ha^{-1}) systems (Rosales *et al*., 2015). Differences were related to increments in light interception caused by tree growth and the lack of tree pruning in 2015.

The highest values for 100 seeds weight (36 to 38 g) were observed in Pinto Centauro across intercropping systems and fertilizer treatments. Higher seed yield and low reduction in seed weight (size) are desired traits in common bean cultivars used in Durango. Common bean represents an important option in agroforestry systems. Also, farmers would have food production during the tree growth and an additional income generated from the seed commercialization. Results observed in the compost treatment need to be confirmed in order to encourage its utilization in reduced organic matter soils that are actually used for common bean production in Durango under traditional monoculture and agroforestry systems.

Table 1. Traits evaluated in two common bean cultivars planted under different fertilizer treatments in the agroforestry and traditional intercropping systems.

Fertilizer Treatment	Pinto Saltillo			Pinto Centauro		
	Days to Maturity	Yield kg ha^{-1}	100 seeds Wt (g)	Days to Maturity	Yield kg ha^{-1}	100 seeds Wt (g)
	Agroforestry System					
Chemical (CF)	105^{a}	755	33^{b}	98^{b}	558	36^{a}
Compost (CP)	105^{a}	844	34^{b}	97^{b}	419	36^{a}
Average	**105^{A}**	**800^{B}**	**34**	**98^{A}**	**489^{B}**	**36**
	Traditional Intercropping System					
Chemical (CF)	104^{a}	1,464	33^{b}	96^{b}	1,770	37^{a}
Compost (CP)	104^{a}	1,171	33^{b}	96^{b}	1,772	38^{a}
Average	**104^{B}**	**$1{,}318^{A}$**	**33**	**96^{B}**	**$1{,}771^{A}$**	**38**

Letters indicate significant differences according to Tukey´s test ($P \leq 0.05$) between intercropping systems ($^{A-B}$) and cultivars^{a-b}.

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EFFECT OF DIFFERENT PHOSPHORUS LEVELS ON GROWTH AND YIELD COMPONENTS OF COMMON BEAN

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INTRODUTION

Common bean (*Phaseolus vulgaris*) is one of the most important legumes in developing countries because it is the main source of plant proteins and minerals. The large demand for these countries requires continuously increasing productivity and improving the grain quality. The edaphic stress is an important factor that affects the production of common bean, and the most area available for agriculture in tropical America have phosphorus (P) deficiency, delaying emergence, reducing plant growth (BINGHAM, 1966), number of leaves, plant height, stem diameter, leaf area (LEAL; PRADO, 2008) and thus loss in productivity. The existence of considerable genetic variability in *P. vulgaris* allows the selection and development of new cultivars that are more efficient in P use (BEEBE et al., 2006). Different P levels in nutrient solution is an effective way to identify the most efficient cultivars in the absorption and use of P, but those studies normally do not evaluate the yield components once they are discontinued in pre-flowering stage (NAMAYANJA et al. 2014). Therefore, the study aimed to evaluate the effect of different P levels on growth and yield components of the carioca type common bean cultivars.

MATERIALS AND METHODS

The experiment was carried out in the greenhouse at Agronomic Institute of Paraná (IAPAR), Londrina - Parana – Brazil, in September to December 2015. The experimental design was a randomized block with six replicates and treatments arranged in a factorial design with two cultivars (BRS Estilo and IPR Tangará) and five P levels (2, 4, 6, 8 e 10 mg L^{-1}). The seedlings with uniform shoot and roots were transplanted into polyethylene vases with capacity 3.35 L containing nutrient solution Hoagland and Arnon (1950), modified by Pavan and Bingham (1982). The redox potential (pH) and electrical conductivity (EC) of the nutritive solution were monitored daily; the pH was maintained at 5.5 ± 0.2 by addition of HCl or NaOH 0.1M and initial electrical conductivity (EC) of 0.35 ± 0.02 dS cm^{-2}. The solution was aerated continuously and it was exchanged when the electric conductivity reached 40% of the initial value. The assessments at physiological maturity stage were: plant height (PH), root length (RL), number of pods per plant (NP), total grain yield per plant (TP) and total dry mass (TDM). The harvest index (HI) was calculated using: total grain yield per plant (g) / total dry mass (g). The data were subjected to analysis of variance and mean grouping test Scott-Knott ($p \leq 0.05$).

RESULTS AND DISCUSSION

BRS Estilo and IPR Tangará cultivars showed plant height (PH) and root length (RL) similar in different concentrations of P, except for the IPR Tangará that had lower RL in the concentration of 10 mg L^{-1}. Phosphorus levels had no influence on the number of pods per plant (NP) for both cultivars (Table 1), however, Silva et al. (2014) showed that P doses of 0.05, 2.00 and 4.00 mg L^{-1} reduced the number of pods per plant by 90, 54 and 17% respectively. For total grain yield per plant (TP) P levels did not influence BRS Estilo, although, IPR Tangará showed a lower weight when subjected to 2 mg L^{-1} of P, demonstrating that low concentrations of P may decrease the production

of grain. Regarding the total dry matter (TDM) the concentration of 2 mg L^{-1} provided a lower production compared with other concentrations in both cultivars (Table 1). For the harvest index (HI), the BRS Estilo was more efficient than the IPR Tangará to allocate the accumulated dry matter to grain production in the lower concentration of P, however, in others concentrations this genotypes showed no difference. The present study demonstrates that the use of nutrient solution containing 2 and 10 mg L^{-1} of P provided the production of grain in sufficient quantities to perform technological and nutritional quality analysis and discrimination between cultivars.

Table 1. Growth and yield components of two common bean cultivars cultivated in nutrient solution with different phosphorus levels.

P*	PH		RL		NP		TP		TDM		HI	
	BRS Estilo	IPR Tangará	BRS Estilo	IPR Tangará	BRS Estilo	IPR Tangará	BRS Estilo	IPR Tangará	BRS Estilo	IPR Tangará	BRS Estilo	IPR Tangará
2	180.9aA	178.3aA	66.50aA	56.01aA	10.33aA	8.00aA	13.80aA	7.65aB	13.28aA	11.14aA	1.04aA	0.67aB
4	208.7aA	200.3aA	70.17aA	66.42aA	12.17aA	11.17aA	12.91aA	11.27bA	17.48bA	16.14bA	0.73bA	0.72aA
6	218.8aA	201.6aA	71.00aA	63.08aA	10.83aA	10.33aA	13.63aA	13.37bA	17.65bA	18.15bA	0.77bA	0.74aA
8	196.9aA	212.0aA	78.50aA	64.47aA	11.83aA	10.50aA	12.74aA	12.42bA	17.90bA	17.84bA	0.74bA	0.70aA
10	197.7aA	209.1aA	83.36aA	65.25aB	12.00aA	11.17aA	14.12aA	12.95bA	20.86bA	17.18bA	0.68bA	0.76aA
CV(%)	19.94	19.94	19.66	19.66	20.58	20.58	22.44	22.44	21.95	21.95	14.00	14.00

Plant height (PH), root length (RL), number of pods per plant (NP), total grain yield per plant (TP) and total dry matter (TDM), harvest index (HI).
Means followed by the same letter, lower case letter in the column and capital letter in the line, are not significantly different by Scott-Knott test at 5% probability.
*Phosphorus concentration in the nutrient solution (mg L^{-1}).

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EFFECTS OF SELENIUM APPLICATION ON YIELD AND SEED SIZE IN COMMON BEANS GROWN IN DURANGO, MÉXICO

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INTRODUCTION: Selenium has been considered as an important element in healthy and functional foods providing natural nutrition and health for humans. An extradietary supplementation of selenium (200 g per day) is recommended for an adult of average weight subsisting on the typical American diet (Schrauzer, 2001). Selenium importance is controversial due to its low range between the benefic and toxic level in foods used for human and animal consume. In México, selenium levels need to be determined in several crop cultivars grown at different soil types, in order to generate safe recommendations for grain consume and supplement requirements according to Mexican consumption habits. Common bean (*Phaseolus vulgaris* L.) is considered an important staple food showing variations in selenium content between 3.9 to 4.5 ppm (g g^{-1}) in cooked grains harvested in Durango, México (Jiménez *et al*., 2014). The objective was to evaluate effects of selenium foliar application on yield and grain quality in several common bean cultivars grown in Durango, México.

MATERIALS AND METHODS: In July 10th 2015, seven common bean cultivars were sown at INIFAP´s experiment station located in Durango, México (24º 01' N, 104º 44' W). The evaluated germplasm included pinto common bean cultivars such as: Saltillo, Centauro, Bravo, Coloso, Libertad and Centauro and a black seeded cultivar (Negro 80 25). Experiment was planted under a factorial design (seven cultivars and four treatments) with three replications. Experimental plots consisted in four rows 10 m in length and 0.81 cm apart. Selenium treatments included 0 (Control), 10, 20 and 30 g ha^{-1} of sodium selenite (Na_2SeO_3) per hectare, diluted in 18 L of water and applied, at the flowering stage, using a hand pump sprayer. Fertilizer was applied at the rate of 35-50-00 (N, P_2O_5 and K_2O) during the first mechanical weed control. Weeds control included mechanical weeding (2) complemented with two hand weeding. Irrigation was applied twice in order to avoid severe water stress caused by low accumulated rainfall (221.4 mm) combined with high average for maximum (27.6 ºC) and minimum (13.1 ºC) temperatures registered during crop growth cycle. During maturity plant samples, consisting of two rows with 8 m in length by 0.81 m in width (12.96 m^2), were taken for yield determinations. Analysis of variance was obtained under factorial (cultivar x treatment) design with three replications and mean comparisons were performed by Tukey´s test ($P \leq 0.05$).

RESULTS AND DISCUSSION: Significant differences ($P \leq 0.05$) were observed for seed yield among cultivars and selenium doses (Figure 1). Higher seed yield in the 0 g ha^{-1} selenium treatment (control) was registered in Pinto Libertad (2,528 kg ha^{-1}) and Pinto Saltillo (2,475 kg ha^{-1}), showing significant reductions in the highest selenium dose (1,890.9 kg ha^{-1} and 2,204.4 kg ha^{-1}, respectively). Other cultivars, such as Pinto Bravo, registered statistically similar response and higher values for seed yield across selenium doses (2,308.8 kg ha^{-1} to 2,685.4 kg ha^{-1}). Black seeded cultivar Negro 80 25 registered lower seed yield in the 0 g ha^{-1} dose (1,930.1 kg ha^{-1}) and significant increments in 10 g ha^{-1} (2,011.0 kg ha^{-1}) and 20 g ha^{-1} (2,178.0 kg ha^{-1}). Different yield responses

were detected across selenium doses in common bean cultivars grown in Durango. In spite of yield effects, similar response was observed for 100 seed weight among selenium doses and significant differences ($P \leq 0.05$) were detected only among common bean cultivars. Higher average for 100 seeds weight was observed in modern pinto cultivars such as (Table 1): Pinto Libertad (42.8 g) significant higher than Pinto Saltillo (34.3 g) and Negro 80 25 (21.2 g). Additional studies need to be done in order to corroborate effects of selenium on yield and seed quality among cultivars.

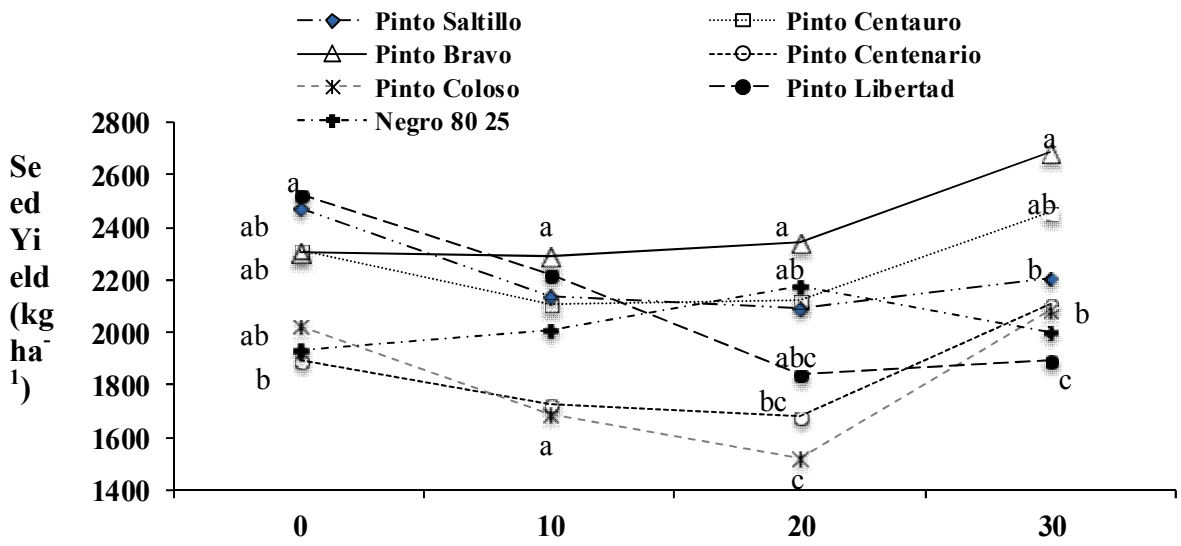

Figure 1. Effects of different selenium doses on seed yield observed in seven common bean cultivars grown in Durango, Méx. Letters indicate significant differences among selenium treatments according to Tukey´s test ($P \leq 0.05$).

Table 1. Average 100 seeds weight in common bean cultivars across sodium selenite doses.

Trait	Cultivars						
	Pinto Libertad	Pinto Bravo	Pinto Coloso	Pinto Centenario	Pinto Centauro	Pinto Saltillo	Negro 80 25
*100S (g)	42.8±1.2[a]	42.7±0.9[a]	42.0±1.3[a]	41.6±0.5[a]	35.8±0.7[b]	34.3±0.5[b]	21.2±0.5[c]

*100S = 100 seeds weight. [a-c]Letters in the row indicate significant differences according to the Tukey´s test ($P \leq 0.05$) among cultivars.

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COMMON BEAN PERFORMANCE EVALUATION (CULTIVAR IPR COLIBRI) IN DIFFERENT SULFUR LEVELS

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INTRODUCTION

Generally speaking, there are few studies on plant response to sulfur (S). Intense cultivation of soils with low organic matter and clay content, the use of concentrated fertilizers, and continuous export of S without replacement may lead to reduction in availability of this element to plants, leading to sulfur deficiency and consequent reduction in crop yield. Although it is possible for plants to absorb S through leaves, most S uptake occurs through the roots. The S found in cells is taken up from the soil solution as sulfate, transported to the root system, mainly through mass flow. Within this context, the aim of this study was to evaluate the effect of S application rates on the development and yield of common bean cv. IPR Colibri.

MATERIALS AND METHODS

This study was carried out in a greenhouse. The soil used was collected from the arable layer (0-20 cm depth), passed through a sieve with a 4 mm mesh, and homogenized, and a sample was removed for chemical analysis. The chemical characteristics of the soil were: pH ($CaCl_2$), 4.8; organic matter, 42.3 g dm^{-3}; P Mehlich, 1.1 mg dm^{-3}; H+Al, 38 mmolc dm^{-3}; K, 0.8 mmolc dm^{-3}; Ca, 8 mmolc dm^{-3}; Mg, 9 mmolc dm^{-3}; S-SO_4, 5.1 mg dm^{-3}, CEC, 55.8 mmolc dm^{-3}, and base saturation (V%), 32.

Soil liming was carried out to raise the V% to 60. The soil was incubated for 7 days, maintaining soil moisture at 60% of water retention capacity. Fertilization at planting was carried out after soil amendment, based on soil analysis.

The treatments consisted of 6 application rates of S (0, 5, 10, 15, 20, and 25 mg kg^{-1}) in the form of agricultural gypsum, with 4 replications per treatment. A completely randomized experimental design was used. Each experimental unit was composed of a pot containing 3 kg of soil. After 7 days of incubation, each pot received 5 common bean seeds of the cultivar IPR Colibri. The plants were thinned 15 days after sowing, leaving 2 plants per pot.

Fertilization at planting consisted of 4 mg kg^{-1} of N, 20 mg kg^{-1} of P_2O_5, and 7 mg kg^{-1} of K_2O. During the experiment, the plants were treated for pest and disease control through spraying of acaricides and fungicides when necessary. Two topdressing fertilizations were made with urea at the rate of 10 mg of N kg^{-1} at 30 and 45 days after sowing.

The common bean was harvested at the end of the cycle, at 71 days after sowing. The plants were then separated into shoots (leaves, branches, and hulls) and grains. The plant samples collected were dried in a laboratory oven (65°C), weighed, and ground for chemical analyses. The samples of the shoots and grains were analyzed in relation to total C, N, and S content through dry combustion in the equipment LECO-S 144DR.

The data were subjected to analysis of variance, and quadratic regression equations were fitted according to the degree of significance. Statistical analyses were carried out using the program Assistat 7.6 beta version.

RESULTS AND DISCUSSION

The dry matter (DM) production of the shoots (SH) of common bean increased as a result of S application. The greatest production of DM of the SH (7.61 g pot^{-1}) was obtained with the application of 16.27 mg kg^{-1}. Grain production increased in a quadratic manner as a result of application rates (Table 1). The greatest grain production (4.5 g pot^{-1}) was obtained with the application of 12.7 mg kg^{-1}.

The S content in the SH of common bean ranged from 1.5 to 3.1 g kg^{-1} as a result of the application rates, and the greatest content (3.06 g kg^{-1}) was obtained with the application of 19.5 mg kg^{-1} (Table 1). In the common bean grains, the average contents were 1.9 g kg^{-1}. The accumulation of S in the SH of common bean increased in a quadratic manner, and the greatest accumulation of S (21.7 mg pot^{-1}) was obtained with the application of 17.23 mg kg^{-1} (Table 1). The values obtained in the N/S and C/S ratio decreased as a result of the S application rates (Table 1).

Table 1. Regression between S rates and the variables obtained.

Regression (X;Y)	Equation	R^2
S rates x Weight matter	$y = -0.0017x^2 + 0.166x + 3.5586$	0.911*
S rates x Grain production	$y = -0.0006x^2 + 0.0457x + 3.629$	0.900*
S rates x S concentration in shoot	$y = -0.0005x^2 + 0.0585x + 1.3539$	0.914*
S rates x S accumulation in shoot	$y = -0.0066x^2 + 0.6834x + 4.0548$	0.982*
S rates x ratio C/S	$y = 0.1974x^2 - 10.752x + 276.27$	0.957*
S rates x ratio N/S	$y = 0.0107x^2 - 0.5165x + 11.849$	0.911*

*Significant at 5%.

A high N/S ratio may lead to accumulation of N in the non-protein form, especially N-NO_3^- and soluble organic N (Haq & Carlson, 1993), reducing plant growth. Plant nutrition studies have shown a positive effect of supplying S on the yield of various crops, with common bean standing out (Furtini Neto et al., 2000).

Reduction in N content and, especially, an increase in S contents in common bean leaves as a result of S application led to a decrease in the values of the N/S ratios. According to Furtini Neto et al. (2000), maximum yield may be related to the N/S balance within the plant. Thus, there is a point or range at which the N/S ratio is considered ideal for maximum yield. Values of the N/S ratio from 13 to 17 have been suggested as ideal for common bean (Aulakh & Parischa, 1977). It may be observed that in the present study, the ratios obtained are below those cited. However, even so, there was a positive effect on plant development as a result of the rates of S applied.

High values of the N/S ratio lead to lower grain yield by common bean (Crusciol et al., 2006), which may be related to the accumulation of N in the non-protein form, especially N-NO_3^- and soluble organic N, brought about by the high N/S ratio (Haq & Carlson, 1993).

CONCLUSION

The rates of S applied under the conditions studied led to an increase in shoot dry matter.

There was a reduction in the N/S ratio, leading to a positive effect in common bean plant development.

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DEVELOPMENT AND REPRODUCTION of *heliothis virescens* (LEPIDOPTERA: NOCTUIDAE) FED ON LEAVES AND FRESH PODS OF BEAN PLANTS

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INTRODUCTION

The cotton budworm, *Heliothis virescens* (Lepidoptera: Noctuidae), is a polyphagous pest with high reproduction capacity (Fitt et al., 1989). This species causes damage to many crops, damaging both vegetative and reproductive structures of the host plant (Fitt et al., 1989; Blanco et al., 2006; Ventura et al., 2015). Some previous studies indicate that *Helicoverpa armigera* (Lepidoptera: Noctuidae) is an important pest of bean plants (Roger & Bries, 2010). Nevertheless, larvae of *Helicoverpa* spp. can be easily confused with those of *H. virescens*, resulting in underestimation of *H. virescens* occurrence in bean fields. Therefore, it is believed that the significance of *H. virescens* for this crop may be underestimated, owing to the possible confusion in identification in the field. Thus, despite its great potential to damage reproductive structures of plants (Fitt, 1989; Cunningham and Zalucki, 2014), information regarding *H. virescens* on bean plants is scarce; its investigation is hence important in order to develop cultural practices of pest management. Therefore, this study aimed to investigate the development and reproduction of *H. virescens* fed on leaves and fresh pods of bean plants.

MATERIAL AND METHODS

This study was carried out at Embrapa Soybean, Londrina, Parana State, Brazil. *H. virescens* was reared under controlled environmental conditions in the laboratory [25 ± 2°C temperature, 70 ± 10% RH, and 14/10 h photoperiod (L/D)]. Bean seedlings were sown in plastic vases (5-L) and kept in a greenhouse. Fertilization with 0-20-20 (N-P-K) was employed 15 days after plant emergence. The leaves and fresh bean pods offered to the budworms were collected from the bean plants at the R_8 phenological stage. Before feeding the caterpillar with leaves or pods, prophylaxis of the plant tissues was performed by submerging the food in a solution of water + 5% sodium hypochlorite for 15 min.

The experiment was carried out in a fully randomized design with two treatments (leaves and pods of beans) and ten replicates per treatment. Each replicate consisted of a pot with seven caterpillars. The insects were placed in an acrylic gerbox with its bottom lined with filter paper. This paper was previously dampened to maintain humidity within the box. When larvae have reached fifth instar the consumption of fresh bean pods was assessed by the weight difference of fresh bean pods before feeding and the weight of these same food 24 hours after the feeding. The pods were weighed before to be offered to fifth instar larvae, aiming to verify the consumption rate, and the weight of food consumed was corrected with the value obtained to the weight of corn grains used in the control treatment (without larva) to assess the weight lost by the grains due to dehydration. Larvae were fed fresh bean pods *ad libitum* until completing their larval phase, i.e., until the pre-pupa phase or died.

During the study were assessed for biological parameters, pod consumption rate (g), larval period (%), pupal biomass (g), longevity (days) and fecundity (total eggs). In each assessment, the insects were touched with a forceps and those that failed to move were classified as dead. Results

obtained were subjected to exploratory analyses to assess the assumptions of normality of residuals, homogeneity of variance of treatments and additivity of the model prior to application of ANOVA. Means were then compared by Student's t test ($p \leq 0.05$).

RESULTS AND DISCUSSION

In general, it was observed that the leaves of the bean plant were not adequate food for development of *H. virescens*, whereas fresh bean pods resulted in faster development and higher larval viability (Tables 1 and 2). Thus, these results suggest that although *H. virescens* larvae consume bean leaves, fresh bean pods are nutritionally more important, indicating a higher potential of causing damage to bean plants during the reproductive stage.

For example, this study showed that cotton budworm consumed a total average of 3.57 g of fresh bean pod, reflecting a pupal biomass of 0.21 g. The moths showed an fecundity (average) of 240 eggs and an eggs viability (average) of 40%. In summary, these results demonstrate that *H. virescens* has the potential to damage bean plants, especially during the reproductive phonological stage of the plants.

Table 1. Larval development of *Heliothis virescens* fed on leaves and fresh bean pods.

Treatment	Larval period (days)						
	1° instar	2° instar	3° instar	4 °instar	5° instar	6°instar	Total
Bean leaves	2.05 a	2.53 a	4.42 a	4.97 a	6.08 a	-	-
Bean pods	1.06 b	2.14 b	2.9 b	2.12 b	2.42 b	2.38	11.54
CV (%)	11.99	14.37	14.66	19.09	21.34		

Treatment	Larval viability (%)						
	1° instar	2° instar	3° instar	4° instar	5° instar	6° instar	Total
Bean leaves	100	84	77	70	44	10 b	2.8 b
Bean pods	98	82	66	60	57	45.5 a	56 a
CV (%)	1.54	8.56	25.82	26.31	32.62	58.49	48.59

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FEEDING OF *HELICOVERPA ARMIGERA* (HÜBNER) (LEPIDOPTERA: NOCTUIDAE) ON GENOTYPES OF *PHASEOLUS VULGARIS* L.

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INTRODUCTION

Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae) is a devastating pest of important plant species (Gordon et al., 2010). In Brazil, larvae of the insect were recorded feeding on cotton, soybeans, and common beans (Ávila et al., 2013; Czepak et al., 2013). Larvae of *H. armigera* feed on leaves and stems of plants, but they show preference for reproductive structures, ultimately causing deformation, rot, and fall of the plant structures (Ávila et al., 2013). This peculiar ability in causing injuries either at the vegetative or reproductive parts of plants associated with the ability of feeding on numerous host plants constitutes a factor that raises the status of the pest (Cunningham et al., 1999).

Given the reports of resistance development of *H. armigera* against several insecticides, efforts should be set on other control methods such as host plant resistance, an important component of integrated pest management. Therefore, it is important to analyze the feeding response of *H. armigera* larvae on pods of bean genotypes in order to determine the genotypes that are less preferred by the insect feeding. In this study we evaluated *H. armigera* larval feeding preference on pods of common bean genotypes.

MATERIALS AND METHODS

Assays were conducted in the Laboratório de Resistência de Plantas a Insetos of FCAV/UNESP, Jaboticabal, SP, Brazil, under environmentally controlled conditions. The following genotypes were evaluated for resistance to *H. armigera* larvae: Pérola, BRS Supremo, IAC Carioca-Tybatã, IAC Galante, IAC Diplomata, IAC Harmonia, IAPAR 81, IAC Una, and IAC Carioca-Eté.

Free-choice and no-choice tests were performed to evaluate feeding of *H. armigera* larvae, both replicated 10 times. Pods at the beginning of seed set were collected from plants and used in both tests. For the free-choice test, glass arenas (26 cm diameter x 5 cm height) lined with moistened filter paper were used, in which pods of the common bean genotypes were distributed, and nine third-instar larvae were released in the center of the arenas (replicates). In the no-choice test, each replicate consisted of one Petri dish (9 cm diameter x 1.2 cm height) lined with moistened filter paper with one pod and one third-instar larva. Attractiveness of pods to the larvae was evaluated at several time points. At the end of the assays, the pods were cut lengthwise to evaluate larval injury among the genotypes. Rates of injury were visually estimated by two evaluators, ranging from zero for uninjured pods to 100% for completely injured pods.

Data recorded from feeding trials were transformed to square root (x+0.5) and data of injury rates on the pods were transformed to arcsine square root (x/100). Transformed data were analyzed by one-way ANOVA, and means were separated by Tukey test (P<0.05).

RESULTS AND DISCUSSION

Differences in attractiveness of *H. armigera* larvae among pods of bean genotypes were observed in the free-choice test at 6h and 12h (Fig. 1A). Genotypes Pérola and IAC Harmonia sustained greatest numbers of larvae, whereas BRS Supremo and IAC Carioca-Eté sustained lowest numbers of larvae. Significant differences were observed for percentage of larval injury in the pods in the free-choice test (Fig. 1C). The genotype IAC Galante was the most injured, while BRS Supremo and IAC Carioca-Eté had the lowest injury rates. Overall, we conclude that genotypes BRS Supremo and IAC Carioca-Eté were the least injured by *H. armigera* larvae, while the other genotypes are susceptible to the insect injury.

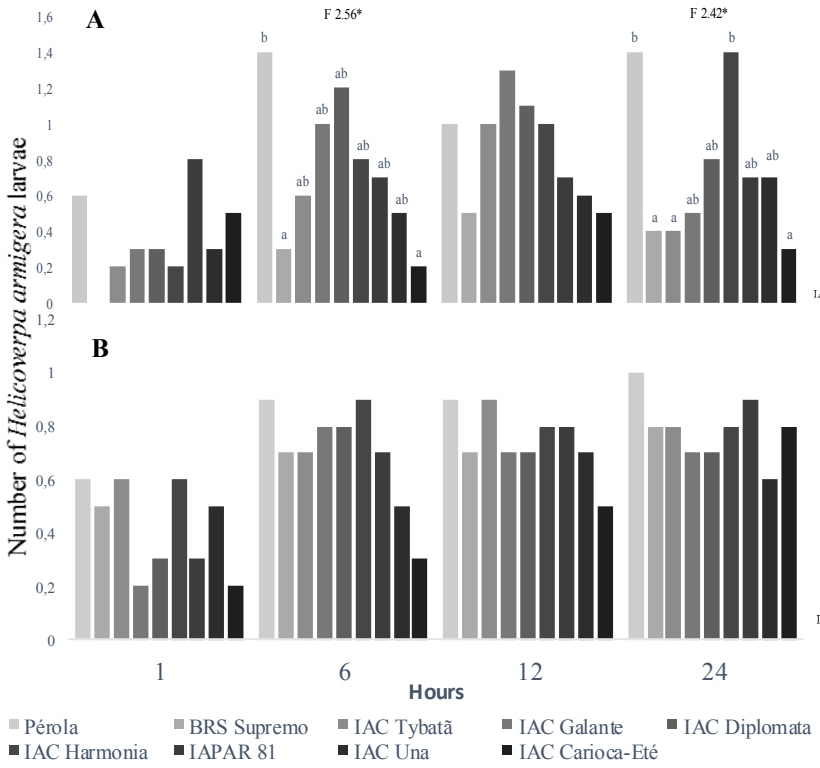

Figure 1. Numbers of *Helicoverpa armigera* larvae feeding on pods of bean genotypes at different hours, in free-choice (A) and no-choice (B) conditions.

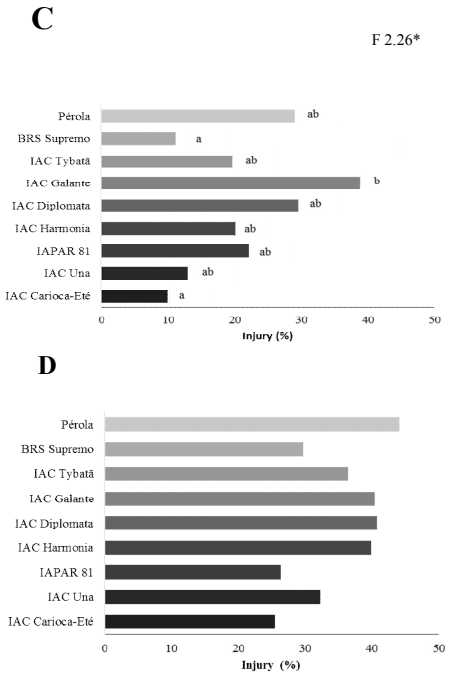

Figure 2. Percentage of *Helicoverpa armigera* larval injury on pods of bean genotypes, in free-choice (C) and no-choice (D) conditions.

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STRATEGIES FOR MANAGING THE WHITEFLY USING NEEM AND CHEMICAL CONTROL ON FIELD-PLANTED COMMON BEANS

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INTRODUCTION

The whitefly *Bemisia tabaci* (Gennadius) biotype B is considered to be the major insect pest of common beans. Its control is based mainly on sprayings of insecticides because of the great availability of registered products and ease of application (Boiça Júnior et al., 2006). However, other alternative control methods such as the use of neem-based bioinsecticides are encourage of being integrated into whitefly management programs in bean crop. Previous studies have reported significant levels of repellency, oviposition deterrence, and nymphal mortality of whiteflies caused by neem-based products applied on common bean plants (Jesus et al., 2009; Quintela and Pinheiro, 2009). We evaluated strategies for managing whitefly *B. tabaci* biotype B based on applications of neem oil, insecticide, and neem oil-insecticide combinations on field-planted common beans.

MATERIALS AND METHODS

Experiment was carried out in an experimental field of the School of Agriculture and Veterinarian Sciences, UNESP, in Jaboticabal, SP, Brazil (21º15'22''S and 48º15'58''W, 595 m altitude). In the experiment, eight strategies were evaluated for whitefly control in a randomized block design with four blocks as replicates. The experiment was set up in the field on 22 December 2012, and planting at this period is referred to as "water season" common beans in Brazil. Seeds of cultivar Pérola were sown at rows spaced 0.5 m apart at a density of 15 seeds per meter, and each plot consisted of four rows 5 m long, with 10 m^2 total area. Plots were fertilized with 430 kg ha^{-1} of 04-14-08 at planting and control of weeds were done manually.

Treatments consisting of eight strategies for whitefly management were based on two sprayings performed at 30 and 45 days after emergence (DAE) of plants in the following fashion: (1) control (no treatment); (2) two sprayings of neem oil at 150 ml ha^{-1}; (3) two sprayings of neem oil at 200 ml ha^{-1}; (4) two sprayings of neem oil at 250 ml ha^{-1}; (5) one spraying of pyriproxyfen at 300 ml ha^{-1} + one spraying of neem oil at 150 ml ha^{-1}; (6) one spraying of pyriproxyfen at 300 ml ha^{-1} + one spraying of neem oil at 200 ml ha^{-1}; (7) one spraying of pyriproxyfen at 300 ml ha^{-1} + one spraying of neem oil at 250 ml ha^{-1}; and (8) two sprayings of pyriproxyfen at 300 ml ha^{-1}. Neem oil was Azamax® and the insecticide pyriproxyfen was Tiger®. We used a CO^2-pressurized backpack sprayer with two hollow cone nozzles regulated to deliver 200 L ha^{-1} at 45 lb pol^{-2}.

Three samplings of whitefly were performed: the first sampling took place the day before the first spraying was performed, and two additional samplings were performed three days after each of the two sprayings. Ten leaflets were collected from plants per plot, kept in paper bags, and in the laboratory the number of nymphs were counted under a stereoscope.

Data of numbers of eggs, nymphs, and adults were analyzed by one-way ANOVA, and treatment means were separated by Tukey's test (α=0.05) when significant. Data were transformed to square root (x+1) prior to analysis but untransformed data are presented in table.

RESULTS AND DISCUSSION

Table 1. Numbers of whitefly nymphs recorded on leaves of common beans before treatment and after sprayings with neem oil, insecticide, and neem oil-insecticide combinations.

Strategies of whitefly control	Before spraying	After 1st spraying	After 2nd spraying*
1. Control (no treatment)	0.75	1.50	2.25 a
2. neem oil 150 ml ha^{-1} + neem oil 150 ml ha^{-1}	0.50	0.75	2.00 ab
3. neem oil 200 ml ha^{-1} + neem oil 200 ml ha^{-1}	0.50	0.25	0.75 ab
4. neem oil 250 ml ha^{-1} + neem oil 250 ml ha^{-1}	0.50	1.00	1.00 ab
5. pyriproxyfen 300 ml ha^{-1} + neem oil 150 ml ha^{-1}	0.25	0.00	0.75 ab
6. pyriproxyfen 300 ml ha^{-1} + neem oil 200 ml ha^{-1}	0.25	0.25	0.00 b
7. pyriproxyfen 300 ml ha^{-1} + neem oil 250 ml ha^{-1}	0.75	0.75	0.75 ab
8. pyriproxyfen 300 ml ha^{-1} + pyriproxyfen 300 ml ha^{-1}	0.50	0.00	1.25 ab

*Means followed by same letters within column are not significantly different by Tukey's test ($P > 0.05$).

Infestation of whitefly populations were still low and was distributed homogeneously in the field plots as observed by the sampling performed prior to treatment spraying (Table 1). After the first spraying at 30 DAE (Table 1), neem oil at any rates or the insecticide pyriproxyfen did not reduce numbers of nymphs, which did not differ from control (no treatment). After the second spraying at 45 DAE (Table 1), the strategy consisting of sprayings with pyriproxyfen at 300 ml ha^{-1} + neem oil at 200 ml ha^{-1} (6) significantly reduced the number of nymphs from 2.25 nymphs per leaflet to none (control). Despite the other strategies reached similar results from strategy 6 they did not differ from control (no treatment).

From this preliminary study we conclude that the strategy based on sprayings of pyriproxyfen at 300 ml ha^{-1} + neem oil at 200 ml ha^{-1} 30 DAE and 45 DAE, respectively, seems to be effective at reducing populations of nymphs of *B. tabaci* biotype B on common beans in the "water season". Further studies are required in order to ensure the replicability of some promising results in the "dry season" and "winter season".

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QTL MAPPING ASSOCIATED WITH MORPHOLOGICAL TRAITS OF BEAN SEEDLINGS AND RESPONSE TO LOW TEMPERATURE IN GERMINATION

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Biparental mapping populations are an interesting resource to investigate the inheritance of complex traits. On the other hand, availability of techniques for massive genotyping allows a quick construction of genetic linkage maps in which can be located the genes associated to the expression of traits. In this study, we investigated the quantitative trait loci (QTL) controlling two morphological traits in bean seedlings (number of secondary roots and root weight) and the response to low temperature (18º C) in germination, a trait associated to adaptation of crops to new environments.

MATERIAL AND METHODS

A population of 113 $F_{2:7}$ recombinant inbred lines (RIL) derived from the cross Xana x Cornell 49242 was used to develop an updated version of the XC linkage map (Perez-Vega et al. 2010). Available genotypic data were supplemented by data supplied by the genotyping platform BARCBean6K_BeadChip (Song et al. 2015). Genotypic data were filtered considering the data completeness (> 90%), deviation from expected genotypic frequency based on chi-square tests ($p < 0.05$), and co-segregations (redundant loci were not included).

Linkage map was constructed using the software OneMap (Margarido et al. 2007). Linkage groups were established with a LOD threshold of 3.0 and a recombination fraction of 0.25. Markers order were estimated based on rapid chain delineation algorithm (Doerge 1996) and ripple analyses. Map distances between loci (cM) were calculated using the Kosambi mapping function.

The traits were assessed in two different experiments. In the first experiment, number of secondary roots and root weight (g) were analyzed in three different tests. Four seedlings per RIL were included in each test. In the second experiment, the response to low temperature in germination (hours to seedling emergence at 18 ºC) was investigated in three test. Eight seedlings per RIL and parental lines were included in each test. The Qgene 4.3.10 software (Joehanes and Nelson 2008) was used to detect QTL using CIM analysis (composite interval mapping) and LOD value > 2.5. Significant QTL were considered when they were detected in at least one test and in the mean of three tests.

RESULTS AND DISCUSSION

A total of 2561 polymorphic SNP were obtained and 1912 were not considered due to distorted segregations or co-segregations. The updated version of the XC genetic map has 11 linkage groups, covers 1393.5 cM, and includes 649 SNPs, as well as 113 INDELs, SSR, and SCAR markers. Several gaps in the distribution of SNP markers across the linkage groups were observed (Figure 1).

Parents Xana and Cornell 49242 showed significant differences for the three analyzed traits. No significant deviations from the corresponding normal distributions were observed in the RIL population for the three traits. Significant correlations among the results of the three tests were observed in the three traits ($p < 0.05$).

A total of eight significant QTL were detected. Figure 1 shows the relative positions of the eight QTL located in the linkage groups Pv01, Pv06, Pv07, Pv10 and Pv11. Five QTL were detected for number of secondary roots, one of them ($NR1^{XC}$) was located in Pv01, a second QTL ($NR6^{XC}$) was located in Pv06, two QTL ($NR7.1^{XC}$, $NR7.2^{XC}$) were detected in Pv07, and the fifth QTL ($NR11^{XC}$) was located in Pv11. Two QTL ($RW6^{XC}$ and $RW10^{XC}$) were detected for root weight in Pv06 and Pv10 respectively. A QTL for seedling emergence at 18ºC ($SE6^{XC}$) was located in Pv06 overlapped the QTL $NR6^{XC}$. In all cases, the alleles contributed by Xana increasing these traits except for, $NR6^{XC}$, which allele from Xana decreases NR values. Considering the mean of three tests, the amount of phenotypic variation (R^2) explained by the individual QTL ranged from 0.1 to 0.21. Results suggest low heritability for the three traits.

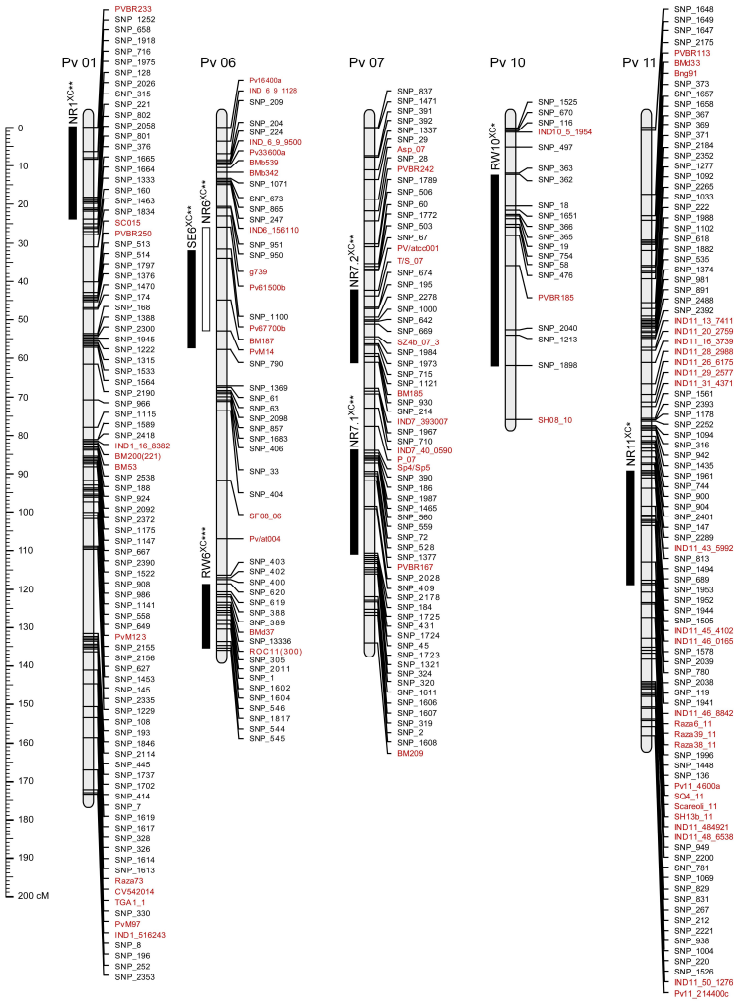

Figure 1. Linkage groups Pv01, Pv06, Pv07, Pv10, and Pv11 obtained in the RIL population developed from the cross Xana x Cornell 49242 showing the location of significant quantitative trait loci for traits in bean seedlings, number of secondary roots (NR), root weight (RW), and response to low temperature (18º C) in germination (SE). Vertical bars to the left of linkage groups indicate significant QTL. Black and white vertical bars represent QTL in which the increase is supported by the alleles of Xana and Cornell 49242, respectively. The number of asterisks near the name of the QTL indicates the number of the tests in which the QTL was detected.

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DOCUMENTATION OF THE GERMPLASM ACTIVE BANK OF *Phaseolus lunatus* L.

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INTRODUCTION: The main center of diversity of *Phaseolus* species is Mexico, where they met about 90% of the 50 known species (Mercado-Ruaro and Delgado-Salinas 2009). It's four (Delgado-Salinas 1985) or five (Debouck 1991) grown, ie, *P. vulgaris*, *P. coccineus*, *P. acutifolius*, *P. lunatus* and *P. polyanthus* (= *P. coccineus* subsp. Darwinian). Delgado-Salinas (1985) estimated that *Phaseolus* contains 36 species in North America and Central America, while Debouck (1991) included 52 species, in the same regions. *Phaseolus lunatus* L. is very important for the Northeast region of Brazil. Research on plant genetic resources involve a number of essential activities that require considerable financial support and, especially, require continuity (Nass et al. 2001). The information obtained from morphological characterizations, cytogenetic, biochemical and molecular contribute to the conservation of the variability and identification of accessions of a germplasm bank.

MATERIALS AND METHODS: The Germplasm Active Bank of lima bean of the Federal University of Piauí (BAG of lima bean - UFPI) is installed on Genetic Resources Laboratory of the Department of Plant Science, the city of Teresina, Piauí, Brazil. The BAG was created in 2005 with the acquisition of landraces in farming communities and markets, by collecting expeditions, which began in September 2004. These expeditions led to the incorporation of 211 lima bean accessions coming from Piauí, Maranhão, Pernambuco and Bahia states. In 2005, it signed a germplasm exchange agreement with the Federal University of Viçosa, Minas Gerais state (Lopes et al. 2010), with the acquisition of 50 lima bean accessions. Many accessions have been incorporated inside the BAG through donations. In 2006, with a partnership between the UFPI and Agricultural Family School Soinho, 118 lima bean accessions were acquired. In 2008 and 2009, we introduced 17 and 54 accessions from CIAT and Paraiba, respectively. In 2011 and 2012, 40 and 14 lima bean accessions collected in Ceará and Bahia states, respectively, were introduced in BAG. These collecting expeditions were funded by FAO in the Global Trust Project. In 2014, we collected 46 lima bean accessions in Paraíba, which is the largest producing state. Currently, lima bean bag - UFPI has 854 lima bean accessions from Piauí, Maranhão, Ceará, Paraíba, Bahia, Pernambuco, Tocantins, São Paulo, Minas Gerais, Espirito Santo states and the Federal District.

RESULTS: In the BAG of lima bean - UFPI, all access belong to the species *P. lunatus* L. The introduction of such accessions in BAG requires documentation in the record book, which contains the following data: access number, name and common collector, date, place and year of collection and color of seeds. In the ten year period (2005-2015), 441 lima bean accessions of the BAG-UFPI were used on agromorphological, physical-chemistry, molecular, for resistance to anthracnose and the potential for nodulation of nitrogen-fixing bacteria characterizations, performed based on descriptors to *Phaseolus lunatus*, published by International Plant Genetic Resources Institute (IPGRI, 2001), currently Biodiversity International. The main quantitative descriptors were: pod length, pod width, pod weight and number of seeds per pod, number of locules per pod, seed length, width of seed, thickness of seed and weight of 100 seeds. The most common qualitative descriptor was the standard seed color.

Table 1. Origin of the lima bean accessions of the Germplasm Active Bank of the Federal University of Piauí.

Origin	Number of accessions (%)
Piauí, Brazil	45,70
Paraíba, Brazil	19,24
Ceará, Brazil	10,29
Minas Gerais, Brazil	7,20
Maranhão, Brazil	5,58
Distrito Federal, Brazil	4,12
São Paulo, Brazil	2,26
Bahia, Brazil	2,06
Pernambuco, Brazil	1,44
Goiás, Brazil	1,05
Espírito Santo, Brazil	0,62
Paraguai	0,23
Tocantins, Brazil	0,21

CONCLUSIONS: The BAG of lima bean - UFPI provides important scientific information about the species *Phaseolus lunatus* L., involving knowledge of multiplication, rejuvenation, characterization, evaluation and accessions storage conditions, as well as biotic and abiotic factors that affect your performance. This information can be obtained through consultation with the scientific research reports, work undergraduate completion of course, dissertations and theses.

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VALIDATION OF ELECTRICAL CONDUCTIVITY TEST FOR BUTTER BEAN SEEDS

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The butter bean (*Phaseolus lunatus* L.) is the second most important legume of this genus due to the protein content and distinctive taste. This bean is used worldwide in the most diverse cuisines, receiving various names, depending on the cultivated area and forms used for human consumption (Martínez-Castillo et al., 2008). In the United States, it is popularly known as lima bean, butter bean, Sieva bean and sugar bean (Oliveira et al., 2004). The butter bean is considered more tolerant to drought, excess moisture and heat when compared to common bean (*Phaseolus vulgaris* L.) and adapting to the most varied environmental conditions (Vieira, 1992). This is important because the predictions of climate change indicate significant changes in rainfall patterns over the coming decades (Borém & Ramalho, 2011). Despite its adaptive potential, related to butter bean crop is still incipient. Therefore, the aim of the present study was to evaluate the adequacy of methodology of the electrical conductivity test for butter bean seed varieties.

Six varieties of butter bean grown in the North of Minas Gerais State, Brazil, were used in this study. The electrical conductivity of the water of hydration was determined from 25 selected beans, discarding malformed and voids. In plastic cups of 200 ml, the beans were immersed in 75 ml of deionized water. Then, the cups were transferred to a germinating chamber under constant temperature of 25°C for 24 hours. The electrical conductivity readings were taken after 4, 8, 12, 16, 20 and 24 hours of soaking. The solutions were gently shaken and the electrical conductivity was determined by conductivity (Model CG1800), and the results were expressed in mS (Dias & Marcos Filho, 1996). The experimental design was completely randomized with six treatments and four replicates per treatment. The data were subjected to analysis of variance and mean to clustering Scott-Knott's test at 5% probability. For validation tests were estimated at different times of soak, linear correlation coefficients (Pearson) between the results of electrical conductivity and other tests related to the physiological quality: Germination (G), First Count Seedling (FCS), Germination Speed Index (GSI), Fresh Mass (FM) and Dry Mass (DM) (Brasil, 2009). All statistical analyzes were done with the program GENES (Cruz, 2001).

In Table 1 we show the results of the electrical conductivity test at different times of soak. In periods of 4, 8 and 12 hours, the varieties 2 and 5 not statistically different from each other and are pointed in the same group. The physiological potential of a seed is inversely proportional to the electrical conductivity values. Therefore, in this period of time, varieties 2 and 5 had lower performance compared to other varieties. Overall, it was inferred that the electrolytes leached by seeds increased linearly in all periods of soaking (Oliveira et al., 2012). During periods of 16, 20 and 24 hours, 6 variety was the most stable, which leads to a good physiological quality, profile maintained since the first hours of evaluation. The simple linear correlation coefficients allowed to set the best time for the electrical conductivity test of butter bean seeds (Table 2). Except for the FM and DM, the results showed a positive correlation in all the tests used to evaluate the physiological quality of seeds in 12 hour of soaking period. Nevertheless, it was possible to organize the varieties into vigor level. In conclusion, in this study, the electrical conductivity test has been validated for the assessment of butter bean seeds. Therefore, it is possible to use such a test for quality control programs in seed laboratories, considering the species *Phaseolus lunatus*.

Table 1. Electrical conductivity test of six varieties of butter bean seeds by soaking period of 25 grains for 4, 8, 12, 16, 20 and 24 hours.

Varieties	Electrical conductivity					
	4 h	8 h	12 h	16 h	20 h	24 h
1	0.77 b	0.78 b	1.83 b	3.32 b	4.46 b	5.79 a
2	1.25 a	2.16 a	2.09 a	4.95 a	5.33 a	5.74 a
3	0.53 b	0.62 b	1.08 c	1.75 c	2.58 c	5.49 a
4	0.61b	0.90 b	1.55 b	2.25 c	2.70 a	5.71 a
5	1.43 a	2.44 a	2.73 a	3.95 b	4.49 b	5.36 a
6	0.48 b	0.76 b	1.23 b	2.20 c	2.24 c	2.48 b
Means	0.84	1.44	1.76	3.08	3.78	5.10
CV (%)	45.13	41.81	31.65	18.84	19.89	15.035

Note: Means followed by the same letter in the column do not differ statistically by Scott and Knott' test at 5% probability; CV: Coefficient of Variation.

Table 2. Coefficients of simple linear correlation between the results of electrical conductivity and physiological quality tests on seeds under different soaking time.

Variables	Time					
	4 h	8 h	12 h	16 h	20 h	24 h
G (%)	0.2992	0.4295	0.4692	0.3871	0.1743	-0.4163
FCS (%)	0.4691	0.4923	0.4920	0.5550	0.4256	-0.3846
SGI	0.3903	0.4452	0.4962	0.3994	0.1997	-0.4559
FM (g)	-0.1280	-0.0600	0.0779	-0.0301	-0.2443	-0.7919
DM (g)	-0.3328	-0.2671	0.0114	-0.2176	-0.3750	-0.8489
G (%)- 4 h	-0.0957	0.2195	0.2030	-0.2737	-0.4328	-0.5856
G (%)- 8 h	-0.3275	-0.2742	0.0921	-0.3329	-0.4864	-0.6348
G (%)- 12 h	-0.2437	-0.2646	0.0307	-0.3624	-0.4412	-0.6543
FCS (%)- 4 h	-0.1110	-0.0289	0.1556	-0.2473	-0.4005	-0.7640
FCS (%)- 8 h	-0.1552	0.1228	0.1228	-0.2475	-0.4462	0.7322
FCS (%)- 12 h	-0.0252	-0.1061	0.1393	-0.3816	-0.4566	-0.3939
FM (g)- 4 h	-0.2958	-0.2359	0.0177	-0.4198	-0.5108	-0.7644
FM (g)- 8 h	-0.3801	-0.3526	0.0354	-0.3944	-0.5556	-0.7605
FM (g)- 12 h	-0.2410	-0.3265	-0.1309	-0.4130	-0.5295	-0.7566
DM (g)- 4 h	-0.3046	-0.2536	0.0469	-0.3788	-0.5237	-0.8453
DM (g)- 8 h	-0.3123	-0.3016	0.1007	-0.3119	-0.4720	-0.7792
DM (g)- 12 h	-0.2901	-0.3282	-0.1245	-0.3884	-0.5120	-0.8126

G: Germination; FCS: First Count Seedling, SGI: Speed Germination Idex; FM: Fresh Mass; DM: Dry Mass.

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PROGENY TESTS FOR COMMON BEAN ISOLINES RESISTANT TO DISEASES WITH THE AID MOLECULAR MARKERS

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INTRODUCTION - Bean (*Phaseolus vulgaris* L.) is one of the most important vegetables in the human diet, mainly due to their nutritional qualities (Maldonado, 2002). One of the major factors limiting their productivity are the fungi that cause rust (*Uromyces appendiculatus*), anthracnose (*Colletotrichum lindemuthianum*) and angular leaf spot (*Pseudocercospora griseola*) in common bean. The aim of this study was progeny tests in segregating populations for resistance genes to these three diseases.

MATERIAL AND METHODS - From the F_2 population [(MAR-138A-1-11-4 x BAT-67-15-8) x Rudá R] carrier resistance genes to angular leaf spot, anthracnose and rust (Sanglard et al., 2007); were advanced two generations in the greenhouse using the pedigree method (*bulk* within families). During this process, tests were conducted for the presence of resistance gene using molecular markers. Evaluations of disease (angular leaf spot, anthracnose and rust) followed the methodology proposed by Pastor-Corrales & Jara (1995), Pastor-Corrales (1992) and Stavely et al. (1983).

RESULTS AND DISCUSSION - Of the 292 individuals F_2 [(MAR-138A-1-11-4 x BAT-67-15-8) x Ruda R], were selected 63 in the presence of SCAR (Sequence Characterized Amplified Regions) molecular markers: $SF10_{1050c}$, $SBA08_{560c}$, $SY20_{830c}$, $SAZ20_{845c}$, $SH13_{520c}$, $SAO12_{950c}$, $SAA07_{950c}$ and $SE04_{640c}$ (Sanglard et al., 2007). For planting the next generation, grains were selected within the standard "carioca"genetic *background* 'Ruda'. According to Santos et al. (2001), the early selection of the characteristic type of grain is very efficient due to its high heritability. In F_3 generation [(MAR-138A-1-11-4 x BAT-67-15-8) x Ruda R] were obtained 50 genotypes carrying all molecular markers amplified in the previous generation. Also in the greenhouse, the F_4 generation [(MAR-138A-1-11-4 x BAT-67-15-8) x Ruda R] was sown in family structure for conducting progeny tests. Fifty families were inoculated $F_{3:4}$ [(MAR-138A-1-11-4 x BAT-67-15-8) x Ruda R] sequentially with 21-3 races of *U. appendiculatus*, 65 of *C. lindemuthianum* and 63.23 of *P. griseola* (Table 1). Nine segregating families were discarded, seven to rust and two for anthracnose. Segregating families to rust have not been evaluated for the other diseases (families underlined in Table 1). In addition the evaluations through inoculations, five plants were chosen randomly from each of 41 families segregating for molecular analysis. Were used the same molecular markers described above. We selected 39 common bean families "carioca" containing resistance genes to angular leaf spot, anthracnose and rust. The combined use of phenotypic and molecular evaluations increases reliability in the selection processes.

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Table 1. Progeny tests performed for families $F_{3:4}$ [(MAR-138A-1-11-4 x BAT-67-15-8) x Ruda R] inoculated with the races 63.23 of *P. griseola*, 65 of *C. lindemuthianum* of 21-3 de *U. appendiculatus*

Families	Rust		Anthracnose		Angular leaf spot		Families	Rust		Anthracnose		Angular leaf spot	
	21-3		65		63.23			21-3		65		63.23	
	R	S	R	S	R	S		R	S	R	S	R	S
R3-4-20-4	15	0	15	0	15	0	R3-14-26-15	13	0	13	0	13	0
R3-4-20-11	13	0	13	0	13	0	R3-15-25-7	12	2				
R3-4-20-16	15	0	15	0	15	0	R3-15-25-10	13	0	13	0	13	0
R3-4-20-27	15	0	15	0	15	0	R3-15-25-13	15	0	15	0	15	0
R3-7-23-10	14	0	14	0	14	0	R3-16-17-4	14	0	13	1		
R3-8-2-5	15	0	15	0	15	0	R3-16-17-9	15	0	15	0	15	0
R3-8-2-18	15	0	15	0	15	0	R3-16-17-12	12	0	12	0	12	0
R3-8-2-22	12	2					R3-16-17-13	13	0	13	0	13	0
R3-8-2-23	10	5					R3-21-18-3	14	0	14	0	14	0
R3-10-11-5	15	0	15	0	15	0	R3-21-18-5	15	0	15	0	15	0
R3-10-11-9	15	0	15	0	15	0	R3-21-18-9	14	0	14	0	14	0
R3-10-11-15	15	0	15	0	15	0	R3-21-18-11	15	0	15	0	15	0
R3-10-11-24	15	0	15	0	15	0	R3-21-18-17	15	0	15	0	15	0
R3-12-42-6	8	5					R3-21-18-19	15	0	15	0	15	0
R3-12-42-14	8	7					R3-21-18-22	15	0	15	0	15	0
R3-12-42-15	14	0	14	0	14	0	R3-21-18-25	11	0	11	0	11	0
R3-12-43-1	0	11					R3-27-11-18	12	0	12	0	12	0
R3-12-43-6	12	0	12	0	12	0	R3-27-11-26	15	0	15	0	15	0
R3-12-43-8	10	3					R3-35-6-2	15	0	15	0	15	0
R3-14-26-2	15	0	15	0	15	0	R3-35-6-8	14	0	14	0	14	0
R3-14-26-8	15	0	15	0	15	0	R3-35-6-10	15	0	15	0	15	0
R3-14-26-9	15	0	14	1			R3-35-6-13	14	0	14	0	14	0
R3-14-26-12	15	0	15	0	15	0	R3-35-6-17	15	0	15	0	15	0
R3-14-26-13	12	0	12	0	12	0	R3-35-6-20	13	0	13	0	13	0
R3-14-26-14	15	0	15	0	15	0	R3-35-6-21	14	0	14	0	14	0

R: resistant; S: susceptible; Underlined lines refer to families segregating.

PYRAMIDED LINES OF "CARIOCA" COMMON BEAN AND THEIR REACTION TO *Pseudocercospora griseola*

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INTRODUCTION: The bean diseases are a major cause of losses of yield of this culture. The angular leaf spot caused by the fungus *Pseudocercospora griseola* (Sacc.) Crous & U. Braun is one of the main diseases of beans. The present work aimed to characterize lines produced by the Breeding Program of Common Bean (PMGF) of the Federal University of Viçosa (UFV), called 'Ruda R3' and 'Pérola R1', in reaction to different races of *P. griseola*.

MATERIAL AND METHODS: The genotypes were lines of F_4 population [(MAR-138A-1-11-4 x BAT-67-15-8) x Ruda R] (Sanglard et al., 2007) and isolines derived from the cross between 'Pérola' and 'Ouro Negro' (Sanglard et al., 2005). The inoculum of each isolate was reproduced according to Sanglard et al. (2009a) and Pastor-Corrales & Jara (1995), respectively. After inoculation, the disease severity was visually assessed at 15, 18 and 21 days using a scale of nine degrees of severity proposed by Pastor-Corrales & Jara (1995). Plants with notes 1 to 3 were considered resistant; between 3 and 6, with intermediate resistance; and between 6 and 9 susceptible.

RESULTS AND DISCUSSION: The results of this work are summarized in Table 1. The line 'Ruda R', the parent lines of group 'Ruda R3', presented susceptibility to 16 of the 17 isolates tested, demonstrating the ineffectiveness of your resistance locus (Carvalho et al., 1998; Ragagnin et al., 2009) compared to the isolates obtained by Balbi et al. (2009). The other parental lines 'MAR-138A-1-11-4' (Oliveira et al., 2005) and 'BAT 67-15-8' (Caixeta et al., 2003) showed resistance to each of eight isolates. The interpolation of the spectra of strains resistant 'MAR-138A-1-11-4' and 'BAT-67-15-8' reactions to generate resistance 12 of the 17 isolates tested, which were found in most strains of the group 'Rudá R3'. If we consider spectra while the resistance of the lines 'MAR-138A-1-11-4', 'BAT-67-15-8', 'COR-25-12-9' and 'MEX-37-3-6-3' (Caixeta et al., 2005; Sanglard et al., 2007), potentially achieve resistance 15 of the 17 isolates tested, except $B_1$46 and $B_7$50, which were classified as race 63.63 (Balbi et al., 2009). In recent years, most of the isolates obtained in this race have been classified, and is considered as one of the most frequent and distributed in Brazil (Balbi et al., 2009). Analyzing the reactions of the parents of the lines of group 'Pérola R1', note that the cultivar 'Pérola' was susceptible or intermediate to 16 of the 17 isolates tested, confirming the trend of susceptibility to angular leaf spot of this cultivar. However, the cultivar 'Ouro Negro' showed intermediate reactions to four isolates and susceptibility to just two. This cultivar has been recommended as a source of resistance to angular leaf spot, with notably lower levels of severity in the majority of the isolates (Sanglard et al., 2009b). In fact it is well-known the relevance of use of the cultivar 'Ouro Negro' as a source of resistance, since it was the only parent to be tolerant to the $B_1$46 and $B_7$50 isolates classified as race 63.63. The 'Pérola R1' lines showed considerable improvement in the spectrum of resistance when compared to the parent lines 'Pérola' with incompatibility reactions to eight isolates. Possibly, these did not reach the same pattern observed in 'Ouro Negro' due to several rounds of backcrossing with 'Pérola' as recurrent parent, which would have led to the loss of smaller effect of genes, and not monitored by molecular markers while dragging (Sanglard et al, 2005;. 2013).

Table 1. Characterization of the parents and selected lines to isolated (races) of *P. griseola*.

Genotypes	Isolates (Races) of *P. griseola*																
	$A_1$13 (15.7)	$A_2$4 (63.7)	$B_1$46 (63.63)	$B_3$8 (63.47)	$B_4$4 (47.39)	$B_4$6 (31.4)	$B_7$50 (63.63)	$C_1$17 (3.23)	$C_1$28 (63.6)	$C_2$10 (23.23)	$CM_1$2 (63.63)	$CM_3$11 (63.31)	Cb20 (63.7)	Cb21 (31.7)	SM32 (63.23)	Vic3 (63.23)	Vic7 (63.63)
MEX-37-3-6-3	*R	R	S	S	R	R	S	R	R	R	S	S	R	R	R	R	S
COR-25-12-9	R	R	S	S	S	R	S	R	R	R	S	R	I	R	R	R	S
MAR-138A-1-11-4	R	I	S	R	S	S	S	S	I	R	S	S	R	R	I	R	R
BAT-67-15-8	R	R	S	R	R	R	S	S	R	S	S	S	R	R	S	S	S
Rudá R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	I
R3-12-42-15	R	R	S	R	R	R	S	S	R	I	S	S	R	R	S	R	R
R3-12-43-6	R	R	S	R	R	R	S	S	R	I	S	S	R	R	S	I	I
R3-15-25-13	R	R	S	R	R	R	S	S	R	R	S	S	R	R	S	I	R
R3-16-17-9	R	R	S	R	R	R	S	S	R	I	S	S	R	R	S	I	R
R3-27-11-26	R	R	S	R	R	I	S	S	R	R	S	S	R	R	S	I	I
R3-16-17-13	R	R	S	R	R	R	S	S	R	R	S	S	R	R	S	I	I
R3-27-11-18	R	I	S	R	I	S	S	S	R	I	S	S	R	R	S	S	R
R3-15-25-10	R	R	S	R	R	R	S	S	R	R	S	S	R	R	S	I	R
R3-16-17-12	R	R	S	R	R	R	S	S	R	I	S	S	R	R	S	I	R
Ouro Negro	R	R	I	R	R	I	R	S	R	R	R	I	R	I	S	R	R
Pérola	I	I	I	I	I	S	I	I	S	I	I	S	I	I	S	S	R
P1-78-23-1	I	R	I	I	R	S	R	I	R	I	R	S	R	I	S	R	R
P1-78-23-2	I	R	I	I	R	S	R	I	R	I	R	S	R	I	S	R	R
P1-88-16-5	I	R	I	I	R	S	R	I	R	I	R	S	R	I	S	R	R
P1-88-16-3	I	R	I	I	R	S	R	I	R	I	R	S	R	I	S	R	R
P1-41-6-7	I	R	I	I	R	S	R	I	R	I	R	S	R	I	S	R	R
P1-41-6-4	I	R	I	I	R	S	R	I	R	I	R	S	R	I	S	R	R

* Classifications obtained by the average rating of 12 plants of each genotype; R: resistant (1 to 3); I: intermediate (3 to 6); S: susceptible (6 to 9). Isolates obtained by Balbi et al. (2009).

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MARKER-ASSISTED SELECTION INTEGRATED TO THE EMBRAPA COMMON BEAN BREEDING PROGRAM

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Several works developed during the last decade report the application of molecular markers in common bean (*Phaseolus vulgaris* L.) research for genetic diversity evaluation, evolution study, pedigree analysis, genetic mapping, gene tagging and breeding, including recurrent parent genome recovering in backcrossing programs and assisted selection of traits with high agronomic interest in segregating breeding populations (Barros & Souza 2012). Initially, codominant RFLP (Restriction Fragment Length Polymorphism) and dominant RAPD (Randomly Amplified Polymorphic DNA) markers have predominantly been used, allowing advances towards identification of molecular tools co-segregating with agronomic traits. Later on, a broad set of SCAR (Sequence Characterized Amplified Regions) markers have been developed from RAPD markers linked to disease resistance genes. Microsatellite or SSR (Simple Sequence Repeats) markers were also being gradually developed and utilized for a wide range of genetic analysis. Currently, many efforts is in course to discovery and use SNP (Single Nucleotide Polymorphisms) markers (Kelly et al. 2003; Barros & Souza 2012).

The establishment of a specific Marker-Assisted Selection Facility at the Embrapa Rice and Beans Biotechnology Laboratory, in 2014, has better supported the routine analysis with molecular markers demanded by the Embrapa Common Bean Breeding Program. In addition, it has also supported other Embrapa plant breeding programs, such as rice and cotton. Depending on the kind of genotypic analysis, DNA extraction is being performed using three different methods: (i) alkaline lysis – a simple and fast protocol widely used by many plant breeding programs worldwide but that results in DNA samples with low quality and, for this reason, it is more suitable for analysis in large scale without the need of DNA storing (Xin et al. 2003; Valdisser et al. 2013); (ii) CTAB (cetyltrimethylammonium bromide) – a very popular protocol that results in DNA samples with high purity, suitable for genotyping based on sequencing, hybridization or enzymatic digestion (Doyle & Doyle 1990; Ferreira & Grattapaglia 1998); and (iii) different commercial kits – fast and easy to use protocols which also results in a high quality DNA, e.g. the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA).

Microsatellite markers have been used for genetic diversity studies (Brondani & Brondani 2006; Cardoso et al. 2014), confirmation of controlled crosses (Morais et al. 2016), marker-assisted backcrossing (Souza et al. 2014a), monitoring of allelic diversity and parental genetic representativeness in breeding populations (Batista et al. 2014), and assessig the genetic purity and identity of cultivars during seed production process (Morais et al. 2016). SCAR, SSR and STS (Sequence-Tagged Sites) markers have been validated and used for marker-assisted selection of genes associated with target traits such as resistance to *Bean golden mosaic virus* (BGMV) (transgenic event), anthracnose, angular leaf spot, rust, as well as to slow darkening of *carioca* grains (Ragagnin et al. 2009; Barros & Souza 2012; Souza et al. 2014a,b). SNP markers for some of these traits, such as resistance to BGMV, anthracnose and angular leaf spot, besides the slow darkening of *carioca* grains, are also being developed, validated and implemented in the pipeline of routine analysis. The consolidation of a genomic platform applied to large-scale genotyping in the Embrapa Common Bean Breeding Program is increasing the efficiency of the selection process of superior genotypes, but reducing time, handwork and datapoint cost.

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THERMAL REQUIREMENTS OF THE MAIN 'PINTO' BEAN VARIETIES GROWN IN DURANGO, MEXICO

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INTRODUCTION

In Mexico, bean (*Phaseolus vulgaris*) is considered as a very important cultivation for consumption due to its high content in fiber, minerals and proteins. At the national level, it is the most cultivated crop on the entire agricultural surface, whether being under irrigation or rainfed modalities. During 2014, the main Mexican federal entities that produced bean were Zacatecas, Durango, Sinaloa and Chihuahua (SIAP, 2015). Due to climatic changes and the great use of diverse bean varieties, is important to know the phenological behavior of each variety in regions where they are cultivated. This is because of the biological phenomena of the plant that happens as part of its phenology that is related to the weather from the area where they are being grown (Escalante and Kohashi, 1993). Therefore, the phenology is a very important discipline to monitor the different stages of the crop, whether they are vegetative or reproductive due to the different phenological changes that can be used as a guide for agricultural and cultural practices (Solórzano, 2007). The objective of this research was to monitor the phenology of different 'Pinto' bean varieties in Durango, Mexico with the goal of estimating their thermal requirements as well as modeling their phenological stages in relationship with degree days (^{o}D).

MATERIALS AND METHODS

During the spring-summer agricultural cycle (P-V) 2014 (LN 23° 59' 23.5", LW -104° 37' 15.6", 1887 m) and in 2015 (LN 23° 59' 21.3", LW -104° 37' 31.9", 1,884 m), experimental plots were established in Durango, Mexico (INIFAP-Valle del Guadiana Experimental Station) with sowing dates going from June 26, 2014 and July 14, 2015, respectively. The surface of each plot consisted of 12 rows with a separation of 0.8 m and a length of 18 m (172.8 m^2). The most used bean varieties was cultivated at the commercial level ('Pinto Saltillo', 'Pinto Libertad', 'Pinto Coloso', 'Pinto Centenario', 'Pinto Libertad' and 'Pinto Centauro') in Mexican Highlands (Aguascalientes, Chihuahua, Durango y Zacatecas). The guide described by Fernandez *et al.* (1982) was used for in field monitoring for each phenological stage of each variety of 'Pinto' bean. The method for daily calculation of degree days (°D) was the one developed by Ojeda *et al.* (2004), in which the following equations were considered: $°D = T_a - T_{c\text{-}min}$, $T_a < T_{c\text{-}max}$; $°D = T_{c\text{-}max} - T_{c\text{-}min}$, $T_a \geq T_{c\text{-}max}$; $°D = 0$, $T_a \leq T_{c\text{-}min}$. In the equations is considered that $T_{c\text{-}min}$ and $T_{c\text{-}max}$ are the daily minimum and maximum temperatures of air respectively, in which the plant is developed in an interval of 10 to 28 ^{o}C for the Durango highland region. To feed the models, weather information was used daily from the Agroclimatic Network Station from INIFAP.

RESULTS AND DISCUSSION

The maturity days and the thermal requirements to get to the last phenological stage varied in respect to the agricultural varieties and cycles (Figure 1 and 2). Definitely, the bean's phenology modeling will direct in the application of agricultural inputs as well as in the development of cultural work since the majority of decisions are taken in regard to the availability of economical resources without considering the advancement of the phenological cycle of the cultivar.

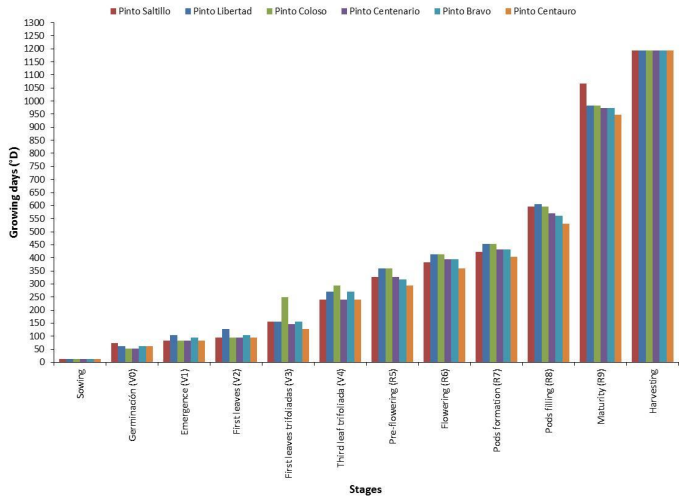

Figure 1. Thermal requirements for the year 2014 of the 'Pinto' bean varieties growing in Durango, Mexico.

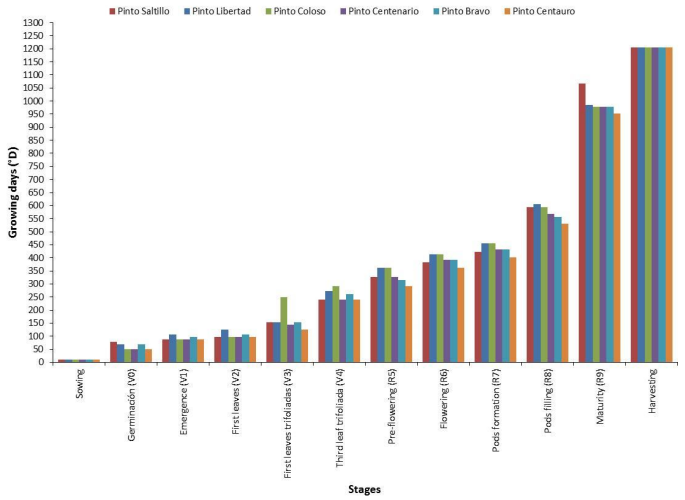

Figure 2. Thermal requirements for the year 2015 of the 'Pinto' bean varieties growing in Durango, Mexico.

The slowest bean variety was for 'Pinto Saltillo' with 107-115 days and a thermal requirement of 1066.08-1067.08 ^{o}D until its maturity with a grain yield average of 2.01 ton/ha. In the two evaluations for agricultural cycles, 'Pinto Saltillo' showed a significant delayed from the pod filling until its maturity in which resulted in a longer cycle with respect to the other evaluated varieties. The earliest bean variety was 'Pinto Centauro' with a duration of 94-97 days until its maturity and a thermal requirement of 948.57-951.72 ^{o}D with a grain yield average of 1.40 ton/ha. The other varieties had duration of 97-101 days until their maturity and a thermal requirement of 973.58-985.92 ^{o}D.

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INDICES OF NODULATION IN DRY BEAN CULTIVARS FOR DIRECT HARVESTING

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INTRODUCTION

In a number of publications (Graham, 1981; Morrison and Baird, 1987; Pereira et al., 1993), the bean cultivars with erect, non-lodging and non-climbing habit type (I and II) are considered to form lower amount of nodules in comparison to the cultivars with habit type III (climbing and lodging). On the other hand, the erect, compact and non-climbing bean habit is incomparably more suitable for mechanized harvesting and simultaneous thrashing, i.e. the harvesting is one-stage and direct. A field trial was carried out with three new cultivars of habit type II^{-a} at Dobrudzha Agricultural Institute with the aim to characterize them for the nodulation ability index.

MATERIAL AND METHODS

The nodulation ability of three dry bean cultivars – Blyan, Ustrem and Vezhen was followed for two years in the trial field of Dobrudzha Agricultural Institute, Bulgaria. These cultivars were developed especially for the trait suitability for direct mechanized harvesting. Their growth habit type is II^{-a} (Debouck and Hidalgo, 1986), with erect, non-climbing plant which forms pods higher on the stem. The first two cultivars are with white seeds, weight per seed 328 mg and 305 mg, respectively, and the third cultivar has colored light brown seeds (Pinto type), with 380 mg weight per seed. These were the followed nodulation indices: life cycle of nodules (beginning and end in days), number and dry weight of nodules per plant at budding stage - R_5 (Fernandes et al., 1986). The soil in the experimental field was leached chernozem (*Luvic phaezem*) with neutral reaction and mass distribution of natural strains of nodule bacteria on bean (*Rhizobium leguminosarum biovar. phaseoli*). This soil type possesses high natural fertility and is very suitable for growing of dry bean.

RESULTS AND DISCUSSION

The first year of the trail was very favorable for the growth and development of bean. The amount and distribution of rainfalls during the growth season was adequate to the physiological requirements of the crop. Yields were high: 2800 – 3000 kg/ha. The second year was significantly less favorable due to the long rainless periods during stages V_4–R_5–R_6. The plants remained with subnormal height and seed yield was with about 45 % lower than the yield from the first year. The data in Table 1 show that in the first favorable year the three bean cultivars nodulated for 30 - 33 days, while in the second unfavorable year the period of nodulation was 26 – 30 days. The number of nodules and their dry weight were also higher in the first year than in the second. All three cultivars had equally good nodulation, with slightly higher values of the followed indices for cultivar Vezhen. The values of the analyzed indices of the three cultivars were similar to the very good nodulation ability of the model cultivar Dobrudzhansky 7 (III^{-b} habit type) which had been grown for many years in this region of Bulgaria. It should be pointed out that this cultivar has later growth cycle (with 12 to 15 days) than the three new cultivars tested, and according to some authors the cultivars with late growth cycle have better nodulation indices (Parck and Byttery, 1981; Brito Fereira et al., 2014).

Table 1. Lifecycle of nodules, days

Cultivars	Begining, DAS#			End, DAS			Total, days		
	2014	2015	Average	2014	2015	Average	2014	2015	Average
Blyan	25^{ns}/a	24^{ns}/a	24.5	55^{ns}/a	50^{ns}/b	52.5	30^{ns}/a	26^{ns}/b	28.0
Ustrem	25^{ns}/a	24^{ns}/a	24.5	55^{ns}/a	51^{ns}/b	53.0	30^{ns}/a	27^{ns}/b	28.5
Vezhen	24^{ns}/a	23^{ns}/a	24.5	57^{ns}/a	53*/b	55.0	33*/a	30*/b	31.5
D-7	24	24	24.5	58	54	56.0	34	30	32.0

- DAS – days after sowing; D-7 - Dobrudzhansky 7
Significant at p ≤0.05, 0.01 and 0.001 respectively: * and *ns* - cultivars differences;
letters *a*, *b* and *c* - years difference

Table 2. Number of nodules (NN) and nodule dry weight (NDW)

Cultivars	NN, plant^{-1}			NDW, mg plant^{-1}		
	2014	2015	Average	2014	2015	Average
Blyan	40.5 ^{ns}/a	24.6 ^{ns}/c	32.5	79.1 ^{ns}/a	31.5^{ns}/c	55.3
Ustrem	41.2 ^{ns}/a	24.1 ^{ns}/c	32.6	80.3 ^{ns}/a	32.1^{ns}/c	56.2
Vezhen	42.3 ^{ns}/a	27.2*/c	34.7	84.5*/a	39.3**/c	61.9
D-7	43.0	28.0	35.5	83.9	38.6	61.2

D-7 - Dobrudzhansky 7
Significant at p ≤0.05, 0.01 and 0.001 respectively: * and *ns* - cultivars differences;
letters *a*, *b* and *c* - years differences

CONCLUSION

The three cultivars formed nodules equally well, with slightly better values of the analyzed indices life cycle, number and dry weight of nodules for cultivar Vezhen. The conditions of the year had significant effect on the investigated indices. The values of the analyzed indices of the three cultivars were similar to the very good nodulation ability of the model cultivar Dobrudzhansky 7 (III^{-b} habit type).

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ADVANCES IN BREEDING STRESS TOLERANT, MARKET DEMANDED CANNING BEANS IN KENYA

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INTRODUCTION

Although canning beans have been grown in eastern Africa since early 1950s, little work has been done to develop improved bean varieties that combine tolerance to biotic and abiotic stresses with canning quality. Consequently, bean varieties such as Mexican 142 (the 'ruling varietiey') still dominate production despite its susceptibility to rust, anthracnose, common bacterial blight and drought. Navy bean has been the main type of canned beans in eastern Africa (Macartney, 1966; CIAT, 2001; Assefa et al, 2007). However, other market classes including the large seeded red mottled, red kidney, small reds, sugars and pinto are being canned to meet diverse consumer preferences (Kimani et al, 2012). Our objective is to develop new bean varieties which combine high yield potential, drought tolerance and resistance to major biotic stresses with high canning quality.

MATERIALS AND METHODS

Study materials were 427 lines representing the Andean and Mesoamerican gene pools and the major market classes grown in east, central and southern Africa. Field trials were conducted for two seasons during the 2012 long-rain and 2012/2013 short-rain seasons. In the first season, 427 locally developed lines from seven market classes and check varieties were planted at Kabete and Thika under irrigated and rainfed conditions in a split plot design. Test lines were also subjected to participatory variety selection with men and women farmers. Selections from the first season were evaluated under rain-fed conditions at four sites (Kabete, Nakuru, Thika and Tigoni) using a 5x5 lattice design. Based on results from the first season and visual selections during second season, 29 lines of different market classes were selected for canning quality evaluation to identify lines combining superior agronomic traits and processing qualities. Mexican142 was used as control. Canning evaluations were conducted following procedures of Uebersax and Hosfied (1996) and Loggerenberg (2004). Laboratory canning tests were conducted at the Pilot Food Processing Plant, University of Nairobi. Data was subjected to analysis of variance using Genstat software (version 15). Fisher's protected least significant difference was used for mean separation.

RESULTS AND DISCUSSION

Results showed there were significant differences in drought tolerance, yield potential, resistance to disease and canning quality among the lines. Drought stress reduced grain yield of the bean genotypes by 18 to 31%. Several new lines out-yielded local and international drought checks by as much as 100% in drought stressed conditions. Grain yield under stress was positively associated with pod partitioning index (r=0.89***), pod harvest index (r=0.40**), and stem biomass reduction (r=0.32**). Fourteen new lines were rated superior to industry standard check variety Mex 142, for agronomic potential, drought tolerance, combined resistance to angular leaf spot, rust, anthracnose, bean common mosaic virus, culinary and canning characteristics (Table 1). These new lines have the potential of increasing productivity, incomes of smallholder farmers, and ensure regular supply and diverse value added products for the processing industry.

Table 1. Grain yield, reaction to diseases, cooking time and water uptake of the new candidate canning bean varieties developed at the University of Nairobi.

Candidate variety	Market class	Mean grain yield	Disease reaction*				Cooking Time (min)#	Water uptake (%)
		kg ha	Root rots	ALS	CBB	Anth.		
KCB13-01	Red mottled	2336	2	3	2	3	29.3	104.0
KCB13-02	Red mottled	2529	3	3	3	5	36.1	115.5
KCB13-03	Red kidney	2617	3	3	2	5	33.9	101.1
KCB13-04	Red kidney	2771	2	2	3	5	34.8	115.3
KCB13-05	Speckled sugar	2732	2	2	2	2	34.6	104.3
KCB13-06	Speckled sugar	2934	2	3	2	3	32.1	101.8
KCB13-07	Small red	2398	3	3	2	5	30.2	105.5
KCB13-08	Small red	2278	2	3	3	3	33.8	93.6
KCB13-09	Navy	2663	2	3	2	2	26.5	99.2
KCB13-10	Navy	2752	2	3	2	2	25.9	129.0
KCB13-11	Navy	3071	3	2	2	3	31.4	112.7
KCB13-12	Navy	2902	2	2	3	3	28.3	102.4
Check								
Mex 142	Navy	1876	7	3	4	2	47.3	89.7

* Disease scores: 1-3=resistant, 4-6 intermediate and 7-9, susceptible; ALS= angular leafspot, CBB= common bacterial blight, and anth.= anthracnose.

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BREEDING RUNNER BEAN FOR GRAIN YIELD, DISEASES RESISTANCE AND SHORT-DAY ADAPTATION IN EASTERN AFRICA

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INTRODUCTION

Runner bean (*Phaseolus coccineus* L), also known as butter bean, is grown in high altitudes of eastern Africa where common bean (*Phaseolus vulgaris* L) is poorly adapted (Suttie, 1969). Productivity of runner bean is poor because no improved short-day varieties are available. Farmers rely on low yielding landraces, which are susceptible to diseases (Kahuro, 1990). The better yielding long-day vegetable type varieties are poorly adapted to tropical conditions (Kimani et al, 2009). Our objectives were to develop breeding populations and select for new short day runner bean lines combining high grain yield potential with resistance to diseases, suitable for cultivation under tropical conditions.

MATERIALS AND METHODS

Four populations were developed from crosses between a long day variety (White Emergo) and four short-day grain type runner bean landraces at Kabete Field Station. The single crosses were advanced to F_5 generation as population bulks at Ol Jorok, Subukia and Kabete. Starting F_5, single plants were selected and advanced through single pod descent method. One hundred thirty-nine $F_{6.8}$ lines were evaluated in a randomized complete block design with three replicates at Kabete (1860 masl) and Ol Jorok (2300 masl) in 2012 and 2013. Data was collected on flower set, duration to flowering, reaction to diseases, pod yield and grain yield and analyzed using Genstat software version 14. Scoring for plant vigour and diseases was based on 1 to 9, where 1-3 is resistant/vigorous, 4-6 intermediate and 7 to 9 susceptible/ poor vigour.

RESULTS AND DISCUSSION

Results showed considerable variation for plant vigour, racemes per plant, grain colour, pod set, pod length, reaction to diseases and grain yield. White Emergo showed delayed second flowering but failed to produce the first flush of flowers (50 days after planting), while all short day parents flowered normally within 36 to 53 days. Number of racemes per plant during the first flush of flowers varied from 3 to 13 with a mean of 7. Each raceme had 10 to 30 flowers. Major diseases observed were rust, common bacterial blight (CBB) and bean common mosaic virus (BCMV) at Kabete and Ol Jorok, and powdery mildew at Kabete. CBB and BCMV were most severe at both sites. However, the test lines showed resistant reactions to rust, CBB and BCMV. Grain yield varied from 2908 to 10,350 kg ha^{-1} with a mean of 6531 kg ha^{-1} (Table 1). The new lines had a yield advantage of up to 67% compared with local short-day checks. Two checks (OLJ DWF 1 and 3) failed to produce a second flush of flowers. Twenty lines and two check varieties (Nyeri1 and OLJ DWF 3) failed to produce the second flush of flowers at Kabete. The poor flower set at Kabete was probably due to severe mid-season moisture stress which caused abortion of flower buds. These results indicate that selection method was highly effective in developing new short-day runner bean lines combining resistance to disease, plant vigour and high grain yield. New high yielding short-day runner bean varieties with resistance to major diseases and tropical adaptation can be developed from these lines and improve productivity in smallholder farms.

Table 1. Plant vigour, reaction to diseases and grain yield of new runner bean lines grown at Ol Jorok (OJ) and Kabete (KAB), Kenya in 2013.

Genotype	Plant vigour		BCMV		CBB		Rust		Grain yield (kg ha^{-1})
	OJ#	KAB#	OJ	KAB	OJ	KAB	OJ	KAB	
SUB-OL-RB13-323-2	1.7	1.7	3.0	1.0	1.7	1.0	1.0	1.0	9908
KAB-RB13-312-160	1.7	2.3	3.0	1.0	2.0	1.0	1.0	1.0	9883
KAB-RB13-310-162	2.3	1.7	3.3	1.0	3.3	1.0	1.3	1.0	9575
KAB-RB13-343-184	2.3	3.0	2.7	1.0	1.7	1.0	1.0	1.0	9514
KAB-RB13-405-196	2.3	3.0	3.0	1.0	3.3	1.0	1.0	1.0	9465
KAB-RB13-364-212	3.7	1.7	2.3	1.0	1.7	1.7	1.3	1.0	9287
KAB-RB13-297-144	3.0	3.0	3.0	1.0	2.3	1.0	2.7	1.0	9199
KAB-RB13-303-146	2.3	3.7	3.0	1.0	1.7	1.7	1.3	1.0	9019
KAB-RB13-46-124	1.7	3.0	2.7	1.0	2.3	1.0	1.3	1.0	8933
KAB-RB13-329-163	2.3	3.7	3.7	1.0	3.3	1.0	1.0	1.0	8910
Checks									
KIN 3	5.7	3.7	4.7	1.0	5.0	1.0	3.0	1.0	3947
KIN 2	2.3	3.7	4.0	1.0	2.7	1.0	2.0	1.0	2573
OLJ DWF 1	3.7	3.7	4.0	1.0	4.3	1.0	2.3	1.3	na
NYERI	3.0	3.0	3.7	1.0	3.0	1.0	1.0	1.3	na
OLJ DWF 3	2.7	4.3	4.0	1.0	3.7	1.0	1.7	1.0	na
Trial Mean	2.1	2.6	2.9	1.0	2.3	1.1	1.4	1.1	6523
$LSD_{0.05}$	1.2	1.2	0.4	0.9	0.6	0.2	0.2	0.1	2469
CV(%)	9.8	9.1	4.0	9.1	6.4	5.4	2.7	4.2	4.4

OJ= Ol Joro Orok, KAB= Kabete. Plant vigour: 1-3 very vigorous, 4-6 intermediate, and 7-9 is poor. Diseases on a scale of 1 to 9, where 1-3= resistant, 4-6 intermediate and 7-9, susceptible.

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GENETIC VARIABILITY IN LIMA BEAN ACCESSIONS OF THE GERMPLASM ACTIVE BANK

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INTRODUCTION: The formation of an germplasm active bank is of paramount importance for plant breeding, since it stores all the genetic heritage of a species (Borem and Miranda 2013). The study of this diversity results in increased genetic variability, which is considered the raw material of all plant breeding program. Thus, this study had as objective the study of genetic diversity in lima bean accessions of the Active Germplasm Bank of the Federal University of Piauí (BAG UFPI), based on agronomically important traits.

MATERIALS AND METHODS: Eight lima bean acesses of BAG UFPI (Table 1) were characterized in the experimental area of the Department of Plant Science in the Centre of Agricultural Sciences of UFPI (located 05º05'S, 42º05'W and altitude of 72.7 m), in 2015. Those accessions were collected in Ceará, Maranhão, Piauí and Paraíba states, Brazil. The experiment was conducted in a randomized block design with four replications. The plots consisted of four rows of 5 m, spaced 0.80 m x 0.70 m. As the accessions have indeterminate growth, corn was used as a tutor. We evaluated the following characters: number of days to flowering, length of the pod (mm), width of the pod (mm), thickness of the pod (mm), number of seeds per pod, hundred seeds weight (g), grain yield (kg ha^{-1}). The genetic diversity among accessions was determined by the average distance method among groups (UPGMA). Furthermore, we estimates the phenotypic correlation coefficient among characters.

Table 1. List of the lima bean accessions from the Germplasm Active Bank of Federal University of Piauí characterized in Teresina, Piauí state, 2015.

Access	Code BAG	Origin	Seed color
1. Fava branca	UFPI 791	Pedra Branca - CE	White
2. Fava branca	UFPI 797	Riachão - MA	White
3. Fava branca	UFPI 798	Riachão - MA	White
4. Fava branca	UFPI 799	Nova Colina - MA	White
5. Boca de moça	UFPI 806	Palmeiras - PI	Variegated (White and brown)
6. Fava branca	UFPI 815	Picuí -PB	White
7. Cara larga	UFPI 817	Remígio - PB	Variegated (White and red)
8. Rosinha	UFPI 832	Remígio - PB	Pink

RESULTS: In the cluster analysis method by the average distance between the groups, the dendrogram (Figure 1) shows the formation of three groups at a level of approximately 30% divergence. The accessions of the same group are genetically similar; in contrast, the accessions of different groups are divergent. Access UFPI 832, named Rosinha, was isolated in a group, showing that differs from the others because it had the lowest grain yield. Estimates of the correlation coefficients between the characters show that the width of the pod (0.7475) thickness of the pod (0.9278), number of seeds per pod (0.8051) and hundred seeds weight are positively correlated and high ($r > 0.7$) with grain yield.

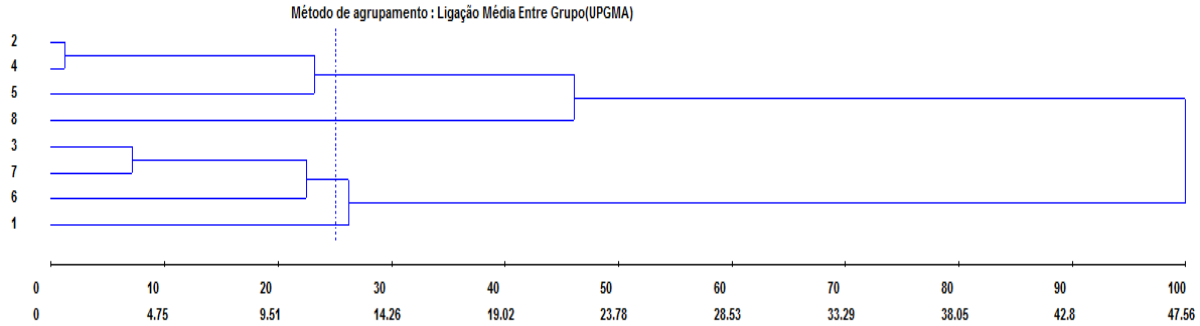

Figure 1 Dendrogram of genetic dissimilarity among eight lima bean accessions (*Phaseolus lunatus* L.) of the Active Germplasm Bank of UFPI obtained by UPGMA method based on quantitative descriptors. Teresina, PI, 2015.

Table 2. Estimates of the phenotypic correlation coefficients between the characters: length of the pod (LP), width of the pod (WP), thickness of the pod (TP), number of seeds per pod (NSP), hundred seeds weight (100SW), grain yield (GY).

	LP	WP	TP	NSP	100SW	GY
Length of the pod	-	0.6911	-0.0177	0.0113	0.5060	0.2283
Width of the pod		-	0.5243	0.4456	0.9073	0.7475
Thickness of the pod			-	0.7797	0.7710	0.9278
Number of seeds per pod				-	0.5772	0.8051
Hundred seeds weight					-	0.8943

CONCLUSIONS: The access of lima bean UFPI 832 differs from the others by having low grain yield. Selection for the grain yield can be made by the production components (width of the pod, thickness of the pod, number of seeds per pod, hundred seeds weight) which are less affected by the environment.

ACKNOWLEDGMENTS
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VIRULENCE DIVERSITY OF *UROMYCES APPENDICUALTUS* IN THE HIGHLANDS OF GUATEMALA

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INTRODUCTION: The common bean is planted throughout Guatemala, especially in the highlands of the South East, North East, and South West regions. In these regions, temperatures fluctuate between 16 y 20 °C and the average rain precipitation is about 1000 mm. These conditions are optimum for the rust disease and bean rust is widespread and recurrent in these regions. The rust pathogen, *Uromyces appendiculatus*, has co-evolved for thousands of years with common beans in Mesoamerica, an area that includes Mexico and countries of Central America. Mesoamerican populations *U. appendiculatus* are characterized by their high virulence diversity and their capacity to produce rust in bean genotypes of Mesoamerican origin (1). Conversely, Andean common beans tend to be resistant to the Mesoamerican virulence diversity (1). Since little is known about the virulence diversity of *U. appendiculatus* in Guatemala, the objective of this study was to collect isolates in the highlands of Guatemala and study their virulence diversity.

MATERIALS AND METHODS: These studies were conducted under field conditions in 12 different locations in the departments of San Marcos, Quetzaltenango, and Chimaltenango of the highlands of Guatemala. The local rust susceptible variety Texel was planted in seven rows, 2 m long, and 0.30 m between rows, in each of these locations. Once the rust disease was visible on Texel, two sets of the 12 differential cultivars were placed between the Texel rows and were left in the field during three weeks. Six of the differential cultivars are of Andean and the other six of Mesoamerican origin. A single seed of each differential cultivar was planted in small Styrofoam cups. When rust symptoms appeared on the differentials, the rust reaction on each of the differential cultivars was evaluated using published methodologies (2).

RESULTS AND DISCUSSION: Eleven different races of *U. appendiculatus* were identified from 12 different locations in three departments of the highlands of Guatemala. Most of these races have not been previously reported from this country. Three races (3-4, 22-37, 22-63) were from Quetzaltenango, four races (13-0, 4-55, 4-62, 4-63) from San Marcos, and four races (4-39, 5-47, 16, 63, 22-61) from Chimaltenango (Table 1).

These results revealed the extensive diversity of the rust pathogen in the highlands of Guatemala. The races that occurred in one department did not occur in the other two departments. Only race 4-51 was found occurring twice in the department of San Marcos. However, the most salient aspect of these results is the confirmation that isolates of the rust pathogen obtained from Mesoamerican beans produce Mesoamerican races that have a strong preference for infecting common bean of Mesoamerican origin (1). Of the 11 races identified, only race 13-0 from San Marcos appeared to be Andean. As reported before, the Mesoamerican races discovered in this study also had a strong preference for infecting Mesoamerican differential cultivars. The Andean differential cultivars were significantly more resistant to these races than the Mesoamerican differential cultivars (Table 1). Furthermore, among the 10 named and mapped genes that confer resistance to the rust pathogen, the genes of Mesoamerican origin have in general much broader resistance to all races of the rust pathogen than the genes of Andean origin. For instance, the Mesoamerican *Ur-5* and *Ur-11* rust resistance genes are resistant to 68, and 87 races of the rust pathogen maintained at Beltsville,

Maryland. On the other hand, the Andean *Ur-4* and *Ur-6* genes are resistant to 35, and 27of the same races. However, in this study involving mostly Mesoamerican races of *U. appendiculatus*, all Mesoamerican rust resistance genes were highly susceptible to the Mesoamerican races from the highlands of Guatemala discovered in this study. The broadly resistant Mesoamerican bean PI 181996 (*Ur*-11) gene was susceptible to 84% of the 11 races from Guatemala. Similarly, the Mesoamerican beans Aurora (*Ur-3*), Mexico 309 (*Ur-5*), and Great Northern 1140 (*Ur-7*) were susceptible to 67%, 92%, and 75%, respectively of the races from Guatemala. Conversely, the Andean bean PI 260418 was resistant to 100% of the same races. In addition, the Andean beans Early Gallatin (Ur-4), Golden Gate Wax (*Ur-6*) and PC 50 (*Ur-9*, *Ur-12*) were resistant to 75%, 67%, and 92%, respectively of the Mesoamerican races from Guatemala.

In conclusion, these results reveal the broad diversity of the rust pathogen in the highlands of Guatemala. They also show that the broadly resistant genes of Mesoamerican origin are vulnerable to Mesoamerican races of *U. appendiculatus*. However, these results also reveal that rust resistance genes of Andean origin confer broad resistance to races of Mesoamerican origin. Finally, these results also indicate that gene pyramiding, that includes Andean and Mesoamerican genes, is an effective way to manage the bean rust disease of common bean.

Table 1. Reaction of common bean cultivars to 12 isolates of *Uromyces appendiculatus* collected from 12 locations in three departments in the highlands of Guatemala

	[1]Reactions of differential cultivars to 12 isolates* of *U. appendiculatus*											
Differentials cultivars	1	2	3	4	5	6	7	8	9	10	11	12
Early Gallatin	5, 4	1, 1	1, 1	4, 4	3, 1	3, 3	1, -	1, 1	5, 5	3, 3	3, 3	3, 3
Redlands Pioneer	1, 1	4,-	4, 4	4, 4	3, 3	3, 3	3, 1	3, 4	3, 1	3, 3	3, 3	1, 3
Montcalm	4, 5	4, 4	4, 4	1, 1	4, 4	4, 4	3, 3	4, 5	5, 4	4, 4	4, 4	4, 3
PC-50	4, 4	1, 1	1, 1	3, 3	3, 3	3, 3	1, 3	3, 3	1, 3	3, 3	3, 3	3, 3
GG Wax	1, 1	4, 4	4, 4	3, 3	3, 3	3, 3	4, 4	4, 4	3, 3	3, 3	3, 3	3, 3
PI 260418	3, 1	1, 1	1, 1	1, 1	3, 3	3, 3	3, 3	3, 3	3, 3	3, 3	3, 3	3, 3
GN 1140	3, 3	4,-	4, 5	1, 3	4, 4	5, 4	5, 5	5, 5	4, 5	5, 4	4, 4	3, 3
Aurora	2+, 2+	5, 5	3, 3	1, 1	4, 4	4, 4	5, 5	3, 3	5, 5	4, 3	4, 4	5, 4
Mexico 309	2++, 2++	4, 4	4, 4	5, 4	4, 4	4, 4	5	5, 5	4, 4	5, 4	4, 4	4, 4
Mexico 235	3, 3	5, 5	3, 3	1, 1	4, 4	3, 3	4, 4	4, 4	4, 4	3, 3	3, 3	5, 5
CNC	1, 1	4, 4	3, 3	3, 3	4, 4	4, 4	4, 4	4, 4	3, 3	3, 3	4, 4	3, 4
PI 181996	1, 1	6,-	4, 4	1, 1	5, 5	4, 4	5, 5	5, 5	3, 4	4, 5	4, 4	5, 5
Races Identified	13-0	22-63	22-37	3-4	4-63	4-55	16-63	22-61	5-47	4-39	4-55	4-62

*Geographical origin of the isolates: 1. Llano Grande, San Pedro de Sacatepéquez, San Marcos; 2. ICTA Labor Ovalle, Quetzaltenango; 3. Municipio de Cajola, Quetzaltenango; 4. Municipio de San Francisco La Unión, Quetzaltenango; 5.Caserio Ixca, San Pedro de Sacatepéquez, San Marcos; 6. Cantón Tojchina, San Antonio Sacatepéquez, San Marcos; 7. Municipio de Parramos, Chimaltenango; 8. Municipio de Tecpan, Chimaltenango; 9. ICTA La Alameda, Chimaltenango; 10. Aldea Piedra Grande, San Antonio Sacatepéquez, San Marcos; 11. Centro, San Antonio Sacatepéquez, San Marcos; 12. Aldea Candelaria Siquival, San Antonio Sacatepéquez, San Marcos.
[1]Standard bean rust grading scale: 2, 2+ = Necrotic spots without sporulation; 3 = Tiny uredinia (sporulating pustules) less than 0.3mm in diameter; f2 = faint and tiny chlorotic spots; 4 = Medium uredinia, 0.3-0.5mm in diameter; 5 = Large uredinia, 0.5-0.8 mm in diameter. HR (2)1, 2, 3, f2 = Resistant; 4, 5, 6 = Susceptible (in gray).

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NEGATIVE IMPACT PROMOTED BY SALT STRESS IN COMMON BEAN SEEDLINGS

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INTRODUCTION

The salinization verified in agricultural soil is a worldwide problem, especially in irrigated areas, where the water is the main agent of salt transport through soil profile. The saline soils present inadequate conditions to seed germination and plant growth, limiting the agricultural production (Melloni et al., 2012). Aim of this study was to determine as salt stress acts in seedlings of common bean.

MATERIALS AND METHODS

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

RESULTS AND DISCUSSION

The IPR-Siriri cultivar presented higher total length in concentrations of 0 and 50 mM of NaCl (Fig. 1 A). Under treatment of 100 mM of NaCl the IPR-139 cultivar has better result, while in concentration of 150 mM the IPR-Siriri returned with higher total length. In concentrations of 0, 50, 100 and 150 mM the IPR-Uirapuru presented higher germination percentages (Fig. 1 B), and only in concentration of 150 mM was obtained significant differences between cultivars. The decrease showed in germination percentage is related to presence of anions and cations, that besides to cause intoxication also will decrease the cell water potential.

The IPR-Uirapuru presented higher tolerance index in total length under concentration of 50 mM of NaCl (Fig. 2 A) and better tolerance index linked to germination when exposed to 150 mM (Fig. 2 B). This higher tolerance to salinity during germination process is associated probably with lower respiratory rates and increase in substance reserves that can be substituted into aerobic respiration process (Rahman et al., 2008). Tolerance index different to each cultivar also were found by Abbas et al. (2013) working with six *Oryza sativa* cultivars.

The reduction in tolerance index to both evaluated parameters reveal that the salt stress is responsible to interfere and consequently to induce negative impact on total length germination of *Phaseolus vulgaris* seedlings.

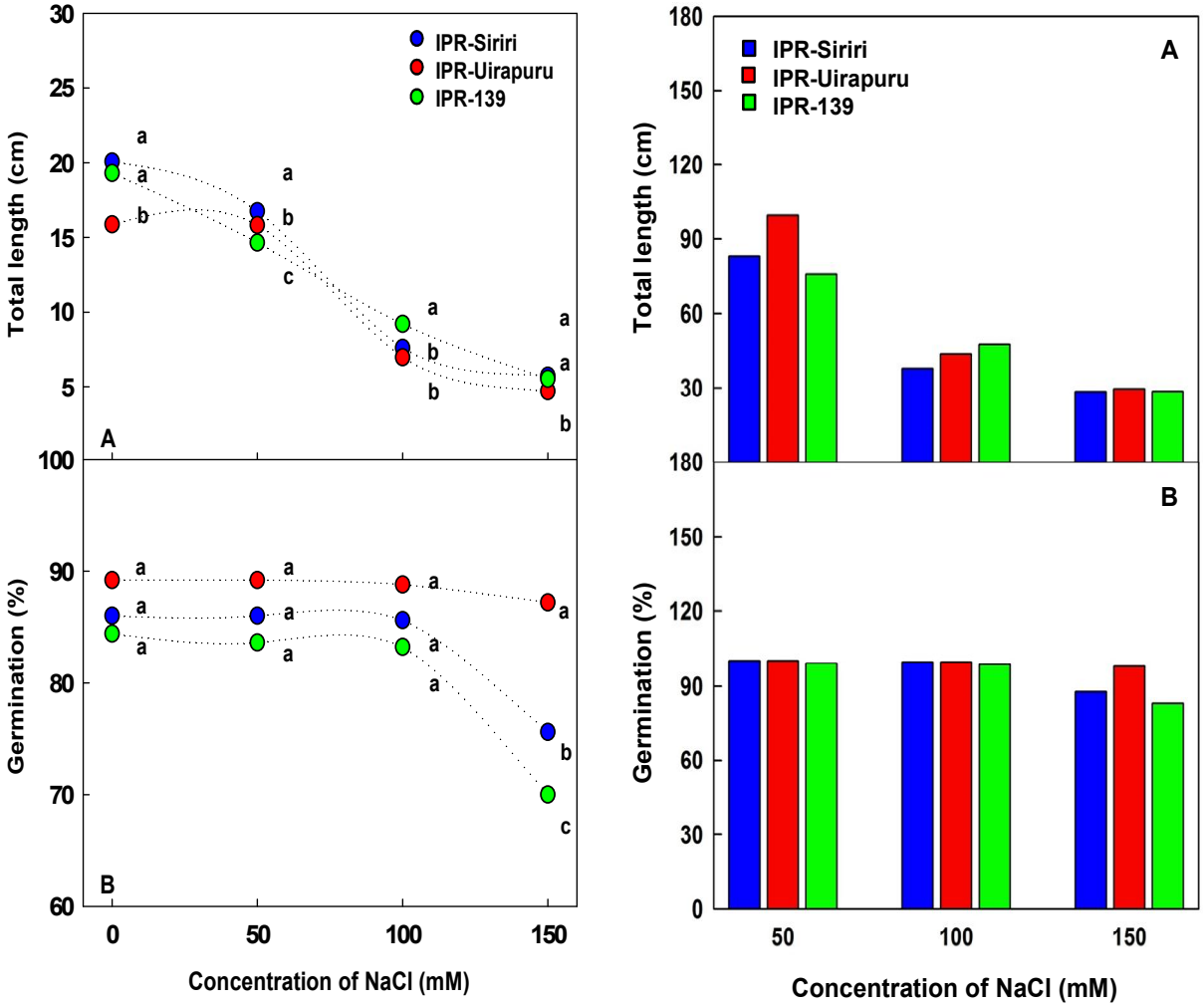

Fig. 1: Total length (A) and germination (B) of three common bean cultivars exposed to concentrations of 0, 50, 100 and 150 mM NaCl. Means followed by the same letter in equal concentrations are not significantly different by the Scott-Knott test at 5% of probability.
Fig. 2: Tolerance index linked to total length (A) and germination (B) of three common bean cultivars exposed to concentrations of 0, 50, 100 and 150 mM NaCl.

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ANTIOXIDANT AND ALPHA-AMYLASE ANALYSIS IN SNAP BEAN GENOTYPES USING THE REML/BLUP PROCEDURE

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INTRODUCTION: The consumption of snap bean (*Phaseolus vulgaris* L.) provides phenolic compounds that act as antioxidants, contributing positively to the health of our body (MORAES; COLLA, 2006; DALLAQUA; DAMASCENO, 2011). Moreover, this food containing alpha-amylase enzyme capable of slowing down the absorption of carbohydrates by inhibiting their digestion, favoring thereby control some diseases. Therefore, it is necessary to develop more genotypes adapted antioxidant capacity and the percentage inhibition of the alpha-amylase. Thus, the objectives of this research were to select snap beans genotypes for traits related to total phenolic compounds (TPC), antioxidant activity (AA) and percentage of inhibition of the enzyme alpha-amylase (% IAA) by REML/BLUP procedure.

MATERIAL AND METHODS: Immature pods from twenty-eight different genotypes of snap bean were used. For extraction of samples evaluated the pods were cut and weighed. Each genotype was separately extracted using distilled water at a ratio of 20 g of the sample (pod) per 50 mL of distilled water. All samples were homogenized, after that centrifuged. The supernatant was collected and stored at 20 ° C during the study period. The analyzes were: TPC, AA and % IAA. Regarding the statistical analysis were carried out individual analyzes referring to the values for genotypes of the characteristics used for screening. Used the SELEGEN program - REML/BLUP (Restricted Maximum Likelihood/Best Linear Unbiased Prediction) to estimate and predict genetic values. The BLUP was used to predict breeding values, using variance estimates obtained through REML (RESENDE, 2007).

RESULTS: The deviances values were 1067.61, 980.10 and 728.39 to TPC; % IAA and AA, respectively, and all significant using the chi-square test ($P < 0.05$). These results showed that there is a wide genetic variability among genotypes. Table 1 shows the genetic parameters and general average of genotypes. Table 2 shows the predicted genetic value, genetic gain and new predicted average of genotypes. The results showed that the genotypes Flo, Gina, Oregon Giant Pole and Trail of Tears are among the top ten orders in all the variables evaluated (TPC, % IAA and AA).

Table 1. Genetic parameters estimated for TPC, % IAA, AA and the general average of genotypes.

Estimates	Variables[1/]		
	TPC	% IAA	AA
Gv	2387.59	600.27	357.83
Ev	94.58	68.50	30.22
Pv	2482.16	668.78	388.06
h^2g	0.96 ± 0.21	0.90 ± 0.20	0.92 ± 0.23
Gcv%	26.10	47.96	31.20
Ecv%	5.20	16.20	9.07
General Average	187.24	51.09	60.63

[1/]TPC= total phenolic compounds; % IAA= percentage of inhibition of the enzyme alpha-amylase; AA= antioxidant activity; Gv= genotypic variance; Ev= environmental variance; Pv= phenotypic variance; h^2g= genetic heritability; Gcv%= genetic coefficient of variation; Ecv%= environmental coefficient of variation.

Table 2. Predicted breeding values (+g), genetic gain (g) and new predicted average (NA) estimated for the selected genotypes for total phenolic compounds (TFC), percentage of inhibition of the enzyme alpha-amylase (% IAA) and activity antioxidant (AA).

Genotypes	Variables[1/]								
	TPC			% IAA			AA		
	g	u+g	NA	g	u+g	NA	g	u+g	NA
Celtic	-17.49	169.74	210.06	29.29	80.38	83.94	10.55	71.18	82.00
Contender	4.34	191.58	236.50	-24.25	26.83	58.58	6.95	67.58	78.58
Flo	43.34	230.58	273.99	31.29	82.38	85.13	25.13	85.76	87.13
Fury	-3.63	183.60	227.82	24.48	75.57	81.64	-13.07	47.55	70.50
Galveston	-39.93	147.30	195.89	21.64	72.73	79.53	-14.77	45.86	68.16
Gina	57.57	244.81	284.85	17.83	68.91	77.56	26.12	86.75	87.59
Provider	6.30	193.54	241.49	7.34	58.42	73.47	19.99	80.62	84.97
Royal Burgundy	120.73	307.97	314.90	-35.70	15.38	53.99	28.13	88.76	88.76
Seabiscuit	-66.30	120.93	187.23	3.14	54.23	72.10	-35.82	24.80	60.63
Serengeti	-32.57	154.66	202.55	19.34	70.43	78.52	-4.96	55.66	74.55
Seville	-46.07	141.17	191.68	-12.61	38.48	64.66	-12.33	48.30	71.78
Slenderella	-32.33	154.91	204.94	-9.51	41.58	67.73	-28.72	31.90	61.95
Spartacus	-11.24	176.00	220.69	27.42	78.51	82.85	7.29	67.91	79.58
Speedy	-45.94	141.29	193.70	-28.67	22.42	57.07	-15.61	45.01	66.17
Stayton	-39.93	147.30	198.00	11.42	62.51	76.19	8.89	69.51	80.75
Storm	-34.05	153.19	200.31	-32.47	18.62	55.54	-18.97	41.65	65.15
Strike	-49.38	137.86	189.70	-38.84	12.25	51.09	-24.99	35.63	63.11
Stringless F. Fillet	6.55	193.78	247.49	-36.64	14.45	52.53	11.37	72.00	83.35
Tapia	-2.04	185.20	231.84	7.53	58.61	74.72	0.72	61.34	75.81
Titan	-7.43	179.80	224.12	-3.04	48.05	69.36	5.85	66.48	77.65
Top Crop	22.86	210.10	263.34	1.36	52.45	70.79	20.04	80.67	85.69
Ulysses	-24.36	162.88	207.58	-21.70	29.39	60.02	-15.03	45.60	67.13
Unidor	-11.97	175.26	215.02	-12.36	38.73	66.12	-18.99	41.63	64.21
Venture	18.81	206.05	255.16	38.49	89.58	89.58	5.78	66.41	76.85
Widusa	-11.85	175.39	217.67	-18.19	32.89	63.08	-9.81	50.81	73.16
Zodiac	-16.14	171.09	212.43	-21.48	29.61	61.48	-14.74	45.89	69.27
Oregon Giant Pole	134.59	321.83	231.83	22.56	73.65	80.50	24.35	84.98	86.70
Trail of Tears	77.56	264.80	298.20	32.34	83.42	86.50	26.63	87.26	88.01

[1/]TPC= total phenolic compounds; % IAA= percentage of inhibition of the enzyme alpha-amylase; AA= antioxidant activity.

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EFFECT OF DEFOLIATION ON YIELD OF SNAP BEAN

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INTRODUCTION

All influences that would reduce the photosynthetic area of cultivated plants will be responsible for low crop yield. The leaf area reduction in snap beans affects yield components, due to the changes caused in the physiological activity finally reflecting themselves in yield. So it makes it important to define genotypes with a higher tolerance to defoliation and the degree of defoliation. In the literature, authors describe the yield loss being influenced by the developmental stage (Campelo & Sediyama, 1999). However, there is no more critical stage, but a period from flowering to filling the pods where the degree of defoliation very marked will influence directly in the pod bean yield (Moura 1999). On the influence of defoliation in the production of bean pod yield, was carried out this study in order to quantify the reduction in grain production due to the increase of defoliation.

MATERIAL AND METHODS

We evaluated four pod bean genotypes, Alessa, Feltrin Amarelo® (FA) and Feltrin® undergoing four levels of defoliation (0, 33%, 66% and 100%) applied at R7 stage, which corresponds to pod formation. The experiment was conducted at Londrina, Paraná, Brazil, from March to May of 2015. Were used 36 plots in randomized blocks, constituting 12 plots for cultivating with three replicates for each level of defoliation.

Each plot was two meters long with four lines and spacing of 0.50 cm. At harvest were determined agronomic parameters: number, diameter and length of pods and productivity.

The data obtained were submitted to ANOVA to verifythe significance of the interaction between the levels of defoliation and genotypes, and the effects of each individual factor. Means were then compared by Tukey test ($p \leq 0.05$).

Data were subjected to analysis of variance to verify the significance of the interaction between the defoliation levels and genotypes, as well as the individual effects of each factor. The Tukey test was used to compare means, at 5% of significance.

RESULTS AND DISCUSSION

The number of pods was lower in 100 % of defoliation level in relation to other levels for all genotypes. Among the genotypes was observed similar behavior between them. The length and diameter of the pods following results similar to those observed in variable yield per area (Table 1). The levels of 33 and 66% caused percentage decreases in grain yield of approximately 30% compared to no defoliation. These results agree with those obtained by Costa et al. (2003) in soybeans, which observed that the plants were most affected by defoliation of 33%, 67% and 100%.

The lack of sheets decreases the supply of carbohydrates, that both stems and petioles are unable to meet their demand for vegetables and grains developing. More research is needed even with other genotypes, as this study indicates that there is a difference between materials in relation to tolerance to defoliation. Reichert (2001) described some soybean cultivars more tolerant to continuous and sequential defoliation. In addition, these studies should be done with all registered genotypes and new cultivars should already have this information at the time of registration.

Table 1. Number of pods, pod length and productivity four genotypes pod due to the defoliation levels.

Genotypes	Defoliation levels(%)			
	0	33	66	100
	Number of pods			
ALESSA	266.00 aA	157.25 bB	114.00 bC	99.50 aD
F.A.	241.75 aA	224.25 abB	186.25 aC	95.50 aD
FELTRIN	262.50 aA	236.50 aB	104.00 bC	100.50 aC
	Longpod(cm)			
ALESSA	16.55 aA	14.91 aA	13.34 aAB	12.21 aB
F.A.	13.98 bA	13.31 aA	13.30abA	12.73 aA
FELTRIN	13.21 bA	12.75 aA	12.59 bA	12.22 aA
	Diameterpod(ø)			
ALESSA	1.20 aA	1.06 aB	1.01 aB	0.95 aB
F.A.	0.80 bA	0.79 bA	0.73 bcA	0.69 bB
FELTRIN	0.71 bA	0.71 bA	0.68 cA	0.62 bAB
	Yield (t ha^{-1})			
ALESSA	5438.07 aA	2992.39 bB	2026.60 aBC	1527.53 aC
F.A.	4544.08 aA	4085.60 abB	2819.00 aC	1311.17 aD
FELTRIN	4514.27 aA	3275.08 bB	2930.88 aC	1789.64 aD

Averages followed by the same small letter in the column and capital letter in the row do not differ from each other by the Tukey test ($p<0.05$).

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SNAP-BEAN GERMINATION RATES: A COMPARISON OF WHITE, PERSISTENT COLOR AND COLORED-SEEDED LINES

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A study was conducted at the Oregon State University Vegetable Research Farm in July 2012 to examine snap bean germination rates of different seed color types, with and without Captan fungicide. Seven lines with normal chlorophyll expression and retention (*Pc)* and five lines with the recessive persistent color *(pc)* allele were planted in a completely randomized design with three replications. Among the seven *Pc* types, four had colored seed and three had white seed conditioned by *p* (Table 1). . Seed of the twelve snap bean lines were left untreated, or were treated with Captan 50-WP fungicide (Bonide - active ingredient at 50% is N-Trichloromethylthio-4-cyclohexene-1,2-dicarboximide) 50% applied as a slurry. The seeds were tumbled in the mixture, removed and allowed to dry prior to planting). Plots were evaluated for emergence at approximately two and four weeks, and percent germinated seedlings were recorded. Data were analyzed by SAS using the GLM procedure for analysis of variance. Mean separation was achieved with Fisher's Protected LSD.

The analysis of variance (ANOVA) for percent germination revealed that genotype (entry), fungicide treatment and entry x treatment interaction were all highly significant.

In general, *pc* lines showed a greater treatment effect from fungicides than did non *pc* lines (Table 1, Figures 1 and 2). Untreated seed showed lower germination rates than treated seed in most cases. Among non *pc* types, lines with colored seeds showed less difference between treatments than lines with white seed. In some cases ('Roc D'or', 'OR91G-djv', and 'Titan'), there was no significant difference in treatment effect for percent germination. This is the most likely source of the significant genotype x treatment interaction.

It has been generally recognized that colored snap bean seed has higher levels of germination and emergence under commercial field conditions than white seed. Researchers have speculated that the anthocyanin and other flavonoids present in the testa inhibit fungal attack and colonization, and that the lack of these compounds allows more aggressive attacks by soil borne pathogens. Hagerty et al. (2015) found a QTL for resistance to fusarium root rot associated with the *p* locus. In addition, white-seeded varieties have thinner testae and lack lignin, which may make the seed more susceptible to mechanical damage and imbibitional injury. Before the 20th century, few white-seeded snap bean varieties were used commercially. The rise of commercial canning and freezing in the 20th Century increased the demand for white-seeded varieties. Water-soluble anthocyanins present in color-seeded varieties leaches into and discolors the liquid in the canned product, and is considered commercially undesirable. In a frozen product, cut beans may reveal a colored ring on the seed testa. With the requirement of white seed for commercial planting, the use of fungicidal seed treatments have become widespread.

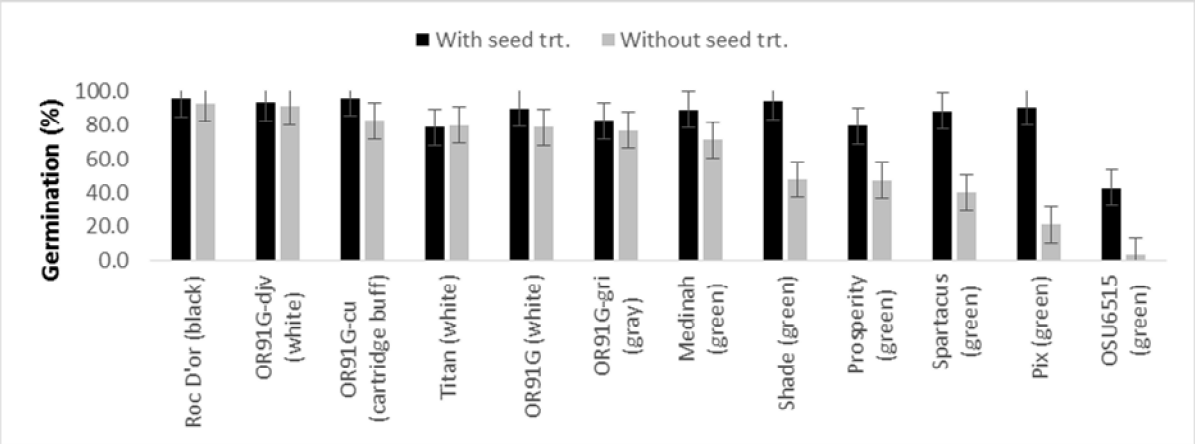
Based on our data, persistent color types show even more severe reductions in germination percentage than do white-seeded types. The reason for the reduced germination percentages of pc types relative to others is not known, but may be due to a thinner testa that is more susceptible to mechanical damage and allowing points of entry for pathogens. Fungicides compensate for the increased susceptibility of *pc* types. While not significantly different in this trial, there is a trend towards reduced germination for *pc* types even with fungicides compared to non *pc* types.

Table 1. Percent germination of selected snap bean cultivars in a trial conducted at the OSU Vegetable Research Farm, Corvallis OR in 2012.

			Percent germination			
Fungicide:			Treated		Untreated	
Cultivar	Seed color	Genotype[z]	Mean	Std Dev	Mean	Std Dev
Roc D'or	black	*P V B Pc C D J*	95.7	4.0	93.0	2.6
OR91G	white	*p V b Pc C D J*	90.0	8.7	79.0	2.6
OR91G-cu	cartridge buff	*P V b Pc c^{u} D J*	96.0	3.6	82.7	5.8
OR91G-djv	white	*P v b Pc C d j*	93.3	2.9	91.0	1.0
OR91G-gri	gray	*p^{gri} V b Pc C D J*	82.7	4.6	77.0	0.0
Prosperity	white	*p* ? ? *Pc* ? ? ?	79.7	4.7	47.3	5.9
Titan	white	*p* ? ? *Pc* ? ? ?	79.0	13.5	80.0	10.1
Spartacus	green	*p* ? ? *pc* ? ? ?	88.3	9.1	40.0	28.8
Medinah	green	*p* ? ? *pc* ? ? ?	89.3	4.6	71.3	8.1
Shade	green	*p* ? ? *pc* ? ? ?	94.0	3.6	48.0	13.7
Pix	green	*p* ? ? *pc* ? ? ?	90.7	5.1	21.0	12.1
OSU6515	green	*p* ? ? *pc* ? ? ?	43.0	3.0	3.0	3.6
LSD 0.05[y]				10.6		

[z]? indicates that a gene is masked by the expression of others and cannot be inferred by test crosses
[y]Fisher's protected least significant difference test ($P \leq 0.05$).

Figure 1. Differences in germination of snap bean lines carrying different seed testa colors when planted with or without fungicide in a trial performed at the OSU Vegetable Research Farm in 2012.



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NUTRITIONAL QUALITY OF BUSH SNAP BEAN IN CONVENTIONAL AND ORGANIC PRODUCTION SYSTEMS

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INTRODUCTION: In terms of nutritional quality, the pods evaluation in different production systems is important when is desired to compare cultivars and identify advantages of one related to another, aiming a more nutritive food. The conventional system is based in the fertilizers and chemical pesticides intense utilization. The organic farming is ruled by the environment stability,by means of the organic matter and natural resouces utilization. Do not exists disponible reports which compare the bushingsnap bean quality in different production systems.So, was aimed to evaluate the nutritional quality of bushing snap bean in conventional and organic production systems.

MATERIAL AND METHODS: In Londrina, Parana State, were evaluated the genotypesIsla ManteigaBaixo®, Isla MacarrãoBaixo®, Feltrin Vicenza AmareloBaixo®, FeltrinMacarrão Napoli®, UEL 1 e UEL 2, in the conventional and organic systems. After harvest, conventional and organic pod sampleswere dried and grinded.Were determinate the levels of calcium, magnesium, phosphorus, potassium, sulfur, iron, cooper, zinc, manganese and crude protein.The experimental design was the completely randomized, with three repetitions for each sample.The variance analysis was conducted applying the F test, with Tukey test at 5% for means comparison.

RESULTS AND DISCUSSION: In the conventional system, the genotype FeltrinMacarrãoNapoli most accumulated potassium in the pod. In the organic, the same performance was observed in UEL 2. Comparing systems, UEL 1 e UEL 2 differed, with more potassium in the organic,which, in general, was more efficient in potassium providing (Table 1).For iron levels, UEL 1 showed higher level in conventional pods, while the genotypes had no difference in organic system. Between systems, the iron concentration was higher in organic for Isla ManteigaBaixoand for UEL 1 in the conventional. For manganese, FeltrinMacarrão Napoli presented higher level in the conventional and was no difference between genotypes in the organic.The conventional system was more efficient in the supply of manganese (Table 1). UEL 2 genotype most accumulated phosphorus. The organic have better efficiency in phosphorus providing.Feltrin Vicenza AmareloBaixo and FeltrinMacarrão Napoli most accumulated magnesium, while UEL 2 and Isla MacarrãoBaixomost accumulated cooper.The conventional system provided more cooper to the plants. FeltrinMacarrão Napoli and the conventional systemwere more efficient in the protein production. Conventional system supplied more calcium to the plants (Table 2). The differences among genotypes in the accumulation ofnutrients certainly are related to genetic load. The superiority in potassiumavailabilityin the organic system can be attributed to the higher level of this nutrient in the soil, verified in the soil chemical analysis. For phosphorus, the biggest quantity of organic matter and your better mineralization rate contributed to help its liberation to plants (Araújo and Machado, 2006).The superiority of the conventional in supplying manganese and calcium probably occurred due to a bigger quantity of those elements in the chemical fertilizer.To cooper, the biggest conventional pods accumulation can be related to the lower organic matter amount in the soil, thus a lower cooper

fixation and higher availability(Dechen andNachtigall, 2006). The bigger protein amount in the conventional is due to the mineral fertilizers utilization, which supply nitrogen in a quicker way then the organics. The nutritional quality pods were higher in the conventional system.

Table 1. Levels of macro and micronutrients in function of genotypes and production systems for bushing snap bean. Londrina, 2014.

Genotypes	Macronutrient		Micronutrients			
	K		Fe		Mn	
	----- g kg^{-1} -----		---------- mg kg^{-1} ----------			
	Conv	Org	Conv	Org	Conv	Org
Isla Manteiga Baixo	25,08 bcA	28,60 bA	206,37 bB	355,40 aA	89,87 bcA	46,97 aB
Feltrin Vicenza Amarelo Baixo	30,45 abcA	33,98 bA	209,40 bA	239,67 aA	109,47 bA	45,57 aB
UEL 2	30,62 abB	43,21 aA	317,57 bA	227,17 aA	71,60 cdA	47,97 aB
Isla Macarrão Baixo	24,24 cA	28,10 bA	218,70 bA	255,77 aA	57,57 dA	38,90 aB
UEL 1	25,59 bcB	29,95 bA	464,97 aA	232,77 aB	66,20 dA	46,17 aB
Feltrin Macarrão Napoli	32,47 aA	33,97 bA	197,87 bA	261,20 aA	180,97 aA	53,87 aB
General mean	28,07 B	32,97 A	269,14 A	261,99 A	95,94 A	46,57 B
CV (%)	8,09		21,14		11,88	

*Means followed by equal letters, lowercase in columns and uppercase in rows, do not differ by Tukey test, at 5% probability.

Table 2. Levels of macronutrients, micronutrients and crude protein of bushing snap bean genotypes. Londrina, 2014.

Genotypes	Macronutrients		Micronutrient	Crude protein
	P	Mg	Cu	
	--- g kg^{-1}---		--- mg kg^{-1} ---	%
Isla Manteiga Baixo	2,66 b	2,48 bc	5,98 ab	22,24 ab
Feltrin Vicenza Amarelo Baixo	3,24 ab	3,08 a	6,25 ab	20,53 bc
UEL 2	3,77 a	2,79 ab	7,57 a	20,94 abc
Isla Macarrão Baixo	2,91 b	2,36 c	6,83 a	19,04 c
UEL 1	3,35 ab	2,53 bc	6,05 ab	21,81 ab
Feltrin Macarrão Napoli	3,18 ab	3,10 a	5,03 b	23,43 a
CV (%)	13,58	7,51	15,65	7,14
Production systems	Macronutrients		Micronutrient	Crude protein
	P	Ca	Cu	
	--- g kg^{-1}---		--- mg kg^{-1} ---	%
Conventional	2,74 b	8,16 a	7,02 a	25,34 a
Organic	3,63 a	6,29 b	5,55 b	17,32 b
CV (%)	13,58	22,07	15,65	7,14

*Means followed by equal letters, lowercase in columns, do not differ by Tukey test, at 5% probability.

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PATH ANALYSIS OF YIELD AND ITS PRIMARY COMPONENTS IN SNAP BEAN

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INTRODUCTION: Correlation among characteristics knowledge has a great importance in breeding programs, mainly when the characteristics simultaneous selection is desired. According to Vencovsky and Barriga (1992), correlations studies do not permit to have conclusions about causes and effects between variables, being the correlation just an associative measure. However, the path analysis method permits to have those conclusions, because it studies the direct and indirect characters effects on one basic variable, whose estimates are obtained by regression equation (Cruz; Regazzi; Carneiro, 2012). The objective of this study was to investigate the correlations among the yield of pods (YOP) and its primary components: pods average number per plant (PANP), pods average weight (PAW), pods length (PL) and pods diameter (PD) in direct and indirect effects, to determine the relative importance of each one of them.

MATERIAL AND METHODS: The experiment were carried at Londrina, Paraná, in southern Brazil, from March to June 2015. Were evaluated six snap beans genotypes of determinate growth habit (Isla Manteiga Baixo, Isla Macarrão Baixo, Feltrin Vicenza Amarelo Baixo, Feltrin Macarrão Napoli, UEL 1 and UEL 2) in the complete randomized block design with three repetitions. The evaluated characteristics were the yield of pods (basic variable) and its primary components (explanatory variables): pod average number per plant, pods average weight, pods length and the pods diameter. Path analysis was performed using genotypic correlation. The multicollinearity diagnosis and the paths analysis were done with GENES software (Cruz, 2007).

RESULTS AND DISCUSSION: The multicollinearity diagnosis involving the five variables resulted in a weak collinearity. The results of the direct and indirect effects of the primary components on the basic variable, the coefficient of determination (R^2) and residual variables effect are represented at Table 1. The value R^2 (99,24%) indicates that the explanatory variables determined almost entirely the basic variables variation. Among the primary components, can be inferred that the PANP and PAW variables magnitude showed positive and high direct effects (0.529058 and 0.455889, respectively), overcoming the residual effect estimative (0.086702). Besides the direct effects, those same variables, showed positive and high indirect effects in PANP via PAW (0.432001) as much as PAW via PANP (0.501335). This way is evident that those variables have great influence in YOP basic variable, thus indicates that the indirect YOP selection through the PANP and PAW variables is effective. Similar results to PAW also were found in common beans (*Phaseolus vulgaris* L.*)* by Furtado et al. (2002).

CONCLUSION: The variables PANP and PAW can be used as auxiliary character in snap beans breeding programs which aim the obtainment of more productive cultivars.

Table 1. Estimates of the primary components direct and indirect effects (PANP: pods average number per plant, PAW: pods average weight, PL: pods length and PD: pods diameter) on yield of pods (YOP).

Characteristics		Estimative
PANP		
	Direct effect on YOP	0.529058
	Indirect effect via PAW	0.432001
	Indirect effect via PL	-0.00012
	Indirect effect via PD	0.011163
	Total	0.9721
PAW		
	Direct effect on YOP	0.455889
	Indirect effect via PANP	0.501335
	Indirect effect via PL	-0.00021
	Indirect effect via PD	0.037387
	Total	0.9944
PL		
	Direct effect on YOP	-0.00265
	Indirect effect via PANP	0.024125
	Indirect effect via PAW	0.036745
	Indirect effect via PD	-0.04172
	Total	0.0165
PD		
	Direct effect on YOP	0.042802
	Indirect effect via PANP	0.137978
	Indirect effect via PAW	0.398219
	Indirect effect via PL	0.002584
	Total	0.5816
Coefficient of determination (R^2) = 0.9924		
Residual effect = 0.086702		

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AGRONOMIC PERFORMANCE OF SNAP BEAN IN CONVENTIONAL AND ORGANIC SYSTEM

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INTRODUCTION

Is increasing the development of organic agriculture, which aims to reduce the adverse effects of the use of chemicals in the ecosystem, through alternative methods of pest and disease control, preservation of the properties of the soil, weed management, mulch and crop rotation (Souza *et al*., 2008; Gliessman 2009; Bettiol *et al*., 2014). However, there are still restrictions on the implementation of organic farming due to lack of information on the agronomic performance of cultivated species, especially with regard to the cost of production and the final yield.

Therefore, this study aimed to compare agronomic aspects and yield of four snap bean genotypes on conventional and organic farming system.

MATERIAL AND METHODS

Were evaluated snap bean genotypes, UEL 2 Feltrin®, Feltrin Amarelo® (FA) and Alessa in organic and conventional systems in the state of Parana, Brazil. The experiments were conducted in March 2015 until December of that year. Used plots with two meters long with four lines and spacing of 0.50 cm, an area with 10 years of organic farming and other conventional cultivation in a randomized block design with four replications. At harvest were determined agronomic parameters: number of plants, number, diameter and length of pods, plant height and yield. Results obtained were subjected to exploratory analyses to assess the assumptions of normality of residuals, homogeneity of variance of treatments and additivity of the model prior to application of ANOVA. Means were then compared by Tukey test ($p \leq 0.05$).

RESULTS AND DISCUSSION

In Figure 1 are some differences between organic and conventional systems bean pod. In terms of evidence between genotypes there is practically no difference between the two systems, and has been a variable behavior in the evaluated parameters.

With respect to the yield observed that the average body system productivity was higher when compared with the conventional FA and UEL 2. However, for Alessa was not observed differences and Feltrin the conventional system was superior.

From a technical point of view, organic farming depends primarily the generation of genotypes with higher levels of hardiness and resistance to diseases and pests to obtain better yields, it is necessary that these materials tolerate these hardships in the field without depending on use pesticides. Preliminary results show that the organic system can and should be adopted , because the second Darolt (2002) combined with the productive performance , the economic indices of the organic system leave no doubt as to its viability. Especially the prices received for organic producers that are almost twice the conventional associated with less variation and more stable over time. Moreover, it is necessary to expand the varieties of research adapted to the organic system.

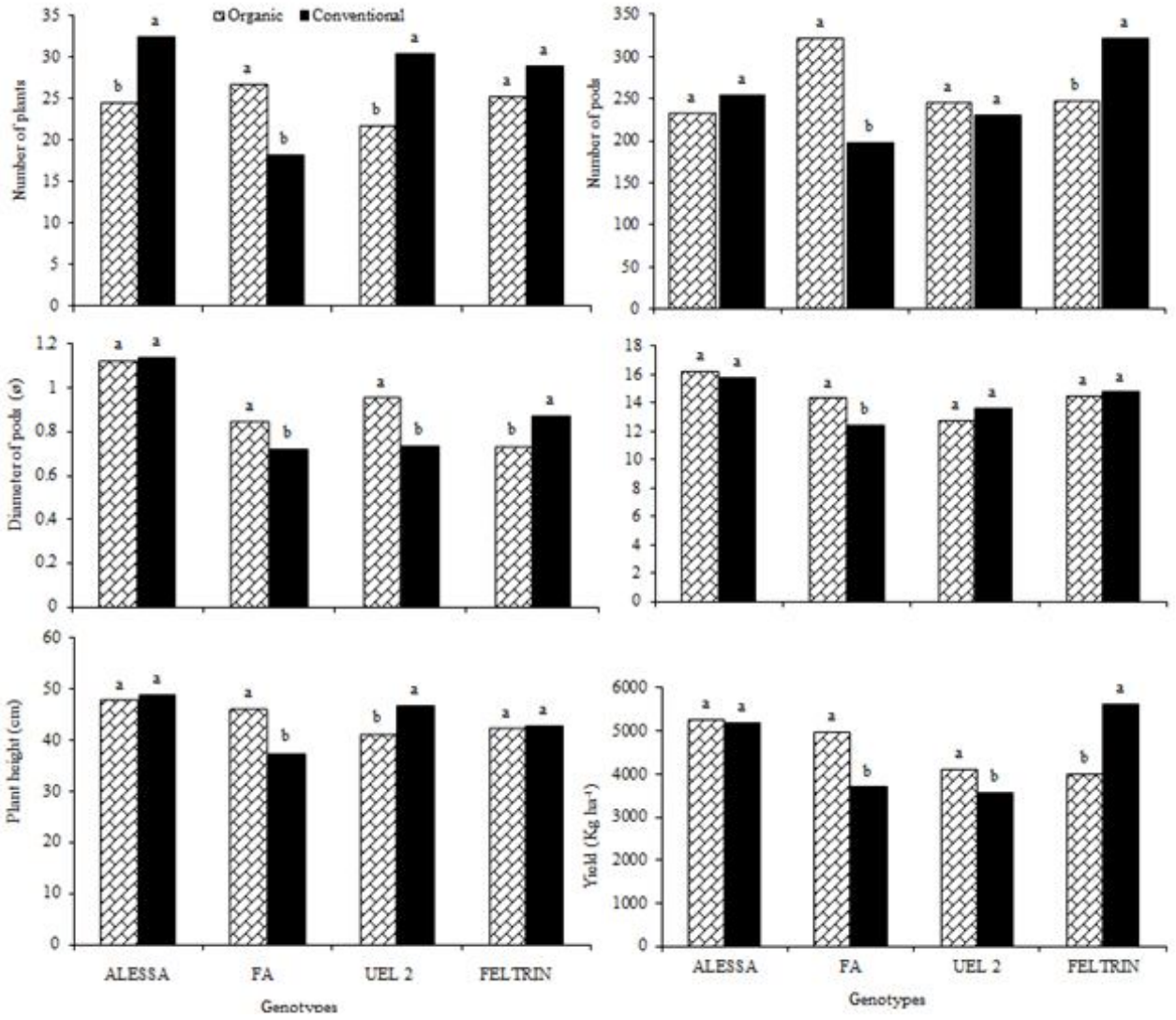

Figure 1. Agronomic performance between snap bean genotypes grown under organic and conventional systems.

Means followed by the same letter info each genotypes are not significantly different by the Tukey test at 5% if probability.

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DEVELOPMENT AND VALIDATION OF NEW SNAP BEAN VARIETIES FOR EASTERN AFRICA

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INTRODUCTION: Breeding for high pod yield, disease and pest resistance, tolerance to abiotic stresses, general adaptation to tropical conditions and acceptable market quality is a critical component of an integrated strategy to address constraints to snap bean production in the east, central and west Africa. In 2006, ASARECA initiated a regional programme to stimulate the development of improved snap bean varieties for smallholder production. The program was based at four institutions in Kenya, Uganda, Rwanda and Tanzania (Kimani, 2006). However, due to funding constraints and lack of skilled personnel, activities slowed down, and eventually stopped in June 2007. However, activities continued at the University of Nairobi, where segregating populations were developed (Kimani et al, 2009. In 2010, promising lines and early generation populations from this program were distributed to programs in five East and Central Africa Bean Research Network (ECABREN) countries (Tanzania, Uganda, Burundi, Sudan and Kenya) and three countries (Cameroon, Togo and Senegal) in West African Bean Research Network (WECABREN). In Kenya, more than 647 single plants with combined resistance to rust, angular leaf spot and anthracnose were selected from crosses and backcross bulk populations in 2010 (Wahome et al, 2011). The selected plants were used to establish $F_{5.6}$ and $F_{6.7}$ progeny rows during the 2011 long rain season (April-August). The objective of this study was to determine the yield potential, pod characteristics and harvest frequency of advanced lines in Kenya.

MATERIALS AND METHODS: Starting from short rain season of 2011 (October- January), 160 $F_{5.6}$ lines with multiple resistance to rust, angular leaf spot and anthracnose were evaluated for marketable pod traits, harvest frequency and pod yield at Thika and Mwea, using participatory variety selection methods with farmers, traders, consumers and exporters. In 2012, promising lines were evaluated for pod yield and pod quality in intermediate and advanced yield trials at the two sites. Trials were laid out in a randomized complete block design with three replicates. A plot consisted of four, 3 m rows. Pods were harvested every other day each week on the inner two rows for up to six weeks. Data collection for disease severity, pod yield, pod quality and grade distribution was performed following procedures described by Wahome et al (2011 and 2013). Data was analyzed using Genstat software, 14th edition.

RESULTS AND DISCUSSION: Results showed that bush snap beans can be harvested three times a week, for up to six weeks. Yields were highest during the first two weeks and gradually decreased later in the season. However, the harvest period can be reduced by environmental stresses such as moisture stress (as happened at Thika) or severe disease attacks, which was observed in susceptible varieties. Results also indicated that harvest period varied with genotypes. New lines were better yielding and more resistant to diseases compared with commercial varieties (Table 1). Number of harvests varied with locations, from 13 at Thika to 18 at Mwea during the 2012 short rain season. There were significant differences in grade distribution across locations, seasons and genotypes

(Table 1). At Mwea, the proportion of extra fine beans was higher during the long rain season (32.7%) compared with 28.3% for the short rain season. However, the fine and bobby market classes were comparable for the two seasons. Mwea produced higher proportion of extra fine and fine beans compared to Thika for the two seasons. This study has demonstrated that local breeding can lead to development of better adapted snap new lines with high pod yield potential, market preferred pod quality characteristics and harvest duration comparable to, or better than the commercial cultivars grown in the region. Ten new candidate varieties are being validated in national performance trials before formal release. These will be the first fully locally developed snap bean varieties which meet market demanded traits.

Table1. Disease scores, pod yield and quality distribution of new snap bean lines grown at Thika and Mwea in Kenya during the 2012/2013 short rain season.

Line/Genotype	Disease score					Total Pod yield (kg ha-^{1})	Extra fine (%)	Fine (%)	Bobby (%)
	ALS*	Anth*	Rust	CBB*	Root rot				
KSB 13-01	3	2	1	3	3	9,835	44.5	19.9	35.5
KSB 13-02	3	2	3	3	1	9,904	58.8	36.1	5.1
KSB 13-03	3	3	2	3	1	9,626	42.4	26.8	30.8
KSB 13-04	3	2	3	2	2	6,329	39.6	40.8	19.6
KSB 13-05	3	2	1	4	1	9,233	54.4	24.7	20.9
KSB 13-06	1	1	1	2	2	11,583	32.8	34.5	32.7
KSB 13-07	3	2	2	3	2	6,837	56.2	32.7	11.1
KSB 13-08	3	3	1	3	1	5,818	56.0	37.0	7.0
KSB 13-09	3	3	1	3	2	7,351	54.5	33.3	12.2
KSB 13-10	3	2	1	3	1	7,235	57.2	31.3	11.5
Paulista	3	3	9	5	1	2721	17.9	16.9	65.2
Amy	7	5	7	5	3	2600	12.5	24.1	63.4
Samantha	6	5	9	3	2	1831	13.9	19.5	66.6
Trial mean	-	-	-	-	-	6992			
LSD 0.05						733.4			

* ALS = angular leaf spot, Ant = Anthracnose; CBB = common bacterial blight and RR = root rot complex.

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NUTRITIONAL CHARACTERIZATION OF SNAP BEAN GENOTYPES USING PRINCIPAL COMPONENT ANALYSIS

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INTRODUCTION: The multivariate analysis techniques are important tools to classify germplasm, order variabilities embedded in accesses, as well as analyze genetic relations among characteristics and improved vegetal material (Iqbal et al., 2008). Between the multivariate techniques, the principal component analysis (PCA) is a powerful methodology to discriminate accesses, which reduces the number of variables to a limited new variables number: the Principal Components (PC). This way, this study objective was nutritionally characterize leafs and pods from six snap beans genotypes by the principal component analysis.

MATERIAL AND METHODS: The experiment was conducted in the city of Tamarana, in the Paraná state, in the south of Brazil, with a complete random blocks design with three repetitions. Were evaluated six snap beans accesses from the Londrina State University breeding program. At the R8 growth stage, leaf and pods samples were collected and were determined the levels of macronutrients: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S). After checking the residual variances homogeneity and the normality, the data were submitted to an analysis of variance (ANOVA), followed by a comparison of the averages by the Tukey test and PCA by means of the R statistical software.

RESULTS AND DISCUSSION: The macronutrients, in leafs and in pods, were significant by the ANOVA with 5% of probability level, indicating that the genotypes presented genetic variability to the evaluated characteristics. Through Table 1, can be verified that the access gen31 presented the highest levels of N, P and K, the access gen11 the highest values of Ca and Mg and the access gen40 the highest level of S in the leaf. However, the access distinction by the pod chemical analysis was reduced, with an exception in the macronutrients N and S, once that the genotypes were assigned in just two groups by the Tukey test. At Figure 1, can be verified the access discrimination in three distinct groups. The first group was formed by the accesses gen40, gen03 and gen31, the second group by the accesses gen25 and gen34 and the third group by the access gen11. The first group showed bigger relation with the levels of N and P in the leafs and P in the pod. The second group presented a positive relation with S in the leafs and in the pod as well as P in the pod. The Nutrients N and Mg in the pod and Ca in the leaf showed a positive relation with the third group. This way, it could be concluded that the principal components analysis permitted the identification of three groups among the accesses, as well as their relations with the studied macronutrients, enabling possible genotypes selections with higher nutritional indexes, besides has proven more efficient in the genotypes discrimination in comparison with the Tukey test.

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Table 1. Average levels of nitrogen (N) phosphorus (P), potassium (K), magnesium (Mg) and sulfur (S) from six genotypes of snap beans.

Genotypes	Leaf[1]					
	N	P	K	Ca	Mg	S
	----------g kg^{-1}----------					
gen 03	33.53 c	2.95 b	17.30 e	9.80 b	1.95 c	0.08 c
gen 11	24.69 f	1.84 cd	31.06 c	10.71 a	2.08 a	0.05 f
gen 25	27.98 e	1.87 c	31.47 c	9.87 b	1.82 d	0.10 b
gen 31	37.62 a	3.40 a	34.97 a	9.24 d	1.98 b	0.06 e
gen 34	28.81 d	1.84 d	32.58 b	9.62 c	1.94 c	0.06 d
gen 40	35.69 b	1.79 e	29.35 d	9.85 b	1.95 c	0.10 a
Genotypes	Pod[1]					
	N	P	K	Ca	Mg	S
	----------g kg^{-1}----------					
gen 03	23.76 b	2.61 b	27.45 a	10.17 ab	1.97 ab	0.06 c
gen 11	39.66 a	1.67 b	10.79 b	10.61 a	2.15 a	0.05 d
gen 25	25.81 b	3.88 a	30.56 a	9.52 b	2.19 a	0.11 a
gen 31	23.76 b	2.46 b	28.80 a	10.38 a	1.91 b	0.06 cd
gen 34	25.40 b	3.88 a	31.06 a	10.35 a	2.06 ab	0.07 b
gen 40	23.76 b	2.09 b	24.57 a	10.62 a	1.99 ab	0.06 cd

[1]Averages followed by the same lower case letter, in the column, are not different by Tukey test at 5% of probability.

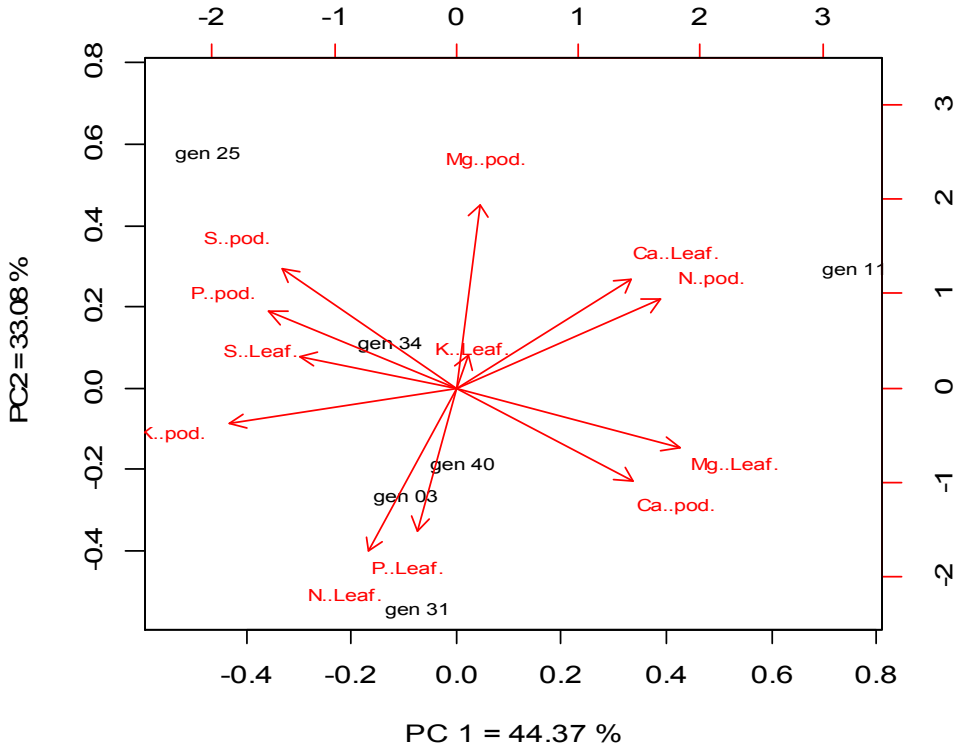

Figure 1. Analysis by principal components for the macronutrients N, P, K, Mg, Ca and S in leafs and pods of six snap beans genotypes.

PHENOLOGY AND GROWTH OF CULTIVATED AND WILD *Phaseolus vulgaris* L.

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INTRODUCTION: The cultivated plants (cultivars) are the product of a process of domestication from wild ancestors carried on by man (anthropogenic). One of the characters commonly sought is an increase of size of those organs of economic importance (the seed in the present case). In contrast, in nature the wild counterpart (without human intervention) produces a large number of smaller seeds which enable it to survive as a species. This basic difference should be reflected in the phenological and morphological traits (Schwanitz, 1966). The wild ancestors of cultivated plants are an important reservoir of germoplasm for plant breeding. The present work is aimed at the phenological and growth patterns. Hydroponic raising of plants will be employed to enable the display of the maximum expression of the genetic potential of the plants.

MATERIALS AND METHODS: The study was conducted in 2014 in a greenhouse of Campus Montecillo, Colegio de Postgraduados (19°30' N, 98°51' O). Flor de Mayo X16441 (CIAT, 1982) a cultivated (C) and S13 a wild (W) bean (registered as the series G24449) provided by Dr. Jorge A. Acosta Gallegos of Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) were employed. Both (C and W) are of indeterminate climbing type of growth. In contrast to the C form, the pods of the W are dehiscent (Delgado *et al*., 1988) and the seeds smaller than those of C. A completely randomized design with two treatments (C and W) and five replications were employed. Steiner nutritive solution (Steiner, 1984) and 18 kg of "tezontle" (inert volcanic cinder substratum) per container were employed. The experimental unit was represented by a container with one plant. Destructive samplings of five plants of each treatment were performed: at the beginning of flowering stage (R6), of pod filling (R8) and at the end of the plant maturity (R9) (CIAT, 1982). At each sampling the root was separated carefully from the substratum and severed from the shoot. Root volume was determined by water displacement. The shoot was separated into its organs and the area of leaflets was registered. Dry weight of root and organs of shoot were obtained after drying at 70 °C during 72 h, in an oven with forced dry air (except for the seeds from R9, since we wanted to maintain their viability). Seed yield and some of its components were determined. Statistical analysis of the data was performed using the *t of Student* with the Infostat program.

RESULTS: Both C and W were epigeous. The vegetative and the reproductive period of C were shorter (12 and 7 d) than those of W (Figure 1), a characteristic often displayed by cultivated forms as indicated by Schwanitz (1966). The ripening of their fruits and seeds in the W extended over a long period. Volume and dry weight of C root were bigger only in R6. In later stages there were no difference between C and W (Table 1). Although the leaflets were smaller for W, the leaf area was larger and the leaf area duration (data not included) longer for the W than those of the C at the R9 sampling. The number of flower buds and the number of flowers and normal pods were higher for W in R8 and R9 samplings (Table 1). The pods of W were smaller than those of C. On the other hand, the number of seeds was also higher in the W type as compared to the C (3067 *vs* 698). The individual seed weight was lower for W than that of C as expected (48.2 *vs* 346.6 mg). However, the seed yield per plant (at 13 % moisture) was higher for the C (243 *vs* 147 g) which compensated for the smaller size of the individual seed.

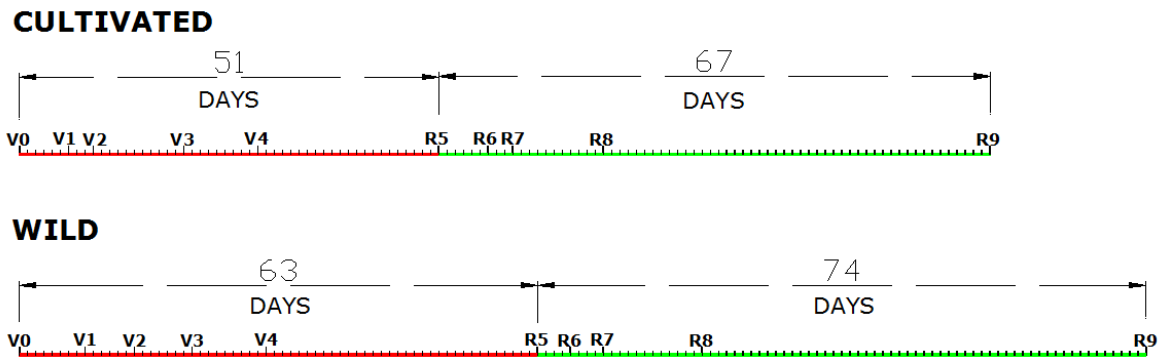

Figure 1. Phenology of *Phaseolus vulgaris* L. cultivar Flor de Mayo X16441 and wild S13. Montecillo, México, 2014. **V0**=germination; **V1**=emergence; **V2**=simple leaves; **V3**=first compound leaf; **V4**=third compound leaf; **R5**=preflowering; **R6**=flowering; **R7**=pod formation; **R8**=pod filling; **R9**=maturation.

Table 1. Comparison of cultivated Flor de Mayo X16441 and wild S13 *Phaseolus vulgaris* L. Montecillo, México, 2014. The data are per plant.

Phenological stage	Type of bean	Leaf area (dm^2)	Shoot dry weight (g)	Root dry weight (g)	Root vol. (cm^3)	Number of flowers buds	Number of flowers	Number of normal pods
Flowering (R6)	**C**	61.83 a	39.49 a	4.55 a	78.7 a	122 a	7.8 a	5.6 a
	W	54.89 a	36.71 a	3.34 b	58.8 b	56.8 b	6.8 a	4.2 a
Pod filling (R8)	**C**	172.45 a	207.28 a	13.73 a	186.5 a	41 b	16.2 b	197.4 b
	W	195.34 a	177.14 b	13.43 a	201.6 a	498 a	180.8 a	453.6 a
Maturation (R9)	**C**	66.08 b	299.17 a	18.95 a	163.2 a	26.2 b	8.2 b	142 b
	W	161.12 a	344.29 a	16.83 a	152.6 a	116.5 a	39.8 a	1092.6 a

C=cultivated; **W**=wild; **vol.**=volume. Different letters in each pair of treatments indicate statistical difference.

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TEPARY BEAN AS A DONOR OF ABIOTIC STRESS RESISTANCE GENES IN COMMON BEAN

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INTRODUCTION

Common beans have limited yield in many locations due to climatic constraints. Modern production has restricted the genetic base and other avenues to gain genetic variability need to be examined in order to improve desirable characteristics such as tolerance to abiotic stress (Kelly, 2010). It is predicted that the bean industry will need to increase bean yield by 30% to keep up with population growth. To do so, untapped wild relatives and closely related species need to be explored to uncover novel genetic variation that may assist with this goal (Porch et al., 2013). Tepary bean, a relative of common bean, is more resistant to many abiotic stresses, including high temperature stress (Gaur et al., 2015), sub-zero temperature stress (Balasubramanian et al., 2004), salt stress (Goertz and Coons, 1991), and drought stress (Beebe et al., 2013). The objective of this study was to determine if genotypes in a population of interspecific hybrids of common bean x tepary bean demonstrate tolerance to water limitation stress and sub-zero temperature stress similar to tepary beans.

MATERIALS AND METHODS

A population of common beans, tepary beans and common x tepary bean (NY5-161*2/W6 15578; BC_2F_6) was used in two different field trials to determine abiotic stress responses in the population.

To determine tolerance to water limitation, 89 interspecific hybrids, 3 common beans and 4 tepary beans were grown in a group balanced split plot design in the winters of 2013-2015 in Isabela, Puerto Rico. Irrigation was provided throughout the growing season, and discontinued to half of the plots at the beginning of flowering to induce a terminal drought stress. Days to flowering, leaf ureide content (a possible proxy for stress), above ground biomass, yield, 100 seed weight and seed germination percentage were all recorded.

The second field trial was conducted in Saskatoon, SK, to determine tolerance to sub-zero temperature stress at the early seedling stage. 116 interspecific hybrids, 3 common beans and 18 tepary beans were seeded in a randomized complete block design bi-weekly starting in August such that at least one cohort would experience a mild frost event as V1 (first trifoliate leaf unfolded) seedlings. Frost events were -1.8°C, -2.0°C and -3.5°C. Following frost, the V1 plants were sampled and germination, leaf ureide content and survival following frost were all recorded.

RESULTS AND DISCUSSION

The tepary bean checks performed better than the common bean checks and the interspecific hybrids, as determined by drought tolerance calculations ($TOL=Yield_{non\text{-}stress}-Yield_{stress}$) (Rosielle and Hablin, 1981). Although a range of responses were observed in the interspecific hybrid population, they were roughly equally distributed to either side of the common bean parent (NY5-161), performing both better and worse under drought stress (Figure 1).

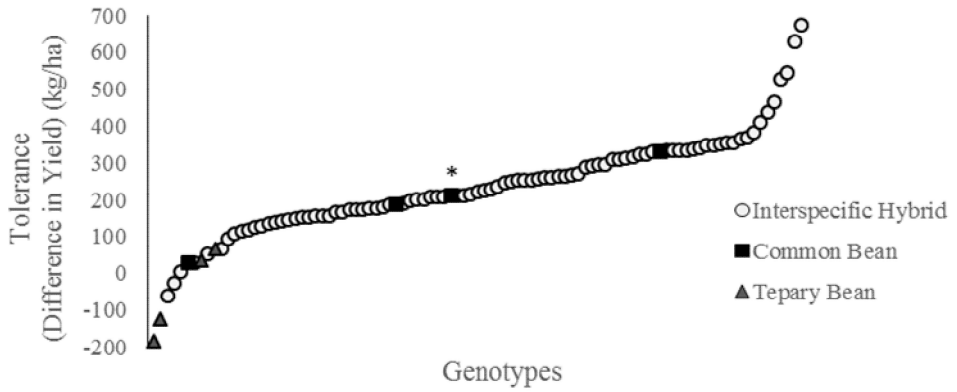

Figure 1: Terminal water-limitation stress in a population of common beans, tepary beans and interspecific hybrids. Tolerance is depicted as the difference between non-stressed yield and stressed yield. Interspecific hybrids are represented as open circles, common bean as closed squares and tepary bean as closed triangles. NY5-161 (the common bean parent) is denoted by an asterisk.

Tepary beans survived the sub-zero temperature stress better than both the common beans and the interspecific hybrids. Sixteen interspecific hybrids had a statistically significant survival rate greater than the common bean parent, determined by a post hoc LSD test (Figure 2).

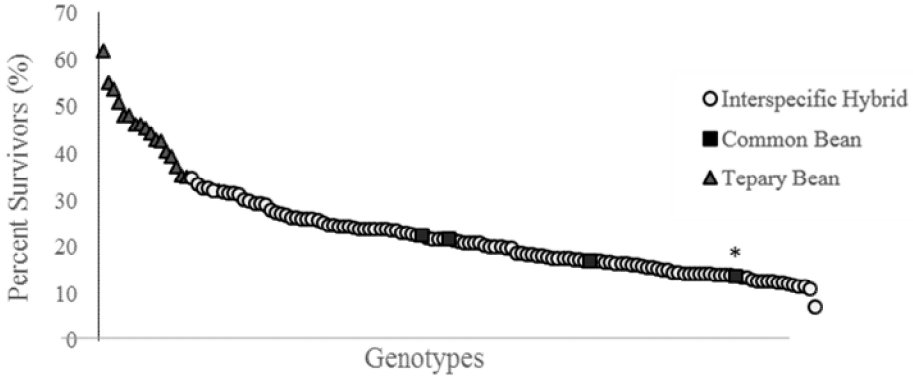

Figure 2: Survival after a sub-zero temperature stress in a population of common beans, tepary beans and interspecific hybrids. The surviving plants were counted a week after the frost. Symbols as in Figure 1. NY5-161 is denoted by an asterisk.

CONCLUSION

The common bean x tepary bean interspecific hybrid population contains genotypes that are more tolerant than the common bean parent to water limitation and sub-zero temperature stress, pointing to a promising outcome from this approach. Many agronomic traits, including yield potential, are lacking in the population, however. The current hybrids which show potential were returned to the breeding program with the intention of improving agronomic traits in the progeny. New hybrids are also being developed.

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YIELD COMPONENT RESPONSE TO WATER STRESS AMONG SIX DRY BEAN GENOTYPES

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INTRODUCTION: To improve the prospects for the sustainability of dry bean production in the Intermountain West, genotypes need to be screened for tolerance to water stress. In other dry bean regions, characterization of yield components in response to drought demonstrated important correlations with yield and drought susceptibility index (Assefa et al., 2013). The objective of this study was to quantify the relationships between yield components and drought tolerance in six Intermountain West cultivars.

METHODS: On 19 May 2015, seed of six genotypes of dry bean were sown in 3-gallon pots (six pots per genotype) in a greenhouse. Soil mix was 33% sand, 33% soil amendment, and 33% native soil. Seed were inoculated with a commercial inoculant. Seedlings were thinned to three per pot and a split-plot design was used with three replicates (drought main factor, genotype subplot). Pots were watered daily or twice daily as needed until the first cultivar started flowering. Soil test indicated N and K deficiency and on selected days, throughout the season, each pot was supplemented with 260 mg of KNO_3 delivered in 100 ml of water. Once the first cultivar exhibited flowers, water-stress was imposed on half the pots. Post-bloom, water-stressed pots received half the water provided to the well-watered. Chlorophyll (via SPAD, third uppermost fully expanded leaf) and relative water content (RWC) were measured periodically and biomass and plant height were determined at maturity. Brown pods were harvested on alternate days as they appeared, kept separated, and hand shelled for determination of seed per pod (data transformed using Box-Cox prior to analysis), seed number and weight, and pod wall weight. Drought Intensity Index (DII) and Drought Susceptibility Index (DSI) were calculated from seed yields per Fischer and Maurer (1978).

RESULTS AND DISCUSSION: Genotype-by-drought interactions, although anticipated, were conspicuously absent for nearly all variables (Table 1). RWC at 46 dap averaged 0.84 and at 53 dap averaged 0.86 but treatment effects were absent. Treatment effects on leaf chlorophyll at 28 dap and 36 dap were absent but at 62 dap, leaves of well-watered plants were significantly ($P = 0.01$) greener (44.8) compared to drought-stressed leaves (39.3). Across dates, chlorophyll concentration of CO-46348 was typically the highest and Long's Peak was typically the lowest. Two cultivars scored as being drought tolerant (CO-46348, DSI = 0.61 and Croissant DSI = 0.54) and four other cultivars were drought susceptible (Bill-Z Centennial, UI-537, all 1.11) and Long's Peak (1.68). CO-46348 has been reported in the literature as being drought tolerant (Brick et al., 2008; Muñoz-Perea et al., 2006). Regression for seed per pod vs. day of pod maturity indicated no differences due to drought or to cultivar but averaged across all factors, seed per pod was reduced by 0.068 seed for each day pod maturity was delayed (data not show). Seed size did not demonstrate any reduction due to delayed pod maturity (data not shown). Pod harvest index (PHI) did not differ across water levels nor was there a genotype-by-water interaction but PHI was significantly higher in UI-537, Bill-Z, and CO-46348 than Long's Peak, Centennial, and Croissant. A genotype-by-drought interaction affected maturity.

Table 1. Yield and yield components of six dry bean cultivars subjected to two watering regimes.

Cultivar	Watering Treatment	Pod Number per Pot	Seed Number per Pot	Seed Weight per Pot (g)	Seed Size (mg)	Pod Harvest Index	Maturity (dap for 65% brown)
Bill Z	Stressed	33	134	38.0	284	77	75
Bill Z	Full	37	162	49.4	304	76	71
Centennial	Stressed	25	101	33.7	336	71	73
Centennial	Full	35	160	49.0	310	73	70
Croissant	Stressed	25	113	37.4	330	72	79
Croissant	Full	35	168	50.9	305	73	80
CO 46348	Stressed	25	100	35.8	356	77	76
CO 46348	Full	19	131	42.1	321	77	78
Long's Peak	Stressed	22	97	31.3	323	73	79
Long's Peak	Full	28	129	41.1	315	72	79
UI 537	Stressed	36	125	40.6	330	77	75
UI 537	Full	41	195	55.9	292	79	70
LSD (0.05)		9	38	2.5	42	4	1.3

At maturity (averaged across genotypes), well-watered plants exhibited greater plant height (101 cm vs. 87 cm), seed number per pot (157 vs. 112), seed yield per pot (48.0 vs. 36.2 g), number of seed per pod (4.7 vs. 4.3), and mature dry root mass (4.3 g vs. 3.6 g per pot) but seed size was unaffected (327 vs. 308 mg). Mature plant root mass in Croissant (6.0 g per pot) exceed that of Centennial (4.5 g) with Bill-Z and CO-46348 exhibiting the least root mass (2.7 and 2.5 g, respectively). In summary, due to the lack of significant genotype-by-drought interactions, this experiment was somewhat inconclusive. However, differences in DSI among these genotypes that matched their available results in field studies, suggests that greenhouse screening for drought tolerance is effective.

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DEVELOPING A WATERPROOF DRY BEAN (*PHASEOLUS VULGARIS* L.): IDENTIFYING GENOTYPES AND GENOMIC REGIONS ASSOCIATED WITH WATERLOGGING TOLERANCE

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INTRODUCTION: Dry bean (*Phaseolus vulgaris* L.) is one of the most sensitive crops to water-stress conditions. Excess water affected ~14% of North Dakota and Minnesota in 2013 (Knodel *et al*., 2014). Waterlogging occurs whenever the soil is so wet that there is insufficient oxygen in the soil pore space for plant roots to adequately respire. Other gases detrimental to root growth, such as CO_2 and ethylene, also accumulate in the root zone and affect the plants (Bailey-Serres, and Voesenek, 2008). Crops differ in their demand for oxygen, so there is no universal level of soil oxygen that can identify critical waterlogging conditions. In addition, a plant's demand for oxygen in its root zone will vary with its stage of growth. Therefore, the extent of crop damage is influenced not only by the crop developmental stage but also by the amount of time the plants are under low-oxygen conditions. On the other hand, flood-tolerant plants survive temporary waterlogging by complex morphological, anatomical, and physiological interactions. Some of the physiological effects of flooding and waterlogging resemble what it has been seen under drought conditions. The objective of this study was to develop a reliable greenhouse procedure to identify the tolerant and susceptible genotypes to waterlogging stress at early growth stages.

MATERIALS AND METHODS: We screened the Middle-American Diversity Panel (MDP) and Andean Diversity Panel (ADP), which consisted of 272 and 280 genotypes, respectively. We employed a Randomized Completely Block Design (RCBD) with a split plot arrangement, in which the stress and non-stress conditions were two levels of the main plot and the genotypes were considered as sub-plots. Four replications and two samples per replication were measured for this experiment. Seeds from each line were planted in small pots, filled with loamy sand soil. All the lines were germinated in a well-drained condition. Upon their start of V2 phenological stage, the stress-designated plots were exposed to flooding stress. To mimic the flooding conditions in the stress plots, pots were placed in flat storage containers and filled with the water until the water level reaches 4-5 cm above the soil level. Stress-designated plots were kept in flooding conditions for 10 d, while the control plots were grown under well-drained conditions. After 10 d, the water in stress plots was drained. Traits such as chlorophyll content (using SPAD 502) and adventitious root scores were measured at this stage. The whole plant including the above ground tissues and roots are carefully harvested and washed and stored in the paper bags then dried in 100^0F for two weeks. Upon drying process, other traits are measured as: 1) Total weight (g): dry matter weight of whole plant (g). 2) Shoot weight (g): the above-ground tissues including stem and leaves 3) root weight (g): total root tissue after careful removal of soil 4) shoot percentage: the above ground portion of the whole plant which was calculated by shoot weight/total weight 5) root percentage: the root portion of the whole plant and 6) hypocotyl length (cm).

RESULTS AND DISCUSSION: Although total weight and shoot weight of both gene pools were reduced in response to waterlogging (Figure 4), these reductions were more drastic in the Middle-American (63% reduction) gene pool compared to the Andean (43% reduction). For shoot weight, 56% and 28% reductions were detected for MDP and ADP, respectively (Figure 1). For root weight, however, a 76% reduction was observed for both MDP and ADP gene pools.

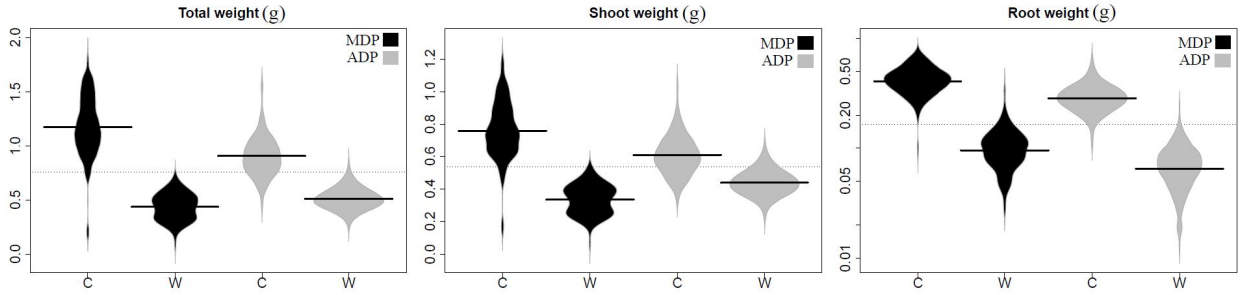

Figure 4. Beanplots indicating the frequency distribution of two gene pools in control (C) and waterlogged (W) conditions for three traits. The mean of each distribution is represented by the solid line and the overall mean of each trait across all gene pools and conditions is indicated by the dashed lines.

Results suggest that different gene pools are using different mechanisms to cope with waterlogging. In the MDP, the susceptible genotypes, although most remained alive, showed chlorosis symptoms. However in the ADP, sensitive genotypes did not survive regardless of chlorophyll content. Based on the SPAD index and survival rate, we were able to choose the potential most tolerant lines (lines with extreme responses to stress) within both MDP and ADP (**Error! Reference source not found.** and 2, respectively). Based on this analysis, the most tolerant lines among MDP were identified within the pinto and great northern market classes (bolded and underlined), however the most tolerant genotypes in Andean gene pool are from North America.

Table 1. The most tolerant lines in each MDP market class based on SPAD index. Highlighted lines were the best, overall.

Pinto		Great Northern		Pink		Red		Black		Navy	
Line	SI[a]	Line	SI	Line	SI	Line	SI	Line	SI	Line	SI
CDC_Camino	48	**Sawtooth**	45	S08418	48	USRM_20	40	black diamond	47	Schooner	30
Arapaho	46	BelNeb_rr_1	51	Roza	42	UI_228	38	Shiny crow	37	Verano	32
Chase	46	Emerson	45	AC_Early_Rose	42	Amadeus_77	34	CDC Jet	34	Oac_rex	33

a SPAD index

Table 2. The most tolerant lines in each origin of ADP based on survival rate. Highlighted lines were the best, overall.

East Africa			Caribbean			North American			Africa		
ID	Line	SR[a]	ID	Line	SR	ID	Line	SR	ID	Line	SR
ADP460	PI331356B	2.6	ADP431	Gurabo5	3.1	**ADP604**	**1062V98**	**4.4**	ADP30	RH6	3.6
ADP470	PI527508	2.6	ADP436	JB178	2.9	**ADP603**	**Wallace773V98**	**3.9**	ADP124	Maini	3.4
ADP471	PI527537C	2.6	ADP429	PR9920171	2.6	**ADP650**	**K42**	**3.9**	ADP54	W6_16447	3.2

a Survival rate

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SUSTAINABLE SYSTEM FOR DRY BEAN PRODUCTION UNDER DRYLAND CONDITIONS

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INTRODUCTION. Soil erosion is a major cause of environmental degradation and one of the most serious limiting factors facing rainfed agriculture to achieve agricultural sustainability in the semiarid region of North Central Mexico. In this region, 1.2 million hectares are involved to produce dry beans, without any agronomic practice which helps harvest, retain, and use rainwater efficiently. It is known that the establishment of living hedgerows, terraces contour, and conservation tillage are effective practices to conserve soil and water by catching runoff, sediment, and nutrients. When these practices are integrated to the dry beans production system, they are an alternative to improve efficiency in the use of rainwater, reduce soil erosion, and allows to modify the production techniques to increase production in a sustainable manner the productivity and profitability of agriculture system in rainfed critical environments.

MATERIALS AND METHODS. During the summer of 2015, an experiment with rainfed dry bean was conducted in Sandovales, Aguascalientes, Mexico, which is located at an altitude of 2040 m above sea level, with 466 mm of rainfall in the growing season, and an anual average temperature of 16.5 °C. Soils has 0.3 m depth, 1.0% organic matter, sandy clay loam texture, 2% slope, and pH of 6.8 (Arellano *et al.*, 2015). Under this soil and climate condition, a sustainable production system was evaluated. It was was composed by contour strips, 15 m wide, planted with dry bean on beds of 1.60 m width and four rows in each bed. The distance among rows was 0.3 m and 0.14 m between plants. Three lines of native nopal (Opuntia sp) and leucaena (leucocephala Glauca) where intercalated to dry bean contour strips. Nopal and leucaena were plated at high densities (1800 to 2500 plants ha^{-1}) at quincunx and counter slope, in order to form living hedgerows as support to avoid sheet erosion and promote the maintenance of organic matter and soil fertility. Five varieties of beans with early and intermediate growing cycle were evaluated: Pinto Centenario, Pinto Saltillo, Flor de Mayo Dolores, Flor de Junio Dalia, and Azufrado 2. All materials came from the breeding program of INIFAP. The experimental unit was a strip of 160.0 m length with 8 planting beds per bean variety. Plant density was 180,000 plants ha^{-1}. Sowing was on July 8 in moist soil and a versatile precision mechanical planter was used. Planter was designed to sow beds 1.60 m wide with four rows, and was coupled to a rainfall harvesting system (Rojas et al., 2013). At planting time, row dikings were constructed in the side furrows of each planting bed in all materials. Seed varieties was inoculated with the strain of INIFAP (*Glomus intraradices*) at doses of 350 g ha^{-1} of mycorrhiza substrate. There was an application of foliar fertilization during grain filling, with urea and phosphoric acid 2 and 1%, respectively. To determine grain yield and its components of the varieties evaluated, five random samples of 1.20 m wide by 5 m length within each experimental unit were taken. Data was analyzed based on a randomized complete block design. Statistical Analysis Systems, version 8 (SAS, 2008) was used and when statistical difference among varieties was detected, Tukey's test was applied ($P \leq 0.05$).

RESULTS AND DISCUSSION. Results of statistical analysis indicated that there were significant differences (Tukey, $p \leq 05$) for grain yield and its components. Bean varieties that had better production based on grain yield (t ha^{-1}), taking as a criterion an average overall grain production of 1.5 t ha^{-1}, were Flor de Junio Dalia (2.03), Pinto Saltillo (1.8) y Pinto Centenario (1.74). The other varieties were below the average. These varieties were Flor de Mayo Dolores (1.34) and Azufrado 2 (0.60) (Table 1). These results exceed those reported by Osuna (2014), who mentioned that using populations of 180,000 plants ha^{-1} and four rows bed planting, grain yield was 1.43 t ha^{-1}.

Table 1. Mean values of grain yield (t ha^{-1}) and straw yield (t ha^{-1}), number of pods $plant^{-1}$, number of grain pod^{-1}, and a hundred seeds weight (g) of five varieties of beans in Sandovales, Aguascalientes, Mexico. 2015.

Variety	GY	SY	NPP	NGP	W100S
Pinto Saltillo	1.80 a	1.03 ab	9.8	3.81 ab	27.2 c
Pinto Centenario	1.74 ab	0.77 c	9.0	2.81 c	33.74 a
Flor de Mayo Dolores	1.34 b	0.87 bc	9.4	3.52 abc	29.00 bc
Flor de Junio Dalia	2.03 a	1.18 a	8.8	3.99 a	29.22 b
Azufrado-2	0.60 c	0.76 c	7.6	3.03 bc	28.72 bc
Mean:	1.50	0.92	8.9	3.43	29.58

GY= grain yield (t ha^{-1}); SY= straw yield (t ha^{-1}); NPP = number of pods $plant^{-1}$; NGP = number of grain pod^{-1}, and W100S = a hundred seeds weight (g).
(a, b, c) Treatments with the same letter have no statistical difference ($p \geq 0.05$).

As it regards straw yield (t ha^{-1}), the overall average obtained was 0.92 t ha^{-1}. Varieties exceeding this average were only Pinto Saltillo and Flor de Junio Dalia. Flor de Mayo Dolores, Pinto Centenario, and Azufrado 2 had straw yield below average (Table 1). High density planting significantly affects some grain yield components of bean varieties evaluated. In the number of pods per plant, there was not statistical difference between varieties (Table 1). However, the number of grains per pod was 3.99, 3.81 and 3.52 for Flor de Junio Dalia, Pinto Saltillo, and Flor de Mayo Dolores were higher than that for Pinto Centenario and Azufrado 2 ($p \leq 05$). The highest weight of 100 seeds was obtained with Pinto Centenario, then with Flor de Junio Dalia, Flor de Mayo Dolores and Azufrado 2, and finally Pinto Saltillo. Average weights were 33.7, 29.2, 29.0, 28.7 and 27.2 g, respectively.

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WATER REGIME EFFECTS ON PHENOLOGY AND SEED YIELD OF COMMON BEAN IN DURANGO, MÉXICO

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INTRODUCTION: In the State of Durango, México, common bean (*Phaseolus vulgaris*) is the most important food product used for direct human consumption after grain cooking. Despite of its decisive role in local economy, human nutrition and nutraceutical properties; common bean is planted in rainfed areas. Under rainfed conditions water shortage (intermittent drought) and yield losses are the main factors causing negative impacts in the farmer´s economy. Some attempts have been made to use common beans under irrigated conditions in order to improve and stabilize seed yield. Modifications in phenology and low water requirements were introduced in modern common bean cultivars. Characterization of modern cultivars is required in order to implement the use of technological tools for irrigation scheduling; as well as phenology and yield prediction. Growing degree days (°D), which are based on actual temperatures, are considered as a simple and accurate way to predict when a certain plant stage will occur (Miller *et al*., 2001). The objective of this study was to evaluate effects of three water regimes on phenology and seed yield of common bean at two locations in the State of Durango, México.

MATERIALS AND METHODS: In 2015, an irrigation experiment was conducted at INIFAP´s experimental stations located in Durango and Canatlán, México. Two common bean cultivars (Pinto Saltillo and Pinto Centauro), both differing in maturity, were included in the study. The cultivars were sown in July 10th (Durango) and July 13th (Canatlán) using randomized block design with split plot arrangement and four replications. In Durango, the experimental plot consisted of 32 rows with 10 m in length and 0.81 m apart. In Canatlán, experimental units consisted of 16 rows with 8 m long separated by 0.81 m. Fertilizer was applied during the first mechanical weeding at the rate of 35-50-00 (N-P_2O_5-K_2O). Data was collected for phenological stages, minimum and maximum temperatures, amount of water (rainfall + irrigation), seed yield and seed weight. Degree days (°D) were also estimated using the same method including the following conditions: $°D = T_a - T_{c\text{-}min}$, $T_a < T_{c\text{-}max}$; $°D = T_{c\text{-}max} - T_{c\text{-}min}$, $T_a \geq T_{c\text{-}max}$; $°D = 0$, $T_a \leq T_{c\text{-}min}$. Where T_a is daily mean temperature, $T_{c\text{-}max}$ and $T_{c\text{-}min}$ represent the air maximum and minimum temperatures related to the range required for plant growth in common beans (10 ºC and 28 ºC).

Three irrigation treatments were applied (100, 80 and 60 % of plant available water) in order to avoid severe water stress in plants and determine precise amounts of water required to optimize yield in common bean. At maturity, three plant samples were taken for yield determination, and then the average value was obtained by replication. Plant samples consisted of two rows with 5 m in length by 0.81 m in width (8.1 m^2). The analysis of variance was obtained under randomized complete block design with split plot arrangement and four replications. Mean comparisons were performed using Tukey´s honestly significant difference test ($P \leq 0.05$).

RESULTS AND DISCUSSION: Low rainfall levels were registered in Durango and Canatlán; therefore, intermittent drought was observed during crop season causing severe yield losses in commercial plantings. The total amount of water applied across water regimes showed fluctuations between 377 mm (60 %) and 765 mm (100 %); while in Canatlán, variations were observed between 401 mm (60 %) and 631 mm (100 %). Pinto Centauro showed a delayed seedling emergence at both locations due to its larger seed size compared to Pinto Saltillo; which affected water imbibition and the germination process. In the vegetative period, Pinto Saltillo showed lower number of days and ºD to reach some phenological stages such as seedling emergence that was registered 7 to 8 days after planting (DAP) (68 to 84 ºD) and third trifoliate leaf (19 to 22 DAP, 171 to 218 ºD). On the other hand, an early response was observed for Pinto Centauro during the reproductive period in stages such as the number of days to flowering (40-41 DAP; 406 to 427 ºD) and physiological maturity (96-99 DAP; 923 to 967 ºD) (Table 1).

In Durango, both cultivars showed similar seed yield response across water regimes (2,665 kg/ha to 3,129 kg/ha). High yield was associated with intermediate level of applied water due to the 100 % treatment (765 mm) that exceeded theoretical crop water requirements (300-362 mm), thus causing seed yield reductions. In Canatlán, significant differences ($P \leq 0.05$) were observed for seed yield among water regimes with variations between 1,645 kg/ha and 3,344 kg/ha. The highest seed yield observed in Pinto Saltillo (3,344 kg/ha) and Pinto Centauro (3,157 kg/ha) was obtained in the 100 % treatment (631 mm).

Results suggest that common beans grown in Durango require a water supply ranging from 425 to 631 mm in order to achieve an increase in seed yield. Additional studies are necessary to accurately identify water requirements in order to optimize common bean production without excessive irrigation. Pinto Centauro registered the highest seed weight (size) across both locations and water regimes (34.5 g - 38.8 g) compared to Pinto Saltillo (30.4 g - 36.5 g). These traits (high yield and larger seed size) observed in Pinto Centauro are necessary to improve acceptance of common bean in domestic and international markets.

Table 1. Phenology and seed yield observed in two locations and three water regimes applied to two common bean cultivars.

Variety	Durango			Canatlán		
	Days to Flowering	Maturity	Yield kg/ha	Days to Flowering	Maturity	Yield kg/ha
	*100 % (765 mm)			100 % (631 mm)		
Pinto Saltillo	$43_{(448)}$	$103_{(1,015)}$	3,129	$45_{(459)}$	$101_{(950)}$	3,344[a]
Pinto Centauro	$41_{(427)}$	$97_{(967)}$	2,546	$40_{(406)}$	$99_{(937)}$	3,157[a]
	80 % (425 mm)			80 % (497 mm)		
Pinto Saltillo	$42_{(438)}$	$102_{(1,007)}$	3,380	$45_{(459)}$	$99_{(937)}$	1,946[b]
Pinto Centauro	$41_{(427)}$	$96_{(958)}$	2,890	$41_{(416)}$	$98_{(930)}$	2,190[b]
	60 % (377 mm)			60 % (401 mm)		
Pinto Saltillo	$43_{(448)}$	$103_{(1,015)}$	2,665	$44_{(447)}$	$100_{(943)}$	1,645[c]
Pinto Centauro	$41_{(427)}$	$97_{(967)}$	2,686	$41_{(416)}$	$97_{(923)}$	1,705[c]
Mean	$42_{(438)}$	$99_{(986)}$	2,883	$43_{(436)}$	$99_{(937)}$	2,331

*Relative value based on plant available water. Letters in each column indicate significant differences according to Tukey´s test ($P \leq 0.05$) between water regimes [a-c].

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WATER REQUIREMENTS OF 'PINTO SALTILLO' BEAN GROWN IN DURANGO, MEXICO

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INTRODUCTION

Seasonal and space variability of the weather generate uncertainty in the distribution and behavior of the different climatic variables that are of agricultural interest. In Mexico, there is a diversity of climates, in which a great variety of crops take place. The big irrigation areas are located especially in arid and semi-arid climates. They are constantly looked into for alternatives of production that allow to increase the productivity and yield of crops with the purpose of getting better revenues with a reduction of production costs. In Durango settles down in most of the agricultural surface, the crops of corn (*Zea mays*) and common bean (*Phaseolus vulgaris*), this last one prevails under rainfed conditions but for their nutritious kindness and the social preference, it's important to maintain their production under a sustainable and conservation of natural resources since this leguminous has been relegated to be grown in climates and on not very capable soils for its production. The objective of this research was to determine the water requirement of the bean variety 'Pinto Saltillo' with the use of forecast irrigation in real time, considering climatic variables, soil characteristics and phenology of the crop being grown in Durango, Mexico.

MATERIALS AND METHODS

During the spring–summer agricultural cycle (P-V) 2014 (LN 23° 59' 23.5", LW -104° 37' 15.6", 1887 m) and in 2015 (LN 23° 59' 21.3", LW -104° 37' 31.9", 1,884 m), experimental plots were established in Durango, Mexico (INIFAP-Valle del Guadiana Experimental Station) with sowing dates going from June 26, 2014 and July 14, 2015, respectively. The surface of each plot consisted of 12 rows with a separation of 0.8 m and a length of 18 m (172.8 m^2). The 'Pinto Saltillo' bean variety was cultivated. An integral irrigation programming developed for potatoes (Ojeda *et al*., 2004, Flores *et al*., 2012) and for corn (Ojeda *et al*., 2006) were used, in which models that are tied to degree days (^oD) concept were considered:

$°D = T_a - T_{c\text{-}min}$, $T_a < T_{c\text{-}max}$;

$°D = T_{c\text{-}max} - T_{c\text{-}min}$, $T_a \geq T_{c\text{-}max}$;

$°D = 0$, $T_a \leq T_{c\text{-}min}$.

In the equations is considered that $T_{c\text{-}min}$ and $T_{c\text{-}max}$ are the daily minimum and maximum temperatures of air respectively, in which the plant is developed in an interval of 10 to 28 ^oC for the Durango highland region. Also, it is considered the estimate and use of other parameters such as; crop coefficient (Kc), radical depth (Rd) and a maximum deficit allowed or a dejection factor (MDP).

$$K_c = K_{max}\ \mathrm{erfc}\{(\frac{X \quad X_{max}}{\alpha_1})^2\}$$

If $K_c < K_{co}$, therefore $K_c = K_{co}$. Where K_{max} is the maximum crop coefficient, K_{co} is the initial crop coefficient. X is an auxiliary variable that depends in the $\sum°D$ and erfc is a complemental function of error and $\alpha 1$is a regression parameter.

$$R_d = R_{do} + (R_{dmax} \quad R_{do})\{1 \quad \exp(\quad \frac{(\Sigma°D)^2}{\alpha_2})\}$$

Where R_{do} is the initial depth of the root, R_{dmax} is the maximum depth of the root, α2 is a regression parameter and ∑°D are the accumulated degree days.
MDP = α3 + α4 K_c. Where α3 and α4 are regression parameters, respectively.

RESULTS AND DISCUSSION

During the agricultural cycle P-V 2014, there was a precipitation of 341 mm and 220 mm in 2015. This allowed saving an application of irrigation in the first cycle of the crop (Table 1 and 2). For the Durango region, the water requirement for bean ranges is about the 368 mm (ETc), but considering that the irrigation applications have an application efficiency of 80% with the surface irrigation system, leading to an increment of irrigation sheets to satisfy the water demand for the crop.

Table 1. Water requirements for the year 2014 of the 'Pinto Saltillo' bean variety growing in Durango, Mexico.

Irrigation number	Days to irrigation	Irrigation interval	Net depth	Gross depth
1	0	0	121.77	143.26
2	35	35	54.95	64.64
3	52	17	80.57	94.79
4	68	16	92.00	108.24
	Total		349.29	410.93

Table 2. Water requirements for the year 2015 of the 'Pinto Saltillo' bean variety growing in Durango, Mexico.

Irrigation number	Days to irrigation	Irrigation interval	Net depth	Gross depth
1	0	0	117.40	138.11
2	35	35	53.08	62.45
3	50	15	73.44	86.40
4	68	18	88.21	103.78
5	96	28	100.31	118.01
	Total		432.44	508.75

The defined parameters to forecast the irrigation for bean are the followings: T_{max}= 28, T_{min}= 10, K_{max}= 1.25, K_{co}= 0.2, XK_{max}= 0.59, R_{do}= 0.1, R_{dmax}= 1.0, α0= 0.45, α1= 437, α2= 1055, α3= 0.9 y α4= 0.05. Because there is more bean varieties used in the influenced area of Durango, it is necessary to consider studies that allow determination of water requirements for bean under their different varieties. This is because they have different vegetative cycle that impacts the consumable inputs and their agricultural use.

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CONSEQUENCES RELATED TO WATER LIMITATION ON COMMON BEAN SEEDLINGS

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INTRODUCTION

During evaluation of water status in plants exposed to water limitations are frequently showed negative effects, such as in common bean plants that normally suffered significant reductions in leaf water potential, stomatal conductance and transpiration rate, with consequent increase in leaf temperature (Mendes et al., 2007). The aim of this study was to evaluate the total length and germination rate, as well as were calculated the tolerance index to each variable in three Brazilian cultivars of common bean.

MATERIALS AND METHODS

Study was implemented in Núcleo de Pesquisa Vegetal Básica e Aplicada of the Universidade Federal Rural da Amazônia, Brazil with seeds of *Phaseolus vulgaris* cvs. IPR-Siriri, IPR-Uirapuru and IAPAR 81. Experiment was organized in a factorial with four concentrations -0.6, -0.4, -0.2 and 0.0 (control) MPa of polyethylene glycol 6000 (PEG 6000) combined with three cultivars (IPR-Siriri, IPR-Uirapuru and IAPAR 81), being used five repetitions, and each repetition with 100 seeds. The seeds were placed in germitest paper with dimensions (length×width; 38×30 cm), being prepared rolls, and it were kept in plastic container. These seeds were soaked with distilled water and PEG 6000 solutions in concentrations previously described. The Nine days after experiment implantation (Brazil, 2009), the parameters evaluated were total length and germination rate, as well as were calculated the tolerance index to each variable. An analysis of variance was performed, and when significant differences were present, a Scott-Knott test with a 5% level of error probability was used.

RESULTS AND DISCUSSION

The water deficit reduced the total length in common bean seedlings (Fig. 1 A). Under concentration 0.0 MPa of PEG the cultivars presented not significant differences. To concentration -0.2 MPa of PEG the IAPAR 81 cultivar obtained higher values. The water deprivation occasioned reduction in total length of common bean seedlings, and this fact probably is related to decrease in cell turgescence that will generate a reduction in cell expansion, because the seedling is highly sensitive to water deficit especially during the growth phase (Kappes et al., 2010).

The results related to germination percentage (Fig. 1 B) demonstrated that under concentrations 0.0, -0.2 and -0.4 MPa of PEG, the better results were showed in IPR-Siriri. The water deficit negatively affected the germination index. An effect induced by water restriction is the decrease in germination index caused frequently by reduction in metabolic activity, such as lower activity of amylase enzymes (Carvalho, 2005). Similar results were described by Nascimento et al. (2011) studying *Vigna unguiculata* seeds submitted to water deficiency.

Under concentration -0.6 MPa of PEG, the IPR-Uirapuru cultivar presented higher tolerance index in relation to total length (Fig. 2 A), while in concentrations of -0.4 and -0.2 MPa of PEG the cultivar that was verified better results was IAPAR 81. A physiological mechanism that confers tolerance to water deficit to one cultivar, it is the capacity to keep open the stomatal by more time, when compared with other cultivars, under lower water potential (Sousa et al., 2009).

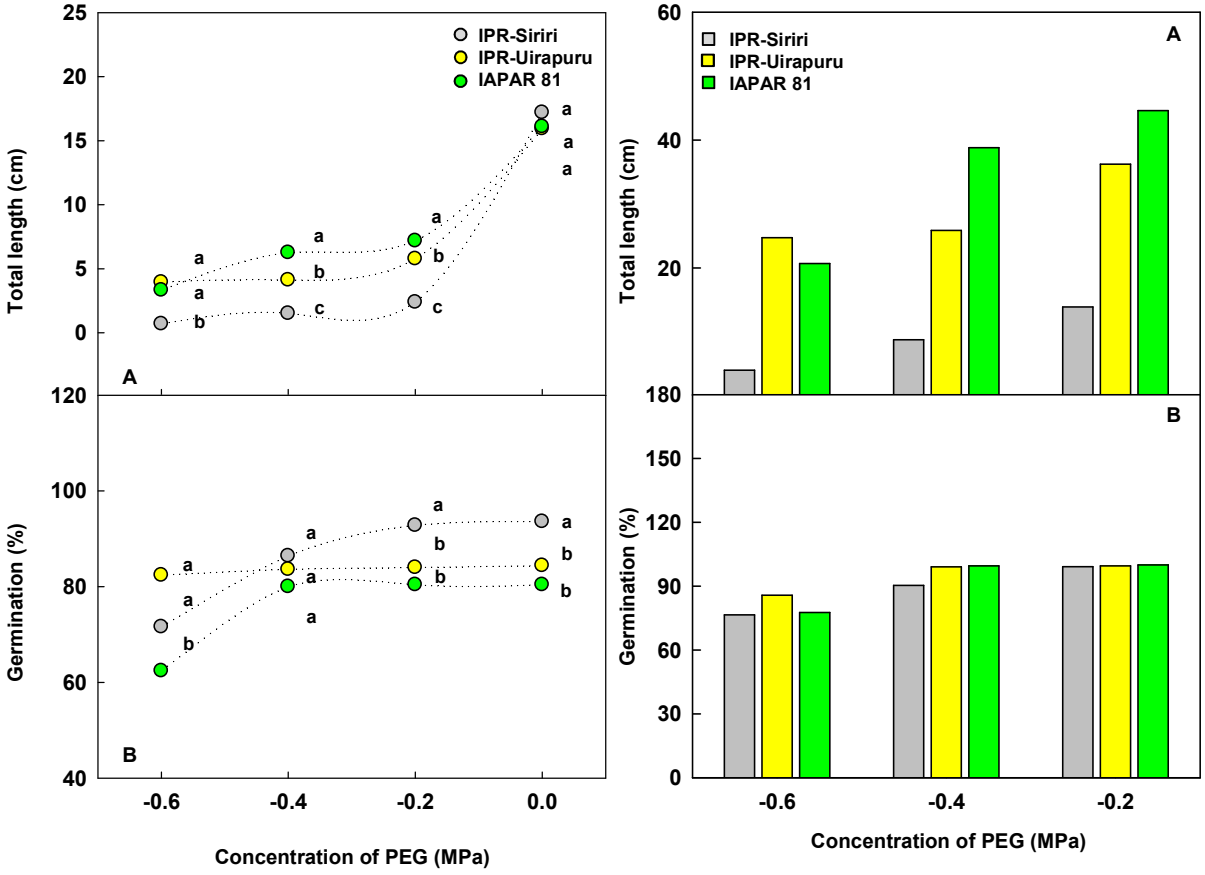

Fig. 1: Total length (A) and germination (B) of three common bean cultivars exposed to concentrations -0.6, -0.4, -0.2 and 0.0 (control) MPa of PEG 6000. Means followed by the same letter in equal concentrations are not significantly different by the Scott-Knott test at 5% of probability.

Fig. 2: Tolerance index linked to total length (A) and germination (B) of three common bean cultivars exposed to concentrations -0.6, -0.4, -0.2 and 0.0 (control) MPa of PEG 6000.

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SEED YIELD PERFORMANCE AND REACTION TO DISEASES OF BLACK BEAN CULTIVARS GROWN UNDER RAINFED AND RESIDUAL SOIL MOISTURE CONDITIONS

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INTRODUCTION: The small, opaque black bean is a seed class under high demand by consumers of the states of Chiapas, Veracruz and Puebla, Mexico. Such dry bean is grown in both cropping seasons, under rainfed condition during summer (June-October) and with residual soil moisture in fall-winter (October-January). In both soil moisture conditions, common bean diseases mainly caused by fungi [(*Uromyces appendiculatus* var. *appendiculatus* Unger, *Pseudocercospora griseola* (Sacc.) Ferraris and *Colletotrichum lindemuthianum* (Shear)], virus (BCMV, BCMNV and BGYMV) and bacteria [(*Xanthomonas axonopodis* pv. phaseoli (Xap) (Smith) Dye)], are the main biotic factors that significantly reduce grain yield (Lopez *et al*., 2002; 2015). A yield trial was designed with a group of small black elite cultivars and breeding lines developed by INIFAP regional bean breeding programs of Cotaxtla (humid tropics), Guanajuato (sub humid highlands), Zacatecas (semiarid highlands) and Sinaloa (dry tropics), Mexico. A field study was carried out in order to assess seed yield performance and reaction to common bean diseases of a group of elite breeding lines grown under rainfed and residual soil moisture conditions in the states of Chiapas, Veracruz and Puebla, Mexico.

MATERIALS AND METHODS: A yield trial was conformed prepared and consisted of 16 entries, 11 common bean cultivars and five breeding lines arranged in a 4x4 lattice design with four replications. Bean genotypes were planted in five environments, three on summer season (June-October) 2008 under rainfed conditions, and two under residual soil moisture on the fall-winter (October-January) 2008-09. Table 1 shows the main environmental and soil characteristics of the field evaluation sites. During the growing season reaction to anthracnose, angular leaf spot and bean common mosaic virus (BCMV) was determined using the 1-9 scale (CIAT, 1987) and seed yield performance was quantified. ANOVA was performed for each disease reaction and seed yield, and correlation analysis between these variables was done. A combined analysis of variance was done for each growing condition (rainfed and residual soil moisture) and the combined ANOVA across all environments. LSD (0.05) was used as a separation of means test.

Table 1. Climate conditions and soil characteristics of experimental locations.

Location/State	Altitude (m)	Annual precipitation (mm)	Mean temperature (°C)	Soil moisture conditions	Soil	
					Texture	pH
Ocozocoautla, Chiapas	864	898	23.6	Rainfed	Clay	6.5
Orizaba, Veracruz	1248	2035	19.0	Rainfed	Loam-sandy	6.6
Tecamachalco, Puebla	1980	573	16.4	Rainfed	Sandy-loam	7.2
Medellin, Veracruz	15	1337	25.4	RSM[†]	Loam	6.4
San Andrés T. Veracruz	84	1750	23.8	RSM	Loam	5.6

[†]RSM, Residual soil moisture.

RESULTS AND DISCUSSION: Seed yield varied significantly among genotypes in both field environments, rainfed and residual soil moisture and Negro Papaloapan was the most productive genotype in both soil moisture conditions. The average seed yield of Negro Papaloapan across environments was much higher than the other genotypes evaluated (Table 2). The occurrence of anthracnose (r = -0516 *) in Orizaba location, (rainfed) and angular leaf spot (r = -0528 *) in San Andres Tuxtla experimental site (residual soil moisture) significantly reduced bean seed yields, and Negro Papaloapan had excellent level of resistance to both bean diseases. There was also BCMV incidence at same locations, but the virus did not significantly affect seed yield (r = -0.22 and -0.37); however, Jamapa-Cora 1, Jamapa-Cora 2 and Jamapa-Cora 3 breeding lines derived from Negro Jamapa cultivar were susceptible to BCMV with a >6.0 virus reaction, while the rest of the genotypes showed low disease symptoms (<2.0).

Table 2. Average seed yield and disease reaction of elite common bean breeding lines and cultivars evaluated under rainfed and residual soil moisture.

Genotype	Summer (June-October) rainfed		Fall-Winter (October-January) residual soil moisture		Mean seed yield (kg ha^{-1})
	Seed yield (kg ha^{-1})	Anthracnose[1]	Seed yield (kg ha^{-1})	Angular leaf spot[2]	
Negro Papaloapan	1,753.3 *	3.1	1,333.5 *	1.0	1,585.0 *
Jamapa Plus	896.0	2.0	792.5	2.7	854.0
Negro 8025	884.7	3.3	687.0	4.8 *	805.0
Negro San Miguel	856.7	3.7	1,097.5	5.9 *	953.0
Jamapa-Cora 3	824.7	4.9	957.5	6.1 *	877.0
Negro Guanajuato	822.7	2.5	975.5	5.0 *	883.0
Jamapa-Cora 1	818.3	5.9	1,069.0	3.4	918.0
NGO 99279	804.0	5.4	938.0	5.5 *	857.0
Negro Medellin	802.3	4.1	894.0	2.7	839.0
Negro Tropical	798.0	3.5	865.0	2.3	824.0
Negro Pacifico	781.7	4.1	1,012.0	1.7	873.0
Negro Tacaná	778.0	3.5	1,063.5	1.4	892.0
Negro INIFAP	711.0	3.5	1,108.5	1.0	870.0
Negro Zacatecas	651.7	4.0	866.0	1.0	737.0
Jamapa-Cora 2	638.3	5.1	953.0	4.1	764.0
Negro Citlali	514.3	4.5	830.5	6.3 *	640.0
Mean	833.5	3.9	965.2	3.4	886.2
ANOVA	**	ns	**	**	**
C. V. (%)	31.8	41.7	20.8	40.5	26.6
LSD (0.05)	93.8		199.3	2.0	344.3
Corr. (r): SY vs D[§]		-0.516*		-0.528*	

[1] Orizaba. [2] San Andrés Tuxtla. **,* Significant at 0.01 and 0.05 of probability, respectively.
[§] SY= Seed yield and D-= Disease reaction.

CONCLUSIONS: Negro Papaloapan was the most productive bean cultivar under both conditions, rainfed and residual soil moisture; under such field environments, Negro Papaloapan was resistant to BCMV, angular leaf spot and anthracnose diseases.

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GROWTH ANALYSIS OF EARLY GENOTYPES OF COMMON BEANS

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INTRODUCTION: The common bean (*Phaseolus vulgaris* L.) has economic importance in several countries and is considered an important source of protein for the people. Embrapa Rice and Beans developed earlier cycle genotypes of common bean to allow achieving high grain yields in shortest time (Nascente & Melo, 2015). These genotypes have life cycle of 65-75 days, while traditional cultivars have 90-100 days. Therefore, this new materials should be characterized in more detail in order to develop a management system that allows fully exploit their genetic potential. Growth analysis is a technique which details the allocation of photosynthate partition as a function of the age of the plant. Determination of dry matter (plant and its parts: stems, leaves and pods) is the most suitable for the growth analysis (Taiz & Zeiger, 2004). With the results of the grow analysis we can verify the material more adapted material for the management systems used (Andrade et al., 2009). The aim of this study was to characterize the agronomic performance of three elite genotypes of common bean with early cycle by growth analysis technique.

MATERIAL AND METHODS: The irrigated field experiment was performed on autumn/ winter (May to July) in 2015 at the Capivara farm from Embrapa Rice and Beans in Santo Antônio de Goiás, GO, Brazil. A field experiment with a randomized block experimental design with eight replications was conducted in Brazil. The treatments consisted of common bean genotypes with early maturity, CNFC 15873, CNFC 15874 and CNFC 15875, we also included the check cultivar 'Colibri'. Sowing of common bean was mechanically held on May 29th, 2015, spaced 0.50 m between rows and with 15 viable seeds per meter. Fertilization in sowing furrows in all treatments was 45 kg ha^{-1} of N as urea and 60 kg ha^{-1} of P_2O_5 as simple superphosphate. In the V4 vegetative stage of the common bean (four trifoliate leaves), a topdressing fertilization of 45 kg ha^{-1} of N as urea was performed for all plots. Other cultural practices were performed according to the recommendations of the crops to keep the area free of weeds, disease and insects. It was collected plants periodically in a linear meter in each plot for the realization of the growth analysis. Bean plants were separated into stems, leaves and pods. We made the mass accumulation graphs of dry matter of each plant structure and total. At harvest time it was made the evaluation of the yield and yield components of each genotype. Data were subjected to an analysis of variance, and the means were compared by Tukey's test at $p<0.05$.

RESULTS AND DISCUSSION: The CNFC 15874 genotype showed the highest dry matter mass of pods (97.0 g m^{-1}) and total (165.6 g m^{-1}) in relation to genotypes 'Colibri' (88.3 g m^{-1} and 163.1 g m^{-1} for pods and total, respectively), CNFC 15873 (62.6 g m^{-1} and 126.5 g m^{-1} for pods and total, respectively) and CNFC 15875 (82.1 g m^{-1} and 133.5 g m^{-1} for pods and total, respectively) at 67 days after sowing (Figure 1). This better development of CNFC 15874 also allowed the highest grain yield (2784 kg ha^{-1}), which differed significantly from genotypes CNFC 15873 (2268 kg ha^{-1}), Colibri (2027 kg ha^{-1}) and CNFC 15875 (1807 kg ha^{-1}) (Table 1). The highest dry biomass accumulation for pods and total of CNFC 15874 could be related to the genotype potential. Nascente & Melo (2015) that performed the trial on the growing season 2014 also related that CNFC 15874 was the more productive genotype. It was observed that all genotypes accumulated dry biomass in the leaves and stems until 60 days after sowing. After this period, leaves and stems dry matter

started to decline indicating that there was translocation of their photoassimilates to the pods. According to Wien et al. (1976) during the period of formation and filling of seeds there is translocation of photoassimilates from the leaves and stems to pods. From the results, we can observed that the use of growth analysis technique allow explaining the better development of the genotype CNFC 15874, which resulted in higher grain yield in relation to the others tested genotypes.

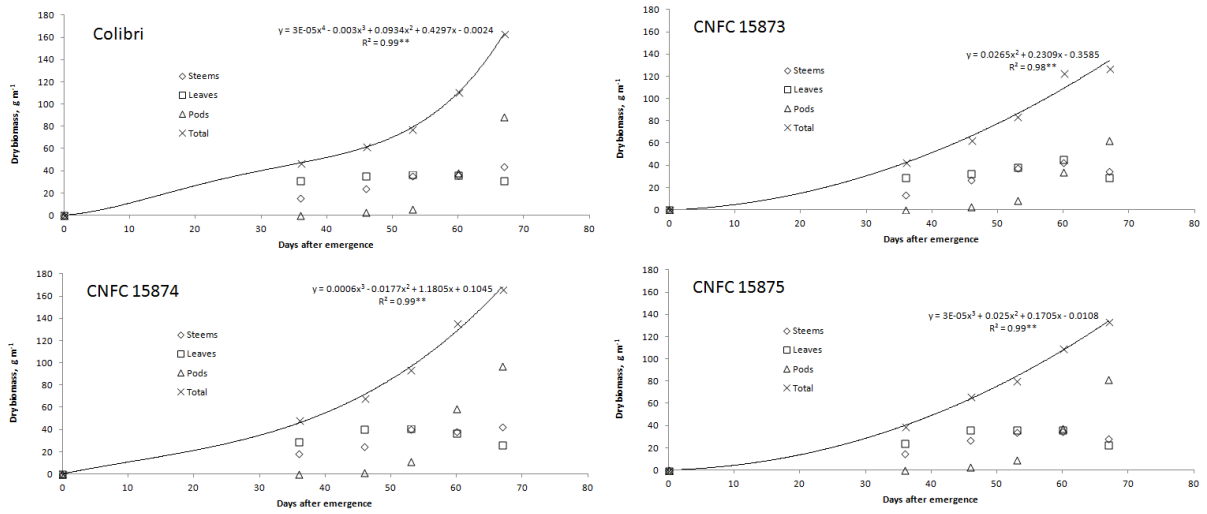

Figure 1. Growth analysis of four common beans genotypes ('Colibri', CNFC 15873, CNFC 15874 and CNFC 15875) cultivated in Santo Antônio de Goiás, Goiás State, Brazil in the growing season 2015.

Table 1 – Number of pods per plant (NPP), number of seeds per pods (NSP), mass of 100 seeds (MASS) and yield of early genotypes of common beans. Santo Antônio de Goiás, Brazil, growing season 2015.

Genotypes	NVP	NGV	MASS	YIELD
Colibri	11 b[/1]	5 a	21 a	2027 bc
CNFC 15873	14 a	5 a	18 b	2268 b
CNFC 15874	15 a	4 a	22 a	2784 a
CNFC 15875	10 b	4 a	22 a	1807 c
Factor	ANOVA (F probability)			
Genotype	<0.001	0.4376	0.0382	<0.001
Coefficient of variation (%)	8.47	10.17	2.83	12.91

[/1] – means followed by the same letter are not significantly different at p<0.05 according to Tukeys's test.

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PRODUCTIVITY, ADAPTABILITY AND STABILITY OF PRODUCTION OF SPECIAL GRAIN COMMON BEAN LINES IN DIFFERENT ENVIRONMENTS OF MINAS GERAIS, BRAZIL

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INTRODUCTION: Minas Gerais is the second largest state producer of common bean in Brazil, with a production over 970 thousand tons per year (EMBRAPA, 2014). Besides commercial classes "Carioca" and "Black", which predominate in the Brazilian market, other ones, as "roxo", "mulatinho", "rosinha", "vermelho" e "manteigão", known as "special grain", supply much of the Brazilian market and represent good alternative for export. The selection of lines with high adaptability and production stability is important for recommending more productive and stable cultivars in different growing regions. Thus, this study aimed to select special grain bean lines with high productivity, adaptability and stability of production, evaluated in different environments of the Minas Gerais State, Brazil.

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

RESULTS AND DISCUSSION: The CNFRx 15275 line showed the highest productivity. The BRS VEREDA, JALO EPP and BRS RADIANT cultivars had the highest yield. The CNFRx 15275 (Wi = 121.12) and VR-18 (Wi = 112.02) pre-commercial lines, and the BRS VEREDA (Wi = 104.43) cultivars showed the greatest adaptability and stability, given their values of genotypes recommendation index (Wi) indicate that they can produce 21.12, 12.02 and 4.43% more than the overall average of the lines studied (Table 1). That indicates they may be released as commercial cultivars in the future.

Table 1: Grain yield (GY), genotype recommendation index (Wi) and classification of special grain common bean lines grown in different environments of Minas Gerais State, Brazil.

Lines	Yield (kg ha^{-1})	Wi[2]	Classification[3]	Commercial Class
CNFRx 15275	2084 a[1]	121,12	1	Purple (Roxo)
BRS Vereda	1836 b	112,02	2	Rosinha
VR-18	1791 b	104,33	3	Red (Vermelho)
Jalo EPP	1731 b	94,31	6	Large-seeded (Manteigão)
RC2RAD-155	1698 b	98,94	4	Large-seeded (Manteigão)
BRS Radiante	1652 b	86,57	9	Large-seeded (Manteigão)
VR-16	1634 b	96,72	5	Red (Vermelho)
PT-68	1629 b	85,85	10	Rosinha
VR-14	1556 c	90,88	7	Red (Vermelho)
BRS Timbó	1543 c	89,11	8	Purple (Roxo)
CNFJ 15288	1534 c	81,99	14	Large-seeded (Manteigão)
VR-17	1468 c	85,24	11	Red (Vermelho)
PT-65	1452 c	79,81	15	Rosinha
Ouro Vermelho	1442 c	84,81	12	Red (Vermelho)
VR-15	1428 c	82,13	13	Red (Vermelho)
RAD/E550-284	1276 c	65,64	16	Large-seeded (Manteigão)

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

CONCLUSIONS: The CNFRx 15275 and VR-18 lines stand out as the most productive and stable ones, with good potential to be released as cultivars of special grain bean for Minas Gerais State, Brazil.

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RELEASE OF PINTO 'RARÁMURI' DRY BEAN FOR THE SEMIARID HIGHLANDS OF CENTRAL MEXICO

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The pinto bean class is widely grown under rainfall conditions at the Semiarid Highlands of Mexico, a region prone to intermittent drought stress. During the last decade the production of this market class have been dominated by a single cultivar, Pinto Saltillo, a drought resistant cultivar of slow darkening grain. Due to the high risk of growing a huge extension of land to a single cultivar, new cultivars are being released to display variation in this market class and reduce the risk of an epidemic disease. However, any new cultivar in addition to outstanding agronomic traits must carry the slow darkening trait since that is what growers and consumers demand.

Rarámuri was developed from a single cross between P02447 / Pinto Saltillo made in 2004 at the Bajío Experimental Station, Celaya, Guanajuato, Mexico. P02447 is an erect type II growth habit, regular darkening seeded line developed by the bean-breeding program of MSU and Pinto Saltillo is a slow darkening cultivar developed by the bean program of INIFAP (Sánchez *et al*., 2004). During the selection process, two growth cycles were conducted per year, one during the dry season under irrigation, a cycle that takes place from the end of winter to the end of the spring season, and the second cycle during summer and fall. During its development selections were made for earliness, disease resistance and slow darkening pinto seed color.

Rarámuri has an indeterminate semi-prostrated growth habit type III with medium length vine and short-day response. Rarámuri is resistant to BCMV (carries de *II* gene), rust and anthracnose and tolerant to common and halo blights. Average 100-seed weight of Rarámuri is 38 g as compared to 32 g for Pinto Saltillo. In the semiarid bean growing areas of Guanajuato and San Luis Potosí Rarámuri displays higher disease resistance and similar seed yield than Pinto Saltillo. Rarámuri carries the molecular markers linked to the following rust resistance genes *Ur 4, Ur 3, Ur 5, Ur 6, Ur 7* and *GB*; and to anthracnose: *Co 1, Co 2, Co 4, Co 4*^{2} and *Co 6.* These markers suggest a strong resistance of Rarámuri against these two diseases.

During the 2015 rainfed season at the semiarid areas of Guanajuato and San Luis Potosí, a severe rust outbreak damaged most commercial cultivars in all seed classes, including Pinto Saltillo and other light colored and black seeded dry-bean landraces, and Rarámuri was resistant and its yield in a commercial demonstration plot conducted under rainfed conditions was 960 kg ha^{-1}. Samples of Rarámuri for research purpose can be obtained from the main author.

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2015 FINANCIAL STATEMENT
BEAN IMPROVEMENT COOPERATIVE

BALANCE AS OF January 1, 2015		**$ 13,684.64**
INCOME		
	2015	
2015 Dues	$ 5400.00	
Extra Articles for 2015 Report	$ 50.00	
2016 Dues prepaid	$ 80.00	
Back Issues	$ 75.00	
Bank Interest	$ 93.12	
TOTAL INCOME	$ 5698.12	
EXPENSE		
Labor Charges	$ 525.00	
Postage, Copy Charges and Office Supplies	$ 1,229.64	
Printing and shipment – Volume 58	$ 1,690.78	
PayPal Fees	$ 197.45	
TOTAL EXPENSE	$ 3,642.87	
BALANCE AS OF December 31, 2015		**$ 15,739.89**