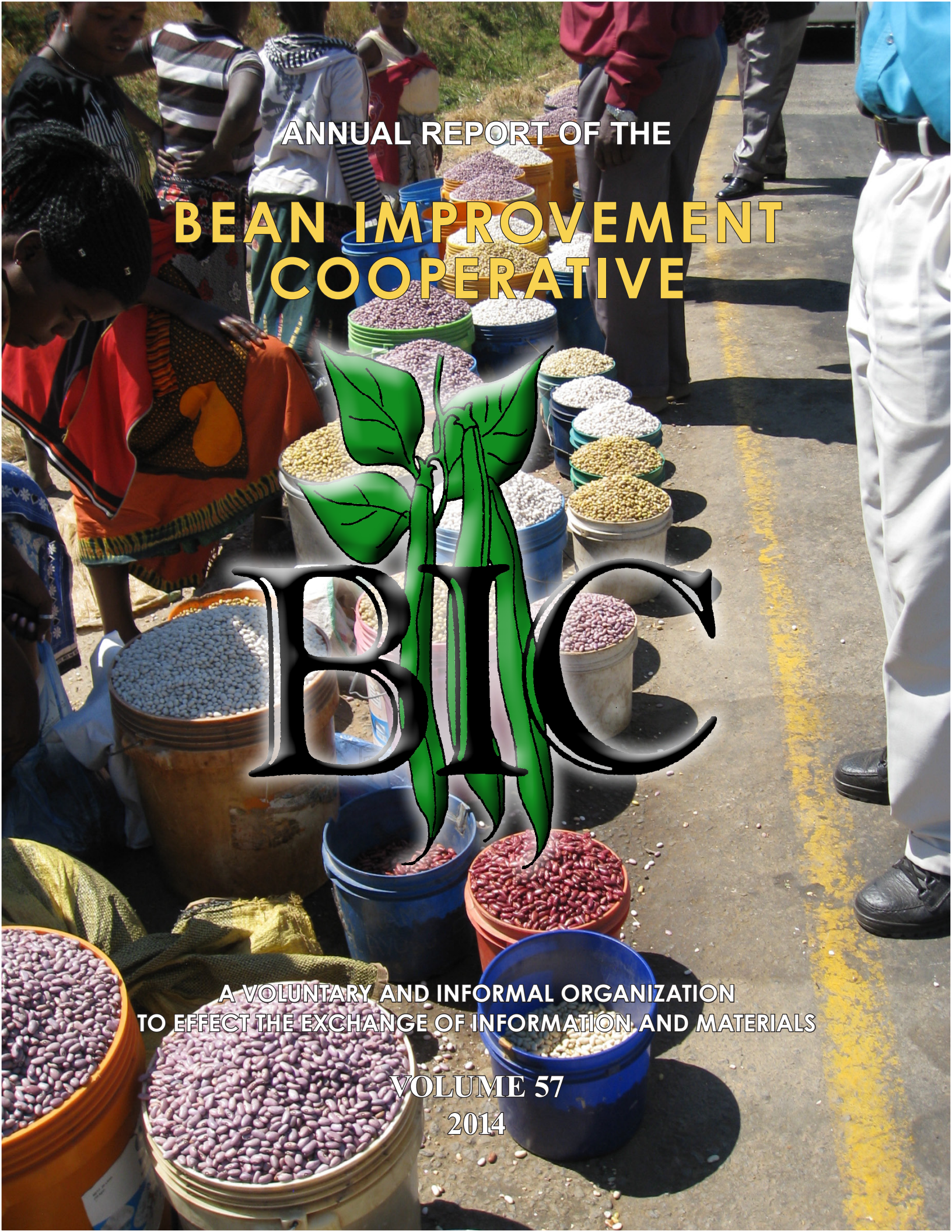

ANNUAL REPORT OF THE

BEAN IMPROVEMENT COOPERATIVE

BIC

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

VOLUME 57
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THE LVII

Report of The

BEAN IMPROVEMENT COOPERATIVE

No. 57 March 2014

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SITE REGULARLY UPDATED

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TABLE OF CONTENTS

RESEARCH PAPERS FOR 2014

Oral Presentations (2014 BIC Meeting):

2014 Articles:

Cover: Roadside bean market in the Southern Highlands of Tanzania (photo courtesy of P. Miklas)

THE 57th ANNUAL REPORT OF THE BEAN IMPROVEMENT COOPERATIVE

The Bean Improvement Cooperative (BIC) celebrated the twenty-seventh Biennial Meeting in Portland, Oregon. This BIC meeting had the highest attendance in recent memory with ~180 registered participants which contributed to a vibrant and exciting atmosphere. The meeting began with Frazier-Zaumeyer Distinguished Lectureship entitled '*The changing face of bean breeding; past and future*' which was presented by past BIC President, Dr. James Kelly, Professor and Bean Breeder at Michigan State University. A special session '*New Landscapes in International Bean Research*' highlighted a new era of deserved attention that dry beans are receiving from national and international donor agencies. The meeting ended with back-to-back sessions on Disease Resistance. Overall there were 41 oral and 65 poster presentations.

The meeting received generous support from numerous donors - Platinum: American Pulse Association, Basin Seed Company, Del Monte, Monsanto/Seminis, Syngenta; Gold: ADM, Pop Vriend, US Dry Bean Council; Silver: Brotherton Seed Company, Crites, Moscow, Harris Moran, Idaho Bean Commission, Pro Vita, Seneca; and Bronze: Ag Biotech and Central Bean. On behalf of the BIC, I wish to acknowledge the substantial role of the organizing committee, James Myers 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, the Meritorious Service Award was presented to Dr. James Nienhuis and Dr. K. Peter Pauls, the Distinguished Achievement Award was presented to Dr. Kirstin Bett, and the first awardee for the Technical Merit Award was Mr. Thomas Smith. Two student awards were presented for the best oral and poster presentations at the BIC meeting.

The outstanding student oral presentation entitled: *'Molecular Genetic Analysis of the Phaseolus vulgaris P Locus'* was presented by Samira Mafi Moghaddam from the Department of Plant Sciences, North Dakota State University – Dr. Phil McClean, advisor [p.14-15].

The outstanding poster presentation entitled: '*Identification of QTL for Drought Tolerance and Characterization of Extreme Phenotypes in the Buster X Roza Mapping Population'* was presented by Jennifer Trapp, from USDA-ARS and Department of Crop and Soil Sciences, Washington State University, - Dr. Phil Miklas, advisor [p.87-88].

The BIC Coordinating Committee directed the President to: i) set more stringent publication standards, ii) consider an electric online version only for the annual report; and iii) give the webpage some needed attention.: Dr. James Beaver was appointed the new chair for the BIC Awards Committee replacing Dr. Howard Schwartz after 15 years of service, and Dr. Carlos Urrea replaced Dr. Shree Singh after nine years of service. The BIC Genetics Committee elected a new chair Dr. Kirstin Bett who replaces Dr. Tim Porch after eight years as chair, and added: Dr. Juan Jose Ferreira (Spain), Dr. Maria Celeste Goncalves-Vidigal (Brazil), Dr. Val Kalavacharla (Univ. of Delaware), and Dr. Juan Osorno (North Dakota State Univ.). Dr. Ted Kisha, new Bean Curator in Pullman, WA, was added as an ex Officio member. The BIC community applauds this service which contributes to sustained success of the organization.

The next BIC Biennial Meeting is planned for Niagara Falls, Canada in October, 2015. The local organizing committee consists of Alireza Navabi, K. Peter Pauls, and Chris Gillard. As the 2015 BIC meeting approaches, details will be posted on the BIC Web page www.css.msu.edu/bic.

Dr. Phillip Miklas, BIC President

BIC COMMITTEE MEMBERSHIP - 1957 to 2014

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**
Antonius, Beaver, **Kelly**, Kotch, Miklas, Myers, Park, Riley, Schwartz (ex officio), Singh, Vandenberg
Antonius, Beaver, **Kelly**, Kotch, Miklas, Myers, Park, Riley, de Ron, Schwartz (ex officio), Vandenberg
2003 Beaver, **Kelly**, Kmiecik, Kurowski, Miklas, Myers, Park, Riley, de Ron, Schwartz (ex officio), Vandenberg
2005 Beaver, **Kelly**, Kmiecik, Kurowski, Miklas, Myers, Park, Riley, de Ron, Schwartz (ex officio), Vandenberg
2007 Beaver, **Kelly**, Kmiecik, Miklas, Myers, Park, Riley, de Ron, Schwartz , Shellenberger, Vandenberg
2008 Beaver, **Kelly**, Kmiecik, Miklas, Myers, Pauls, Riley, de Ron, Schwartz , Shellenberger, Vandenberg
2010 Beaver, Kelly, Kmiecik, **Miklas**, Myers, Pauls, Riley, de Ron, Schwartz , Shellenberger, Vandenberg
2011 Bett, Kelly, Kmiecik, **Miklas**, Myers, Osorno, Pastor-Corrales, Pauls, Riley, de Ron, Wahlquist

Awards Committee:

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

Genetics Committee

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

BIC GENETICS COMMITTEE MINUTES

Meeting location: DoubleTree Hotel, Portland, Oregon
Date: Oct. 30, 2013
Time: 7:30 to 9 PM

Committee Members – all present:

Kirstin Bett	Phillip McClean
Paul Gepts	Tim Porch (Chairperson)
James Kelly	Phil Miklas (BIC president)
Carlos Urrea	

Additional guests:
M.-C. Goncalves-Vidigal, Peter Pauls, Ali Navabi, Steve Beebe, Juan Osorno, Steve Noffsinger, Jim Beaver, Juan Jose Ferreira, Shree Singh, Marilda Cainceta, Giseli Vanentini, Sandra Lima Castro, Danielle Nanamim Maria de Souza, Vanusa Martins, Ted Kisha, Julie Thayer, Jeff Parker, Weidong Chen, Clarice Coyne

Old Business
Approval of the Genetics Committee meeting minutes at the Niagara Falls Marriott Gateway Hotel, Niagara, Canada on Nov. 5, 2012.
JK/PG - AIF

Gene symbols approved between meetings:

bc-3^{2} Conditions resistance to BCMV and allelic with *bc-3, cyv,* and *desc*; Located on Pv06 and linked to PveIF4E.
Co-3^{4} Renaming of anthracnose locus on Pv04; Acceptance of *Co-10* as an additional allele of the *Co-3* locus and its renaming as *Co-3*^{4}.
Pse-6 Resistance to halo blight identified in BelNeb-RR1; Located to Pv04 in gene cluster and linked to SB10.550 SCAR marker.

Xap-1 gene symbol: Single dominant gene resistance from PR0313-58; Co-segregated with SAP6 on Pv10.

Discussed a recommendation to add strain super-script (e.g. *Xap-1*^{3353}).
Since there are no systematic collections of strains of Xap, it was decided to leave the symbol as published and without the superscript. The strain information will be included in the gene symbol description.
TP/KB – passed

New Business
Updated "List of Genes- *Phaseolus vulgaris*"
Motion: Move that we add the new sections [b iii and iv] to the guidelines.
The evidence must include:
data from one generation to formulate an hypothesis
data from subsequent generations to test that hypothesis

for hyper-variable pathogens: family mean testing, and use of multiple, specific races of the pathogen to separate effects of individual genes in gene clusters
molecular marker data and map position when available

JK/PM - passed
Also, move the guidelines to top of the document after the editorial paragraph and include in the editorial paragraph a note about where the SCARs and maps can be found on the BIC website.

Use of new genomic tools with the genetic map (Phil McClean)
Discussion:
How does having physical map affect our genetic map?
Where is this "living map"? The one on BIC site is good and needs to continue to be updated.
Physical map is based on Stampede x Redhawk so it is not fixed – this is important to keep in mind when making comparisons.
Would be good to have a physical location for markers – but how to define the location? Group of linked SNPs - use peak? Trouble with AFLPs.
Could be challenging in R-gene rich regions.
Phaseolus Genes where CMAP feature has markers placed on map and most link out to genome browser - good for now.
In future it would be highly preferable to have physical locations for markers.

Presentation on the *Co-16* gene symbol (Maria Celeste): *Co-16* confers anthracnose resistance from cultivar 'Crioulo 159' to multiple races including 2047. It is located on Pv04 and linked to g2685.
Paper received by committee members this morning.

Jim Kelly: note that most of the differentials are not segregating for just one gene as was originally thought. Need to put the race tested next to the gene symbol otherwise we don't know. Concerned that these should be added as part of a cluster rather than as another Co-gene.

Discussion to continue after the meeting. Find out what is being done in other species.

Membership:
Motion to add: Juan Osorno, Kal Kalavacharla, Juan Jose Ferreira and Celeste Gonçalves-Vidigal
TP/PMc - passed

New Chairperson: Tim Porch stepping down as Chair – thanks for many years of dedication to the position. Kirstin Bett nominated as new Chair.
PM/JK - passed

Next meeting: Puerto Rico, Feb. 2015 during the W2150 meeting.

2013 AWARD RECIPIENTS

THE BEAN IMPROVEMENT COOPERATIVE

Proudly Presents the

Frazier – Zaumeyer Distinguished Lectureship

to

James D. Kelly
Michigan State University
East Lansing, MI

Meritorious Service Award

to

James Nienhuis

University of Wisconsin

Madison, Wisconsin

and

K. Peter Pauls
University of Guelph
Guelph, ON, Canada

Distinguished Achievement Award

to

Kirstin Bett
University of Saskatchewan
Saskatoon, SK, Canada

Technical Merit Award

to

Thomas Smith
University of Guelph
Guelph, ON, Canada

JAMES D. KELLY

Dr. James D. Kelly is nationally and internationally recognized for his body of sustained scholarly research and creativity that is focused on the improvement of dry edible beans. He received his B.S. and B. Agr. degrees in Agricultural Botany from Queens University of Belfast, N. Ireland, in 1968-69 followed by Masters and Ph.D. degrees in Plant Breeding and Genetics from the University of Wisconsin in 1971 and 1974. Jim spent some of his time during his graduate studies working with the bean breeding program at CIAT which cemented his long term affair with dry bean improvement. While at CIAT he became fluent in Spanish which facilitated productive collaborations across Latin American, Brazil, and Spain, throughout his career.

The breadth of his career, from 1980 to present, has been with Michigan State University where he rose through the ranks of Assistant to Full Professor and most recently was promoted to University Distinguished Professor in 2013. A short stint at UC-Davis as a visiting scientist in 1989 whetted his appetite for generating molecular markers with application for bean breeding. His lab, in 1992, was the first to identify a molecular marker linked to a disease resistance gene in dry bean, and 12 additional R-gene linked markers were subsequently identified and applied toward marker-assisted breeding. Along the way, gene nomenclature was improved upon for naming rust and anthracnose R genes, new anthracnose resistance genes were discovered, and many QTL were identified and characterized for disease resistance, drought tolerance, and yield.

Dr. Kelly's first job in 1975 was with the research division of Campbell Soup Company in Ohio, which started his career as a bean breeder, and which he has now excelled at for 38 years. Jim has developed and released 41 new dry bean varieties in 11 different bean classes, and cooperated with others in releasing 68 germplasm lines and 14 international cultivars. More important than the numbers is the breakthroughs that many of the cultivars provided: 'C-20' and 'Mayflower' were the first high yielding upright navy bean cultivars which provided the background for today's high yielding cultivars with upright growth habits amenable for direct harvest. The same breakthroughs were generated for the pinto 'Sierra' and 'Aztec', great northern "Alpine' and 'Matterhorn', black 'Jaguar' and 'Zorro', and most recently the pink 'Sedona' and 'Rosetta' bean market classes.

Significant improvements for disease resistance, yield, upright architecture, and other traits in dry bean culminate many years of successful and dedicated breeding and research efforts made by Dr. Kelly. His 141 publications and 7 book chapters document these efforts and provide the bean community with vital formation that is needed to understand the bean plant and bean genome. His 1987 paper in TAG is one of the earliest papers where agronomic and yield related traits in beans were studied in relation to plant growth habit. He was an early adaptor of marker-assisted selection, and wrote many of the first papers in bean detailing discovery and utilization of molecular markers in breeding for resistance to biotic and abiotic traits. Significant contributions toward genetic characterization of drought tolerance, canning quality, white mold resistance and other traits were made.

Equally impactful has been his contribution in mentoring agricultural scientists (30 graduate students, 5 post docs, 12 visiting scientists, 90 graduate student committees, and numerous international partners) in crop science, bean breeding and genetics. His contributions as an educator are highlighted by the Distinguished Faculty Award in 2007 and promotion to University Distinguished Professor in 2013.

Colleague recognition is exemplified by service as BIC President from 1998 to 2009, being made Honorary Member of the Michigan Crop Improvement Association in 2003, reception of Fellow by the Crop Science Society of America in 2008, and receiving the BIC Distinguished Achievement Award in 1989 and Meritorious Service Award in 1997.

JAMES NIENHUIS

Professor Jim Nienhuis grew up on a vegetable farm just north of Chicago. He milked cows and hoed vegetables for most of his youth. He was the first person in his extended family to attend college; with a scholarship to the University of Illinois where he studied Agronomy, and graduated with honors in 1976. However, before completing B.S., he worked for two years as a Peace Corps Volunteer in Costa Rica in Agricultural Extension, met his lovely wife, and learned the Spanish language.

He earned his M.S. in Statistics from the North Carolina State University in 1979 and Ph.D. in Plant Genetics and Plant Breeding from University of Wisconsin in 1982. Jim was a Post-Doc in Bean Breeding at CIAT, Cali, Colombia in 1983 and 1984; then worked at the NPI Biotechnology in Salt Lake City, Utah from 1984 to 1990 before joining University of Wisconsin in 1990. Since then Jim has conducted breeding and genetics in snap bean.

While at CIAT, Jim determined the extent of heterosis among intra- and inter-racial and inter-gene pool crosses, conducted quantitative genetic analyses of seed yield and its components, and assessed the productivity potential of newly developed determinate and indeterminate plant types at different plant population densities and locations. While at the NPI, Jim was among the first to apply biotechnology to quantitative genetics, as he reported an RFLP marker associated with a QTL for insect resistance in tomato in 1987 (Crop Sci. 27:797-803).

During his tenure at UW-Madison, Jim developed the first molecular marker linkage map in common bean based on RAPD markers in Eagle x Puebla RIL population and contributed >700 polymorphic markers that were integrated with the UC-Davis RFLP map of BAT 93 x Jalo EEP 885 in mid-1990's. He pioneered identifying and mapping of resistance to bacterial brown spot and root rots. He also mapped some white mold resistance QTL. Recently, he completed mapping of pod sugars in snap bean.

He has authored and co-authored more than 50 scholarly refereed publications. Three of those articles relied on molecular markers to measure genetic distances and to better understand the structure of common bean germplasm collections; and were honored in 1997, 1998, and 2000 with the ‘Crop Science Award for Outstanding Paper in Plant Genetic Resources’.

Jim teaches ‘World Vegetables’ and the “Principals of Plant Breeding’ at the UW-Madison. In addition, he teaches courses on Tropical Horticulture and on Organic Vegetable Production. Recently, he developed an Organic Workshop offered in Spanish. Jim has received several teaching awards from both the College of Agriculture and Life Science, as well as at the University level at UW-Madison. He has trained more than 25 graduate students from around the world. Jim developed and released four cultivars, one of which 'Accelerate', was recently PVPP with specific resistance to root rot and licensed to a private company.

K. PETER PAULS

Dr. Pauls received his Ph.D. in Plant Physiology from the University of Waterloo in 1981; his B.Sc. and M.Sc. were also from the University of Waterloo. Following his Ph.D., Peter was awarded the prestigious NSERC (National Scientific and Engineering Research Council of Canada) post-doctoral research fellowship, which he held from 1981 to 1983 in biophysics at the University of British Columbia in Canada.

He continued on and worked as an NSERC Research Fellow in the Crop Science Department at the University of Guelph for three years and was then promoted to Assistant Professor in 1986, to Associate Professor in 1989, and to Professor in 1996. From 1987 to 1990, Peter served as the director of Guelph/Waterloo Plant Biotechnology Centre. Peter took a sabbatical leave in 1991 at the Laboratoire de Bologie Cellulaire, Versailles. He served as the acting Associate Dean, Academic, Ontario Agriculture College (OAC) from 2006-2009 and has been Chair of the Department of Plant Agriculture, University of Guelph since 2010.

Peter has advised 35 graduate students: 23 M.Sc. (4 co-advised) and 12 Ph.D. students (2 co-advised); and is currently advising 7 Ph.D. (2 co-advised) and 4 M.Sc. students (1 co-advised), plus supervising the research of 5 post-docs, 2 technicians and 1 research project manager. He has published 132 refereed papers and 17 book chapters; selected 19 bean lines (*Phaseolus vulgaris)* supported for variety registration during 2006 to 2011; disclosed a DNA array consisting of approximately 35 bean phenypropanoid pathway gene fragments to the University of Guelph; and submitted more than 200 gene sequences to Genbank.

His research funding has averaged ~$400,000/y from various sources including: NSERC Discovery, NSERC CRD, NSERC Strategic, Ontario White Bean Producers, Ontario Coloured Bean Growers, Ontario Soybean Producers, CanAdapt, OMAFRA, and the Wheat Producers. He has coordinated successful ORF application entitled “Phaseolus Genomics for Improved Bio-Product Development” ($3.7 million over 5 years); developed nondarkening cranberry bean germplasm; coordinated successful CFI application to establish an Agricultural Plant Biotechnology Centre at the Univ. of Guelph ($4.7 million); demonstrated the use of molecular markers for estimating genetic relatedness in bean, corn and soybean plant breeding populations; and identified molecular markers for bacterial blight disease resistance genes in beans.

Professional honors include: President of the Canadian Association of Plant Physiologists, Associate Editor of the Canadian Journal of Plant Science, CSGA Registered Plant Breeder, Ontario Agricultural College Distinguished Researcher Award, University of Guelph Distinguished Professor Award, NSERC University Research and Postdoctoral Fellowships, and University of Waterloo Pearson Medal in Biology for outstanding scholarship in research.

KIRSTIN E. BETT

Kirstin Bett has had a life-long passion for plant breeding and genetics, especially for the bean crop. She started her first research on beans during her M.Sc. at University of Guelph. During that time she attended the 1993 BIC meeting in Boise, Idaho and caught the bug. In 2001 she completed her Ph.D. in canola genetics at the University of Saskatchewan while working at Agriculture and Agri-Food Canada in Saskatoon. Soon after that she joined the faculty of the Department of Plant Sciences at the University of Saskatchewan. During her first year there she decided, thankfully, to devote her research commitment to pulse crops, especially beans.

Apart from breeding and the world of beans, Kirstin has a wide range of interests and skills. She has published many high quality scientific articles on a wide range of topics and disciplines spanning genomics, genetics, biochemistry, crop quality and breeding techniques. She has provided dozens of contributions in the BIC publication, and too numerous to count conference abstracts, proceedings and presentations. Kirstin also has a heavy teaching load, and has taught almost as many different courses as are required for an entire degree in plant biology including population genetics, advanced plant breeding, plant genomics, applied plant biotechnology, bioinformatic techniques and plant cytogenetics.

She has supervised dozens of Ph.D. students, M.Sc. students and post-docs in her lab and has participated in a large number of supervisory committees spanning many disciplines in several colleges at the University of Saskatchewan. She also has an eye for scientific talent and regularly supervises undergraduate student research projects. She has served as the mentor to high school students who have placed in the top three in national and international competitions like the Sanofi BioGENEius Challenge.

Kirstin joined the BIC executive committee in 2011, and through her association with the BIC over the years has developed collaborative relationships with many BIC member scientists. At the U of S, Kirstin has led the effort of the pulse research group to integrate genomic approaches into applied breeding, and was recently the co-lead author on a landmark publication on SNP discovery and mapping for the lentil crop. Kirstin was also the force behind development of the Knowpulse web portal for pulse crop genomics. She has been the scientific lead or co-lead on many major grants focused on developing genomics integration for crop improvement in several crops, including tepary bean for which she has actually attracted research funding. She will publish the first modern genetic map for tepary bean.

As far as bean breeding and genetics goes, Kirstin has made great contributions in the area of seed coat genetics and quality, particularly in research and development of slow darkening pinto bean varieties. She has released commercially successful pinto bean varieties (CDC White Mountain series). She also developed the first successful northern adapted yellow bean variety (CDC Sol), and most recently CDC Superjet black bean. I am also happy to report that Kirstin regularly eats her own research outputs. She has extensive personal interest and scientific knowledge in the area of gastronomy, oenology, nutrition and the potential for genetically improving the nutritional profile of pulse crops.

THOMAS SMITH

Mr. Tom Smith is currently a senior research technician with the Collaborative AAFC/University of Guelph Bean Breeding Program. Tom was raised in southern Ontario and obtained his B.Sc. (Agr.), with a major in Crop Science, in 1984 from the University of Guelph. Tom first worked in the Bean Breeding Program as a summer student in 1983. Later on, after his B.Sc. graduation, he worked as a contract technician under Ken Hugh (former Bean Technician at Guelph) and Dr. Tom Michaels (former Bean Breeder at Guelph).

Tom was appointed the head technician in the Bean Breeding Program in 1986 and is currently the senior technician responsible for organization and implementation of all field and growth room experiments. In the winter semester Tom also runs the crossing program. Tom is a CSGA (Canadian Seed Growers' Association) recognized plant breeder, and is responsible for managing and inspecting dry bean breeder seed production in Idaho. Tom received his Professional Agrologist (PAg) designation in 2007. Tom currently serves as the secretary of the Ontario Pulse Crop Committee.

Tom has been involved in the development of many dry bean varieties, including OAC Thunder, OAC Silvercreek, OAC Rex, OAC Tomahawk (pinto), Lightning, Rexeter, OAC Spark, OAC Dublin, Lighthouse, Mist, Bolt, Yeti, OAC Redstar, OAC Derkeller, OAC Lyrik, Dynasty, and OAC Inferno.

In addition to his role in support of the day-to-day operations of the breeding program, Tom also takes part in the publication and extension of research findings by being responsible for growing bean variety display plots each year. His involvement in the education of highly qualified personnel is evidenced over the years by his great assistance to and support for many graduate students in the Bean Breeding Program and supervising summer students; many of whom moved on to pursue their graduate studies within the breeding program.

IN MEMORY OF JOHN W. AYLESWORTH

John W. Aylesworth died June 29, 2013 on this 96th birthday. He was born in Windsor Ontario, he married Jean Monk and together they raised one son and three daughters. John received his B.S. from the Ontario Agricultural College in Guelph, Ontario and his M.S. and Ph. D. degrees from the University of Minnesota. Soon after graduation Dr. Aylesworth began a storied career in bean breeding and genetics at the Canada Department of Agriculture, Harrow Research Station, in Harrow, Ontario when the research station's dry bean breeding program was established in 1956. As Ontario's bean-growing acreage increased in the late 1960s, the breeding program expanded to include work on addressing more complicated diseases and production problems. From 1966 to 1983, eight new white bean varieties were introduced to southwestern Ontario by the team of Drs. G.M. Clark, John Aylesworth and Dick Buzzell. The team approach to bean breeding involved the expertise of crop physiologists, pathologists and entomologists.

The legacy that Dr. Aylesworth left us with was a full coffer of important varieties that have been widely grown in both Canada and the U.S. These varieties included early bush navy bean Kentwood, followed by the high-yielding variety Fleetwood. Dr. Aylesworth retained the 'wood' name in his varieties in recognition of his home in Woodslee Ontario. In 1984, Dr. Aylesworth left public service with Agriculture Canada to form his own research company Gen-Tec Seeds Ltd., with the focus to breed beans under contract. The company was unique in that it was headquartered in Ontario but was partnered with Clyde Butcher who managed the seed production and research in Twin Falls, Idaho. The company retains the slogan: "If You Don't Know Us...You Don't Know Beans".

The breeding philosophy was to introduce disease resistance traits into widely grown bean varieties. He released Harokent, Harofleet and Seaforth navy beans all with additional anthracnose resistance as the new delta race of anthracnose had appeared in Ontario in the 1980s. When the industry moved from bush to upright short vine navy bean varieties Dr. Aylesworth led the way with the release of Vista navy bean that was the leading variety grown in Ontario and Michigan for many years. Other varieties that were released under the Gen-Tec Seeds Ltd., label were Envoy, Cargo, Crestwood, Fleetside, Scepter, Reliant, Regent, GTS 544, Reliant navy beans, Black Jack, Millenium, GTS 1103 black beans, Rally, GTS-900, GTS 904 pinto beans, Cran 09 cranberry bean, GTS 104 and 106 dark red kidney, GTS 401 white kidney and GTS 1701 yellow eye.

John was a member of Bean Improvement Cooperative and attended many of the meetings in the course of his career. He is survived by his wife Jean, three daughters, his son Stuart who now manages the Gen-Tec Seeds Ltd., Company, 11 grandchildren and 12 great-grandchildren.

IN MEMORY OF CÉSAR CARDONA MEJÍA

Dr. César Cardona, who died 19 June, 2013, in Cali, led a life full of scientific and personal success. The news of his passing caused sadness but also brought forth praise from his many colleagues and friends at CIAT, in Colombia, and throughout the region. He was a giant of CGIAR entomology and made a unique contribution to CIAT's research in Colombia and Latin America. Cardona built his extraordinary career on a solid academic foundation. After receiving an undergraduate degree in agronomy from the National University of Colombia in Palmira, he did his master's degree in entomology at the University of California, Santa Barbara, where he also completed his Ph.D. In the years that followed, Cardona led many projects, contributing importantly to agricultural science and rural development, and he published numerous journal articles and other documents, many of which received national and international recognition. He shared his knowledge in other ways as well, participating in conferences, workshops, and seminars and directing the thesis research of M.Sc. and Ph.D. candidates. Many students who were privileged to work under Cardona's guidance have gone on to gain recognition of their own. Cardona joined the Bean Program in 1978, where he pursued several lines of outstanding research, with emphasis on developing genetic resistance to pests together with simple but effective strategies for integrated pest management, which farmers could readily adopt. He's especially remembered for his pioneering work on resistance to *Zabrotes* bruchids, an important pest of beans in storage, whose damage forces farmers to sell beans soon after harvest rather than wait for a better price. Cardona also led work on resistance or tolerance to the bean pod weevil in Central America and to *Empoasca* leaf hoppers as well as *Thrips palmi*. In addition, he spearheaded work on integrated management of snap beans in Colombia and Ecuador. But CIAT was not the only research organization that benefitted from Cardona's expertise and erudition. He began his career with the Colombian Agriculture and Livestock Institute (ICA), where he formed part of a group called "ICA's seven samurai." He also worked with the National Cotton Growers Federation and served for 4 years with the International Center for Agriculture in the Dry Areas (ICARDA). In addition, he was a founding member of the Colombian Entomological Society (SOCOLEN). All of these organizations mourn his passing. Another of Cardona's passions besides entomology was soccer football; he was an avid fan of the Deportivo Cali team. When the FIFA World Cup games were underway, it wasn't unusual to see him taking a portable television somewhere to watch the games. This facet of Cardona's personality contrasted strikingly with that of the serious and somewhat hot-tempered scientist. Those who worked with him agree, though, that he mellowed significantly after becoming a grandfather. In times of budgetary stress, when staffing cuts seemed imminent, César suffered at the thought of having to terminate the contract of any of his workers. Outwardly tough, César won the loyalty and affection of all his staff and colleagues without exception." Five years ago, Cardona retired from CIAT. Just before his departure, he ordered dozens of books on history, which he read while enjoying the view of Cali from his home on the outskirts of the city. After a prolonged illness, the researcher took his leave – he who never settled for mediocre work, who always expected 100 percent commitment, who shared every prize with the whole team, and who, when he needed to concentrate, put a sign on his door saying, "I'm here but I'm not here."

IN MEMORY OF MAURILIO ALVES MOREIRA

Maurilio A. Moreira died October 27, 2013. He was 64 years old. He was born in Aguanil, Minas Gerais state, Brazil, on May 15, 1949. His first marriage was with Sandra Maria Couto with whom he raised three daughters. His second marriage was with his partner Rita Maria de Morais with whom lived the last years of his life. Dr. Moreira received his B.S. (Agronomy) and M.S. (Plant Sciences) degrees from the Federal University of Viçosa, Viçosa, Minas Gerais state, in 1971 and 1975, respectively. He earned a Ph.D. degree in Plant Biochemistry and Genetics from Purdue University (1980) under the supervision of Dr. Brian A. Larkins. Soon after he receiving his B.S. degree Dr. Moreira was hired by the Federal University of Viçosa (UFV) as a teaching assistant. In 1980, when he finished his Ph.D., he initiated his research on soybean breeding for quality and common bean breeding for disease resistance as an associate professor. His career at UFV spanned 41 years. He served as professor in the Department of Chemistry and the Department of Biochemistry and Molecular Biology and held various administrative positions, such as Pro-Chancellor of Research and Graduate Studies, coordinator of graduate programs in Agricultural Chemistry and Agricultural Biochemistry and Head of the Department Chemistry. In 1992, he founded with other researchers, the Institute of Biotechnology Applied to Agriculture (BIOAGRO/UFV), which he directed for 11 years.

Dr. Moreira´s research group made many important contributions along three decades of hard work. Dr. Moreira published 291 papers in refereed journals most of them related to the improvement of soybean and the common bean. He supervised 77 master´s and 63 Ph.D. students along with a large number of undergraduate students.

The other legacy that Dr. Moreira and his group left was a series of cultivars and an improved common bean germplasm with important resistance genes identified and incorporated into elite lines via gene pyramiding assisted by molecular markers. These materials harbor alleles for resistance to anthracnose, angular leaf spot and rust. These lines are today part of the Common Bean Breeding Consortium involving UFV, EPAMIG and EMBRAPA. The resistance alleles and linked molecular markers identified at UFV are recognized and used in Brazil and in other countries. His group also developed several soybean cultivars and a special soybean germplasm which incorporates alleles for quality traits, such as flavor, protein and oil content, and fatty acid composition.

Dr. Moreira was also member of the Board of Trustees from FAPEMIG for 4 years. He was a member of the Minas Gerais State Council of Science and Technology (CONECIT) for 4 years and member of the National Biotechnology Committee. He was awarded the Medal of Merit in Research at UFV and on August 23, 2013 he received the title of Professor Emeritus of UFV. He was a dedicated father to his three daughters and four grandchildren. He worked at BIOAGRO until the day previous to his death regardless of the severe health problems inflicting his body. He will certainly be remembered with love by the many friends he left in Viçosa, and in other parts of Brazil and in the U.S., especially by his former students – his *'jovens'* to whom he used to refer in this friendly way.

IN MEMORY OF RAFAEL A. SALINAS PÉREZ

Rafael Atanasio Salinas Pérez was born on October 2, 1952, in the City of Monterrey, Nuevo León, Mexico. He completed his undergraduate studies at the Faculty of Agriculture in the Autonomous University of Nuevo León earning him the title of Agronomist in July 1975. He joined the Edible Legumes Program as a researcher in the Valley of Santo Domingo Experiment Station in Baja California Sur, in the former National Institute for Agricultural Research (INIA), one of the predecessors of the current National Institute for Forestry, Agriculture and Livestock (INIFAP). Subsequently he obtained in 1980 a Master's of Science at the Instituto Tecnológico de Estudios Superiores de Monterrey (Campus Monterrey) in the area of Plant Breeding. He rejoined the institute as a researcher of the same Edible Legumes Program in the Valle del Fuerte Experimental Station (CEVAF). In 2013 he was concluding a Doctoral Program at the University of the West-Pacific in Los Mochis, Sinaloa when he passed away at the age of 61. He literally died working, few hours after practicing selection in segregating populations of beans, after 38 years of dedicated service to INIFAP. He is survived by his wife and two sons.

During his career Rafael Salinas conducted research on genetic improvement of edible legumes. He was leader of the interdisciplinary bean group in CEVAF from 1980 to 1983, and Research Coordinator of Edible Legumes for the North Pacific Region of INIFAP from 1984 to 1992, and the National Network Coordinator of beans and other legumes in INIFAP from 2008 to 2010. He authored and co-authored numerous scientific and technological publications and his accomplishments to the genetic improvement of grain legumes in Mexico include the following: i) he actively participated in the development of bean varieties: Azufrado Peruano-87; Azufrado Regional-87, Pinto Anzalduas-91; Azufrado Noroeste; Azufrado Higuera, Negro Pacifico; Aluyori, and the recent varieties Azufrasin and Janasa. Azufrado Higuera has been the leading cultivar in the yellow bean class for more than a decade; ii) he introduced high levels of resistance and/or tolerance to virus diseases, rust and white mold into bean lines with superior culinary quality and highly acceptable commercial seed types; and he and his colleagues in the region developed kabuli chickpea varieties widely adapted in northwestern Mexico, with high levels of tolerance to root-rots, erect growth habit, combined with large seed size, suitable for export markets. Varieties released include: Blanco Sinaloa 92, Progress 95, Jamu 96, Évora 98, Suprema 03, Costa 2004, Jumbo and Blanoro. For more than two decades Blanco Sinaloa 92 has been the leading chickpea cultivar in Mexico.

The work of our colleague Rafael Salinas who was fondly called "El Patron" (The Boss) leaves a very important legacy for regional legume producers in Mexico and an everlasting example of dedication for his colleagues and researchers in INIFAP.

THE CHANGING FACE OF BEAN BREEDING; PAST AND FUTURE

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Major changes have taken place in bean breeding programs to confront greater competition from major commodities, to gain better access to new technologies and funding opportunities, respond to the expanding scientific information and collaborations and meet the increased demand for different seed types suited to different management systems. Despite these changes, and the important international role that beans play in food security, productivity gains have been modest and have failed to keep pace with cereals. How can bean breeders respond to those external issues that drive change in agricultural systems?

- Change is driven by economics, costs, market opportunities, competition, convenience, consumers, climate change, population growth, and loss of agricultural land
- Loss of productive land to major commodities and high value horticultural crops have forced beans to be grown on marginal lands in all continents
- In order to reverse this trend, can researchers increase the value and demand for the crop
- Breeders are important drivers of permanent genetic change but breeders can only work within limits of genetic variability available, as genetic modification is still not attainable or desirable in the market place
- The criticism leveled at bean breeders as to why they have not kept pace with progress made in other major commodities is unfair as progress is mainly a funding shortage, or a crop displacement issue beyond the control of researchers.
- Past international funding for research on grain legumes was limited; emphasis was on placed on cereals, but new funding opportunities now exist for climate resilient legumes
- Future progress will require access to new variability and ways to identify useful germplasm, including access to wild bean germplasm that may lack speciation barriers
- Related cultivated species offer limited potential as species divergence has resulted in a loss of recombination or compatibility with common bean, despite the successful introgression of major genes for common bacterial blight resistance from tepary bean
- In the bean research community there exists a willingness to work with inferior progeny of interspecific crosses (embryo rescue) to attain a goal, but not to work to enhance similar inferior progeny resulting from inter gene pool crosses
- One weakness is the inability to effectively introgress the broad agronomic strengths of the Middle American gene pool into Andean germplasm
- The existing genetic incompatibility may be the result of contrasting physiological processes resulting from sub species divergence
- Breeding efforts to force recombination between gene pools are lacking
- Bean breeders should focus more on cyclic intermating to generate new genetic recombinations; these recombinations are less likely to emerge from single crosses
- Climbing beans offer potential to increase yields but not in mechanized systems
- Climbing beans may provide a genetic bridge to enhance bush types, JeMa variety in Ecuador

- Calima germplasm offers a better gene pool bridge that does US kidney germplasm
- Climbing beans are high yielding, locally adapted with high consumer preference for seed/pod types, but they lack resistance to seed borne pathogens, and local races of anthracnose, rust, angular leaf spot and common bacterial blight
- Marker-assisted backcrossing could be used to introgress major gene resistance with minimal genetic change, to correct major deficiencies caused by biotic factors
- Robust markers based on bean genomic sequence information would bring efficiency to the process if SNP markers can be converted to more breeder friendly SSR markers
- Bean breeders should enter the genomics era with caution
- Most economic traits are not under single gene control, so the haste to identify underlying genes controlling these traits is unlikely to contribute to yield improvements
- Breeders still have to effect change and develop new genetic combinations
- The goal of RNA seq data and differential display data is to sequence genes not breed new varieties
- Association mapping is a static approach to crop improvement as it does not generate new genetic recombinations, but identifies linked markers that might have future use
- Underlying assumption that new markers linked to key traits translates into new varieties
- Experience shows that new varieties come from extensive crossing and field testing of unique recombinants, not from a battery of linked markers
- Breeders should continue to give increased priority to introgress novel variability
- Continue trait improvement in commercial beans and the training of local scientists
- Unfortunately, short term funding does not support long term cyclic intermating schemes that are needed to meet these goals
- The integration of SNP platform and new genomic tools and information into breeding programs is important but not at the cost to traditional breeding strategies outlined above

In conclusion, some of the most dramatic changes in bean production are driven by genetic changes made to bean plant architecture. Few traits other than architecture have had a major influence on the successful production of beans. Changing the climbing habit that requires either a physical or biological support for growth to the short bush habit or upright short vines suited for direct harvest has ensured success of different seed and pod types in diverse production areas. Plant breeding science is dominated with papers on breeding for yield and for resistance to an array of pests, diseases and stresses but all have alternative management strategies to correct or ameliorate them. Since the green revolution, changes in plant architecture are rarely given the importance they disserve. Plant architecture helped drive the green revolution not only in partitioning efficiency but in the development of upright varieties that did not lodge and were better suited for direct harvest in areas where labor was scarce. Plant architecture was the basis of domestication of many crops including the determinate habit in beans. At its basis, ideotype breeding is founded on changes in plant architecture that lead to more efficient partitioning and spawned a plant breeding approach that has led to yield improvement in many major crops. Beans have been a remarkable model for changes in plant architectural improvement - changes from climbing pole blue lake beans where production was limited to small garden plots to the large scale commercial mechanized production systems where beans are successfully grown and compete with major commodities for limited land resources in a changing agricultural climate….to a return to climbers in highland East Africa.

ENHANCING GLOBAL FOOD SECURITY THROUGH INTERNATIONAL LEGUMES RESEARCH

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Feed the Future is the U.S. Government's global hunger and food security initiative that aims to reduce hunger, poverty, and undernutrition by advancing broad-based growth in the agriculture sector. The initiative emerged from the 2009 G8 Summit in L'Aquila, Italy, where President Obama called on global leaders to reverse a three-decade decline in agricultural investment and commit to putting food security and nutrition high on the international agenda once again. In May 2012, during the Camp David G-8 Summit, President Obama launched the New Alliance for Food Security and Nutrition, a shared commitment to achieving sustained and inclusive agricultural growth and raising 50 million people out of poverty by 2022. Feed the Future is the principal vehicle through which the U.S. contributes to the New Alliance.

Feed the Future, which is led by USAID, focuses on smallholder farmers, particularly women, and supports partner countries in developing their own agriculture sectors to generate opportunities for economic growth and trade, which can help reduce poverty and hunger. To achieve impact, Feed the Future focuses on cost-effective results; aligns with country-led plans; embraces innovative partnerships; fosters a policy environment that enables private investment; helps build resilience; integrates nutrition, climate change, and women's empowerment into programming; and works to increase smallholders' adoption of transformative technologies. The initiative is driving real impact through research and development, which is driven by a comprehensive research strategy informed through robust consultations with USAID, USDA, partners from U.S. universities, international research organizations, and the private sector.

Recognizing the potential of science and technology to contribute to global food security, the Feed the Future initiative invests significantly in agricultural research for development. Given the lags in legume productivity gains relative to yield improvements in cereals over the past few decades, legume research is a priority research area under Feed the Future. In the countries where Feed the Future operates, legumes contribute to nutritional quality in the diet, are a source of income for farmers, especially women farmers, and legume production contributes to greater sustainability of low-input farming systems partly due to nitrogen fixation.

Common bean plays a particularly important role in our research portfolio, as it is produced throughout countries where Feed the Future is focused. Through Feed the Future, USAID invests in bean research through collaborative research efforts among scientists in U.S. universities, host-countries, USDA, and in the international agricultural research centers. These research activities span the value chain, including genetics and genomics, on-farm productivity, as well as social science research on farmers' adoption of new technologies and access to market opportunities. Emerging efforts on technology scaling will facilitate availability of and access to bean technologies for smallholders to help increase yields and improve their livelihoods
Beans have a significant role in improving protein and micronutrient intakes among target beneficiaries of Feed the Future – specifically, women and children. However, there are ways in which research on beans and bean production can contribute to nutritional outcomes beyond the nutrient density of the bean. Hence, the need to Think Beyond the Bean!

Nutritional status in children results from a number of interacting factors, including the food they eat, their health status, and the care they receive. Agricultural livelihoods impact on all these factors. Nutritional outcomes can be affected – and potentially improved – with attention to how agricultural activities affect these underlying elements. Through the integration of a nutrition-sensitive perspective among bean researchers, new innovations and technologies to enhance bean production and productivity could contribute to improving the nutritional status of children in bean producing areas. The 'who', 'how' and 'when' of changes in labor requirements for bean production are critical considerations for ensuring that new approaches and technologies do not adversely affect the care of children, and ultimately, their nutritional status. Women's involvement both in bean production and in childcare can be a source of both potential improvements and threats to child nutrition. Women's enhanced bean production and productivity can support child nutrition through greater availability of beans, potentially increased income, and more income in women's control, which is associated with improved child nutrition. However, when women's time allocations or labor requirements are changed during the agricultural cycle, this can have impacts on child caring and feeding practices. New technologies and approaches should evaluate/consider potential effects on women's and men's time allocation and incomes to ensure that new approaches and technologies do not adversely affect the care of children, and ultimately, their nutritional status.

In addition, as new bean varieties are developed, understanding the market context in which these crops would fit is critical to understand the potential for nutrition impacts. Understanding whether adoption of the new bean variety could lead to increases in total production, as opposed to less land allocated to a higher-yielding variety, and whether that increase in production will be consumed at the household level or sold in the market, particularly if the variety commands a price premium, can inform the framing of technology scaling efforts to facilitate the greatest potential broad-based benefits from technology adoption. Similarly, technology improvements can lead to shifts in production of other crops or may change labor requirements at key points in the production cycle – all of which can be considered to design strategies to optimize benefits to farmers and their households. If nutrition considerations are reviewed during technology development, there is significant opportunity to contribute towards improvements in nutritional outcomes at the household level. Accomplishing this requires close collaboration between biophysical and social scientists across disciplines in the agricultural and nutrition sciences to develop technologies and knowledge that fit the socio-economic context of communities.

The recent increases in resources for bean research, and legumes research broadly, affords an unprecedented opportunity for scientists to develop collaborations to consider these additional factors in their research. While nutrition outcomes are a function of many factors, it is incumbent upon the agricultural research community to consider how their approaches may impact nutritional outcomes and think of the ways they can modify their efforts to achieve even greater impacts on nutrition than simply from bean consumption. There is opportunity for the bean research community to lead efforts in this area and galvanize interest from researchers in other fields to contribute to the broader effort of engaging agriculture in more comprehensive ways to improve nutritional status among children and women in the developing world.

LEGUME INNOVATION LAB PROGRAM EXTENSION – STRATEGIC OBJECTIVES AND RESEARH PORTFOLIO

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USAID's Bureau of Food Security extended, through September 2017, the Feed the Future Innovation Lab for Collaborative Research on Grain Legumes (Legume Innovation Lab), previously branded as the Dry Grain Pulses CRSP, administered by Michigan State University.

Grain legumes are a priority crop in USAID's Feed the Future Global Food Security Research Strategy because of their multi-functional roles as nutrient-dense staple foods, and as a crop that generates income for smallholder farmers, improves the livelihoods of rural women and contributes to the sustainability of agricultural systems.

The technical vision of the Legume Innovation Lab is to support collaborative research and institutional capacity building in alignment with USAID's Feed the Future Research Strategy, to provide international leadership to research on common bean and cowpea, to utilize innovative cutting edge research approaches so as to make substantive advances, to be accountable for generating outputs that lead to quantifiable and scalable intermediate development outcomes, to complement legume research of the CGIAR Grain Legume program and of other research investments of USAID and the USDA/ARS, and to engage and mentor a new generation of international scientists in grain legume research.

The Strategic Objectives of the Legume Innovation Lab for the period 2013-2017 are to (1) advance the productivity frontier for grain legumes, (2) transform grain legume systems and value chains, (3) enhance nutritional quality and diversity of diets of the poor, and (4) to improve outcomes of research and capacity building investments. Under these four strategic objectives, the Legume Innovation Lab will be supporting a total of nine collaborative projects that focus on: genetic improvement of common bean and cowpea (four projects); use of biologicals for effective insect IPM in cowpea (one project); improving smallholder farmer decision making for more sustainable soil fertility management (one project); enhancing bean value chain performance through improved understanding of consumer behavior (one project); understanding the role of bean consumption on micro-biome ecology, gut health and function and child growth (one project): and improving research outcomes through impact pathway development and ex-post impact assessments (one project).

In accord with USAID strategic priorities, the Legume Innovation Lab projects will be engaging collaborating agriculture research institutions in eleven FTF focus countries in three regions to transform priority legume-based cropping systems; the West African Sahelian cowpea-cereal cropping system, the Eastern and Southern Africa bean-maize cropping system, and the Latin American/Caribbean hillside bean-maize cropping system. Scientists from nine U.S. universities will be providing technical leadership to Legume Innovation Lab research and capacity building activities including Iowa State University, Kansas State University, Michigan State University, North Dakota State University, University of California-Riverside, University of Illinois, University of Hawaii, University of Nebraska- Lincoln, and the University of Puerto Rico. As a

whole of government initiative, the Legume Innovation Lab is also supporting the research of three USDA/ARS bean scientists.

The Legume Innovation Lab is also a "strategic partner" in the CGIAR's Consortium Research Program (CRP) on Grain Legumes. In this role, Legume Innovation Lab research projects will seek to be complementary and synergistic to those of the Grain Legume CRP plus also address technical gaps in areas where U.S. universities have comparative research capacity (e.g., human nutrition).

Game changing outputs from Legume Innovation Lab collaborative research projects include:

- Molecular markers for drought, CBB resistances and cooking time in common bean from the Andean gene pool
- Genetically improved tepary bean lines for increased seed size, yield and agronomic traits
- Bruchid resistance genes introgressed into improved varieties of important bean market classes for Central America and Haiti
- Genetically improved Middle-American climbing bean lines with disease resistances and agronomic traits for production in highland milpa production systems
- Traits for increased resistances to aphids, thrips, and pod-sucking insects discovered and bred into elite lines of cowpea
- Sustainable IPM solutions utilizing biological controls for insects in West African cowpea systems developed, validated and scaled-up
- Models for smallholder bean farmer decision making regarding sustainable soil fertility management developed and tested
- Diagnostic and decision support aids for improved smallholder soil fertility management
- Solutions to gaps in bean/cowpea production, marketing and distribution systems developed, validated and scaled-up to strengthen value chains
- Knowledge of how regular consumption of beans and cowpeas will influence the incidence of environmental enteropathy, contribute to changes in gut microbial , and improve nutrition and growth of children over 6 months of age.

Although the bean research community is currently being afforded unprecedented funding opportunities, it is imperative more than ever that research efforts be prioritized, coordinated and sustained so that they lead to outputs that substantively improve bean productivity and enhance the livelihoods of farmers both in the U.S. and developing countries.

INHERITANCE AND PERFORMANCE OF BRUCHID RESISTANCE INTO FARMERS' PREFERRED COMMON BEAN (*P. VULGARIS*) VARIETIES IN TANZANIA

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INTRODUCTION: Significant dry bean losses occur during storage as a result of damage by bean bruchids. In Tanzania, two bruchid species occur: *Acanthoscelides obtectus* Say and *Zabrotes subfasciatus*. The two species cause 38-48% reduction in quality and quantity of dry beans (Slumpa & Ampofo, 1991), which worsens the longer the bean seeds are stored. High levels of resistance to *Z. subfasciatus* and moderate resistance to *A. obtectus* have been characterized (Cardona et al., 1992). Resistance is associated with lectin-like seed storage proteins (LLPs), particularly, arcelins (ARL) (Osborn et al., (1988) and alpha amylase inhibitor (α-AI). Along with phytohaemagglutinin (PHA), genes for these closely related seed storage proteins make up the complex ARL-PHA-α-AI (APA) locus (Lioli et al., 2003).

Different allelic combinations of arcelins give varying levels of resistance to the two-bruchid species. Some (*Arl-1*, *2*, *4* and *5*) confer strong resistance to *Z. subfasciatus*, but weak resistance to *A. obtectus* (Cardona et al., 1992). Furthermore, Hartweck et al., (1997) systematically combined several different ARL alleles, with and without phaseolin. *Arl-2* and *Arl-4* without phaseolin gave high levels of resistance to *Z. subfasciatus*.

High levels of bruchid resistance have been demonstrated in *P. acutifolius* and with purified *P. acutifolius* LLPs fed in artificial seeds to *A. obtectus*. We verified that resistance in *P acutifolius* is associated with APA seed storage proteins (Kusolwa & Myers., 2011) by transfering the APA locus to common bean. We hypothesized that combining *P. acutifolius* APA proteins and *Arl-2* in a phaseolin null background would confer high resistance to both bruchid species. We investigated the performance of farmers preferred varieties (FPV) with APA and *Arl-2* introgressions for resistance to bruchids. The objective of this work was to improve bean bruchid resistance among FPV to increase food security.

MATERIALS AND METHODS: We identified four major FPV that were used to introgress APA and *Arl-2* seed storage proteins. 'Kablanketi', 'Soya Njano' (or 'Kigoma'), 'Punda' (also known as 'Jessica'), Bwana Shamba (red kidney) and 'Soya Ndefu' were selected. Plants possessing APA and the *Arl-2* proteins were identified using DNA gene specific primers for ARL-3 and ARC-2. Plants were backcrossed three times followed by selection based on seed types and the presence of insecticidal seed storage proteins. Bruchid feeding trials were established using three replicated of vials containing 20 seeds of each inoculated with 15 adult bruchids (A. *obtectus* and *Z. subfasciatus*) with higher female:male ratio. Data on number of days to bruchid emergence, total number of emerged bruchids and number of damaged seeds were recorded for 70 days from the inoculation date.

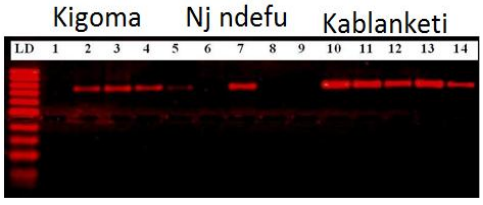
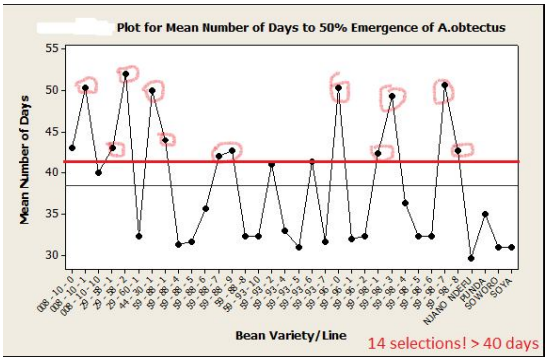


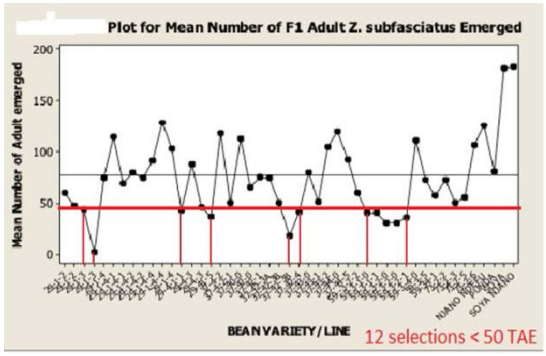
Figure 1. DNA markers for ARL3 used for introgression of APA proteins into farmers preferred varieties.

RESULTS AND DISCUSSION: The arcelin and arcelin-like seed storage proteins were transferred into

farmers preferred varieties based on DNA markers (Fig. 1). All lines possessing DNA markers for bruchid resistance were advanced for bruchid feeding trials. Selected resistant lines are shown in Figures 2a & b. Selection was based on threshold of days to 50% adult emergence and total number of adult bruchids emerged per sample. Low bruchid emergence and delayed emergence were the correlated criteria for bruchid resistance associated with the presence of arcelin (ARC-2) and arcelin like proteins (APA) described in Table 1. These lines were advanced for variety selection for isogenic lines to FPV indicated in Table 1. This work presents the first transfer and utilization of bruchid resistance arcelin based mechanism into FPV in Tanzania.



2a



2b

Figures 2. Graphs showing selection thresholds for bruchid resistant isogenic lines based on **2a)** number of adults emerging and **2b**) days taken for 50% bruchid emergence in a given seed sample.

Table 1. Performance of ARC-2 and APA introgression lines of different farmers preferred varieties (FPV) for bruchid resistance based on total adult emergence and days to 50% adult emergence (DAE 50%).

ARC-2 Introgression			APA Introgression			
Lines	**DAE 50%**	**Recurrent FPV**	**Lines**	**50% DAE**	**Adult emerged**	**Recurrent FPV**
29-1-2	45.80	Njano Ndefu	44-30-1	50	1	Njano Kigoma
29-1-2	47.00		008-10-10	40	4	Punda
37-42-0	48.33	Bwana Shamba	008-10-1	50	3	
37-42-1	45.67		29-58-2	52	22	Njano Ndefu
37-42-4	48.00		29-58-1	43	22	
37-44-5	50.00		59-98-3	49	1	Kablanketi
59--3-2-2	52.67	Kablanketi	59-88-1	44	6	
59-3-1-0	48.33		59-93-6	41	7	
59-3-1-1	45.33		59-93-2	41	2	
59-3-2-0	54.00		59-96-0	50	16	
59-3-2-1	49.00		59-98-8	43	15	
59-34-1	47.33		59 - 98 - 1	42	13	
73-2-2	47.33	Punda				
73-2-3	50.67					
73-2-5	50.33					

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BEHIND THE SCENES OF THE COMMON BEAN GENOME

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The recent release of version 1.0 of the common bean (*Phaseolus vulgaris* L.) genome sequence (http://phytozome.net/) provides an unprecedented opportunity for the bean community to better understand the form and function of this essential societal crop and to use the sequence as a tool for crop improvement. While the sequence information and its assembly are valuable, they are essentially devoid of functional content. By analyzing the associated gene models and annotation it is now possible to begin creating a functional landscape of the genome. A long term effort of the entire community will be to mine this rich genotypic resource for those genes associated with the important phenotypes traits that will improve common bean. Here we provide a first glimpse at a few features of the genome.

The assembly defined 11 pseudochromosomes. Utilizing recombination rate data across the genome obtained from a detailed genetic map, two of the eleven were characterized as acrocentric chromosomes (Pv06, Pv09). The initial annotation of the genome defined 27,197 gene models and 4,491 alternate transcripts. As with most genomes, the majority of the gene models are located in the arms of each chromosome where the recombination rates are the highest. In the arms of the chromosomes the physical to genetic distance ranged from 95-1,156 kb/cM while the distance ranged from 2,118-9,208 kb/cM in the low recombination central portion of the chromosomes.

Another important structural feature is the relatively high (50%) repeat composition of the genome. This is important because non-gene based molecular marker would be enriched for these elements. For example, the sequence of three previously discovered markers linked to seed color or patterning genes (*Bip*, *Z*, and *P*)[1,4] are repeat elements. While these function well as genetic markers, the number of homologous repeat sequences in the genome make it difficult to use these markers to begin the search for candidate genes.

Common bean is susceptible to many different plant pathogens. Extensive molecular mapping experiments have placed these genes in specific genetic positions. One feature is the apparent genetic clustering of some of these genes. To investigate this further, we used the annotated gene models and performed a Pfam analysis to discover those sequences containing a nucleotide binding site (NBS) sequence. This sequence was selected because many of the cloned resistance genes contain such a structure. For comparative purposes, we also evaluated two other Phaseoleae species, soybean and pigeon pea. Among these three species, common bean had the most NBS sequences, and of these the Coil-NBS-LRR class was the largest (Table 1). Soybean, in contrast, had 25% less NBS sequences. This is surprising because given its ancestral relationship to common bean and the relatively recent tetraploidization of soybean, it would be expected to have significantly more NBS sequences. This suggests different evolutionary patterns for NBS-containing genes in these two species. The reduced number of pigeon sequences may reflect upon the quality of its reference genome sequence.

Table 1. Distribution of putative resistance gene analogs in specific classes.

Class	Common bean	Soybean	Pigeon pea
Coil-NBS	8	2	4
Coil-NBS-LRR	115	30	38
NBS	2	12	31
NBS-LRR	77	78	78
RPW8-NBS	1	1	0
RPW8-NBS-LRR	5	11	6
Toll-NBS	10	14	7
Toll-NBS-LRR	74	67	8
Total	**292**	**215**	**172**

There are a number of plants examples where orthologous sequences from two related species were shown to control the same phenotype[3]. This phenotypic synteny may also reflect ancestral sequence relatedness between two species within the same clade. This indeed seems to be the case in the Phaseoleae clade where large blocks of the common bean genome map to two regions of the soybean. For example, long stretches of Pv01 are syntenic with both Gm03 and Gm19 of soybean. These relationship are best represented at the phenotypic level by the determinacy loci in these two species where *Fin* in common bean maps to Pv01[2] and *Dt1* is located on the Gm19 syntenic region[5]. These syntenic relationships are well displayed at the whole chromosome leve in the Phaseolus GBrowse at the Legume Information System (http://phavu.comparative-legumes.org/gb2/gbrowse/Pv1.0/). Comparing the genomic organization of common and soybean will provide insights when attempting to clone genes which control common phenotypes in the two species.

This common bean reference genome can also be used to consider issues related to linkage disequilibrium (LD), the population parameter critical to successful genome-wide association analysis. Such an analysis with the BeanCAP population showed that LD varied not only between chromosomes, but between different regions within a chromosome. Cases of interchromosomal LD were also observed. Collectively, these genomic features, and others yet to be discovered, will drive future common bean improvements.

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DEVELOPMENT AND APPLICATION OF EPIGENOMIC METHODS TOWARDS A BETTER UNDERSTANDING OF COMMON BEAN GENOME

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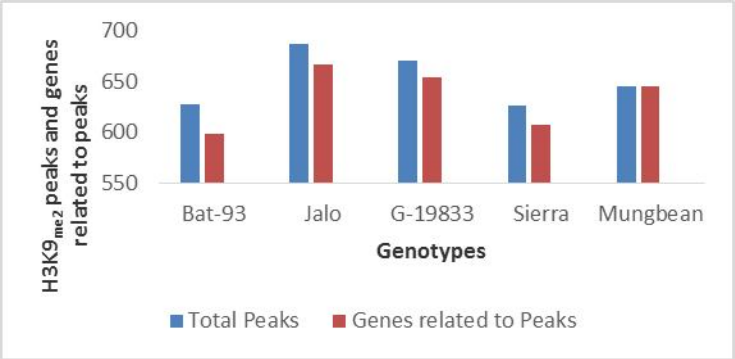
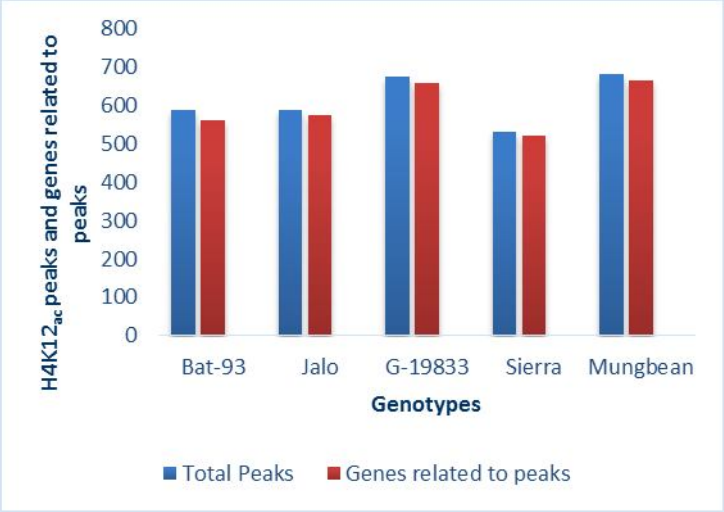
INTRODUCTION: Epigenetics refers to changes in gene expression due to perturbations in the environment, including abiotic and biotic stresses, without a change in the inherent DNA sequence. Genome-wide investigation of protein-DNA interactions and mapping of epigenetic marks is essential for understanding transcriptional regulation. Recent progress in massively parallel sequencing has allowed the characterization of DNA associated with proteins using the combination of chromatin immunoprecipitation and sequencing (ChIP-seq). ChIP-seq has become an indispensable tool for studying gene regulation and epigenetic mechanisms. A number of studies have reported efforts on the global identification of DNA-histone interactions in several model organisms. This technology has been proved as an efficient tool to generate high-resolution epigenomic maps of mammalian genomes, and plants such as *Arabidopsis*, rice, maize, *Brassica*, soybean and poplar. Common bean (*Phaseolus vulgaris* L.) represents the most important legume species for direct human consumption and meets important nutritional needs as a source of protein, fibers, minerals, and vitamins. Until now, there is no report available for next generation sequencing in common bean using ChIP-seq. We have developed a method for chromatin immunoprecipation sequencing (ChIP-seq) of common bean in order to develop a genome-wide survey of the location of two epigenetic marks ($H3K9_{me2}$ and $H4K12_{ac}$, repressive and activation marks, respectively) in several genotypes (viz, Bat-93, Jalo, G19833, and Sierra) of common bean and one locally bought mungbean genotype.

MATERIALS AND METHODS: ChIP antibodies ($H3K9_{me2}$ and $H4K12_{ac}$) were validated by dot blot analysis and ChIP assay was performed as described previously by the Lam Laboratory (Rutgers, The State University of New Jersey), and modified for common bean. ChIP DNA was prepared for Illumina sequencing at the Delaware Biotechnology Institute sequencing facility. An inventory of the raw sequencing data was initially prepared and the quality of reads was assessed using FASTQC. Raw sequencing data was cleaned to filter out low quality data, and the mapped reads were obtained by mapping the cleaned reads (with 50 bases) to *Phaseolus vulgaris* G19833 genome (Phytozome version 1.0), with a criteria of no more than two mismatches by Bowtie (Version 1.0; Langmead and Salzberg 2012). To identify genes, which were differentially enriched for $H3K9_{me2}$ and $H4K12_{ac}$, we used Model-based Analysis of ChIP-seq (MACS, version 2.0.10; Zhang et al. 2008) on the data from respective genotypes. $H3K9_{me2}$ and $H4K12_{ac}$ peaks were identified in MACS with the following parameters: effective genome size = $2.7e^{-9}$, tag size = 20, bandwidth = 300, mfold = 10, 30 and p-value cutoff =$1.00e^{-05}$. The peaks were then annotated with Hypergeometric Optimization of Motif Enrichment (HOMER; Vire et al. 2006). A gene was regarded as being $H3K9_{me2}$-modified gene or $H4K12_{ac}$-modified gene if the gene region had more than 50% overlap with modification peaks of genes.



(A)



(B)

Figure 1. Total peaks and genes related to peaks with (A) $H3K9_{me2}$ and (B) $H4K12_{ac}$ modifications in common bean and mungbean.

RESULTS AND DISCUSSION

The common bean genotype G-19833 associated genomic dataset was used as a reference. We used a MACS model-based algorithm for identification of significant clustering of windows with enriched ChIP signals, called "peaks" of $H3K9_{me2}$ and $H4K12_{ac}$. We filtered data based on a quality score >20 and a distance of 10 Kb from TSS. In total, 628, 687, 671, 627 and 645 peaks for $H3K9_{me2}$ and 599, 667, 654, 607 and 629 known genes related to peaks in $H3K9_{me2}$ were identified. Similarly, 589, 588, 677, 533 and 682 peaks for $H4K12_{ac}$, and 563, 574, 660, 520 and 665 known genes related to peaks in $H4K12_{ac}$ have been identified for Bat-93, Jalo, G-19833, Sierra and mungbean respectively (Figure 1). This study will help in understanding the role of $H3K9_{me2}$ and $H4K12_{ac}$ in the regulation of individual gene expression and will facilitate systematic insight into the overall structure and function of chromatin.

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ASSOCIATION OF GENES CODING FOR PHENYPROPANOID PATHWAY ENZYMES AND REGULATORY ELEMENTS WITH FLOWER AND SEED COAT COLOUR IN *PHASEOLUS VULGARIS*

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INTRODUCTION: Seed coat colour is a defining characteristic of bean market classes and genes involved in the phenylpropanoid pathway are thought to correspond to some of the classical seed coat colour genes (Reinprecht et al. 20013). The locations of 18 phenylpropanoid pathway genes were mapped with the BAT93 × Jalo EEP 558 RIL population and 5 were mapped with an OAC Rex × SVM Taylor RIL population. The study identified some interesting co-localizations between phenylpropanoid pathway genes and seed coat colour genes that warrant further investigations to determine if they are related.

MATERIALS AND METHODS: Polymerase chain reaction (PCR) and restriction fragment length polymorphisms (RFLP) were used to map the phenylpropanoid pathway genes in two RI populations derived from BAT93 × Jalo EEP 558 (Freyre et al. 1998) and OAC Rex × SVM Taylor Horticulture (Larsen, MSc thesis, 2005). The segregation patterns of 18 phenylpropanoid pathway genes (IFR, FBP4, CHR, LAC, IFS, 4CL, AS, CAD, F5H, Myb transcription factor, RT, IOMT, VT, CCR, F3'H, LAR, PAL1, PAL2) in the BAT93 × Jalo EEP 558 recombinant inbred population were analysed and their locations in the bean linkage map were determined using JoinMap analysis. Ten SSR markers and 89 SNP markers were scored for the 89 inbred lines derived from cross between 'OAC Rex' and 'SVM Taylor'. Their map locations were added to the existing genetic map of this population.

RESULTS AND CONCLUSION: Seven genes out of 18 genes were mapped within 2-17 cM

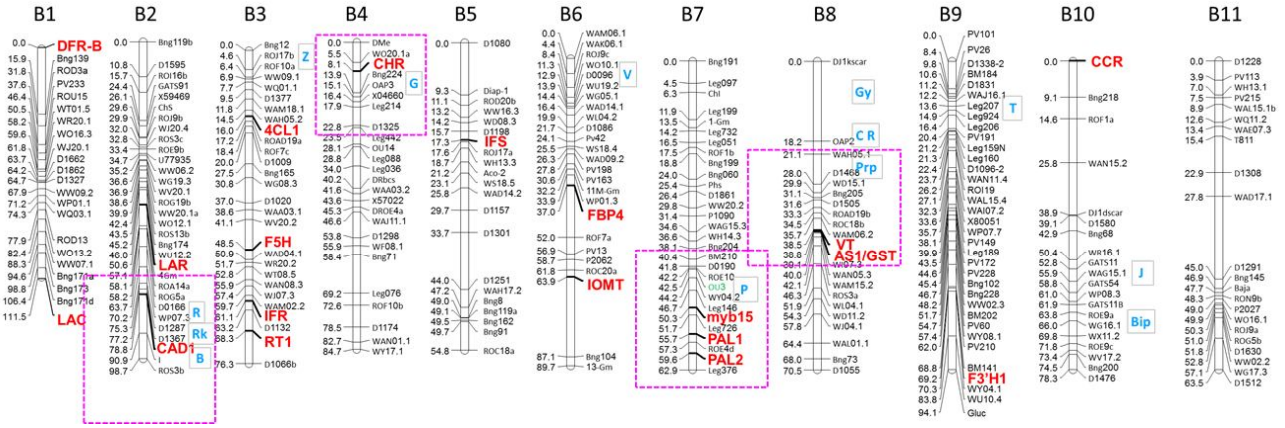

Figure 2. Core Linkage map of the common bean recombinant inbred population. Symbols for colour genes are boxed and blue and those for phenylpropanoid pathway genes are in red. Centimorgan (cM) distances between markers ordered at a LOD score 4.0 are shown to the left of each linkage group.

of colour gene loci. In particular, associations were found between PAL1 (13.2 cM), PAL2 (17.1 cM) and Myb transcription factor (7.8 cM) to P, CHR (7 cM) to G, CAD (2.1) to B and VT and AS1/GST (10.5 cM) to Prp.

Polymorphisms for five phenylpropanoid pathway genes in OAC Rex × SVM Taylor were used to place them on the linkage map of this population. DFR1 was mapped 13.9 cM from a flower colour locus and the marker for this gene was significantly associated with this trait.

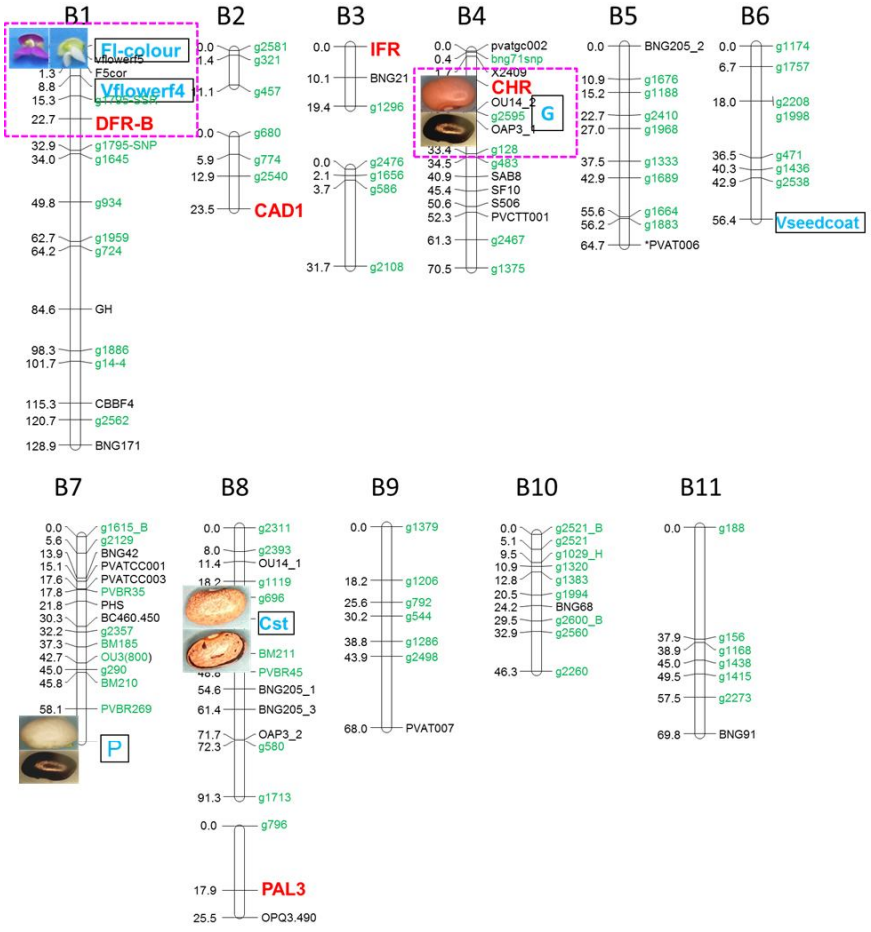

Figure 3. . 'OAC Rex' × 'SVM Taylor' linkage map of the common bean recombinant inbred population. Phenylpropanoid pathway genes are in red and colour phenotypes mapped in the population are boxed and blue. Centimorgan (cM) distances between markers ordered at a LOD score 3.0 are shown to the left of each linkage group.

Further studies are needed to confirm the roles of the phenylpropanoid genes as potential colour genes.

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MOLECULAR GENETIC ANALYSIS OF THE *PHASEOLUS VULGARIS P* LOCUS

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INTRODUCTION: Common bean market classes are distinguished by their many seed colors, patterns, and size[11]. At least 23 genes, acting independently or in an epistatic manner, affect the seed coat color and pattern[3]. The *P* locus which is described as the "ground factor" by Emerson[4], has multiple alleles[2,3] and controls all color expression. The presence of its recessive allele *p* results in white seeds and flowers due to its epistatic effect on the expression of the other color/pattern genes. Different molecular markers mapped the *P* locus on Pv07[5,8]. The availability of a reference genome (Schmutz, McClean et al. submitted) facilitates the identification of a candidate gene based on comparative genomics approach. Previous studies in Arabidopsis indicate indicates that multiple mechanisms are involved in seed coat color expression[1,9,11]. One possibility is that the *P* locus encodes a transcription factor that regulates the flavonoid biosynthesis pathway in a manner similar to the Arabidopsis *TT8*[9] or pea *A*[6] genes. Our objectives were to 1) physically fine-map the *P* locus using known linked markers, 2) use a comparative genomics approach to find a candidate bean ortholog to *AtTT8* and *A*, and 3) characterize different alleles of the *P* gene candidate. We also performed an independent genome wide association study (GWAS) to find the genomic regions associated with the seed coat color.

MATERIALS AND METHODS: The RAPD marker $OU3_{2300}$ [5] and the g2416 and g1175markers[8] that co-segregate with the *P* locus were mapped to the bean draft genome (V1.0) using BLAST. RNAseq data were used to validate the bean predicted gene models that were orthologous to *AtTT8* and *A*. The BeanCap Mesoamerican Diversity Panel MDP, consisting of 190 white-seed and 90 colored-seed genotypes were used for the GWAS. The 280 lines were genotyped using 34,799 mapped SNPs. The seed coat color was phenotyped as a binary trait (colored/white), and a penalized multiple logistic regression in PUMA7 was used for the GWAS. Principal component analysis was used to control for population structure. A diagnostic marker for the *P* gene candidate, encompassing exon 1, intron 1 and exon 2 of the Phvul.007G171300 gene model, was used to screen: 264 genotypes from BeanCAP MDP (179 colored, 85 white); 148 individuals comprised of various wild, landraces, and genetic testing genotypes; and the F_2 population used by Erdmann et al.[5] to map *P* to chromosome Pv07.

RESULTS AND DISCUSSION: RAPD marker $OU3_{2300}$, which co-segregates with the *P* locus mapped to position 40,472,206 to 40,474,507 bp on Pv07. Gene-based markers g2416 and g1175 flanked $OU3_{2300}$ and mapped to 39,311,484 bp and 42,520,159 bp on Pv07, respectively. In this 3.2 Mb interval, 253 genes reside. Of these, two tandem gene models (Phvul.007G171300 and Phvul.007G171400) were orthologous to *AtTT8* and *A*. BLASTP demonstrated that Phvul.007G171300 and Phvul.007G171400 align to the N-terminal and C-terminal of *AtTT8* and *A*, respectively.

A single RNA-seq contig from flower tissue included contiguous segments of the last exon of Phvul.007G171300 and the first exon of Phvul.007G171400 which indicates the two exons are part of the same gene. The reconstructed gene model is 13,596 bp and comprises 7 exons. The RNA expression pattern was consistent with the phenotype associated with *P*. The gene candidate is highly expressed in flowers, flower buds, and young and green mature pods.

We conducted an independent GWAS to find the genomic regions that control seed coat color. Eight SNPs, within 200 Kb of *P* gene candidate, passed the Bonferroni threshold. Two markers mapped in the *P* gene candidate and four markers were in complete LD with them. The marker in the gene accounted for 37% of the phenotypic variation after controlling for population structure.

To evaluate the nature of mutations associated with *P*, segregating populations were screened for the diagnostic *P* gene marker. All the colored-seed genotypes from the BeanCAP MDP exhibit a 1560 bp fragment and the majority of the white genotypes possess a 270 bp fragment. All (but one) white-seeded great northern genotypes were unique because they contained the 1560 bp fragment. Another exception was NEP-2 (a white-seeded mutant from black bean San Fernando) that showed the 1560 bp fragment. The wild, landraces and genetic testing materials showed the same general pattern. The progeny of the F_2 population used by Erdmann *et al*.[5] (2002) showed the same pattern as their parents where the *PP* parent (5-593) showed the 1560 bp fragment, while the $p^{gri}p^{gri}$ parent (VO400) did not produce any fragment. Other alleles of the *P* gene are yet to be discovered.

Although not definitive, these molecular genetic analyses provide compelling evidence that this gene should be investigated further as a *P* candidate. Additional evidence from VIGS analysis or TILLING mutant screening will provide more definitive proof. Beyond this research, defining the genetic factors controlling other seed coat color/patterning genes and developing markers linked to these genes will provide new tools for targeting breeding efforts of specific market classes of beans.

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DEVELOPMENT OF POTENTIAL BREEDER-FRIENDLY MARKERS FOR THE *I* GENE USING BULKED SEGREGANT ANALYSIS AND WHOLE-GENOME SEQUENCING

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INTRODUCTION

Bean common mosaic virus (BCMV) and its related necrotic species *Bean common mosaic necrosis virus* (BCMNV), are the most prevalent potyviruses in common bean (*Phaseolus vulgaris*). The dominant *I* gene confers immunity or temperature-dependent hypersensitive response to most strains of BCMV, and temperature-independent systemic necrosis to strains of BCMNV (Drijfhout et al., 1978). The *I* gene has been tagged with the dominant SCAR marker SW13, which is commonly employed in breeding programs (Melotto et al., 1996). However, the genomic location of the marker is unknown, and recombination between SW13 and the *I* gene occurs (Vandermark and Miklas 2005), leading to erroneous genotyping. The *I* gene in combination with other recessive genes (*bc-1, bc-2*^{2}*, bc-3*) provides resistance to all strains of BCMV and BCMNV, but due to their epistatic interaction among them and the dominance nature of current markers it is not possible to indirectly select plants with desirable allele combinations. Therefore the development of co-dominant markers linked to BCMV resistance would increase breeding efficiency. The objective of this work was to discover polymorphisms in candidate disease resistance (NBS-LRR) genes at the *I* locus for development of breeder-friendly markers with potential for marker-assisted selection for BCMV resistance.

MATERIALS AND METHODS

One-hundred F_6 derived recombinant inbred lines (RIL) from the cross between the susceptible G122 (*ii*) × resistant Montcalm (*II*) plants were previously inoculated in the greenhouse with the NL-3 strain of BCMNV. Susceptible plants develop mosaic symptoms, whereas resistant plants develop top necrosis which eventually results in plant death. DNA samples from 15 susceptible and resistant plants were pooled to create susceptible (GM-S) and resistant (GM-R) bulks, respectively. The DNA bulks were submitted for whole-genome sequencing using a single lane of Illumina HiSeq2000 at Eurofins MWG Operon. For data analysis, next-generation sequencing (NGS) reads (100 bp paired-end) of each bulk were separately mapped onto a 500 kb region of chromosome 2 from the reference *Phaseolus vulgaris* genome, deposited at Phytozome, using the CLC Genomics software. The genomic region contained a 158 kb sequence flanked by the *Phgp* and Bng45 markers, previously used to genetically map the *I* locus (Vallejos et al., 2006). For this study, polymorphisms (SNP, Indel) within exons/introns and regulatory sequences of candidate genes were detected using CLC Genomics software, and only those that were fixed (100% homozygous) in the GM-R bulk but not in GM-S were targeted for development of agarose-gel based markers. Potential markers were screened accross 92 GM RILS, and other select common bean genotypes with and without the *I* gene.

RESULTS AND DISCUSSION

From this population, 65 resistant and 27 susceptible plants to the NL-3 strain of BCMNV were identified. *In silico* analysis of the region between the *Phgp* and Bng45 marker in chromosome 2 identified seven R genes (Phvul.002G323000- Phvul.002G323500, Phvul.002G323800)

encoding for NBS-LRR disease resistance products. This finding supports the previous notion that the *I* locus contained a cluster of R genes (Vallejos et al., 2006), and encompasses a 73.7 kb region located at the terminal end of chromosome (physical position ~48.2-48.3 Mb).
Bioinformatics analysis of the *I* locus assembly of GM-R and GM-S bulks revealed high level of heterozygosity, and only 8% (138) and 19% (239) of the detected polymorphisms were homozygous in the GM-R and GM-S, respectively. After screening of parents and GM-R and GM-S bulks with potential PCR markers, only five markers (1 co-dominant, 1 dominant for resistance, and 3 dominant for susceptibility) were polymorphic, and each targeted a single R gene (Table 1).

Table 1. Survey of agarose-gel based markers linked to BCMV resistance across the RIL population and cultivars of different market classes and gene pools

		Marker/Gene-based marker					
		SW13 690 bp/NA[b]	Phvul.002G323000 473bp/305 bp	Phvul.002G323300 460 bp/NA	Phvul.002G323400 NA/368	Phvul.002G323500 NA/453	Phvul.002G323800 NA/182
Genotype	***I allele***	***II/ii***	***II/ii***	***II/ii***	***II/ii***	***II/ii***	***II/ii***
GM-R RILs	+[a]	65/0	65/0	65/0	64/1	64/1	64/1
GM-S RILs	-	1/26	0/27	0/27	0/27	1/26	0/27
37-2 (M)	+	+	-	-	+	+	+
Cardinal (A)	+	-	+	+	+	+	+
Dor 364 (M)	+	-	+	+	+	+	+
Eclipse (M)	+	+	+	+	+	+	+
Raven (M)	+	+	+	+	+	+	+
Red Hawk (A)	+	+	+	+	+	+	+
Red Kloud (A)	+	+	+	+	+	+	+
Stampede (M)	+	+	+	+	+	+	+
Aztec (M)	-	+	+	+	+	-	+
Chase (M)	-	-	-	+	+	-	+
Mont #5 (M)	-	-	-	+	+	-	+
Mont-Rose (M)	-	-	-	+	+	-	+
Olathe (M)	-	-	-	+	+	-	+
THort (A)	-	-	+	-	+	+	+
UI-3 (M)	-	-	-	+	+	-	+
UI-537 (M)	-	-	-	+	+	-	+

[a]Indicates presence or absence of the *I* allele as determined by greenhouse inoculation with the NL-3 strain of BCMNV.
[b]Indicates no amplicon detected.

There was a high level of agreement between the assignment of plant genotype and plant phenotypic characterization in the greenhouse. The presence of two recombinants in the GM population narrows down the location of R gene conditioning resistance to BCMV between Phvul.002G323000 and Phvul.002G323400. Based on the genotyping of the other plant genotypes, it seems that markers in Phvul.002G323000 and Phvul.002G323500, in combination, might be useful for marker-assisted selection. Work is ongoing to test these markers in a much broader set of common bean germplasm and their potential for marker-assisted selection for BCMV resistance within and across gene pools.

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WHAT'S SO DIFFERENT ABOUT TEPARY BEANS?

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INTRODUCTION: Limits to adaptation of common bean (*Phaseolus vulgaris*) are largely due to a lack of stress tolerance, particularly sensitivity to drought and extremes in temperature both hot and cold. Tepary bean (*Phaseolus acutifolius*) is another domesticated bean crop, grown originally in the arid regions of the south-western USA and into Mexico and Central America. Being much more stress tolerant than its common bean relative, it has also been cultivated in marginal areas of South America and Africa where common bean cannot be grown as successfully. There has been a resurgence in interest in this crop recently as it is viewed as a potential source of genes for stress tolerance for common bean breeding or as a crop in its own right.

Tepary bean is in the tertiary gene pool of common bean and the first few generations following hybridization generally require embryo rescue to be successful. Tepary bean is also the source of tolerance to common bacterial blight found in many cultivars grown today and is being explored as a source of tolerance to stress[1].
While developing SNP resources for common bean, we decided to include tepary beans to compare the two species and gain a better understanding of the similarities and differences between these important bean species.

MATERIALS AND METHODS: cDNA libraries from two tepary bean lines (W6 15578, PI 430219) were sequenced using a strategy already used in lentil[2]. SNPs were called relative to the Pv0.9 version of the common bean genome[3] and a 768-SNP Illumina GoldenGate OPA was developed for tepary bean SNPs.
Tissue was collected from individual plants from an F_2 mapping population derived from a cross between the two wild tepary bean lines as well as a collection of 88 diverse domesticated tepary beans, two *P. parvifolius* accessions from the collection at CIAT and four tepary beans from Prairie Garden Seeds in Saskatchewan. DNA was extracted using a modified CTAB procedure, quantified, genotyped using the GoldenGate assay following standard procedures and the data were processed as in Sharpe *et al*.[2]
Genotypic data from the mapping population were used to generate a map of the genome in JoinMap4.0. The flanking sequences for each SNP in the tepary bean map were compared to the common bean pseudomolecules from Pv1.02[3] using MUMmer4. A dendrogram representing genetic relationships among the tepary accessions was developed using the neighbor joining tree function in DARwin.

RESULTS AND DISCUSSION: 679 SNPs were scored in the mapping population and could be resolved into 11 linkage groups covering 1103 cM with an average of 1.8 cM between the loci. Because the common bean homologue of the tepary bean genes from which the SNPs were derived could be identified, it was possible to compare the two genomes (Fig. 1). All 11 tepary bean linkage groups corresponded to the 11 common bean pseudomolecules with only a few internal inversions and translocations.

The domesticated tepary beans formed a cluster quite distinct from the two *P. parvifolius* accessions (Fig. 2). One of the teparies sourced in SK, Mitla Black, separated out distinctly from the other tepary beans suggesting it is not in fact a tepary bean at all. A query of GRIN revealed a *P. vulgaris* accession with the same name, suggesting it is likely a common bean that has been misclassified as a tepary bean. Based on its small seeds, and leaves and pods that more resemble tepary bean plants, this is not unlikely. Within the domesticated tepary beans there were several large clusters and a few more diverse lines but there was no clear pattern based on origin (data not shown).

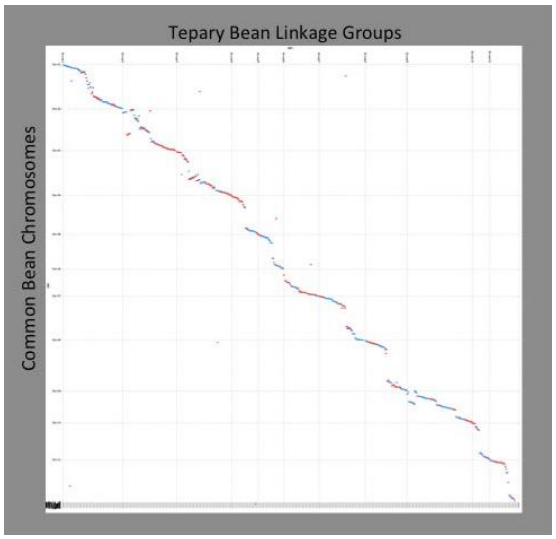

Figure 1. Dotplot comparison of tepary bean linkage groups (left to right) with common bean chromosomes (top to bottom) based on homology between the tepary sequences from which SNP markers were derived and the common bean reference sequence Pv1.0.

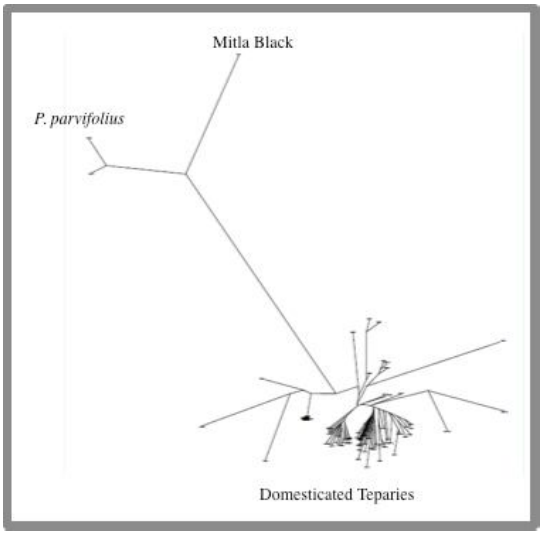

Figure 2. Genetic distance among 92 domesticated tepary beans and two *P. parvifolius* accessions. Distances calculated from 391 polymorphic SNPs.

The tepary bean SNP resources described here will greatly facilitate further genetic characterization of tepary germplasm as well as mapping populations from tepary bean crosses and interspecific hybrid populations derived from crosses between tepary and common bean. The high level of shared synteny between the two species suggests that introgression from one to the other should be possible and could be tracked using these genomic resources.

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GARDEN-BASED BIOLOGY AND NUTRITION EDUCATION TO PROMOTE THE CONSUMPTION OF COMMON DRY BEANS

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INTRODUCTION: Fewer than 10% of children (ages 4 to 13 years) in the U.S. meet USDA recommendations for consumption of fruits and vegetables (Heim et al., 2009). Concurrently, the leading cause of death in the U.S. is heart disease, and diabetes is seventh (Hoyert and Xu, 2012); both diseases are primarily a result of poor diet and lack of exercise (Adams et al., 2005). Despite the evidence that incorporating dry beans into the diet can reduce the incidence of heart disease and diabetes, pulses remain an underutilized food in the U.S. Garden-based nutrition education can be an effective tool for teaching K-12 students healthy eating habits, and children who observe and participate in growing and tasting demonstrations are more likely to regularly consume the targeted food (Comstock and Symington, 1982; Heim et al., 2009; Kirks and Wolff, 1985; Lineberger and Zajicek, 2000; McAleese and Rankin, 2007). The objective of this project is to determine if garden-based dry bean biology and nutrition education at the K-12 level has an effect on improving knowledge and food choice of dry beans in targeted students.

MATERIALS AND METHODS: The project is being carried out in Whatcom County in cooperation with one fourth grade class at Roosevelt Elementary School, Bellingham School District, and one ninth grade class at Windward High School, Ferndale School District. Three lessons that include biology, nutrition, and school gardens have been developed for each grade; all lessons are Science, Technology, Engineering and Math (STEM)-based and also meet the new Next Generation Science Standards. The dry bean biology component of the curriculum includes growth cycle, nitrogen fixation and point of origin (Watrin, 2011). The dry bean nutrition component includes health benefits of fiber, protein, low-fat content, vitamins and minerals (Howarth et al., 2001; Patwardhan, 1962). For the school garden component, students planted bean seed 28 May at Windward high school and 6 June at Roosevelt elementary. Both classes harvested their beans 1 October and threshed dried beans that were grown at WSU Mount Vernon NWREC (beans harvested at the schools were not dry enough to thresh the same day).

Dry bean consumption is being measured through a plate waste study of meals made with dry beans in the school cafeteria before and after the education program (Kock et al., 2006). Four recipes were developed for the school cafeteria, two hot and two cold. The School District Food Service Directors selected one for the school food service staff to prepare: 'cheese beans'. Dry beans were provided by regular food supply channels (Sysco) to Windward High School, and WSU Mount Vernon NWREC provided beans to Roosevelt Elementary School. Both schools soaked the beans overnight and cooked them on the stovetop to prepare their dishes. Consumption of the meal was measured for two classes from each school (the class that is receiving dry bean education and one control group) (Adams et al., 2005).

RESULTS: There was a 6% increase in consumption of 'cheese beans' by the ninth grade class that received the garden-based biology and nutrition education, while the control group showed a 4% increase (Fig. 1). In the fourth grade class that received the education, there was a 32% decrease in consumption, while the control group showed only a 12% decrease. For the plate waste study for the ninth grade class, beans were served the same way both pre and post-education, as a side dish to their meal. In contrast, for the fourth grade class plate waste study,

beans were served as the main dish pre-education and as a side dish post-education; thus, the two consumption measurements were not comparable. It was difficult to coordinate this study with the school food service staff, as food is prepared and portioned at a central kitchen and not at each school.

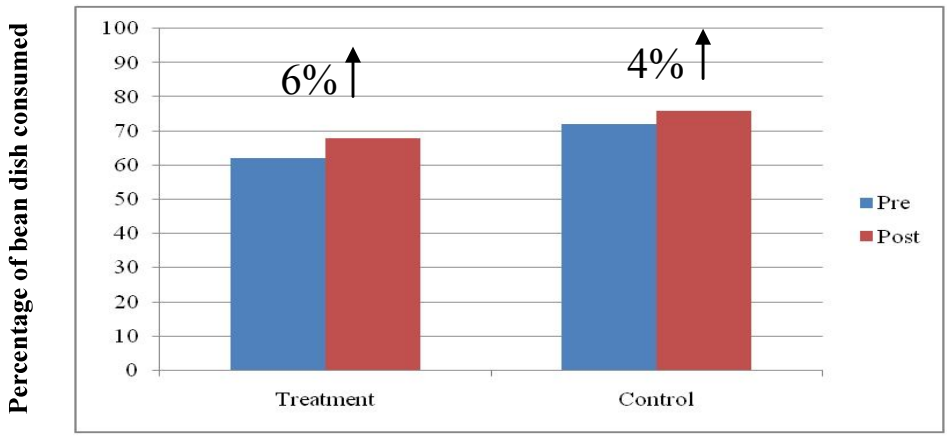

Fig. 1. Average amount of dry bean dish consumed by ninth graders before and after dry bean education.

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OVERCOMING DRY BEAN PRODUCTION CONSTRAINTS IN WESTERN WASHINGTON

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INTRODUCTION

In order to increase dry bean (*Phaseolus vulgaris*) production and profitability for diversified direct-market producers in western Washington, this project seeks to:
1.) Identify dry bean production constraints and opportunities in western Washington;
2.) Evaluate the potential of local heirloom varieties to meet the needs of regional producers relative to commercially available varieties.

MATERIALS AND METHODS

Dry Bean Producer Survey: We distributed an on-line survey in spring 2013 through Washington State University Extension personnel in 12 western Washington counties. Extension personnel distributed the survey via email to more than 700 producers.

Field Trials: May 2013 we planted a replicated field trial with 20 bush varieties including 9 local heirloom varieties that have been cultivated in western Washington for 20-120 years and 11 commercially available check varieties. Trial plots consisted of four 3.04 m rows on 76 cm centers, Rhizobium-inoculated seed was placed every 5 cm within rows. Organic fertilizer (Proganic 8-2-4) was applied at the rate of 56 kg/ha. Weeds were controlled by cultivating between rows two times, and hand weeding within rows twice. No supplemental irrigation was applied. At maturity, whole plants were harvested from the center 1.52 m of two center rows (3.04 m total), dried at 40.6 ^{o}C for 36 hrs, and threshed.

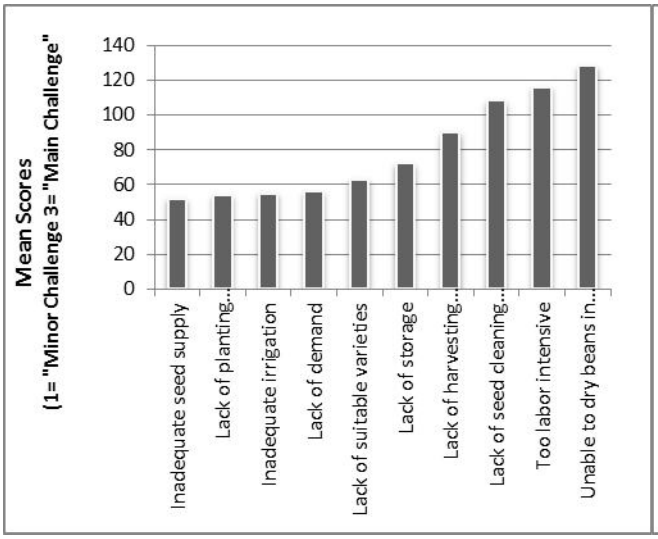

Figure 1: Mean scores of challenges to dry bean production N=63.

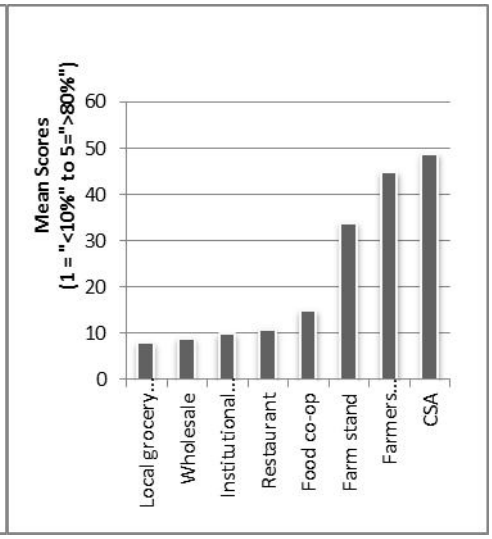

Figure 2: Mean scores of market outlets N=30.

RESULTS

Of the 119 survey respondents, 108 are growing or would like to grow dry beans. Being unable to dry beans in the field was ranked as the main production challenge (Figure 1). Community supported agriculture (CSA) was ranked as the primary market outlet for dry beans (Figure 2). Mean days to harvest was 112 and ranged from 101 to 124. Mean yield was 2592 kg/ha and ranged from 1922 kg/ha to 3468 kg/ha (Table 1).

DISCUSSION AND CONCLUSIONS

Early maturing varieties and improved access to scale-appropriate equipment will address dry bean production constraints in western Washington. Local heirloom varieties represented a wide range of market classes and varied greatly in agronomic performance. Opportunity exists to test and/or breed new early maturing, high yielding varieties with unique seed coat colors or patterns suitable for niche markets.

Table 1: Dry bean variety trial planted May 21, 2013 at WSU-Mount Vernon NWREC. Values are means ± standard error. Letter subscripts indicate significantly different means based on Tukey's pairwise comparison.

Variety	Market Class	Source	Days to Maturity	Yield (Lbs/Acre)
Black Coco	Black	Heirloom	101 ±0.0^{ab}	2218 ±96.0^{ab}
Black Coco	Black	Commercial	109 ±1.5^{abc}	2343 ±149.6^{ab}
Eclipse	Black	Commercial	120 ±5.8^{bc}	3467 ±629.9^{a}
Skyriver Black	Black	Heirloom	122 ±1.3^{bc}	2704 ±55.5^{ab}
Swedish Brown	Brown	Commercial	115 ±7.0^{abc}	2100 ±112.6^{ab}
Youngquist Brown	Brown	Heirloom	119 ±3.4^{bc}	2928 ±277.6^{ab}
Calypso	Colored Patterned	Commercial	110 ±0.0^{abc}	1922 ±103.5^{b}
Orca	Colored Patterned	Commercial	117 ±3.4^{bc}	2175 ±244.0^{ab}
Rockwell	Colored Patterned	Heirloom	107 ±1.7^{abc}	2689 ±186.4^{ab}
Bale Cranberry	Cranberry	Heirloom	104 ±0.0^{ab}	2933 ±207.2^{ab}
Decker	Cranberry	Heirloom	101 ±0.0^{ab}	2724 ±133.2^{ab}
Etna	Cranberry	Commercial	113 ±2.8^{abc}	2697 ±242.2^{ab}
Kring Cranberry	Cranberry	Heirloom	104 ±0.0^{ab}	2288 ±139.4^{ab}
Silver Cloud	Kidney	Commercial	119 ±3.0^{bc}	2243 ±275.1^{ab}
Lariat	Pinto	Commercial	114 ±6.4^{abc}	3371 ±187.4^{a}
Soldier	Soldier	Commercial	117 ±4.0^{bc}	2250 ±341.4^{ab}
Hutterite	Yellow	Heirloom	124 ±4.2^{bc}	2113 ±428.6^{ab}
Hutterite	Yellow	Commercial	123 ±3.8^{bc}	2683 ±305.0^{ab}
Ireland Creek Annie	Yellow	Heirloom	101 ±0.0^{ab}	2909 ±178.9^{ab}
Ireland Creek Annie	Yellow	Commercial	104 ±1.5^{ab}	3079 ±180.2^{ab}
Overall Mean			**112**	**2659**
p-value			<0.0001	0.003
Heirloom Mean			**110**	**2659**
Commercial Mean			**114**	**2593**
p-value			0.097	0.628

DIVERSITY FOR COOKING TIME IN ANDEAN DRY BEANS

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Dry beans (*Phaseolus vulgaris* L) are a nutrient dense, low cost food and therefore are an excellent value for consumers (Drewnowski and Rehm, 2013). In spite of this value, long cooking times limit bean consumption. This is true in developing countries where cooking fuel is sometimes scarce and in developed countries where consumers don't have time to invest in cooking (Brouwer. et al. 1989). Understanding the genetic variability for cooking time in beans would help efficiently breed fast cooking bean varieties. The objective of this study was to evaluate the cooking time of a panel of Andean bean lines from diverse market classes and seed types important in major bean growing and consuming regions of Africa and the Americas.

MATERIALS AND METHODS: A subset of 250 bean lines of the Andean Diversity Panel (ADP) was grown in 2012 at the Montcalm Research Farm in Entrican, MI. Two replications were planted per entry in a randomized complete block design. The cooking time of each entry was then determined using a modified Mattson-type cooker (Mattson 1946) on 25 pre-soaked bean seeds in DI water for 12 hrs. Weight differences between raw and soaked seeds were measured to determine water uptake. The optimum cooking time was recorded as the time it takes for 80% of the plungers to pierce the seeds (Wang, 2005). Cooked seed was freeze dried and minerals were measured using the Inductively Coupled Plasma Emission Spectroscopy (ICP-ES) at A&L Great Lakes Laboratory, IN. Pearson correlations were conducted among the seed variables.

RESULTS AND DISCUSSION: Cooking data was collected on 250 bean genotypes representing diverse Andean germplasm from eight major market classes. The cooking time ranged from 17 min to 90 min and the fastest and slowest cooking beans were both cranberry types (Figure 1). As a group, the white beans were the fastest cooking and also had the least amount of diversity for range of cooking time. A faster cooking time was also correlated with a number of other seed characteristics, most notably, higher levels of boron and potassium in the cooked seed (Table 1). This diversity analysis will be useful to identify parental materials, to understand the genetics control of cooking time, and to breed fast cooking beans in diverse Andean market classes.

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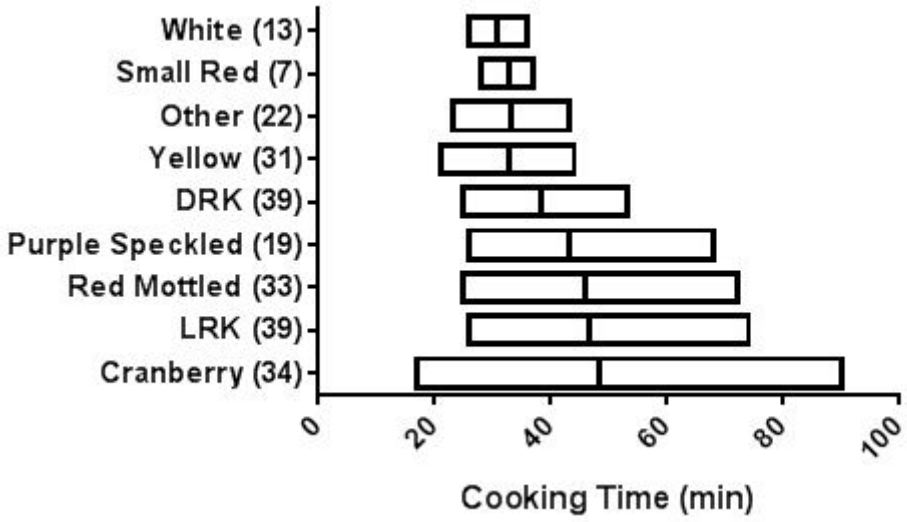

Figure1: Range in cooking time of 250 bean genotypes grouped by seed type. Numbers in parentheses represent how many of genotypes in each market class. The black line in each bar indicates the mean cooking time of the samples.

Table 1. Correlations between cooking time and cooked bean nutrient levels of 250 Andean bean genotypes grown at the Montcalm Research Farm, MI, 2012

Variables	R	P value
Market class	0.142	0.002
Dry seed size (g)	0.040	0.388
water uptake	-0.193	< 0.0001
P_percent	-0.232	< 0.0001
K_percent	-0.404	< 0.0001
S_percent	-0.186	< 0.0001
Zn_ppm	0.128	0.006
B_ppm	-0.458	< 0.0001

MINERAL ALLOCATION TO POD WALLS AND SEEDS IN COMMON BEAN

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INTRODUCTION: Common bean (*Phaseolus vulgaris* L.) is an important source of dietary nutrients, energy, and fiber for humans. Because we are interested in improving the mineral nutritional quality of bean seeds, we have investigated various temporal and spatial aspects of whole-plant mineral transport in an attempt to identify rate-limiting steps. For this study we focused on the role of pod walls in the trafficking of minerals to seeds. We analyzed final mineral content in seeds and pod walls of greenhouse-grown Andean bean lines, in order to identify possible limitations in this process. We also measured the dynamics of mineral flow between pod walls and developing seeds. These profiles are used to discuss strategies for manipulating pod and seed nutrient allocation in bean, in order to enhance seed nutritional quality.

MATERIALS AND METHODS: Twenty-five diverse Andean lines of common bean were grown in pots (five plants per 8 L pot) with soilless media in a greenhouse (Houston, TX). Plants were provided full nutrients (including nitrogen) throughout plant growth via a drip-line system with daily delivery of water and nutrients. Pods were harvested at maturity for the measurement of pod wall and seed minerals (mineral allocation study). An additional set of plants (cv. Micran) were grown as described above for a pod developmental study. For this study, flowers were tagged at anthesis and pods were harvested at five day intervals (15-40 days after anthesis; DAA) and immediately dried in a 60 C oven.

Dried pods from both studies were separated into pod walls and seeds. Average pod wall weight per seed position was calculated by dividing the measured pod wall weight (both halves) by the number of filled seed positions. Average seed weight was calculated by weighing seeds and dividing the weight by the seed number. Mineral contents were determined by multiplying mineral concentration (see below) times tissue mass. Mineral allocation percentage to seeds (percent per whole pod unit) was calculated as:

$$\frac{\text{(mineral content of unit seed)} \times 100}{\text{(mineral content of unit seed + mineral content of unit pod wall unit per seed position)}}$$

Mineral analyses were carried out by inductively coupled plasma – optical emission spectroscopy as previously described (Farnham et al., 2011).

RESULTS: Mineral allocation to seeds (as percent of total mature pod unit) varied by element, with values ranging from 42% to 98%. Specific values (% $\pm$ SD) for macroelements were: Ca, 42 $\pm$ 6; K, 56 $\pm$ 5; Mg, 55 $\pm$ 6; P, 98 $\pm$ 0; S, 96 $\pm$ 1; and for microelements were: B, 64 $\pm$ 5; Cu, 94 $\pm$ 4; Fe, 98 $\pm$ 0; Mn, 79 $\pm$ 5; Mo, 93 $\pm$ 2; and Zn, 96 $\pm$ 1. As comparison, dry matter allocation to seeds in this same group of lines averaged 77% ($\pm$ 2%).

Temporal data on pod mineral content demonstrated that pod walls are a dynamic system, with net turnover (remobilization) observed for certain minerals. In studies with cultivar Micran, unit pod wall content of Fe, Mo, and Zn reached a peak at roughly 20 to 25 DAA (Figure 1) and then demonstrated a net decline by the end of pod development. Phosphorus was the only macroelement that exhibited the same trend. All other microelements and macroelements showed increasing or stable contents in pods, beginning around 25 DAA (Figure 1).

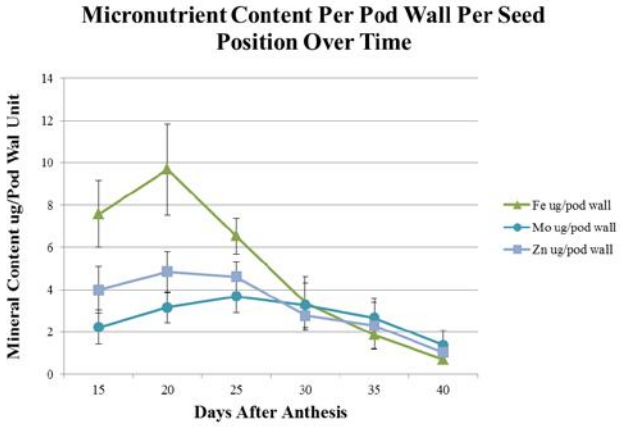

Figure 1. Microelement content in pod walls (per unit seed position) over the course of pod development in cultivar Micran.

DISCUSSION: For minerals that are delivered to the developing pod over the course of its nutrient and biomass gain, several were demonstrated to be highly allocated to seeds (>90% allocation), with these amounts being higher than the dry matter allocation (77%) to seeds. For these highly allocated elements (P, S, Cu, Fe, Mo, and Zn), efforts to increase seed mineral concentrations would require increased import over the course of pod development, because there is almost nothing left in the pod wall to be remobilized. For the other macroelements (Ca, K, Mg) and microelements (B, Mn), it is feasible that some moderate seed mineral concentration gains could be achieved by altering the existing allocation patterns between pod wall and seeds. And, our developmental study (Figure 1) demonstrates that net remobilization of certain minerals is possible. Nonetheless, even for these other minerals, additional transport to the whole pod unit would be warranted to achieve significant gains in seed mineral concentrations. For all elements, this would require increased uptake at the root level to provide more minerals to the leaves and pods via xylem transport, and possibly increased capacity of source leaf-to-pod transport of minerals via the phloem pathway. Further work will be needed to establish whether pod walls have the capacity to move higher quantities of minerals to the seeds, if the walls could be fed these nutrients at higher levels. This work was supported in part by funds from USAID to the Feed the Future Grain Legumes Project.

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GENOMIC ANALYSIS OF MUTATIONS CONFERRING STORAGE PROTEIN DEFICIENCY IN COMMON BEAN

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INTRODUCTION: Storage proteins contribute to the nutritional quality of common bean (*Phaseolus vulgaris*). A set of genetically related lines (SARC1, SMARC1-PN1 and SMARC1N-PN1) integrate a progressive deficiency in major storage proteins (Osborn et al. 2003). The deficiency in phaseolin and lectins is associated with an increased concentration of sulphur amino acids, cysteine and methionine, and increased levels of sulphur-rich proteins (Taylor et al. 2008; Marsolais et al. 2010; Yin et al. 2011; Liao et al. 2012). This property is of interest to improve protein quality. The changes in protein composition are also associated with differences in protein solubility. Phaseolin and most lectins are encoded at two unique loci. A combination of approaches is being used to determine the precise composition of phaseolin and lectin isoforms in these lines, including proteomics, genomic sequencing and PCR.

MATERIALS AND METHODS: 1. Protein analysis by spectral counting: Total protein was extracted from mature seeds of SARC1 and SMARC1N-PN1 (n = 3), and 75 μg separated on a 7 to 15% polyacrylamide gel, at the McGill University and Génome Québec Innovation Centre (Montréal, QC). Each lane was excised into 15 bands followed by tryptic digestion. Samples were analysed by mass spectrometry (Marsolais et al. 2010). Data was reanalyzed with SCAFFOLD (Proteome Software, Portland, OR) against the UniProt database. **2. Genome sequencing:** To understand the genomic basis for variations in protein profiles, the three lines and parental genotype Sanilac were submitted to short-fragment genome sequencing using an Illumina HiSeq 2000 platform at the Clinical Genomics Centre (Toronto, ON). Genomic DNA was isolated from leaf tissue from a single plant and genotyped by PCR. Reads were aligned to reference sequences from the genomes of G19833 (www.phytozome.net/commonbean.php), BAT-93 (http://www.genoma-cyted.org/), and OAC-Rex, and a BAC clone of the APA locus from an arcelin-5 genotype (Kami et al. 2006), and visualised with Tablet and Integrated Genomics Viewer. **3. Promoter analysis:** Reads were carefully joined and the resulting promoter sequences from different mutants were analysed using plant *cis*-acting regulatory DNA elements in the PLACE database.

RESULTS AND DISCUSSION: 1. Differentially expressed storage proteins in mature seeds of SARC1 and SMARC1N-PN1: Spectral counting results confirmed the deficiency in phaseolin, phytohemagglutinin and arcelin in SMARC1N-PN1 (Table 1). The deficiency is compensated by large increases in residual lectins, particularly the α-amylase inhibitor 1, α-amylase inhibitor like protein, mannose lectin FRIL and leucoagglutinating phytohemagglutinin. **2. Polymorphisms associated with protein expression:** In SMARC1N-PN1, a lectin gene sharing the location of pha-L in SARC1 has a characteristic 1 bp deletion resulting in a premature stop codon, previously characterized as *Pdlec1* in Pinto bean (Voelker et al. 1986), in agreement with the fact that Great Northern 1140, the parent of SMARC1N-PN1 has US 5 Pinto in its pedigree (Bean Genes database). When reads were aligned with BAT-93, the lectin gene

lec4-B17 and *pha-E* appear completely absent in SMARC1N-PN1. The polymorphism associated with the lack of α-phaseolin expression remains to be identified.

Table 1. Differentially expressed proteins quantified by spectral counting (n = 3) assigned with 95% confidence

Protein (gene)	Accession	Total spectra	
		SARC1	SMARC1N-PN1
Phaseolin precursor (*Phs*)	Q43632	826	40
Alpha-phaseolin	Q41115	799	0
Phaseolin, alpha-type	P07219	713	0
Arcelin-1 precursor (*ARC1*)	P19329	955	0
Arcelin (*arc3-II*)	Q8RVY3	101	0
Arcelin-like protein 4 (*arl4*)	Q8RVX7	90	0
Arcelin (*arc4-I*)	Q8RVX4	73	0
Lectin precursor (*lec4-B17*)	Q8RVX5	27	0
Phytohemagglutinin (*pha-E*)	Q8RVX6	73	0
Phytohemagglutinin (*pha-L*)	Q8RVH2	44	7
Leucoagglutinating phytohemagglutinin (*PDLEC2*)	P15231	38	121
Alpha-amylase inhibitor like protein	Q9SMH0	0	150
Alpha-amylase inhibitor-1	A0V2T3	0	311
Mannose lectin FRIL	Q9M7M4	0	116

3. Differences in expression of β-phaseolin: Low expression in SMARC1N-PN1 is associated with a single nucleotide substitution in the promoter region, affecting an ABRE element required for high expression in seed (Fig. 1) (Chandrasekharan et al. 2003).

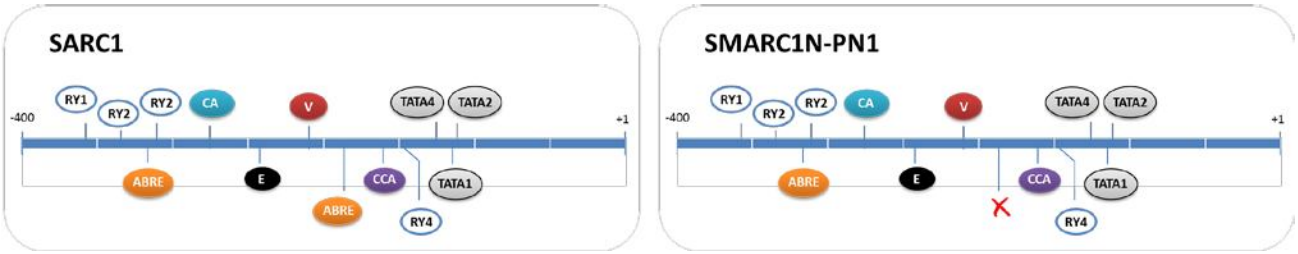

Fig. 1. Reduction in β-phaseolin protein levels is associated with the lack an ABRE in the promoter of SMARC1N-PN1.

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WHAT EVER HAPPENED TO GENERAL PUBLIC LICENSE?

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At the 1999 BIC meeting in Calgary, AB, Michaels proposed a germplasm exchange agreement called General Public License for Plant Germplasm (GPLPG) to protect the unencumbered and free exchange of bean germplasm for plant breeding purposes (Michaels, T. E. 2000). Under this proposed release mechanism plant germplasm designated as GPLPG could be used for plant breeding without encumbrance except that that any novel germplasm resulting from use of a GPLPG parent must also be released under GPLPG and itself available for use in plant breeding without encumbrance. The motivation for developing such an exchange agreement was the concern that some mechanisms for intellectual property protection that at that time were being applied to plant germplasm were resulting in restricted availability of germplasm for breeding purposes. The primary germplasm pool was in danger of shrinking and stratifying into small islands of permissible breeding germplasm, not due to biology, but due to intellectual property protection.

In the years since the first proposal for GPLPG, public and private institutions alike have intensified the intellectual property protection of their plant germplasm releases (Ghijsen, H. 2009; João Carlos da Silva Dias 2010). For most vegetable cultivars other than corn, intellectual property protection has been primarily accomplished in the United States through Plant Variety Protection (PVP). As of January 2012, 394 garden beans and 190 field beans had been protected by PVP (Anonymous 2013a). Since the PVP Act includes a research exemption allowing use of protected varieties in breeding, PVP does not restrict the availability of germplasm for breeding purposes (although the PVP holder is not required to make the seed available for research or breeding). Holders of utility patents, on the other hand, may and typically do include claims that exclude other breeders from using the protected germplasm for breeding. Again in the case of bean, a recent search identified 29 garden beans and 9 dry, popping or field beans that are covered by utility patents (Anonymous 2013b). Intellectual property protection of plant germplasm is also extended to non-commercialized releases through the use of contract law tools such as Material Transfer Agreements (MTA), Technology Transfer Agreements, and contracts affixed to the seed package known colloquially as “bag tags” and “shrink wrap” licenses. These contracts specify allowed and disallowed uses. A typical MTA would either not permit use in breeding or only permit such use if the recipient agrees to enter into revenue sharing negotiations should any of the resulting germplasm be commercialized. The number of these contracts is not known, but is likely much higher than the numbers of PVP and patent applications since germplasm releases and exchanges outnumber cultivars.

Heald and Chapman (2009) argue that for vegetable crops other than corn, intellectual property protection has not had a marked impact on diversity as measured by numbers of commercially available cultivars. While diversity of very high value and GMO crops like corn may have benefitted from strong intellectual property protection, the diversity of modest value and specialty vegetable crops, including beans, has not.

After a period of quiescence, the GPLPG concept was revived in 2003 when Dr. Janet Hope, then a Ph.D. student at the Australian National University, cited the proposal in her thesis and in her 2008 book on open source biotechnology (Hope, J. 2008). Kloppenburg read the reference to GPLPG, contacted Michaels and together in 2011 they assembled a broad-based group of plant breeders, agricultural policy activists, farmer advocates and students to revive the free exchange concept underlying the GPLPG proposal. This group named themselves and their effort the Open Source Seed Initiative (OSSI). OSSI promotes innovative plant breeding that produces resilient and productive cultivars adapted to a multiplicity of sustainable agroecosystems. It works to encourage and reward the sharing rather than the restriction of germplasm, to revitalize public plant breeding, and to integrate the skills and capacities of farmers with those of plant scientists.

OSSI's initial activity was to draft maximally defensible licenses enforcing the underlying concept that new germplasm developed using OSSI germplasm must itself be released as OSSI. However, we quickly found that the actual process of fashioning an effective open source license for plant germplasm was more complicated than initially hoped. At a practical level, OSSI has encountered a variety of technical, legal obstacles to drafting workable licenses that has made us rethink our relative emphasis on the normative goal of reintroducing an ethos of sharing for germplasm exchange versus the pragmatic goal of creating a legally enforceable mandate for sharing. While the future direction of OSSI is yet to be determined, current discussions point to several possibilities, some of the most prominent of which are:

- Promoting and supporting the release of open source germplasm through a simple, short, affirmatively phrased statement expressing a commitment to allowing unrestricted use of the seed and its derivative progeny lines,
- Drafting an "OSSI clause" that could be inserted into existing institutional MTAs
- Focusing attention on public advocacy, contact, cooperation and education surrounding open source seed,
- Enhancing and strengthening farmer participatory plant breeding projects.

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TOWARD A BETTER BEAN: IMPROVING GENETIC, GENOMIC, BREEDING, AND CROP MANAGEMENT RESOURCES FOR LIMA BEAN (PHASEOLUS LUNATUS)

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Lima bean is a cornerstone crop of the Mid-Atlantic vegetable processing industry, centered in Delaware, and extending into Maryland, New Jersey, and Virginia. There are multiple challenges to production in the region including diseases and lack of adapted cultivars. A sponsored Specialty Crop Research Initiative in lima bean is generating genomic tools to support the lima breeding program, developing improved identification, assessment and predictive tools and developing crop management practices to reduce the need for pesticides for four important diseases of lima bean: downy mildew (*Phytophthora phaseoli*), root knot nematode (*Meloidogyne incognita*), *Phytophthora capsici,* and white mold (*Sclerotinia sclerotiorum*). A summary of grant research and progress follows.

***P. phaseoli* population genetics/analysis.** Eleven isolates of *P. phaseoli* have been genotyped using a genotyping by sequencing (GBS) protocol. This technique reduces the genome of the organism through the use of restriction enzymes and size selection. These steps are followed by sequencing using the Illumina NGS sequencing platform. Sequencing data is being analyzed for SNPs. Eight isolates obtained from the field in 2013 have been cultured and are being maintained. In addition, harvested cells have been lyophilised and stored. In 2014, the pathogen will also be isolated directly from the field for DNA prep and processing through the GBS pipeline, rather than trying to isolate in pure culture, which is very difficult.

Genetic resource building: Characterizing and developing germplasm resources to aid in the breeding of new varieties. A total of 250 diverse lima bean accessions will be used for genetic analysis to assess the structure of genetic diversity of lima bean. GBS is being employed to characterize this lima bean germplasm, including representatives from diverse geographic regions. Currently, DNA has been extracted from 50 accessions. Accessions from these diverse lines will be selected after genotyping analysis to create a nested association mapping (NAM) population which will be used to characterize traits responsible for *Phytophthora phaseoli* resistance. In another area of genetic research, 128 lima bean lines from single seed increases have been selected for GBS Nested Association Mapping development. Approximately 2000 RILs from 20 to 40 individual crosses will be used for mapping. Currently, crosses have been made between 10 presumed Andean diverse lines and the Andean reference parent and 10 presumed Mesoamerican diverse lines and the Mesoamerican reference parent. In March, F1 seed will be planted in the greenhouse and F2 seed will be planted in the field in June 2014. In addition, one Mesoamerican and one Andean RIL population with indeterminate individuals will be included. Additional crosses in Fall 2014 will be made with parents selected based on GBS results. To accomplish this work GBS is being optimized for lima bean. A goal of this portion of the project is to use is to use nested association mapping (NAM) to dissect more complicated genetic traits important to lima bean production stability, such as yield and heat-tolerance. Lines

will be phenotyped for seed size, shape & color, growth habit, photoperiod response, cyanide production, heat tolerance, nitrogen fixation, and disease resistance.

Other research areas: Downy mildew epidemiology and improved prediction models for the disease. In 2014, field experiments were conducted on pre-emergence infection potential from prior plant residue, the effects of rotation on pathogen survival to determine how long do oospores survive in the soil and a study on the leaf wetness duration range and optimum for disease development. Leaf wetness research, initial disease observation in the field, and weather data prior to disease development will be used to develop an improved disease prediction model for downy mildew.

Assessing the prevalence of *P. capsici* (Pod Blight) and developing a risk assessment tool. Region- wide surveys of lima bean fields are being conducted over 3 years. *P. capsici* samples are being collected from lima bean plants in the field, soils, and irrigation water; isolates are being cultured; and *P. capsici* mating type and Ridomil sensitivity are being determined. DNA will be extracted and genotyped using genetic markers to characterize diversity of isolates. Other susceptible crops in rotation with lima beans such as cucumbers will also be sampled, analyzed and compared with P. capsici isolates from lima bean fields. Field information including topography, rotations, cultural practices, and weather will be used to develop a Pod Blight Risk Assessment Tool

***P. capsici* control studies.** Chemical fungicide efficacy trials were conducted in 2013 and will be repeated in 2014 and 2015. Trials include biological and biorational fungicides. In parallel, alternative control studies are being conducted including the use of biofumigant mustards and sorghums, compost and other soil amendments, and biological controls (biocontrol fungi and bacteria). A third area of control research is on tillage practices and residue effects on disease incidence and severity.

White mold research. White mold research will be conducted to develop risk assessment guidelines, determine the optimum time to spray for white mold under continuous pressure, and to quantify the short and long term benefits of *Coniothyrium minitans* as a biological control.

Root Knot Nematode (RKN) prevalence and risk model development. In this area of research, the prevalence of RKN in the region is being evaluated by detailed surveys of lima bean fields for quantification and distribution of RKN. Research on enhanced sampling strategies and detection methods is being conducted using samples from these surveys. This includes soil extraction and counts, bioassays, and genetic methods for root knot estimation involving DNA extraction from the soil and qPCR using RKN specific primers. A major goal is to develop predictive tools for RKN using field information including soil characteristics, rotations, cultural practices, and weather.

Evaluation of chemical and alternative control measures for RKN in lima bean. Evaluations of alternative control measures for RKN in lima bean started in 2013 and included commercially available biological control organisms, compost amendments, and biofumigants. Commercial nematicides were also tested.

UREIDE ACCUMULATION IN BEAN LEAVES DURING CONDITIONS OF ABIOTIC STRESS

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Ureides in Plants

Ureides, primarily allantoin and allantoate, have been quantified in breeding programs to assess the efficiency of nitrogen fixation since the late 1970s. As nitrogen transport molecules in fixing tropical legumes, ureide levels are compared to nitrate and amino-N concentration to determine the contribution of nitrogen from symbiotic fixation (Herridge, 1994).

In addition to synthesis in the nodules, ureides are regularly generated in plant cells *in situ* during purine turnover. Inosine, guanosine, xanthosine and adenosine are converted to xanthine then broken down using the same ureide catabolic pathway as fixed-N, forming allantoin and allantoate as intermediates and ultimately releasing ammonia and glyoxylate for re-assimilation (Zrenner et al. 2006).

More recently, ureides have been suggested as having a role as Reactive Oxygen Species (ROS) scavengers. Ureides accumulate in soybean plants during drought stress in both shoots and nodules (de Silva et al. 1996, Serraj et al. 1999, Vadez et al. 2000). In Arabidopsis, allantoin accumulates during periods of extended darkness or with increased leaf age and Arabidopsis xanthine dehydrogenase mutants accumulate ROS (Brychkova et al. 2008). In addition to nitrogen fixing plants, ureides also accumulate in non-fixing soybean in response to drought and to exogenous reactive oxygen species (ROS) (Souter, unpublished data). Since ureide accumulation occurs both in non-fixing legumes as well as in other species, we suggest that ureide accumulation is a plant response common to different abiotic stressors.

We have assessed ureide accumulation in response to water limitation and the herbicide methyl viologen (MV) -induced oxidative stress. We are also interested in determining if ureide levels increase in response to cold-induced abiotic stress. Experiments by Strauss et al. (2007) demonstrated a decrease in ureide concentration in leaves of nitrogen fixing soybean exposed to cold temperatures, however since nitrogen fixation and ureide export itself may have been affected by the cold stress, the decrease could be attributed to a decrease in fixed ureide-N, potentially masking ureides produced in leaf tissue in response to cold.

Genetic Control

Ureide levels vary by cultivar and have been associated with sensitivity to drought stress (Purcell et al. 2000), but this likely includes differential inhibition of nitrogen fixation. Since ureide accumulation can be affected by both the rate of synthesis and the rate of catabolism we are interested in quantifying ureide levels both before and after an exposure to an abiotic stress in an attempt to determine if ureides can be used to predict general abiotic stress tolerance.

Responses in Tepary Bean and Interspecific Hybrids

Tepary bean has been targeted as a potential source of genes for tolerance to both cold and drought for common bean (Martinez et al. 2007). Using interspecific hybrids, we are determining

if there are correlations between ureide accumulation and response to both drought and cold stress. We see ureide accumulation as a potential test for general abiotic stress tolerance that could be used to track introgresion of this trait into common bean.

Leaves of 30 tepary bean lines from the CIAT germplasm collection were treated with MV to induce ROS. There was variability in ureide accumulation, with both differences in overall ureide accumulation and differences in accumulation of individual ureides. This variation holds promise for tepary bean as a genetic donor with the intent of introgressing stress tolerance associated with ureide metabolism into common bean.

Interspecific hybrids of tepary bean and common bean, along with accessions of tepary bean and common bean were analyzed for ureide accumulation as it relates to cold tolerance during a late fall frost event in Saskatoon, Saskatchewan. There was variability in response, with the two tepary bean accessions showing consistently lower levels of ureides, pre-and post-frost, compared to the interspecific hybrids. Genotypes varied as to whether allantoin or allantoate was the dominant ureide accumulating. Interestingly, the two tepary accessions also accumulated uric acid.

Variation among genotypes indicates ureide accumulation has a genetic component and therefore can be selected for. Better understanding the relationship between ureides and abiotic stress has the potential to benefit breeding programs addressing abiotic stress tolerance. Ultimately, we aim to address not only the relationship between ureides and stress, but also the underlying mechanism conferring tolerance.

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QTL MAPPING FOR DROUGHT TOLERANCE USING A RIL POPULATION OF BUSTER x SER 22

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The identification of loci associated with drought tolerance is an important strategy for implementing Marker Assisted Selection (MAS). A population of 335 recombinant inbred lines (RIL) was derived from the cross between 'Buster', a drought-susceptible pinto cultivar, and SER 22, a drought-tolerant small red germplasm line developed at the International Center for Tropical Agriculture (CIAT). Evaluation in replicated non-stress (NS) and drought stress (DS) trials using an alpha design with commercial and local checks ('Beryl-R', 'Buckskin', 'Matterhorn', 'Morales', 'Raven', 'Verano', and 'UI 114') was performed at two locations: the University of Nebraska-Lincoln, Panhandle Research & Extension Center, Mitchell, NE (41°56'.6''N, 103°41'.9''W, 1240 m elevation), and the Experimental Station of the University of Puerto Rico in Juana Diaz (18°01'.81''N, 66°31'.713''W, 23 m elevation) over three years (2011-2013), for a total of twelve environments. Both locations have drought and heat stress, allowing screening for abiotic stress tolerance in multiple environments.

To evaluate plant response to drought, physiological and agronomic data were collected under both DS and NS environments as described by Rao et al. (2008). At the flowering stage, leaf temperature (^{0}C), days of flowering (d), and stomatal conductance ($mmolm^{-2}s^{-1}$) were collected, while at harvest time, days to maturity (d), 100-seed-weight (g), biomass (kg ha-^{1}), seed yield (kg ha^{-1}), pod harvest index, and harvest index were collected.

High yielding, drought-tolerant RILs with broad adaptation have been identified even though the levels of drought stress in Mitchell, NE and Juana Diaz, PR were not the same across years. These lines could be used in future crosses in breeding programs.

The linkage map comprised a total of 378 single nucleotide polymorphism (SNP) markers for a total map distance of 778.4 cM divided across twelve linkage groups representing 47% of the estimated genome size of the 'Redhawk'/ 'Stampede' (1665.5 cM) consensus map. The number of markers per linkage group varied from 5 to 99. The average distance between markers was 2.06 cM. The largest interval between markers was 23.5 cM, located on Pv7. Gaps in the genetic map are present on Pv9, and Pv10. Transgressive segregation was observed for all traits under study. Three QTL for seed yield were found on two linkage groups using composite interval mapping (Table 1). Additional QTL were identified for agronomic and physiological traits, six for 100-seed weight and ten for leaf temperature in five environments (Table 1). A considerable set of SNP markers linked to drought-tolerant genes have been identified. With the results from this study, plant breeders will have a better understanding of the genetics and possible mechanisms behind drought tolerance, as well as the possibility of the use of these QTL for genetic improvement. Validation studies are going to be performed using extreme phenotypes.

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Table 1. QTL information for seed yield, seed weight, days to maturity, days to flower, and leaf temperature over 12 environments in the 'Buster'/SER 22 RIL population.

Trait	Environment	Pv[†]	Peak position (cM)	LOD score[‡]	Additive effect	Allele[§]	R^2
Seed yield	NEDS11	3	03.31	1.92	23.00	S	0.09
	NENS11	7	99.81	1.66	111.97	B	0.03
	NEC11	3	39.51	2.92	104.16	B	0.05
Seed weight	NEC11	8.1	00.01	2.12	1.30	B	0.03
	PRDS12	2	23.61	3.42	1.03	B	0.05
	PRC12	2	23.61	3.61	0.79	B	0.05
	PRC12	8.2	59.51	3.02	1.18	S	0.06
	NENS13	2	12.51	2.91	0.93	S	0.04
	NEC13	11	60.91	3.54	0.95	S	0.06
Days to flower	NEC11	6	21.51	2.95	0.60	B	0.05
	PRNS12	8.1	3.91	2.14	0.32	B	0.03
Days to maturity	NEPRNS1112	2	82.01	2.92	0.37	B	0.04
Leaf temperature	NENS11	1	73.71	5.71	0.28	S	0.08
	NENS11	1	84.21	5.52	0.30	S	0.10
	NENS11	2	76.91	3.79	0.28	S	0.05
	NEC11	2	66.61	1.04	0.41	S	0.01
	NEC11	3	76.11	1.35	0.71	B	0.02
	NEC11	3	101.810	1.07	0.48	B	0.01
	PRDS12	2	00.01	3.27	0.31	B	0.04
	NEPRDS112	1	44.71	1.45	0.26	B	0.02
	NEPRNS1112	5	77.81	1.64	0.30	S	0.02
	NEPRNS1112	5	13.21	1.73	0.42	S	0.02

[†]Pv = *Phaseolus vulgaris* chromosome based on consensus map. [‡]Significant cut off value based on 95% permutation test. [§]Allele contribution 'Buster' (B) and from SER 22 (S).

EVALUATION OF THE ANDEAN BeanCAP LINES TO TERMINAL DROUGHT IN WESTERN NEBRASKA

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INTRODUCTION

Although dry beans are 100% irrigated in the Great Plains, drought is the most limiting abiotic stress affecting yield production. In recent years, ground water decline due to overuse has resulted in pumping restrictions in many areas of Nebraska. Identification of high-yielding, drought-resistant dry bean lines is needed in order to reduce dependence on irrigation water, lower costs of production, and increase profit margins for dry bean growers in western Nebraska. The objective of this study is to identify sources of drought tolerance.

MATERIALS AND METHODS

In 2013, 49 Andean Bean Coordinated Agricultural Project (Bean-CAP) lines were evaluated in replicated trials under drought (DS) and non-drought stress (NS) at Mitchell, NE (41°56.6′ N, 103°41.9′ W, 1240 m elevation), in irrigated and non-irrigated plots adjacent to each other. Within each block, the Andean lines were assigned to experimental units using a 7 x 7 lattice design with 2 replicates. Each plot consisted of two 7.6 m rows spaced 0.6 m apart. Targeted plant density was 200,000 plants ha^{-1}. Both NS and DS blocks were irrigated until flowering to ensure good plant establishment and normal vegetative growth. Thereafter, the stressed block was not irrigated. Daily rainfall during the growing season was recorded. In addition to seed yield (kg ha^{-1}), data was recorded for 100-seed weight (g), number of days to flowering and maturity. To quantify drought severity, the drought intensity index (DII), geometric mean (GM), the drought susceptibility index (DSI), and percent yield reduction (PR) were determined to predict the performance of a line under DS and NS conditions. The same set of lines was evaluated in North Platte, NE to common bacterial blight.

RESULTS AND DISCUSSION

Maximum and minimum average temperatures were 29.3 and 18.4 0C, respectively. The NS and DS plots received 453.0 and 248.2 mm, of total water, respectively. A total of 63.2 mm of precipitation occurred after flowering. Drought stress was moderate (DII = 0.47). Yield under NS and DS ranged from 1402 to 4011 kg/ha, and from 682 to 2847 kg/ha, respectively. 100-seed weight under NS and DS ranged from 33.4 to 60.8 g and 28.8 to 551.0 g, respectively. Days to maturity under NS and DS ranged from 81 to 100 and 71 to 90 days, respectively. Under DS conditions, yield and 100-seed weight were reduced an average of 47.0 and 12.5%, respectively, relative to NS conditions. Using GM as the major selection index, Wallace 773-V98 was found to be well adapted to both NS and DS environments. Kardinal Kidney had a GM of 2787 kg ha^{-1} and the lowest yield reduction (8.8%). Cardinal had the lowest CBB (4.0) followed by VA-19 (4.9), and Capri, Michigan Improved Cran, Myasi, and Red Kanner with a score of 5.0. Fiero and Drake hat the highest scores to CBB of 8.3. Drake, K-42, UC Canario 707, Sacramento, Beluga, Red Kote, USDK-CBB-15, Silver Cloud, Charlevoix, USCR-9, CDRK and UC Nichols had a GM of 1803, 1803, 1782, 1752, 1650, 1617, 1605, 1530, 1482, 1343, 1313, and 1313 kg ha^{-1} (data not shown).

AndeanBeanCAP lines tested to terminal drought at Mitchell, NE during 2013.

ID	Geometric Mean Kg ha^{-1}	Yield Reduction %	Drought Intensity Index	CBB (1-9)†
Wallace 773-V98	3019	20.5	1.7	7.3
Krimson	2806	37.8	1.3	5.3
Kardinal Kidney	2787	8.8	1.9	7.3
G-122	2740	35.5	1.4	5.3
VA-19	2701	53.8	1.0	4.3
Chinook 2000	2673	50.0	1.1	6.0
Capri	2662	36.0	1.4	5.0
Dolly	2612	53.8	1.0	5.3
Pink Panther	2608	38.2	1.3	7.3
Hooter	2555	44.5	1.2	6.3
Cardinal	2543	28.3	1.5	4.0
Red Rider	2524	49.1	1.1	6.3
Montcalm	2484	57.4	0.9	6.0
Royal Red	2464	26.5	1.6	7.3
Etna	2451	36.6	1.3	5.3
Bellagio	2424	32.5	1.4	5.3
CELRK	2293	50.9	1.0	7.3
Red Kanner	2244	68.7	0.7	5.0
Isabella	2234	59.4	0.9	6.0
Red Kloud	2223	48.7	1.1	6.0
Blush	2182	43.3	1.2	6.3
MiCran	2181	58.6	0.9	5.0
UCD 0801	2179	20.0	1.7	5.3
Fox Fire	2166	17.7	1.8	6.0
Myasi	2062	27.5	1.5	5.0
Fiero	2039	61.4	0.8	8.3
Pompadour B	2012	38.3	1.3	6.3
USWK-CBB-17	1993	17.6	1.8	6.3
Litekid	1990	72.4	0.6	6.3
K-59	1943	60.0	0.9	7.3
02-385-14	1921	37.8	1.3	5.3
ND061106	1877	61.6	0.8	5.3
Jalo EEP558	1860	43.6	1.2	6.3
Red Hawk	1855	60.5	0.8	6.0
USCR-CBB-20	1847	23.2	1.6	6.3
Lassen	1816	57.9	0.9	6.0

† 1, 2, 3= Resistant; 4, 5, 6= Intermediate; 7, 8, 9= Susceptible. CBB was inoculated in North Platte, NE with 2 Nebraskan isolates.

IDENTIFYING HEAT TOLERANT LIMA BEAN (*PHASEOLUS LUNATUS*) GERMPLASM AND DEVELOPMENT OF HEAT TOLERANCE FIELD SCREENING TECHNIQUES

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INTRODUCTION

Heat stress reduces yields of May and early June planted lima beans (*Phaseolus lunatus*) on the Delmarva Peninsula. High night temperatures during flowering and pod set reduce or delay set and can result in later harvest, lower yield and split set. Breeding heat tolerant baby and Fordhook type lima beans is one goal of the University of Delaware lima bean breeding program. To increase the efficiency of heat tolerance breeding efforts, experiments were undertaken in 2013 to identify heat tolerant germplasm, establish field screening methods for this trait and investigate physiological effects of heat stress on lima bean.

MATERIALS AND METHODS

A total of eighteen genotypes, including both known heat susceptible and reported heat tolerant varieties, were evaluated using several approaches to field heat tolerance screening. Eleven of the genotypes have a determinate growth habit and were evaluated in three adjacent successive plantings with flowering during periods of low and high levels of heat stress. The seven genotypes with indeterminate growth habit were planted on a single date in a separate trellised plot. Pod set ratings and yield component measurements were made for all of the genotypes.

Genotypes Evaluated in 2013 Heat Tolerance Screening at Georgetown, Delaware

Cultivar/Accession Name[1]	Growth Habit	Seed Source
Cypress	determinate	ADM Seedwest
C-elite Select	determinate	Ben Fish
Concetrated Fordhook	determinate	Charter Seed
Fordhook 242	determinate	Seedway
Jackson Wonder	determinate	Seedway
Dixie Butterpea	determinate	Seedway
Dixie Speckled Butterpea	determinate	Baker Creek Heirloom Seeds
PI 549509 (Bush Florida Butter)	determinate	USDA Collection[2]
G27525 (1102-6)	determinate	James Beaver, University of Puerto Rico[2]
G27529 (1102-10)	determinate	James Beaver, University of Puerto Rico[2]
UC Luna	determinate	Paul Gepts, UC Davis
Dr. Martin	indeterminate	Rohrer's Seed[2]
Sieva	indeterminate	James Beaver, University of Puerto Rico[2]
Florida Butterbean	indeterminate	Vermont Bean Seed Company
Alabama Blackeye	indeterminate	Baker Creek Heirloom Seeds
Worchester Indian Red Pole	indeterminate	Southern Exposure Seed Exchange
Violet's Multicolored Butterbean	indeterminate	Southern Exposure Seed Exchange
PI 347786	indeterminate	USDA Collection[2]

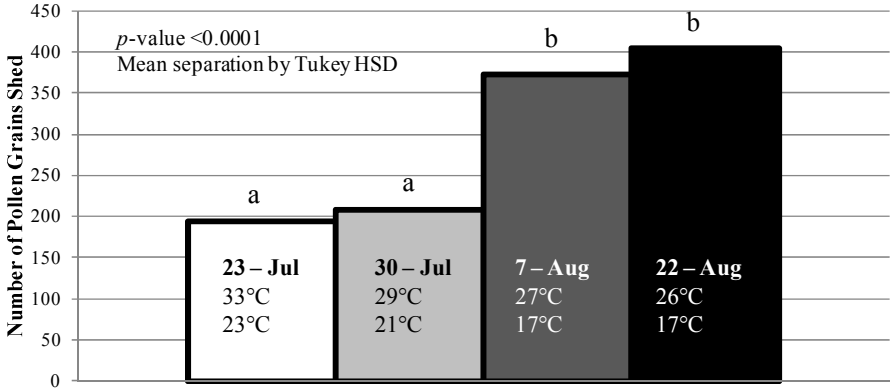
[1]Reported heat tolerant lines are highlighted. [2] Seed increased in Delaware.

For select genotypes, counts were made of pollen grains adhering to the style and stigma for flowers collected on four dates. Styles were removed from the flower, stained with acetocarmine, mounted on a microscope slide and stained pollen grains were counted. For the determinate genotypes, an OS1p chlorophyll fluorometer from Opti-Sciences was used to measure photosynthetic yield Y(II). Fluorometer measurements were taken on seven dates, each approximately a week apart. Readings were taken between 10 a.m. and noon on clear days.

RESULTS AND DISCUSSION

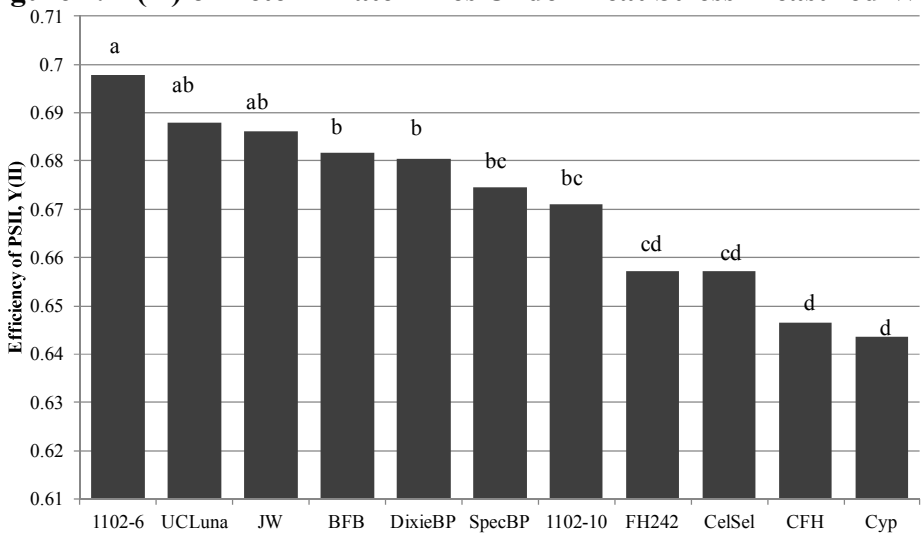
Pollen counts differed significantly between the genotypes tested and also by date. Low pollen counts were associated with days where low temperatures for the previous ten days averaged at or above 21°C (Fig. 1). The genotypes with the greatest amount of pollen shed during high temperature conditions were Bush Florida Butter, Sieva, 1102-6 and 1102-10.

Figure 1. Average Pollen Count by Date for All Lines and Previous 10-Day Average High and Low Temperatures



Fluorometer readings differed significantly between genotypes tested (Fig. 2) and also by date. There was no genotype by date interaction, which means that genotypes ranked in a consistent order over the seven testing dates.

Figure 2. Y(II) of Determinate Lines Under Heat Stress Measured Weekly Over 7 Weeks



Source of Variation	*p*-value
genotype	<.0001
block(date)	<.0001
date	<.0001
genotype*date	0.3195

Alabama Blackeye, Violet's Multicolored Butterbean, Dixie Speckled Butterpea, 1102-6 and Dixie Butterpea had the least yield depression under heat stress, as indexed to unstressed yield. The proposed strategy for achieving high yield under heat stress is to combine vegetative and reproductive heat tolerance traits.

GENETIC VARIABILITY FOR NITROGEN FIXATION IN THE ANDEAN DIVERSITY PANEL OF *PHASEOLUS VULGARIS*

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Common bean, *Phaseolus vulgaris* is considered poor in biological nitrogen fixation (BNF) when compared with other legumes (Bliss, 1993). However, adequate genetic variability for BNF has been reported in the Mesoamerican gene pool in varieties such as Puebla 152 (Graham, 1981; Attewell and Bliss 1985). Challenges exist in moving desirable BNF traits from Mesoamerican genotypes into the Andean genetic background because of linkage drag and incompatibilities. Identification of Andean genotypes with enhanced BNF could circumvent these limitations and provide Andean sources for breeding programs. Prior efforts have concentrated on identifying superior BNF genotypes from the Mesoamerican gene pool and less in the Andean gene pool. We have assembled and evaluated an Andean diversity panel of 300 genotypes for BNF and associated traits. We demonstrate the existence of adequate genetic variability for these traits.

MATERIALS AND METHODS

An Andean diversity panel (ADP) of 300 genotypes comprised of diverse varieties, elite lines and landraces from both the American and African continent was assembled. This panel was evaluated for BNF and associated traits in the greenhouse and field in 2012. In the greenhouse evaluation, two plants of each genotype were grown in a one gallon size plastic pot filled with perlite and vermiculite in the ratio of 2:1 by volume. Two non-nodulating mutants were also included in the experiment. The seeds were inoculated at planting with a *Rhizobium tropici* strain USDA 9030. To increase the levels Rhizobium, a second inoculation of 1ml of *Rhizobium* broth per pot was added. From planting to flowering when the plants were harvested for evaluation, only nitrogen (N) free nutrient solution was applied. At flowering, plants were removed from the pot, the root system cleaned and nodules collected. The shoot and nodules were oven dried at 60^{O}C for 3 days, weighed, ground and total N measured. Chlorophyll content was also evaluated at flowering using a SPAD meter. Total N fixed by each genotype was estimated as a product of N concentration in the shoot and the dry weight shoot biomass at flowering. Nitrogen difference method was used to estimate the amount of nitrogen fixed by each genotype by subtracting the amount of N in the non-nodulating mutant from the fixing genotypes. In 2012, the ADP was planted in the field at the Montcalm research farm located in the region of Michigan where Andean beans are predominantly grown. The location has sandy loam soil and the field had an average of 25 ppm of residual soil N at the time of planting. Each genotype was planted in two row plots of 4.5 m long and experiment had two replications. Chlorophyll content was measured at 30 and 40 days after planting using the SPAD meter. At flowering, four plants were randomly selected from each plot and nodulation was ranked on a scale of 0-6, with zero being no nodulation and six being well nodulated plant with over 80 nodules. The shoots of these four plant samples were oven dried at 60^{O}C for three days, weighed, ground and total N measured. The N difference method was used to estimate N fixed by each genotype.

RESULTS AND DISCUSSION

Significant differences ($p<0.001$) for nodulation, chlorophyll content, shoot biomass, shoot N concentration and total N fixed were observed in both greenhouse and field experiments. Some of the genotypes that showed superior N fixation in the greenhouse included TZ-37 (from

Tanzania), CC-22 (Mecosta from Michigan, USA) and Silver Cloud (from USA) (Figure 1). These genotypes also had higher shoot biomass and nodule weight as reflected in the significant Pearson correlation among total N fixed, shoot biomass and nodule weight. Preliminary studies on seed N has also shown TZ-37 to have higher seed N suggesting that it is superior in both N fixation and partitioning of the N to the seed. There were significant correlations between chlorophyll content measured by the SPAD meter and total N fixed suggesting that chlorophyll content is an effective predictor of N fixation in greenhouse experiments. Since evaluating chlorophyll content is both faster and cheaper that total N, the SPAD meter may be useful in evaluating genotypes for N fixation in greenhouse experiments. Adequate variability for nodulation, chlorophyll content, shoot biomass and total nitrogen was observed in the field experiments. Some genotypes such as TZ-37, CC-22 that had superior biomass weight and total N fixed in the greenhouse also showed consistent superior performance for these traits in the field experiment. These results are from one greenhouse experiment and one field season and they should therefore be treated with caution. Additional greenhouse and field experiments are needed to confirm these results. In this preliminary study we have demonstrated the existence of adequate genetic variability for BNF in the Andean gene pool and identified superior Andean genotypes for N fixation. These genotypes could potentially be used in breeding programs designed to improve N fixation of the Andean germplasm.

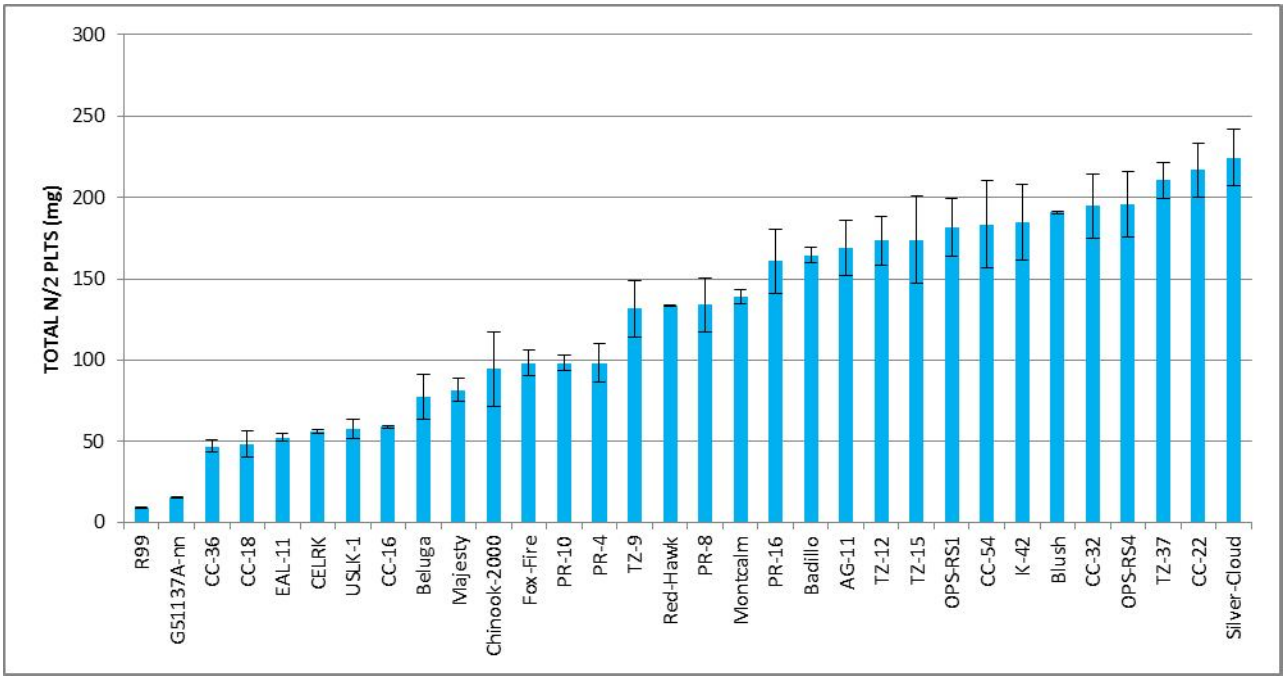

Figure 1. Total N fixed by two plants for selected genotypes of the ADP grown in the greenhouse. R99 and G51137Ann with least N amounts are non-nodulating mutants

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SNAP BEAN (*PHASEOLUS VULGARIS* L.) BREEDING FOR ENHANCED NITROGEN-USE EFFICIENCY

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Wisconsin is the leading producer of processed snap beans (*Phaseolus vilgaris* L.) in the United States. Most of this acreage is found in the central sands where nitrate leaching partially due to over fertilization, soil type, and irrigation is high. Enhancing nitrogen-use efficiency (NUE) for economic and environmental reasons in this crop has for the most part been unsuccessful due to the high environmental influence on this trait. After augmented design field evaluations of 96 total genotypes from two $BC_1SSD_3F_{3-9}$ populations significant genotypic effects for NUE were found over two years. Although significant genotype by environment interactions were found, narrow sense heritability estimates for two different NUE measures presented herein were estimated above 0.50. Selections for eventual cultivar release were made within each of the two populations. This study demonstrated that simple field evaluation with thorough replication was sufficient for making selections of enhanced NUE in common bean.

The Nitrogen Stability Index (NSI) (Smith, 2012) was calculated as a measure of NUE for each genotype as follows: NSI = 1 – [(High N DW) – (Low N DW) / (High N DW)]
A second measure of NUE was used. The NUE measure was calculated using the dry weight for each genotype as a percentage of the corresponding replication of R99 dry weight (DW) under high and low nitrogen treatments: NUE_H = (Genotype High N DW) / (R99 High N DW)
NUE_L = (Genotype Low N DW) / (R99 Low N DW)

Four checks were included in the design: the two snap bean parent cultivars, Eagle and Hystyle; the high NUE donor parent, Puebla 152; and the non-nodulating mutant R99. Using NSI, DW, and stability of NSI across years as criteria, ten lines from both populations were selected as superior performing genotypes in respect to NSI with aboveground biomass accumulations equivalent to that of the two snap bean cultivar check varieties. NSI can be enhanced within common bean leading to superior snap bean cultivars performing equivalently in low and high nitrogen environments. NUE enhancement is dependent on the amount of synthetic nitrogen available. This is likely related to the effects of N fertility on BNF.

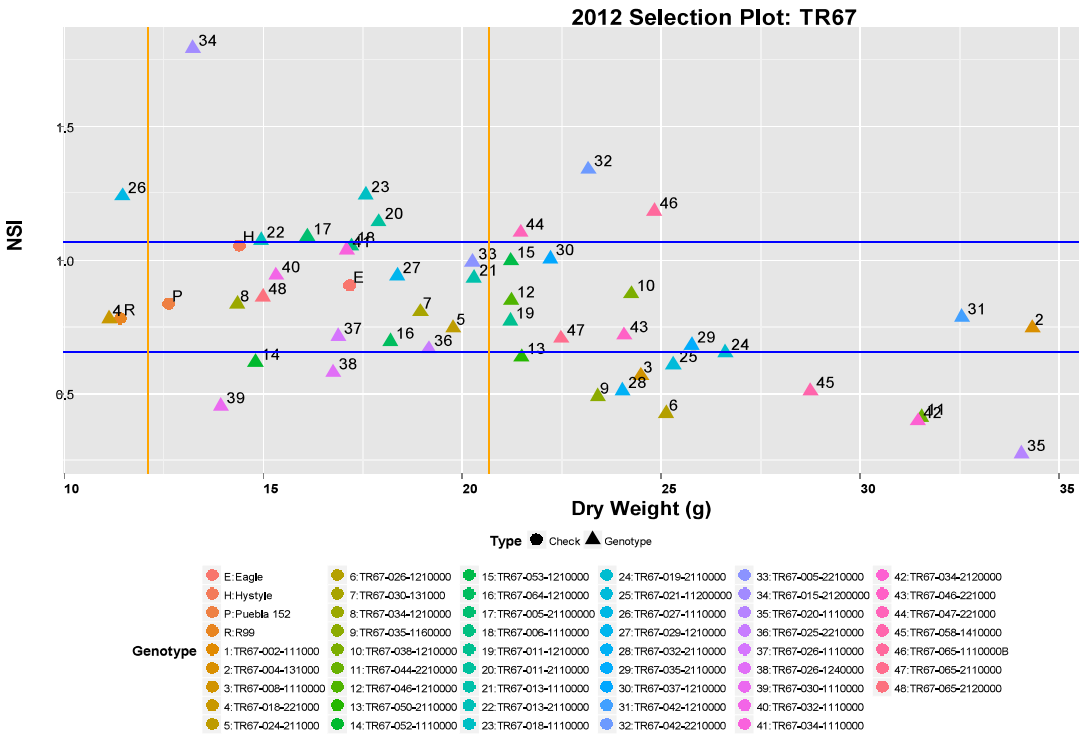

Figure 1 - Scatter plot of one population's geometric LS means plus four check varieties in 2012. NSI is plotted across the y-axis, DW under high N across the x-axis. Blue horizontal lines represent the 95% confidence interval (CI) for the NSI of Puebla 152. Orange vertical lines represent the lower and upper bounds of the 95% DW CIs of Hystyle and Eagle, respectively.

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ESTIMATION OF HERITABILITY FOR POD CHARACTERISTICS IN AN ANDEAN SNAP BEAN X MESOAMERICAN DRY BEAN POPULATION

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INTRODUCTION: In common bean (*Phaseolus vulgaris* L.), the most important characteristics associated with domestication were changes in size, shape, and color of pods and seeds, as well changes in growth habit, suppression of seed dispersal (shattering), and reduced sensitivity to photoperiod (Gepts, 1998). Pod characteristics such as pod length, spur length, and pod shape are important in snap bean quality compared to the dry bean. The objective of this study was to estimate the heritabilities of traits associated with snap bean domestication and pod quality: pod length, spur length, and pod shape ratio in a recombinant inbred line (RIL) population derived from a cross between an Andean snap bean cultivar and a Mesoamerican dry bean landrace.

MATERIALS AND METHODS: A seventy five progeny of RIL population derived from an original cross between 'Eagle' and 'Puebla 152' (EP-RIL) was sequentially evaluated in 2008, 2009 and 2010 in University of Wisconsin ARS at West Madison, WI . 'Eagle' is a white seeded, green podded snap bean cultivar, has determinate plant growth, is predominantly from the Andean gene pool. In contrast, 'Puebla 152' has an indeterminate growth habit (IV), has strong climbing ability, originated from the Mesoamerican, a dry bean, and has a black small seeded type. The experimental design was a randomized complete block design, with two replicates. Five pods in each progeny were harvested randomly in each replication. Pods were harvested at sieve four, which is 8.5-9.7 mm from the 90° off suture of the pod. Total pod length, spur length, pod shape ratio were measured and evaluated. Data were analysed using the statistic package SAS 9.2 and the heritabilites and standard error (SE) based on entry-mean basis were calculated (Hallauer et al., 2010).

RESULTS AND DISCUSSION: There were highly significant differences among the genotypes, and genotype by year (GxY) interactions for all traits: total pod length, spur length, and pod shape ratio (Table 1). Significant GxY interactions for pod length were also observed among bean cultivars evaluated in two contrasting environments in Turkey and Spain (Dursun, 2007; Escribano et al., 1997). The heritability for pod length in this EP-RIL population was 0.93 ± 0.02. The heritability of pod length was generally high, ranging from a low of 0.24 to a high of 0.80, with no clear pattern of differences in crosses between either market classes or gene pools (Bertoldo et al., 2010; Raffi and Nath, 2004). The spur at the distal end of the pod is an important cosmetic trait in snap beans and can be straight or curved; the consumer and processing preference is for short and straight for enhanced fresh market appearance and for ease of removal during processing and packaging (Myers and Bagget, 1999). Although important in both Andean and Mesoamerican snap beans, heritability for spur length has never been documented. In this EP-RIL population, the h^2_N of the spur length was high, 0.92 ± 0.16. Pod shape ratio, measured as the ratio of the pod diameter 90° off the suture relative to the diameter on the suture, determines if pods are flat or round. Pod shape has been reported to be inherited additively with some degree of dominance in crosses within snap bean cultivars, 'OSU 5062' with near-round podded 'Oregon 83' and 'Slenderette', oval-podded 'Bountiful', and flat-podded 'Roma' (Chung

et al., 1991). The heritability for this trait was high in the EP-RIL population, ranging from 0.88 to 0.97 over the years, but moderate as a mean, 0.63 ± 0.28 because of the GxY interaction.

High heritability in domestication traits such as total pod length, and spur length for EP-RIL suggest that phenotypic selection based on those traits could result in gigantism of pod size and improved quality of the common bean. The high heritability observed for domestication reflects the ability of mass selection over time to be effective in dramatically altering the dry bean into a vegetable pod. Snap bean evolution will continue and the results of this study suggest that changes in pod characteristics will continue to transform the snap bean with characteristics that benefit growers, markets, and consumers.

Table 1: Analysis of variance (ANOVA), variance component, and narrow-sense heritability (h^2_N) for domestication traits of Eagle x Puebla 152 recombinant inbred line (EP-RIL) bean population.

Sources	df	Mean square		
		total length (cm)[z]	spur length (cm)[y]	pod shape (ratio)[x]
Year	2	294.6**	0.88	4.49
Rep(year)	3	21.0**	1.07**	3.40**
Genotype	74	85.7**	1.10**	0.40**
Genotype x year (GxY)	140	5.9**	0.09**	0.15**
Error (pooled)	1840	1.4**	0.03	0.02
Variance components				
	σ^2_g	2.89	0.04	0.01
	σ^2_{gxe}	0.48	0.01	0.02
Heritability				
	$h^2_N \pm SE(h^2N)$	0.93± 0.02	0.92 ± 0.16	0.63 ± 0.28
	2008	0.94	0.88	0.88
	2009	0.97	0.96	0.98
	2010	0.96	0.95	0.97

*, ** significant at $p \leq 0.05$ and 0.01 respectively, [z, y] Total length and spur length of five randomly pods were measured using a ruler. [x] Pod shape was measured by having a ratio of 90° off suture and on suture width. Measurements were taken in the middle of the pods.

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UNCOVERING THE GENETIC BASIS FOR SUGAR ACCUMULATION IN SNAP BEAN PODS

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Flavor is an essential component to the marketability of fresh and processed snap bean varieties, and sugar content is one major flavor determinant in the pods. The snap bean's nutritional benefits are also desirable. When these traits are bred in combination, snap beans become more marketable, especially in an increasingly health conscious population. This study explores the genetic basis behind variation in sugar content among green pod-type beans including commercially grown cultivars and inbred backcross (IBC) lines. A key objective of this sugar analysis is to confirm putative QTLs associated with sugar accumulation in snap bean pods using SNP markers. A deeper understanding of genetic variation in pod sugar among these lines will facilitate development of new snap bean cultivars with more desirable sugar content and flavor.

The USDA promotes consumption of foods like green beans. Their nutritional benefits include soluble fiber, carotenoids, flavenoids, and vitamins[1]. Despite their health benefits, many Americans, especially children, have an aversion to vegetables. This is due to several factors including off flavors and low sweetness. Taste panelists preferred sweeter varieties of both edamame and dry beans[2]. Increasing sugar levels of snap beans would likely make them more palatable as well. Because snap beans are considered a very healthy food, the goal is not to increase sugars to an unhealthy level, especially if they are to be marketed toward children. However, even doubling sugar levels in snap bean pods would only bring them to the sugar content of a sweet potato, and would drastically improve desirability and flavor without making them unhealthy[1]. These sweeter beans could be used by fresh market and snap bean processors and marketed to those concerned with both taste and the nutritional benefits of vegetables.

The Nienhuis lab at the University of Wisconsin-Madison ran several experiments to test for genetic variation in sugar content of snap bean pods. These experiments shed light on how different cultivars, IBC lines, and stages of maturity affect sugar in the snap bean pod. The variation could be utilized by bean breeders to improve flavor and marketability. The experiment was repeated over several years, first using recombinant inbred lines (RIL) and then using IBC lines (***Table 1***). The RILs, called ExP, were a cross between a snap bean, and a dry bean. The RILs vary in many traits including sugars. The lines were grown at West Madison Agricultural Research Facility in Madison, WI. In 2010 and 2011, 75 ExP lines were harvested at sieve 4 and evaluated for fructose, glucose, and sucrose using EtOH soluble sugar extraction followed by HPLC[3]. The project evaluated 155 RAPD markers in R-QTL. One QTL significant for fructose and sucrose concentration levels existed on linkage group 1. This QTL showed inverse effects between mono and disaccharide levels. Therefore, it was putatively suggested that this is invertase related. [One separate component of the study included measuring pod sugar levels ($mg \cdot g^{-1}$ dry wt) of check varieties at each sieve size. The study concluded that sieve 3 or sieve 4 contained the highest sugar levels, and as many snap bean processors prefer sieve 4 beans, that size was used in the rest of the analysis of variation.][4]

In 2012 and 2013, a very similar experiment was repeated using IBC lines. ExP lines were crossed to commercial snap bean lines (Eagle and Hystyle) and then selfed to create lines which varied for many traits but were more domesticated than the ExP lines (***Table 1***). The IBC lines are called TR65 and TR67. This newer experiment analyzed 19 commercial checks and 2

TR populations of 48 lines each for sugar accumulation. Again, sucrose, glucose, and fructose were measured at sieve size 4 using the same procedure as the ExP lines. Fructose is the most palatably sweet carbohydrate in snap beans. By focusing on this sugar, the bean will taste the sweetest while adding the smallest number of calories. ***Figure 1*** shows fructose variation in checks and TR lines for 2012. Check mean fructose was 30.7 $mg \cdot g^{-1}$ dry wt compared to 51.2 $mg \cdot g^{-1}$ dry wt and 44.1 $mg \cdot g^{-1}$ dry wt in TR65 and TR67 respectively. The ranges of fructose levels in checks was smaller compared to TR lines (31 $mg \cdot g^{-1}$ dry wt vs. 71.8 $mg \cdot g^{-1}$ dry wt and 45.9 $mg \cdot g^{-1}$ dry wt). The 2012 and 2013 data from this experiment will be analyzed for QTL using SNP markers and integrated with the ExP QTL map in the coming months.

Ultimately, snap beans are grown because they are marketable as a healthy dietary choice. Variation in fructose will facilitate breeding for sweeter beans and this phenotypic variation will be used to confirm the genetic basis of sugar accumulation in snap beans through SNP marker and other analyses. Improved flavor will make snap beans more desirable and easier to sell. Increasing fructose in snap beans will sweeten them and add the fewest number of calories, attracting health conscious people who still want satisfying food choices.

Table 1: Beans Used in 2010-2013 Sugar Variation Analyses

Cultivar	Gene Pool	Origin	Market Class
Eagle	Andean	Asgrow Seed Co. (1972)	Processing snap
Puebla 152	Mesoamerican	Landrace from Mexico (1942)	Dry
Hystyle	Andean	Harris Moran Seed Co. (1985)	Processing snap
ExP	EaglexPuebla 152	Variation for many traits including sugars	
TR65	(EaglexPuebla 152)xEagle	Variation for many traits including sugars More domesticated than EaglexPuebla 152	
TR67	(EaglexPuebla 152)xHystyle		

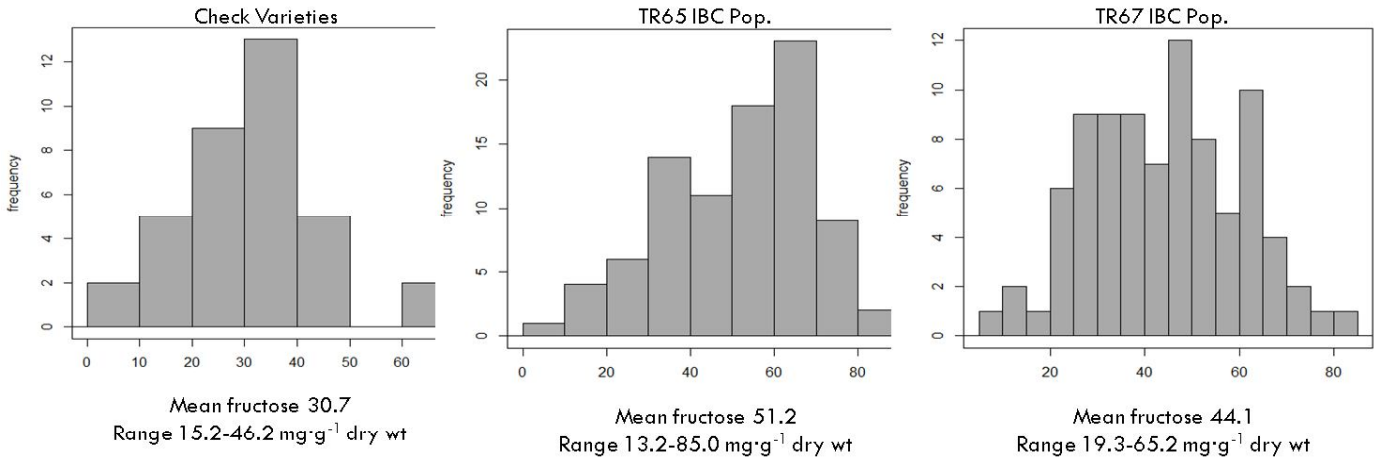

Figure 1: 2012 Fructose Variation

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WESTERN BEAN CUTWORM, *STRIACOSTA ALBICOSTA* (SMITH) LEPIDOPTERA: NOCTUIDAE, OVIPOSITION PREFERENCE, LARVAL SURVIVAL AND INSECTICIDE EFFICACY IN DRY BEAN

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The Western bean cutworm, *Striacosta albicosta* (Smith) Lepidoptera: Noctuidae, is a pest native to North America, but is new to Ontario. Larvae of this insect can feed on both corn and dry bean, though its impact to dry bean producers is not well understood. Studies were performed to examine oviposition preference, larval survival, and insecticide efficacy in dry bean.

The oviposition study was performed in the greenhouse at the University of Guelph Ridgetown Campus. Seven different dry bean market classes (navy, black, pinto, otebo, light red kidney, dark red kidney, and cranberry) were grown to one of three developmental stages (late vegetative, flowering or early pod-set). Five replicates were performed for the late vegetative and flowering developmental stages, while 4 replicates were performed for the early pod-set stage. Cages $1m^3$ were set-up and one plant from each market class was placed inside. Each cage contained plants from only one developmental stage. Five moths (2 female and 3 male) at 2 days of age were added at the beginning of the study. Examination of the plants occurred at 24 h intervals for 3 days, and egg mass presence was recorded. No differences ($P<0.05$) were found for oviposition on the lower leaf surface when examining preference between either plant developmental stage or market class, however egg mass numbers were low in this study.

The larval no choice feeding study was conducted at the University of Guelph Ridgetown Campus and consisted of 12 treatments and 3 replicates. Each replicate was composed of 1 bioassay tray containing 32 individual cells. One 1^{st} instar larva, obtained by hatching out eggs collected from a local corn field, was placed in each cell. Survival at 28 days was examined on three different dry bean market classes (navy, light red kidney, and adzuki) and three different tissue types (leaf, flower, pod), compared to a control diet. Control diet replicates were performed three times and coincided with each of the 3 tissue type replicates. Tissue was changed every 3 days for leaf and pod tissue and every 2 days for flower tissue. Weight of the larvae was recorded on a weekly basis. Survival on adzuki pod tissue was lower than on adzuki and navy leaf tissue or navy flower tissue, though survival on all other treatments were not significantly different. Larval weights were lower in adzuki pod tissue when compared to navy pod tissue, with no other differences found between any other treatment.

Insecticide efficacy trials were conducted in Ridgetown Ontario in 2012 and 2013 as a RCBD with 4 replicates and 8 treatments: untreated, lambda-cyhalothrin (10 $g.ai.ha^{-1}$), chlorantraniliprole (50 $g.ai.ha^{-1}$), lambda-cyhalothrin + chlorantraniliprole (25 + 50 $g.ai.ha^{-1}$), dimethoate (480 $g.ai.ha^{-1}$), thiamethoxam + lambda-cyhalothrin (25 + 19 $g.ai.ha^{-1}$), methoxyfenozide (30 $g.ai.ha^{-1}$), spinetoram (72 $g.ai.ha^{-1}$). Plots were 6 m in length and were composed of four rows of dry bean (cv. T9905) plants. Western bean cutworm egg masses were collected from a corn field near Bothwell ON and 4 egg masses were stapled to the underside of the dry bean foliage in each row with the egg masses facing outwards. Egg masses were

monitored daily until 50% of the eggs had hatched based on visual examination. All chemical products were applied 8 days later. The incidence (%) and severity of pod feeding was determined several times throughout the season. Severity was recorded on a scale of 0-3, with 0 being no damage, 1 being slight superficial feeding ($\leq$ 0.25cm in diameter), 2 being superficial feeding >0.25cm in diameter, and 3 being feeding that entered into the pod. At the end of the season, 5 m from each of the middle two rows in each plot was harvested to calculate yield and a 200 seed sample was removed and examined for insect damaged seed. No yield differences were found between the untreated control and any of the chemical treatments, though damage on pod tissue was quite low (< 1% for each plot). In the 200 seed sample, it was found that the weight of damaged seed in the untreated treatment was significantly higher than all treatments except for dimethoate and spinetoram. However, the weight of the damaged seed in the untreated control was also quite low.

In conclusion, it appears that there is no preference for ovipositioning of WBC moths, though egg mass numbers in the trial were quite low. There was lower larval survival on adzuki pod tissue, when compared to all other treatments. No yield impact was found when examining the different chemical treatments. Insect damaged seed were higher in the untreated, dimethoate, and spintoram treatments, but it is unlikely that this would impact economic return. Further research into the impact of this pest to the dry bean industry should be performed, as low egg laying and minimal damage was found in these studies.

OCCURRENCE OF ENDORNAVIRUSES IN WILD, LANDRACES, AND CULTIVARS OF *PHASEOLUS VULGARIS* FROM THE MESOAMERICAN AND ANDEAN REGIONS

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INTRODUCTION

Based on the type of relationship with the host, plant viruses can be grouped as acute or persistent (3). Acute viruses are well studied and cause disease in plants. In contrast, persistent viruses do not appear to affect the phenotype of the plant host. Persistent viruses include members of the family Endornaviridae which has members with genomes that range from 9.8 to 17.6 kbp; infect plants, fungi, and oomycetes; are transmitted only via gametes; and do not cause apparent symptoms (1). *Phaseolus vulgaris* endornavirus 1 (PvEV-1) and *Phaseolus vulgaris* endornavirus 2 (PvEV-2) have been reported to be prevalent in common bean (*Phaseolus vulgaris*) cultivars of Mesoamerican origin. In contrast, they have been found rarely in cultivars of Andean origin (2, 5). The goal of this investigation was to determine the occurrence of these viruses in a variety of *P. vulgaris* genotypes that originated from the two centers of domestication of common bean: Mesoamerica and the Andes.

MATERIALS AND METHODS

Plant introduction lines of wild, landraces, cultivars, and breeding lines of *P. vulgaris* from various countries of the two centers of domestication of common bean were obtained from the USDA/ARS, National Plant Germplasm System (NPGS), Washington State University Regional Plant Introduction Station (WRPIS), Pullman WA. In addition, common bean cultivars and breeding lines bred in the United States were provided by J. Osorno (North Dakota State University, Fargo, ND) and M. Pastor-Corrales (USDA/ARS, Beltsville, MD). Seeds were planted in the greenhouse and foliar tissues tested for endornaviruses by dsRNA analysis (4). To confirm the identity of the viruses, selected samples were tested by RT-PCR using virus-specific primers.

RESULTS AND DISCUSSION

A summary of the results are shown in Table 1. In the case of wild *P. vulgaris*, 14% (9/62) of the Mesoamerican lines were infected with endornaviruses whereas the 26 lines of Andean origin were virus-free. For the landraces, 39% (14/36) of the Mesoamerican lines and 7% (3/42) of the Andean lines were virus-infected. Testing common bean cultivars and breeding lines from breeding programs in the United States resulted in 94% (66/70) of the Mesoamerican genotypes infected. In contrast, only 11% (5/44) genotypes of Andean origin were infected. Most infections in wild *P. vulgaris* consisted of PvEV-2, whereas most of the infections of landraces consisted of PvEV-1. Mixed infection of PvEV-1 and PvEV-2 were more prevalent in USA-bred cultivars of Mesoamerican origin.

The data support earlier reports on the common occurrence of these viruses in *P. vulgaris* from Mesoamerica. It appears that virus-infected wild *P. vulgaris* from Mesoamerica was not spread to the Andean region or that the original source of these viruses was not present there. Alternatively, these viruses may have been in wild *P. vulgaris* of both regions but had a negative

effect on the plants in the Andean region whereas they provided an evolutionary advantage to plants in the Mesoamerican region. These two viruses were not detected in four other domesticated *Phaseolus* species or in 14 other non-domesticated *Phaseolus* species. Nevertheless, we have detected an unrelated endornavirus in several *P. lunatus* cultivars. The role or effect that these endornaviruses have in common bean is not known.

Table 1. Occurrence of PvEV-1 and PvEV-2 in wild, landraces, and cultivars/breeding lines of Mesoamerican and Andean origin

***P. vulgaris* genotype**	**Mesoamerican**				**Andean**			
	PvEV-1	**PvEV-2**	**PvEV-1 & 2**	**Total**	**PvEV-1**	**PvEV-2**	**PvEV-1 & 2**	**Total**
Wild	0/62	7/62	2/62	**9/62**	0/26	0/26	0/26	**0/26**
Landraces	5/36	1/36	8/36	**14/36**	2/42	0/42	1/42	**3/42**
Cultivars/lines (USA)	1/70	0/70	66/70	**66/70**	2/44	2/44	1/44	**5/44**

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HEATING THINGS UP FOR CONTROLLING SEED-BORNE DISEASE IN DRY BEAN

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INTRODUCTION: In dry bean production, the use of high quality seed is very important as it can account for a large part of the input costs. The presence of seed-borne pathogens can lower dry bean yield and seed quality (Agarwal 1997). Currently several management practices including cultural, chemical, and genetic controls are used to lower disease incidence and severity (Agarwal 1997; Maude 1996; McGee 1997). Even with those control practices, seed-borne diseases are still difficult to manage. Therefore, the use of an alternative control method, microwave radiation, was tested to determine the effect on seed germination and pathogen viability of *Xanthomonas axonopodis* pv. *phaseoli* (Xap), *Pseudomonas syringae* pv. *phaseolicola* (Psp) and *Colletotrichum lindemuthianum*.

MATERIALS & METHODS:

Lab Study: Various microwave radiation treatments were applied to navy (cv. Navigator) and pinto (cv. Pintoba) beans infected with Xap or *C. lindemutianum* and navy (cv. Envoy) and kidney (cv. GTS 402) beans infected with Psp using a home microwave oven (General Electric JES 1142 WPC microwave). Microwave exposures ranging from 0-90 s were applied at 10 s intervals (0-90 s) to each experimental unit. The seed was then planted following the Canadian Germination Commission (CGC) warm germ test protocol and evaluated using the Canadian Food Inspection Agency (CFIA) methods and procedures for seed testing. Disease plating was conducted to evaluate disease control on each seed lot using potato dextrose agar amended with 50% lactic acid for *C. lindemutianum* and Xap and King's Medium B for Psp. From these results, a maximum exposure rate (MER) was determined for each seed lot, based on a minor decrease (<10%) in germination. Using the MER, a maximum rate (1x) and $^1/_2$ x rate for microwave radiation exposure were determined for field trials in Ontario and Manitoba.

Field Study: A randomized complete block design (RCBD) was used to compare the effect of microwave radiation alone and in combination with two chemical seed treatments, a BASF experimental (BAS 720) and an industry standard using copper hydroxide (58.3%) for the bacterial blights and thiamethoxam, fludioxinol, metalaxyl-M, azoxystrobin for anthracnose. Ratings for percent disease severity (leaf and pod) were conducted throughout the growing season. In addition, ratings for plant emergence, vigour, yield, pick and a calculation on return on investment (ROI) were carried out.

Trt #	Treatment
1	Uninfected ('Disease Free') Control (UC)
2	Infected Control (IC)
3	BAS 720
4	Industry Standard
5	$^1/_2$ x Microwave (25 seconds)
6	1x Microwave (50 seconds)
7	$^1/_2$ x Microwave + BAS 720
8	1x Microwave + BAS 720
9	$^1/_2$ x Microwave + Industry Standard
10	1x Microwave + Industry Standard

RESULTS & DISCUSSION:

Lab Study: When microwave radiation was applied at 0-40 s no decrease occurred in seed germination for any of the seed lots. Over 60 s a decrease in seed germination was observed in

all the seed lots. In the 40-60 s range a slight decrease in germination was observed, this length of time was where the MER was determined for each seed lot. For Xap, the MER of navy bean was 60 s for both years and 60 s and 50 s for the pinto beans in 2012 and 2013, respectively. For Psp a MER of 50 s was determined for the navy beans in both years and 50 s and 40 s for kidney beans in 2012 and 2013, respectively. For the *C. lindemuthianum* infected navy beans a MER of 50 s was set for 2012 and 2013 and 50 s and 40 s was set for the pinto beans in 2012 and 2013, respectively. For disease plating a linear decrease in pathogen growth of *C. lindemuthianum* was seen with increased microwave exposure, however, no differences occurred for either of the bacterial blights.

Field Study: The microwave treatment caused a slight decrease in field emergence, ranging between 2-9%, for all seed lots. This loss was considered acceptable based on the MER parameters set in the lab studies. Two other differences were seen in emergence; the NIC was better than the IC for all studies (NIC used uninfected seed) and BAS 720 (92%) treatment had higher emergence compared to the TFMA (84%)in the navy bean trial for *C. lindemuthianum*. No differences were observed in vigour for any of the studies. For disease control in the bacterial blights, no differences in leaf or pod ratings were observed when the microwave treatment was applied. In the *C. lindemuthianum* studies, disease ratings were evaluated using an area under disease progress curve (AUPDC). The NIC had less disease symptoms than the IC and the addition of chemical seed treatment decreased disease symptoms when applied alone or in combination with microwave treatment. The application of microwave treatment did not decrease disease symptoms compared to the IC when applied alone or in combination with chemical treatments for both leaf and pod infection. For seed pick, no differences were detected in the Xap studies. In the kidney bean Psp studies, the NIC, chemical treatments and microwave treatments had lower visible infection compared to the IC. However, no differences were seen between any of the other treatments. In the pinto bean *C. lindemuthianum* studies, the NIC had lower infection compared to the IC and BAS 720 had fewer symptoms compared to the TFMA treatment. Finally for yield, no differences were seen in the Xap studies. In the Psp studies, a slight increase in yield occurred when chemical treatment was applied, however this was only seen at one study for both the navy and kidney beans. For *C. lindemuthianum,* the addition of chemical treatment to the microwave treatment increased yield compared to microwave treatment alone in the navy bean studies. However, only in 2013 did the chemical treatments alone yield higher than the IC. For ROI no differences were detected for Xap. For Psp, differences were only seen at one study for kidney beans, where the application of microwave and chemical treatment in combination lowered ROI compared to chemical treatment alone. Finally, for *C. lindemuthianum* ROI increased in the pinto studies when chemical and microwave treatment were applied together.

CONCLUSIONS: The application of a microwave treatment did not lower seed germination and had little impact on Xap, Psp, or *C. lindemuthianum* in the field. The addition of chemical treatment to microwave radiation or on its own also had limited impact on disease control.

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CHARACTERIZED ISOLATES OF *SCLEROTINIA SCLEROTIORUM* CAN FACILITATE IDENTIFICATION AND VERIFICATION OF RESISTANCE TO WHITE MOLD IN DRY AND SNAP BEANS

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A collaborative project that was a part of the National Sclerotinia Initiative (NSI) facilitated the collection of 366 *Sclerotinia sclerotiorum* isolates from white mold (WM) screening nurseries and bean grower fields across most of the dry and snap bean production areas of the USA as well as a location each in France and Mexico. The screening nurseries provided locations to test bean lines with levels of WM resistance derived from NSI projects. Each annual nursery had nine test entries and three control lines, Beryl (WM susceptible), Bunsi (less susceptible to WM) and G122 (semi-resistant to WM) planted in a randomized block with three replications. This allowed statistical comparisons of isolate characteristics within nurseries, between nurseries and grower fields and between years from 2001-2011. A straw test (Otto-Hanson et al., 2011) was used to evaluate isolate aggressiveness and 16 microsatellite loci defined genetic diversity.

There were no significant differences in aggressiveness between isolates collected from the three control hosts of *S.sclerotiorum* in the screening nurseries in any year of collection. However, there were significant differences in aggressiveness between some screening nurseries located in MN, MI, ND, NE and CA. There were fewer isolate collections from grower fields due to lack of year-to-year establishment of WM. Isolates from NE and ND had higher aggressiveness than WA (Table 1). There were also examples of significant variation in aggressiveness found between isolates collected in Red River Valley compared to Trail County. When isolates collected from nurseries and grower fields within a state were compared, MI isolates did not differ in aggressiveness. However, ND isolates compared in the same way were significantly different, as were those from WA where the year of collection also was a significant factor in aggressiveness variation.

The development of a large dendrogram using 16 polymorphic microsatellites (Sirjusingh & Kohn, 2001) and UPGMA (unweighted pair-group method with arithmetic mean) cluster analysis has enabled us to define clusters using Euclidean distance indices of 32% and compare genetic similarities and differences isolate origin. The 20 clusters generated were similar for the three control hosts in the screening nurseries with 54, 50 and 55 total isolates for Beryl, Bunsi and G122 respectively (Table 2). State or country origin isolates exhibited variability in total clusters and distribution between the 20 clusters (Table 3). Grower field isolates were also variable when compared by state origin, where isolates were distributed in only 10 clusters (Table 4).

The next step in characterization of isolates is comparison of isolate sensitivity to common fungicides. Our goal: isolates with higher or lower levels of aggressiveness and widely or locally distributed will be available for use in WM resistance screening.

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Table 1. Aggressiveness of isolates from grower fields in CO, MI, NE, ND and WA

State	Number of isolates	Mean (1-9 scale)	Standard Dev.
Colorado	41	4.7	0.9
Michigan	17	5.1	0.8
Nebraska	17	5.7	0.8
North Dakota	50	5.9	1.0
Washington	22	4.2	0.4

Table 2. Screening nursery isolates: Host vs. Clusters

Host	Euclidean Distance (32%) Clusters																				
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**Total**
Beryl	1	1	2	1	1	0	1	0	2	1	1	1	2	2	5	0	8	0	5	20	**54**
Bunsi	0	2	2	0	0	1	1	0	3	0	0	5	0	1	3	1	9	2	4	16	**50**
G122	0	1	4	0	2	2	0	1	2	0	1	4	3	0	2	0	9	0	3	21	**55**
Total	**1**	**4**	**8**	**1**	**3**	**3**	**2**	**1**	**7**	**1**	**2**	**10**	**5**	**3**	**10**	**1**	**26**	**2**	**12**	**57**	**159**

Table 3. Screening nursery isolates: State of origin vs. Clusters

State	Euclidean Distance (32%) Clusters																				
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**Total**
CA	1	4	5	1	0	0	0	1	1	1	2	0	0	0	0	0	0	1	1	0	**18**
FRA	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	4	1	1	10	**18**
MEX	0	0	1	0	0	0	0	0	3	0	0	0	0	0	0	0	7	0	7	0	**18**
MI	0	0	0	0	0	2	0	0	1	0	0	0	2	0	0	1	7	0	0	17	**30**
MN	0	0	0	0	2	0	1	0	0	0	0	0	0	0	4	0	0	0	1	1	**9**
NE	0	0	0	0	0	1	0	0	0	0	0	2	2	2	4	0	1	0	0	3	**15**
ND	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	3	**7**
OR	0	0	2	0	0	0	0	0	2	0	0	0	0	1	0	0	0	0	1	10	**16**
WA	0	0	0	0	0	0	1	0	0	0	0	2	1	0	2	0	7	0	0	11	**24**
Total	**1**	**4**	**8**	**1**	**2**	**3**	**2**	**1**	**7**	**1**	**2**	**10**	**5**	**3**	**10**	**1**	**26**	**2**	**11**	**55**	**155**

Table 4. Field isolates: State of origin vs. Clusters

State	Euclidean Distance (32%) Clusters										
	6	**9**	**11**	**13**	**14**	**16**	**17**	**18**	**20**	**21**	**Total**
CO	3	0	0	0	0	5	4	7	3	19	**41**
MI	0	0	1	0	1	6	1	1	1	7	**18**
NE	4	0	0	4	0	8	0	0	0	1	**17**
ND	0	0	2	0	1	6	0	2	0	39	**50**
WA	0	1	0	0	1	0	0	4	0	16	**22**
Total	**7**	**1**	**3**	**4**	**3**	**25**	**5**	**14**	**4**	**82**	**148**

* Only clusters with isolates are presented.

CHANGES IN PRIMARY AND SECONDARY METABOLISM ASSOCIATED WITH TOLERANCE TO SCLEROTINIA SCLEROTIORUM IN DRY BEAN

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INTRODUCTION

Plant metabolic processes are increasingly recognized as central to disease resistance. However, for dry bean (*Phaseolus vulgaris* L.) the molecular and metabolic processes that confer tolerance to white mold (*Sclerotinia sclerotiorum*) are largely unknown. Metabolomics, the comprehensive analysis of small molecules in biological systems, is a complementary analytical approach to genomics and transcriptomics that can help explain mechanisms associated with complex phenotypes. Identifying dry bean metabolites associated with *Sclerotinia* infection may provide novel, non-transgenic targets to breed for enhanced resistance. Here, the metabolic changes that occur during *Sclerotinia* infection of a detached leaf were characterized using a non-targeted metabolomics workflow detecting both primary and secondary metabolites in a resistant and susceptible bean line.

MATERIALS AND METHODS

Detached leaves from a resistant (A195, light brown Andean) and susceptible (Sacramento, light red kidney Andean) bean line were inoculated with *Sclerotinia* isolate S20 for metabolite profiling at 16, 24, and 48hr, and a mock inoculation was used as a control for each line (n=5 leaves per treatment). A pooled sample of leaf punches (n=5 punches per leaf, 1 cm diameter) were taken at the areas adjacent to the site of infection, or adjacent to the mock inoculum plug for control leaves. Samples were frozen in liquid nitrogen and ground to a fine powder using steel beads in a TissueLyser II (Qiagen). Metabolites were extracted by adding 1 mL of methanol:water (80:20, v:v) to the ground tissue, shaking at room temperature for 2 hr, centrifuging at 13,500 *g* for 10 min, and the supernatant was collected and stored at -80 ºC until further analysis. Metabolites were detected using mass spectrometry coupled to ultra performance liquid chromatography or gas chromatography (UPLC-MS, GC-MS). For UPLC-MS, 1 µL of metabolite extract was injected into a UPLC-TOF-MS system using instrumentation and methods as previously described [1]. For GC-MS, 500 µL was dried using a speedvac, derivatized via methoximation and trimethylsilylation in 100 µL, and 1 µL was injected into a GC-MS system using instrumentation and methods as previously described [2]. The resulting UPLC-MS and GC-MS datasets were defined by molecular features (mass signal/retention time pairs) and the corresponding relative quantities observed in each treatment.

RESULTS AND DISCUSSION

The resistant line A195 had noticeably less mycelial growth at 16 hours post inoculation (hpi) than Sacramento. An orthogonal projection to latent structures discriminant analysis (OPLS-DA) was performed and the results support that A195 had a more distinct metabolite profile from its control than Sacramento at 16 hpi (Figure 1A). The UPLC-MS and GC-MS datasets were analyzed by z-transformation of the inoculated sample group for each bean line using each bean line's mock-inoculation as the control. The analysis detected 144 metabolites that varied between inoculated A195 and Sacramento when compared to their controls and included amines/amino acids, organic acids, saccharides, hormones, cell wall-related compounds, and glycerolipids. A195 exhibited a reduction in many amino acids and increased abundance of ureides (Figure

1B), indicating nitrogen metabolism may be a critical component to resistance to white mold in dry bean. A195 also had varying production of several phytoalexins, and this phenotype was more apparent at the 24 and 48 hr timepoints. Additionally, many of the phytoalexins and hormones that varied appear specific to legumes, such as gibberellin A37 glycoside, soyasaponin, and phaseolin. A second experiment was conducted in stems and a similar trend in secondary metabolism was observed.

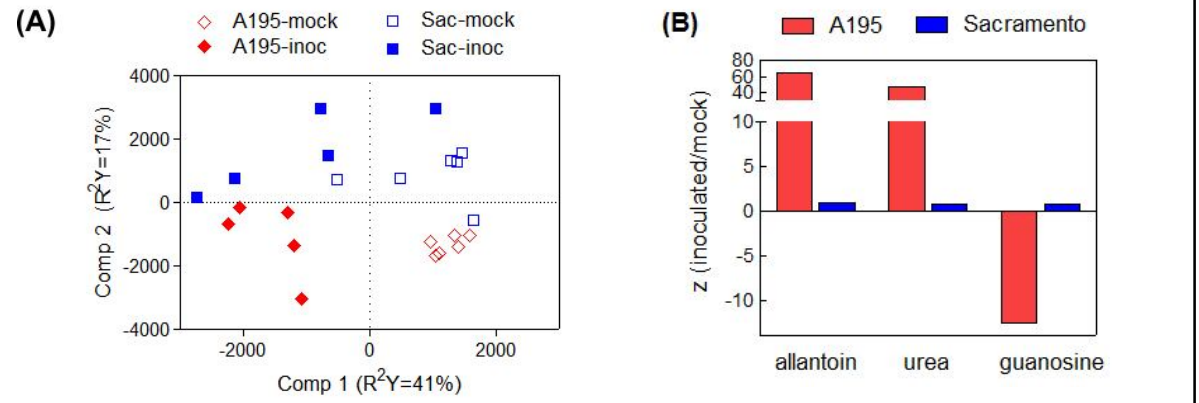

Figure 1. Metabolomic profiling of the resistance response to *Sclerotinia*. (A) An OPLS-DA scores plot of combined UPLC-MS and GC-MS metabolite profiling shows separation of the A195 and Sacramento metabolome following inoculation with isolate S20 at 16 hpi. (B) Metabolites that varied at 16 hpi related to ureide production, whereby data is expressed in z based on the mean values of inoculated samples compared to the mean and standard deviation of the mock inoculated control.

CONCLUSIONS

The diversity in metabolic changes observed in the tolerant cultivar point towards a multi-faceted mechanism for plant resistance to *Sclerotinia* in dry bean.

ACKNOWLEDGEMENTS

This work was supported by the USDA-ARS National Sclerotinia Initiative.

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THE TRANSMISSION AND CONTROL OF ANTHRACNOSE (*COLLETOTRICHUM LINDEMUTHIANUM)* IN DRY BEAN (*PHASEOLUS VULGARIS* L.)

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Material Transmission: Material transmission refers to conidia that adhere to a passing material and are transferred from infected to uninfected plants. A great deal of anecdotal evidence regarding the spread of *C. lindemuthianum* by agricultural equipment and workers exists yet no studies have been undertaken to determine its extent. This led to a study to determine if canopy moisture and inoculum source impact the material transmission of *C. lindemuthianum* in *P. vulgaris*.

Materials and Methods: In 2012 a site was planted in Morden MB (cv. Navigator) while in 2012 and 2013 sites were planted in Ridgetown ON (cv. T9905). An RCBD design was used with plots 3 m by 10 m and 4 rows wide. Each plot was isolated by 3m of soybeans on all sides. Four materials were chosen (leather, denim, metal, and leather) and fashioned into swatches fitting the researcher's leg. Three Race 73 inoculum sources were evaluated, including a 10^5 and 10^7 artificial inoculum as well as a block of infected plants to serve as a natural source of inoculum. At first flower, treatments were applied by spraying the swatch with the artificial inoculum or walking through the naturally infected plants, followed by walking along one row in the plot to be treated. Applications were done in a wet or dry canopy. There were 25 treatments in total, including an uninfected control. Three points along the treated row were evaluated throughout the season, marked at 1-2m, 5-6m, and 8-9 m. Anthracnose infection (%) on leaf veins, stems and pods were rated, as well as on the harvested seed. Statistical analysis was done using SAS and differences were determined using Fishers protected LSD (<0.05). Data was analyzed using area under the disease progress curves (AUDPC) where distance was substituted for the traditional time measurements.

RESULTS: Each of the materials transmitted disease at all inoculum concentrations with significant differences between some treatments. Material transmission through a wet canopy tended to transmit the pathogen a farther distance down the row with leather and denim having the greatest difference in transmission between a wet or dry canopy. The 10^7 inoculum transmitted disease to the greatest degree regardless of canopy moisture. The natural source of infection was affected more by canopy moisture than the artificial inoculants.

Foliar fungicide efficacy: Foliar fungicides are an effective method to control anthracnose. It is imperative that new compounds be tested to provide growers with reliable data on product efficacy for *C. lindemuthianum*. This trial evaluated fungicides registered for *C. lindemuthianum* in Canada, fungicides registered for other bean diseases as well as experimental fungicides being evaluated for various dry bean diseases.

MATERIALS AND METHODS: In 2012 a trial site was planted in Exeter ON and in 2012 and 2013 trials were planted in Ridgetown ON. Race 73 infected seed (cv. T9905) was planted in each study. A RCBD design was used with plots 3 m by 6 m or 4 rows in width. Each plot was isolated by 3m soybean on all sides. There were 15 treatments, including various fungicides and an infected untreated control plot. The fungicide treatments were applied at approximately 5% field infection. Throughout the season, anthracnose infection (%) on leaf veins, stems and pods

were evaluated, as well as seed yield, seed weight and pick. Data analysis was performed using SAS and differences determined using Fishers protected LSD (<0.05).

Results: Differences were seen between treatments at a limited number of sites. The strobulurin formulations and fluopyram+prothioconazole provided the greatest degree of anthracnose control. There were no differences between fluazinam, penthiopyrad and fluopyram and the infected control.

Transmission by Fungicide: A third trial examined the effects of *C. lindemuthianum* material transmission in conjunction with fungicide application. The trial was designed to mimic a grower's field sprayer to evaluate the potential to spread pathogens while applying a fungicide meant to control the pathogen.

Materials and Methods: Field trials were set up in Ridgetown, ON and Exeter, ON (cv. T9905) in 2012 and 2013. Trials used an RCBD design with plots 6m by 10m or 8 rows wide. Plots were isolated by 3m of soybeans on all sides. The foliar fungicides evaluated were thiophanate-methyl, pyraclostrobin and fluazinam and treatments were applied to a wet and dry canopy. There were 7 treatments in total, including a disease free control plot. A rubber swatch was formed to fit over the researchers' leg and a 10^7 spore ml^{-1} solution of anthracnose inoculum (race 73) was applied. Rows 2 and 7 of each plot were treated with inoculum, followed immediately by a fungicide application over row 2. Each treated row was marked at 1-2m, 5-6m, 8-9m. Throughout the season, anthracnose infection (%) on leaf veins, stems, and pods were measured at these points. At harvest seed infection was rated. Data was analyzed in the same manner as the material transmission trial.

RESULTS: Preliminary analysis showed that rubber transmitted disease down the row at both wet and dry canopy conditions. Disease was still transmitted despite the application of fungicides, but fungicide treated rows had lower AUDPC than those treated with inoculum only. Pyraclostrobin controlled the transmission of anthracnose significantly more than thiophanate-methyl and fluazinam. Thiophanate-methyl and fluazinam did not differ in their level of control.

DEVELOPMENT OF MOLECULAR TECHNIQUES FOR DETECTION OF PATHOGENIC ISOLATES OF *RHIZOCTONIA SOLANI* IN BEANS

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INTRODUCTION

Root rot caused by *Rhizoctonia solani* Kuhn is one of the most common root rot affecting beans. *R. solani* is found in most agricultural soils, in different grades of infestation, and can affect many plant species of economic importance. The fungus develops when temperatures are moderate to low—the ideal being 18°C—and soil humidity is moderate to high. The symptoms, known as cankers, are reddish-brown concave lesions that appear on stems and on the main root. The pathogen can eventually cause lodging, a condition also known as *damping off*. Susceptible plants suffer serious damage in the first 2 weeks after planting. Advances in molecular methods allow the accurate detection of microorganisms including pathogenic fungi. In this work we developed a molecular technique to be able to detect *R. solani* associated with bean plants.

MATERIALS AND METHODS

In order to establish a rapid and specific identification method for *R. solani*, 22 isolates pathogenic on beans, 1 on rice and 2 on Brachiaria were tested in a set of bean varieties under greenhouse conditions at CIAT. Inoculum preparation and inoculation was carried out as described by Castellanos et al. (2011). After sowing in this inoculated ground the bean variety, it was possible to establish which of the 22 isolations were really pathogenic to that plant.
The mycelia of the strains was used in DNA extraction for the amplification and later sequencing of internal transcribed RNA ribosomal sequence (ITS), using the primers ITS1 (5´TCCGTAGGTGAACCTGCGG-´3) and ITS4 (5´TCCTCCGCTTATTGATATGC-´3), described by White (1990).

The sequences obtained were purified and analyzed for polymorphic regions that were used for the design of primers able to anneal only to sequences derived from pathogenic for beans.

RESULTS AND DISCUSSION

After examining the sequences, we were able to design two primer pairs, the first one RhP (5'-AGCAGGTGTGAAGCTGCAAT-3')), in combination with the first ITS1, which were able to amplified the expected DNA fragment in all the isolates including the pathogenic on bean, Rice, Brachiaria, but also non-pathogenic strains(Picture 1).
The second primer obtained rhfp(5'-GACCTCCAATACCAAAGCAG-3 '), was designed to be used in conjunction with ITS1, allowed specifically to detect *R. solani* pathogenic isolates belong anastomosis group AG 4 (Picture 2).
The new designed primers were also tested on DNA extracted from bean infected tissue using different isolates of *R. solani.* Expected amplifications were only obtained from DNA coming from plants that presented symptoms of the disease caused by *R. solani*.
Currently, the same primers are being tested to specifically detect the presence of pathogenic *R. solani* using soil samples collected from bean fields.

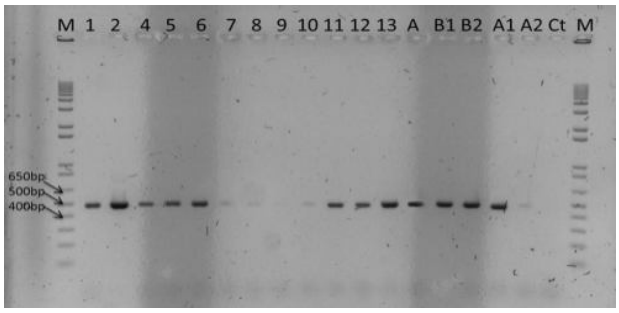
CONCLUSIONS

We were able to design a molecular tool for the detection of ***R. solani*** affecting beans.

Two primers were designed and both showed different specificities, one for pathogenic strains independent of the host, and another one which resulted to be specific for pathogenic strains associated only with beans.

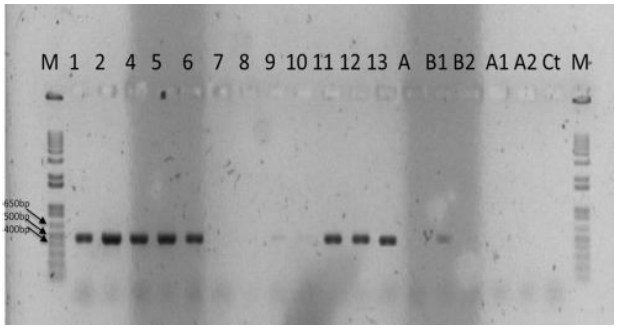
These primers proved to be functional, not only in DNA extracted from strains, but also from infected tissue. The described methodology represents an important advance in detection method for a bean pathogen that could be used as diagnostic method.

Picture 1



DNA from *Rhizoctonia solani* **was isolated and subjected to PCR using ITS primers (**RhP and ITS1**). Electrophoresis of the PCR products produced a 500-bp band. PCR products were separated by electrophoresis in a 1% agarose-TBE gel. The 1-Kb ladder (Promega®) (M) was used to estimate the size of PCR products. Lanes 1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13:** *R. solani* **isolated from bean; Lane A** *R. solani* **isolated from rice;Lanes B1, B2** *R. solani* **isolated from** *Brachiaria* sp.**; lanes A1, A2 DNA from isolates** AG1 y AG2-1 respectively; **Lane Ct** negative control**.**

Picture 2



DNA from *Rhizoctonia solani* **was isolated and subjected to PCR using** RhFP and ITS1**primers. PCR produced a 450-bp band. PCR products were separated by electrophoresis in a 1% agarose-TBE gel. The 1-Kb ladder (Promega®) (M) was used to estimate the size of PCR products. Lanes 1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13:** *R. solani* **isolated from beans; Lane A** *R. solani* **isolated from rice;Lanes B1, B2** *R. solani* **isolated from** *Brachiaria* sp.**; lanes A1, A2 DNA from isolates AG1 y AG2-1 respectively; Lane Ct negative control.**

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Full Text/Texto Completo

A NEW COMMON BACTERIAL BLIGHT RESISTANCE QTL IN VAX DRYBEAN BREEDING LINES AND HOST QTL INTERACTION WITH BACTERIAL STRAINS.

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INTRODUCTION

Resistance to common bacterial blight (CBB) is controlled by more than 20 QTL (Miklas and Singh, 2007). A QTL on Pv10 linked to SAP6 SCAR markers is derived from common bean. Higher levels of resistance associated with BC420 QTL on Pv06 (Yu et al., 2000) and SU91-CG11 QTL on Pv08 (Pedraza et al., 2007) are derived from tepary bean. The BC420 QTL is linked with undesirable seed coat color (Mutlu et al., 2005; Park et al., 1999) and the SU91 QTL is associated with low yield (O'Boyle et al., 2007). The objectives were to (1) identify a new resistance gene/QTL in VAX 1 and confirm it in VAX 3, and (2) examine the interaction between the new QTL, SAP6 and SU91-CG11 against two bacterial strains.

MATERIALS AND METHODS

Sixty one $F_{6:7}$ recombinant inbreed lines (RIL) from Othello/VAX 1 and 100 RIL from Othello/VAX 3 with their parents were screened in the greenhouse at Kimberly, Idaho in 2012. One less aggressive bacterial strain ARX8 and one more aggressive Xcp25 at density of 1.7 x 10^8 CFU/mL were inoculated in each of the primary leaf at 15 days after sowing. The first 3/4th expanded trifoliolate leaf was inoculated with ARX8 at 20 days and the second with Xcp25 at 25 days and two pods/plant at 60 days with each strain. Disease severity was recorded at 21 days post inoculation in leaves and 7 days in pods, using a 1 to 9 scale (1= no bacterial symptoms, and 9=water soaked lesions extended to the leaf/pod margin) (Lema et al., 2007). A total of 1688 single nucleotide polymorphism (SNP) markers in Othello/VAX 1 RIL, and 1493 in Othello/VAX 3 were used to develop 11 linkage groups. Also, SAP6 and SU91-CG11 (only in Othello/VAX3) were assayed. Genetic linkage map was constructed using JoinMap4 and QTL analysis was carried out using the Qgene software.

RESULTS AND DISCUSSION

A new QTL linked with the SNP47467 marker was identified on Pv11 in both populations. SNP47467 QTL had significant effect (P<0.01) in leaves explaining 10-20% of phenotypic variance in the primary leaf in response to ARX8 and 30-40% against Xcp25. The respective values for the trifoliolate leaf were 20% against ARX8 and 30-40% against Xcp25. No significant effect of the Pv11 QTL was noted in pods against both bacterial strains. The Pv11 QTL is very likely derived from tepary bean G40001 which was a common parent in VAX 1 and VAX 3 breeding lines (Singh el al., 2001). Thus, it would be important to confirm the presence of this marker in G40001.

Recombinant inbreed lines with SAP6 QTL had resistant CBB scores in leaves and pods against ARX8 in both populations (Table 1). In contrast, no significant effect of SAP6 was observed in any plant tissue against Xcp25 in both populations. The SU91-CG11 QTL had significant positive effect only in leaves in response to Xcp25. But, it was ineffective against less aggressive

strain ARX8. Thus, the use of new Pv11 QTL would be crucial for breeding for stable and higher levels of foliage resistance to common bacterial blight in common bean, especially in combination with SAP6 QTL.

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Singh, et al. 2001. Crop Sci. 41:275-276.

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Table 1. Mean common bacterial blight scores for the primary and trifoliolate leaves and pods against ARX8 and Xcp25 strains of *Xanthomonas campestris* pv. *phaseoli* in the Othello/VAX 1 and Othello/VAX 3 recombinant inbreed lines evaluated at 21 days post inoculation in leaves and 7 days in pods in the greenhouse at University of Idaho, Kimberly in 2012.

Plant tissue	Othello/VAX1			Othello/VAX 3			
	ARX8						
	None	SAP6	SNP47467	None	SAP6	SU91-CG11	SNP47467
Primary leaf	5.5 a	2.0 c	2.6 b	5.3 a	1.7 c	3.7 b	3.8 b
Trifoliolate leaf	6.8 a	3.4 c	4.5 b	6.6 a	3.0 d	5.4 b	4.9 c
Pods	4.4 a	3.2 b	4.3 a	4.8 a	2.7 c	4.7 a	4.1 b
	Xcp25						
Primary leaf	6.5 a	5.6 b	3.8 c	6.7 a	6.2 b	5.0 c	5.1 c
Trifoliolate leaf	7.8 a	7.1 b	5.6 c	7.4 a	7.4 a	6.7 b	6.0 c
Pods	4.9 a	4.8 a	4.7 a	5.0 b	4.9bc	5.3 a	4.6 c

Scored on a 1 to 9; 1= no symptoms, and 9= water soaking lesions extended to leaf/pod margin

IDENTIFYING CBB-RESISTANCE GENE CANDIDATES THROUGH COMPARATIVE GENOMICS BETWEEN OAC-REX AND G19833

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INTRODUCTION: Common bacterial blight (CBB), caused by the pathogen *Xanthomonas axonopodis* pv. *phaseoli* is a foliar and seed disease in common bean (*Phaseolus vulgaris*) that is endemic in most bean growing regions. Immunity to *Xap* has not been observed in *P. vulgaris*, but members of the secondary and tertiary gene pools, such as *Phaseolus acutifolius*, are resistant (for review see Singh and Schwartz, 2010). OAC-Rex was developed from an interspecific cross (*P. vulgaris* x *P. acutifolius*, Parker, 1985) and was registered as the first bacterial blight resistant cultivar in Canada in 2002 (Michaels *et al.,* 2006). CBB resistance in *P. vulgaris* is a multi-QTL trait, with the region associated with the SU91 marker (GI: 156072919) on chromosome 8 accounting for the strongest resistance in OAC-Rex (Shi *et al.,* 2012; Durham, 2011).

Although the development of molecular markers has aided research on CBB resistance, the lack of genome sequence information for bean has hindered the identification of the resistance genes. With the release of the G19833 genome (*P. vulgaris* v1.0, DOE-JGI and USDA-NIFA, http:://www.phytozome.net/commonbean), and the ongoing efforts to sequence OAC-Rex, direct genomic comparisons between resistant and susceptible *P. vulgaris* lines are now possible. The goal of this study was to identify unique genes surrounding the SU91 marker in OAC-Rex by comparing its sequence to G19833.

MATERIALS AND METHODS: For the full materials and methods, please refer to the complete study, Perry et al., (2013).

RESULTS AND DISCUSSION:

The SU91 marker was identified on a single contig in OAC-Rex (232701). As the SU91 marker is absent from G19833, two SNP markers (c00126p592970 and c00322p82935) were used to select a region from 58,994,870 to 59,444,870bp on chromosome 8, spanning the region predicted to contain SU91 (Xie et al., unpublished results) and 105 predicted genes. In order to identify OAC-Rex contigs homologous to this region, 7 markers from the BeanCAP group were identified in this region in G19833 (Table 1) and used to select 4 contigs from OAC-Rex (231733, 232029, 232701 and 231171). They cover a total distance of 550kb and contain 96 predicted genes. Although the gene order and orientation were highly conserved between G19833 and OAC-Rex, comparisons between the two lines indicated that there were 18 unique genes in OAC-Rex. Of these, 231733-8-004 and 231733-8-005 were of particular interest, because they had high homology to NBS-LRR resistance genes. Also, 232701-8-007 and 232701-8-008 were homologous

Table 1: SNP markers and unique CBB-resistance candidate genes in OAC-Rex

BeanCAP Marker	Designation	Location (bp)
sc00187ln435150_255274_G_A_135407497	M-8-1	59250063..59250183
sc00187ln435150_224424_G_A_135376647	M-8-2	59280917..59281037
sc00187ln435150_172862_C_T_135325085	M-8-3	59331499..59331619
sc00187ln435150_154478_T_C_135306701	M-8-4	59349712..59349832
sc00187ln435150_76559_G_A_135228782	M-8-5	59423268..59423388
sc00187ln435150_54957_C_T_135207180	M-8-6	59444877..59444997
sc01730ln60519_17555_C_T_372768027	M-8-7	59615745..59615865
Unique Genes in OAC-Rex	**NCBI**	**Location (bp)**
231733-8-004 CC-NBS-LRR	KF429160	13860..22394
231733-8-005 CC-NBS-LRR	KF429161	24691..31932
232701-8-007 Niemann Pick Like	KF429165	42491..59132
232701-8-008 Niemann Pick Like	KF429166	62281..69187

to a Niemann Pick transporter (Figure 1b and c).

The unique NBS-LRR genes (231733-8-004 and 231733-8-005) were found to occur just outside of the major QTL associated with SU91 and exist in a cluster of CC-NBS-LRR type resistance genes, which has been enlarged in OAC-Rex relative to G19833 (Figure 1b).

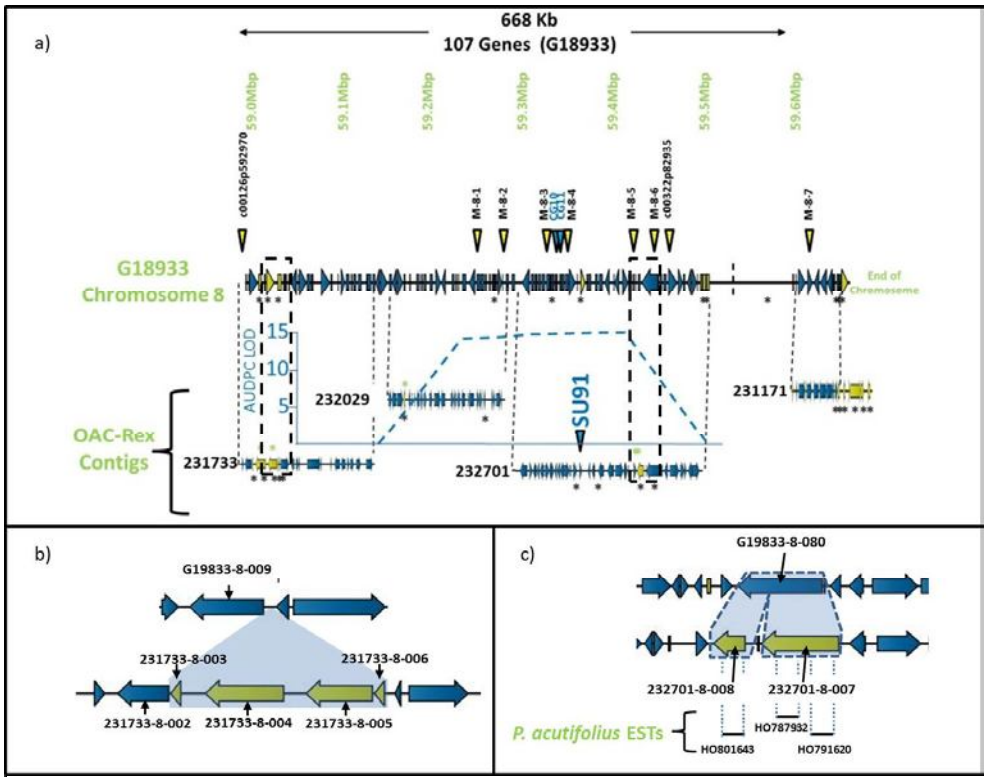

Figure 1: Comparison of the SU91 containing region in G19833 and OAC-Rex. (a) A 668Kb region surrounding the SU91 marker was identified in G19833, and matched with 4 contigs in OAC-Rex by comparison of genomic content. Regions containing the unique genes in OAC-Rex are outlined with dashed boxes. The unique genes in OAC-Rex were identified as CC-NBS-LRR resistance gene homologues (b) and a rearrangement of a Niemann Pick like gene (c), resulting in two independent genes, which appear to be expressed independently in *P. acutifolius*, as determined by homology to EST sequences.

The two Niemann Pick genes were found to be in close proximity to the CBB resistance QTL, and alignment of these genes relative to the G19833 gene showed that the OAC-Rex genes appear to have been derived from different regions of the G19833-8-080 gene, with 232701-8-007 representing the region from the N-terminus to 900 aa, and 232701-8-008 representing the 1100 aa to the C-terminus. Interestingly, these genes appear to be expressed in *P. acutifolius* as ESTs homologous to both the OAC-Rex genes (HO801643, HO791620, and HO787932) were found, indicating that they may have a different function in *P. acutifolius* (and OAC-Rex) than the Niemann Pick gene in G19833 (Figure 1c).

It is not currently known if the CBB resistance in OAC-Rex is due to the action of the unique R-gene candidates or is the result of a unique function of the modified Niemann Pick-like genes. Further molecular studies are needed to determine what role, if any, they play in resistance.

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BREEDING COMMON BEAN FOR QUANTITATIVELY INHERITED DISEASE RESISTANCE

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Several dozen bacterial, fungal, and viral diseases adversely affect common bean production worldwide. Resistance to these diseases may be inherited qualitatively (i.e., by major genes with large all-or-none effects) and/or quantitatively (i.e., by several minor genes or quantitative trait loci, each with small to appreciably significant effects). Some examples of diseases with qualitative inheritance of resistance include angular leaf spot, anthracnose, BCMV, and rust. Although some major resistance gene(s) have also been reported, examples of some diseases of major economic importance with quantitatively inherited resistance include common bacterial blight, white mold, and *Bean golden mosaic virus* (BGMV). One common feature of the latter group of diseases is that there is often only partial or low to intermediate levels of resistance occur in the common bean primary gene pool. In addition, significantly higher levels of resistance occur in the secondary (e.g., *P. coccineus* L.) and/or tertiary (e.g., *P. acutifolius* A. Gray) gene pools. Therefore, there is a strong justification or need for introgression of resistance genes/QTL from these distant relatives. I will briefly describe a commonly used breeding strategy and then give some examples of successful endeavors.

For quantitatively inherited disease resistance breeding in common bean when resistance genes/QTL occur in different races and gene pools of the common bean, and *Phaseolus* species of the secondary and/or tertiary gene pools, often a three-step breeding strategy is used to develop successful cultivars. The three steps are: (1) introgression of resistance genes/QTL from the distant relatives such as another race, gene pool, and secondary and/or tertiary gene pool species into the market class of common bean of interest, (2) pyramiding or combining resistance genes/QTL from across races and gene pools of the common bean, and its primary and/or secondary gene pool species, and (3) development of cultivars, i.e., combining high levels of pyramided resistance to quantitatively inherited disease(s) with resistance/tolerance to other important biotic and abiotic stresses, and other essential/desirable traits such as plant type, maturity, high pod or seed yield, seed and canning quality, etc. into the market class of common bean of interest for commercial production. However, it may be worth noting that no matter how different each of these three activities may be, they are not mutually exclusive, and may be carried out simultaneously in different projects to expedite cultivar development process.

For each of the three above mentioned quantitatively inherited diseases, namely BGMV, bean common bacterial blight, and white mold, useful resistance gene/QTL are found in the common bean and its secondary and/or tertiary gene pool. For example, for BGMV, resistance to plant dwarfing exists in small-seeded (<25 gr 100 seeds) race Mesoamerica beans from Central America (e.g., 'ICA Pijão', 'Porrillo Sintetico', 'Turrillalba'), large-seeded (>40 gr 100 seeds) Andean beans (e.g., 'Cardinal', G 122, 'Royal Red'), and *P. coccineus* (e.g., G 35171, G 35172). For leaf chlorosis or yellowing, resistance is found in race Durango pinto beans (e.g., Garrapato, synonymous with G 2402). And for pod deformation, resistance is found in the race Mesoamerica beans mentioned above. Moreover, resistance to plant dwarfing and pod

deformation are controlled by single dominant genes, whereas resistance to leaf chlorosis is controlled by recessive genes and QTL (see reviews by Singh and Schwartz, 2010). A similar situation occurs for white mold (see Schwartz and Singh, 2013) and common bacterial blight (see Singh and Muñoz, 1999).

Often a recurrent or congruity backcross, or their modifications (Singh, 1982) are used for introgression of resistance genes/QTL from distant relatives. For example, Singh et al. (2009) used a congruity backcross to introgress white mold resistance from *P. coccineus* G 35172 into interspecific breeding lines VCW 54 and VCW 55, and a recurrent backcross to introgress white mold resistance from *P. coccineus* PI 439534 into the SE153 series pinto beans (Singh et al., 2014a) and from *P. costaricensis* G 40604 into VRW 32 (Singh et al., 2012). Similarly, Singh and Muñoz (1999) used a modified recurrent backcross to introgress common bacterial blight resistance from tepary bean G 400001 into interspecific breeding lines VAX 1 and VAX 2. It is worth noting that in case of quantitatively inherited resistance to diseases the donor parents and the F_1 of single-crosses, as well as all subsequent crosses, are variable. Therefore, pure-lining resistance donor parents before crossing and gamete selection in the single-cross F_1 and subsequent backcrosses helps increase the frequency of resistance genotypes (Singh et al., 2014a). For pyramiding high levels of white mold resistance from across *Phaseolus* species and gene pools into common bean, use of multiple-parent crosses and gamete selection was successfully used (Singh et al., 2014b). Similarly, for development of breeding lines with multiple desirable traits including resistance to abiotic and biotic stresses from which then to select successful cultivars, use of multiple-parent crosses and gamete selection is advised. For example, Singh et al. (1998) used multiple-parent crosses and gamete selection to develop upright carioca beans with resistance to angular leaf spot, anthracnose, BCMV, BGMV, common bacterial blight, and leafhoppers. More recently, we have developed cultivars of some Andean beans with resistance to multiple diseases including, BCMV, BGMV, common bacterial blight, and white mold. However, farmers' participation and testing of populations, families, and breeding lines on farms have been crucial for development of successful cultivars, their subsequent adoption, and enhanced production life.

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SCREENING THE ANDEAN DIVERSITY PANEL FOR REACTION TO RUST UNDER FIELD CONDIONS IN CEDARA, KWAZULU-NATAL, SOUTH AFRICA

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INTRODUCTION: The yield and seed quality of large-seeded Andean dry beans grown throughout Eastern and Southern African countries is recurrently and often severely reduced by rust, angular leaf spot (ALS), and anthracnose (1). Although host resistance is the most cost-effective strategy to manage these diseases, only a very small number of the varieties available to growers in this region are resistant to these diseases. Thus, a very important objective of the Grain Legumes Project in Africa supported by the Norman Borlaug Commemorative Research Initiative between USAID Feed the Future and ARS-USDA is to identify Andean dry beans with resistance to these diseases. To that end, we sought to evaluate the Andean diversity panel (ADP) dry bean nursery under field conditions at the Cedara Agricultural Research Station, KwaZulu-Natal, South Africa, where rust, ALS, and anthracnose are recurrent.

MATERIALS AND METHODS: A total of 305 ADP dry bean entries and 29 Andean and Mesoamerican bean disease check cultivars, mostly for rust and ALS, and some local cultivars, were planted at Cedara the first week of January, 2013. Two reps of each cultivar were planted in five meter rows. The disease progress in the field was monitored frequently. An important evaluation was conducted on February 27 and 28, 2013. Many cultivars were flowering while others had green pods at different stages of development. The reaction of the bean cultivars to all diseases was evaluated using the CIAT Standard System for the Evaluation of Bean Germplasm. This scale includes nine categories, where 1, 2, and 3 are resistant; 4, 5, and 6 are intermediate, and 7, 8, and 9 are susceptible. The 1 category includes plants with no visible disease symptoms while the 9 category includes plants with very severe symptoms (See reference on Table 1).

RESULTS AND DISCUSSION: Disease symptoms on the 305 ADP common bean genotypes and the 29 disease check cultivars revealed that rust disease was severe on susceptible cultivars. In addition, rust was widespread and well distributed throughout the nursery. Also, rust resistant, intermediate, and susceptible ADP entries and check cultivars were observed in all sections of the field, indicating that this was an excellent rust epidemic for the identification of ADP lines with rust resistance. Other diseases including anthracnose, halo blight, scab and common bacterial blight were noted in some entries. ALS disease was not observed on any entry.

A total of 73 entries, from a total of 333 planted, were resistant to rust. Of these, 58 entries were from the ADP, while the other 15 entries were check cultivars. Seven of the 58 ADP entries were highly resistant with a 1 rust rating (with no visible rust symptoms), 17 were resistant with a 2 rust rating (with very few rust pustules), and 34 were resistant with a 3 rust rating (having small amounts of small and intermediate size rust pustules). It is important that 19 ADP entries had high levels of rust resistance at Cedara. These and other resistant entries will be evaluated under greenhouse conditions with a diverse group of races of the rust pathogen with the objective of identifying those with broad resistance. Some of the entries may be new and very much needed Andean sources of resistance to the hyper variable rust pathogen.

Because of the informative nature of the check cultivars, we discuss here their specific reaction to rust at Cedara. Based on their gene pool, the 29 check cultivars segregated into two groups: 15 Andean and 14 Mesoamerican. Only two (13%) of the 15 Andean check cultivars were resistant to rust, while 13 cultivars (87 %) were susceptible. Conversely, 13 (93%) of the 14 Mesoamerican check cultivars were resistant, while only one cultivar (7%) was susceptible. Similar results have been observed previously (1, 2, 3). The rust results observed at Cedara in 2013, suggest that Andean cultivars are much more likely to be susceptible while Mesoamerican cultivars are more likely to be resistant to rust in South Africa. These results also suggest that most isolates of the rust pathogen present in the field at Cedara were Andean that usually infect only or mostly Andean but not the Mesoamerican beans (2). Similar results to those observed at Cedara in 2013, are also likely to be observed for rust, angular leaf spot and anthracnose in other countries of Eastern and Southern Africa, where Andean beans are predominate.

Table 1. Reaction of selected ADP common bean genotypes with high levels of resistance to the rust pathogen under field conditions at Cedara, South Africa, March 2013

ADP Identif	Cultivar Name	[1]Rust Rating		ADP Identif	Cultivar	Rust Rating
ADP 97	Bilfa 4	1		ADP 119	A 193	2
ADP 127	Selian 6	1		ADP 126	Selian 05	2
ADP 199	G3452	1		ADP 220	G5625	2
ADP 453	INIAP 428	1		ADP 435	RM-05-07	2
ADP 457	INIAP 481	1		ADP 438	46-1	2
ADP 517	Carioca Kibala	1		ADP 443	Vason 7	2
ADP 33	Kijivu	2		ADP 447	INIAP 414	2
ADP 40	Katwela	2		ADP 449	INIAP 420	2
ADP 87	Kablanketi	2		ADP 462	PI 527540-B	2
Reaction to rust of disease check and local cultivars - separated by their gene pool[2]						
Mesoamerican	Resist Gene	[1]Rust Rating		Andean	Resist gene	Rust Rating
Aurora	Ur-3	3		Early G.	Ur-4	7
Mexico 235	Ur-3+	1		Golden GW	Ur-4	8
Mexico 309	Ur-5	1		P.C. 50	Ur-9, Ur-12	3
G.N. 1140	Ur-7	8		Don Timoteo	Not Known	7
PI 181996	Ur-11	1		Bonus	Not known	7
Redlands P.	Ur-13	2		Kranskop	Not Known	7

[1]Rust rating based on a 1-9 rust severity scale, where 1, 2, 3 = Resistant; 4, 5, 6 = Intermediate; 7, 8, 9 = Susceptible. [2]Check cultivars separated based on the gene pools of the common bean. van Schoonhoven, A. and Pastor-Corrales, M. A. 1987. Standard System for the Evaluation of Bean Germplasm. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia. 54 p.

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MAPPING QTL FOR ROOT ROT RESISTANCE, ROOT TRAITS, AND MORPHOLOGICAL TRAITS IN A COMMON BEAN RECOMBINANT INBRED POPULATION

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INTRODUCTION

Root rot is one of the primary yield limitations of snap bean production in the US (Navarro et al., 2008). QTL for genetic resistance and morphological traits have the potential to increase efficiency of breeding programs developing high yielding material suitable for the processing industry. The objective of this study was to evaluate a recombinant inbred population for Fusarium and Aphenomyces root rot resistance and for root architecture. We also investigated whether the same QTL were involved in resistance to both diseases and we mapped pod traits associated with the snap bean phenotype.

MATERIALS AND METHODS

RR6950 (resistant, small black seeded, indeterminate) was crossed with OSU5446 (susceptible, white seeded, determinate bush blue lake) in 2003. Both parents are of Mesoamerican origin. In the F3, one determinate plant from each F2-derived family was advanced to the next generation. The population was advanced from the F3 to F5, generation using a single plant in each family. In 2008, F5 families were bulked to develop lines for replicated testing. The population had 177 families.

Phenotypic evaluations for root rot resistance were conducted at the OSU Vegetable Research Farm in Oregon and at the Hancock Agricultural Research Site in Wisconsin. OR root rot is predominantly *Fusarium solani,* while WI root rot is predominantly *Aphanomyces euteiches.*

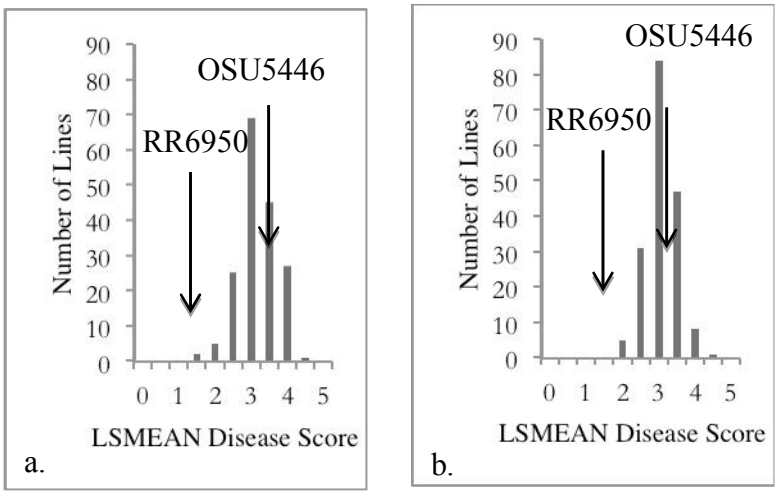

Figure 1a &1b Histograms LSMEANs of disease score for *F. solani* in Oregon (a. OR averages 2010, 2011, 2012) and *A. euteiches* in Wisconsin (b. WI averages Aug & July 2011, July 2012).

The population along with parents and checks were replicated three times in a RCBD. Five-10 plant samples per plot were rated for root rot symptoms on a one – five scale (1=resistant, 5=susceptible). Families were also evaluated for flower color, and pod traits (wall thickness, fiber, strings, cross-sectional shape, length, and color). In 2012 we evaluated taproot diameter, basal root diameter, adventitious roots, shallow root angle, deep root angle and, biomass using a shovelomics protocol (Lynch & Brown, 2013).

The RIL population was genotyped using the Illumina 10,000 SNP BARCBEAN6K_3 Beadchip developed through the BeanCAP. JoinMap 3.0 (VanOoijen and Voorrips 2001) was used to construct a linkage map and standard procedures in QTL Cartographer (Basten et al. 2002) were used to identify QTL.

RESULTS AND DISCUSSION

Resistance showed continuous distribution (Fig. 1a & 1b) indicating quantitative genetic control. The linkage map included 1,689 SNPs spanning 1,196 cM. The map included 14 linkage groups with Pv01 split into three groups and Pv11 split into two groups. Our study yielded several QTL including QTL for *F. solani* root rot, *A. euteiches* root rot, root architecture, pod shape, and strings (Table 1).

Table 1 QTL discovered in the RR138 mapping population.

Trait	Chrom.	Position (cM)	Additive efffect	R^2	Nearest SNP
Taproot Diameter	Pv02	93.6	-0.09	0.10	ss715646264
A. euteiches resistance	Pv02	96.5	0.26	0.15	ss715647851
F. solani resistance	Pv03	22.8	0.23	0.09	ss715641537
A. euteiches resistance	Pv04	4.6	0.18	0.10	ss715647818
Pod Wall Fiber	Pv04	82.9	-0.33	0.21	ss715649259
Pod Height	Pv04	82.9	-0.54	0.26	ss715649259
Pod Width	Pv04	82.9	0.43	0.18	ss715649259
Pod Wall Thickness	Pv04	83.9	0.22	0.16	ss715649259
Shallow Basal Root Angle	Pv05	14.9	-3.28	0.19	ss715645443
A. euteiches resistance	Pv06	12.3	-0.19	0.05	ss715649329
F. solani resistance	Pv07	47.8	0.32	0.22	ss715649511
Pod Length	Pv09	23.6	0.36	0.05	ss715647275

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GENOTYPING-BY-SEQUENCING (GBS) ENABLED MAPPING AND MARKER DEVELOPMENT FOR THE *By-2* POTYVIRUS RESISTANCE ALLELE

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A complex of aphid-transmitted viruses has emerged as a major source of crop damage and economic loss to snap bean production in the Great Lakes Region of the United States (Larsen et al., 2008; Shah et al., 2006). Snap beans are a major vegetable crop in this region and can generate more than $185 million per year in farm-gate revenue (USDA-NASS, 2013). Though the frequency of widespread disease epidemics is sporadic, the recently introduced soybean aphid, as well as numerous additional species of aphid vectors, disperse and transmit the component viruses rapidly and in a nonpersistent manner (Nault et al., 2004). Epidemics cannot be predicted and virus disease incidence cannot be controlled with insecticides. Resistance to the component viruses is the most efficient and effective strategy to attempt to reduce crop damage.

As a component virus, *Bean yellow mosaic virus* (BYMV) induces severe symptoms in susceptible common bean cultivars, particularly when infection occurs early in development. Resistance to the type strain of BYMV has been reported to be conditioned by 2-3 complimentary recessive alleles donated by UI 31 GN (Tatchell et al., 1985), and by a single dominant allele, *By-2*, that was introgressed from *P. coccineus* (Dickson and Natti, 1968).
The objectives of this research were to adapt genotyping-by-sequencing (GBS) (Elshire et al., 2011) to simultaneously discover and genotype single nucleotide polymorphisms (SNPs) in the parents and in recombinant inbred lines (RILs) of a snap bean-*By-2* introgression program, to identify SNPs significantly associated with *By-2*, and to subsequently enable marker-assisted selection (MAS) for *By-2*.

MATERIALS AND METHODS

A series of 42 resistant and 42 susceptible $BC_1F_{5:6}$ RILs were selected as the result of two cycles of line development to introgress *By-2* from the black bean donor line B-21 (Provvidenti et al., 1989) into the processing snap bean cultivar Hystyle. The 'NY' isolate of the type strain of BYMV, originally recovered from a production field in NY in 2007 was employed in resistance evaluations according to standard protocols for mechanical inoculation in the greenhouse. Following the restriction enzyme evaluation and adapter titration experiments to adapt GBS to common bean, DNA of these lines and their parents was digested with *Ape*KI, and prepared for multiplexed sequencing according to the GBS protocol (Elshire et al., 2011). Raw sequence data from a single lane of an Illumina Hi-Seq 2000 instrument was processed with the GBS Discovery Pipeline for species with a reference genome and implemented in TASSEL Version 3.0 (Bradbury et al., 2007). Sequence tags were aligned to the Phaseolus vulgaris V1.0 reference genome (DOE-JGI and USDA-NIFA, 2013) and SNPs were called at their physical positions throughout the genome. A case-control genome wide association study (GWAS) was conducted with GAPIT (Lipka et al., 2012) to discover significant associations between the GBS SNPs and the BYMV resistance conditioned by *By-2*. A subset of the resulting significant SNPs were converted to KASP SNP assays to validate genotype-phenotype cosegregation in an F_2 population of 185 plants derived from a cross between a snap bean breeding line that possessed *By-2* and Hystyle.

RESULTS AND DISCUSSION

Empirical evaluation of the commonly used methylation-sensitive restriction enzymes for GBS (*Ape*KI & *Pst*I) suggested that *Ape*KI digestion produced fragments from repetitive regions of the bean genome. We chose *Ape*KI despite this because its shorter recognition sequence was presumed to occur at a higher frequency and would result in a higher number of SNPs discovered. GBS with this germplasm resulted in the discovery of 7,530 SNPs (MAF $\geq$0.05) across the 11 chromosomes with an overall mean of 29% missing data across the genome. The number of SNPs per chromosome was moderately correlated (r =0.45) with the size of the respective chromosome. The case-control GWAS identified 44 highly significant SNPs ($P \leq 1.3 \times 10^{-6}$) associated with *By-2*. These SNPs delimited a 974 kb region on the distal portion of chromosome 2 (Phvul.002: 47991715 – 48965798). This region encompasses the 81.7 kb region (Chr02: 48183168 – 48264877) that harbors a complex cluster of TIR-NBS-LRR type virus resistance genes associated with the *I* allele for resistance to *Bean common mosaic (necrosis) virus* (BCM(N)V) (Vallejos et al., 2006). Four KASP SNP assays were demonstrated to cosegregate completely with the resistance phenotype in 185 F_2 individuals and validated their use for MAS of *By-2*. Additional SNP assays and populations with enhanced recombination have been developed for the current effort to fine map *By-2*.

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MOLECULAR AND BIOLOGICAL CHARACTERIZATION OF THE RU1-OR STRAIN OF *BEAN COMMON MOSAIC VIRUS*

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INTRODUCTION: *Bean common mosaic virus* (BCMV) exists as a complex of strains that are defined based on interactions with seven resistance genes in common beans (*Phaseolus vulgaris* L.); one dominant *I* gene, and six recessive genes, *bc-u*, *bc-1*, *bc-1*^{2}, *bc-2*, *bc-2*^{2}, and *bc-3* (1). Seven BCMV pathotypes (numbered I to VII) have been identified producing a range of symptoms in 12-14 differential bean lines carrying these resistance genes in different combinations. BCMV strain RU1 belongs to pathotype VI, but does not induce temperature-insensitive necrosis in *I*-gene bearing varieties of common beans, and has a characteristic B-serotype typical of non-necrotic strains of BCMV. Whole genome sequence was determined for two isolates of RU1 (2,4). Strain RU1 was originally found in an intercepted shipment of common beans in mid-1980s (3), and was confined to the lab ever since, it was never reported from the field so far. In 2011, two field isolates of BCMV were collected in the Willamette Valley, OR, and subjected to biological typing on bean differentials, to serological typing, and to whole genome sequencing.

MATERIALS AND METHODS: Two BCMV isolates, later named RU1-OR-B and RU1-OR-C, were collected near Corvallis, OR, from the field-grown common bean line L192 exhibiting mild symptoms of mosaic and leaf deformation. Both isolates were biologically typed on a set of 11 bean differentials according to Drijfhout (1). Four BCMV isolates from the laboratory collection were used as controls, NY15P (pathotype V), RU1P (pathotype VI, non-necrotic), TN1 (pathotype VI, necrotic), and US10 (pathotype VII). Serological typing was conducted using a set of strain-specific polyclonal antibodies against control isolates NY15P, TN1, and US10 in TAS-ELISA and Western-blots. Whole genomes of RU1-OR-B, RU1-OR-C, RU1P, and US10 were amplified using initially potyvirus-specifc primers targeting conserved areas in the HC-Pro, CI, and NIb cistrons, and later with specific primers filling the gaps between initial amplified areas. The very 5'-terminus of each of the four genomes was amplified using the RACE-Kit (Roche). All amplified fragments were cloned into the pGEM-T Easy plasmid vectors (Promega) and sequenced. The four sequences determined have been deposited in the GenBank database under the following accession numbers: KF919297 (RU1-OR-B), KF919298 (RU1-OR-C), KF919299 (US10), and KF919300 (RU1P). All four sequences were subjected to standard sequence analysis, and analyzed for possible recombination using ClustalX and RDP4 programs.

RESULTS: Both OR-B and OR-C isolates exhibited pathotype VII when tested on bean differentials, similar to the control isolate US10, and distinct from isolates NY15P (pathotype V), RU1P (pathotype VI, non-necrotic), and TN1 (pathotype VI, necrotic). When subjected to serological typing, both OR-B and OR-C isolates reacted as typical B-serotype isolates, distinct from the necrotic TN1 (A-serotype). However, both isolates failed to react with the NY15P-specific antiserum which reacted strongly with the US10 isolate exhibiting the same pathotype VII. This serological distinction between OR-B and OR-C, on one hand, and US10 exhibiting the

same pathotype VII, on the other hand, prompted our interest in sequencing all three isolates of BCMV. Initial partial sequencing of segments of the OR-B and OR-C genomes from the HC-Pro, CI, and NIb cistrons produced very close matches with the sequence of the RU1 strain deposited in the GenBank (GQ219793, 98 to 99% identity). In order to understand the molecular basis of the difference between BCMV isolates exhibiting pathotypes VI and VII, we sequenced the whole genomes for OR-B and OR-C (pathotype VII), and for two control isolates RU1P (pathotype VI) and US10 (pathotype VII). Whole genomes for isolates OR-B and OR-C were found 9,984-nt long, excluding poly(A), encoding a polyprotein of 3,196-aa, both had almost identical sequences (only 23 nucleotide differences), and a single name was retained, RU1-OR. Whole genomes of US10 and RU1P were found 9,998-nt and 10,003-nt long, respectively, coding for polyproteins of 3,201 and 3,202 aa, respectively. The whole genomes for RU1-OR, RU1P, US10, as well as two whole genomes of RU1 available in the GenBank database, were aligned using CLUSTALX program and subjected to a recombination analysis using the RDP4 package of programs. Figure 1 shows the patterns of similarity and dissimilarities between different regions of their genomes.

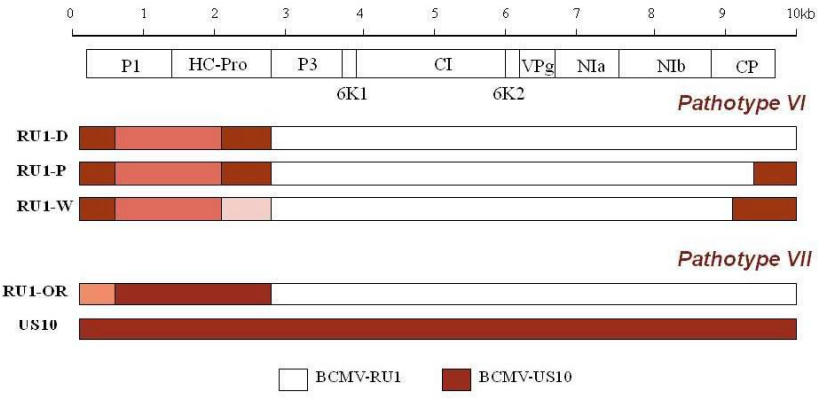

Fig. 1. Schematic diagram showing putative BCMV recombination positions between US10 and an unknown BCMV parent. Putative recombinant junctions are shown as vertical lines.

CONCLUSIONS: Our data suggests that recombination between BCMV strains is quite common, and isolates from BCMV strain RU1 represent multiple recombinants. US10 may be considered the most likely parental strain while other parents have yet to be identified (Fig. 1). The region spanning the C-terminal half of P1 and the N-terminal part of HC-Pro in pathotype VII isolates may interact with the *bc-2*^{2} gene (Fig. 1).

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DREB GENES AS CANDIDATES FOR IMPROVING DROUGHT TOLERANCE IN COMMON BEAN

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INTRODUCTION: The mechanisms underlying drought tolerance in common bean have been analyzed on the genomic, trancriptomic and physiological bases. cDNA libraries have shown differentially expressed genes under water deficit across diverse functional categories (Hernández et al., 2008; Recchia et al., 2013), highlighting among others the *DREB* gene family which has been studied by our group (Caldas et al., 2012). *DREB* (Dehydration Responsive Element-Binding) genes code transcription factors with a single AP2 protein domain. These genes are frequently induced by water deficit, salt, cold, heat, ABA and other abiotic factors. Therefore, *DREB* genes are promising candidates for marker-assisted selection (MAS) aimed at drought stress tolerance. Also *DREB*-specific polymorphisms (SNP markers) have been directly associated with tolerance traits in other crops. This study is an initial step towards the use of *DREB* genes as candidates for MAS for drought tolerance in common bean. Therefore, we aimed at the identification, expression profiling and SNP search for representatives of the *DREB* gene family, hoping to define associations of gene variation and phenotypic traits.

MATERIAL AND METHODS: We performed an *in silico* search on Phytozome and NCBI databases and found 181 sequences containing a single AP2 domain. Out of these, 54 matched one of these specific features of DREB proteins: (1) the 14th (Valine) and 19th (Glutamic acid) aminoacids of the AP2 domain were conserved (Sakuma et al., 2002); (2) protein motifs were conserved; (3) phylogenetic analyses matched DREB sequences from *Arabidopsis* and soybean. All common bean *DREB* genes were categorized into six sub-groups (A-1 to A-6) according to *Arabidopsis* (Sakuma et al., 2002). *In silico* mapping was performed based on the physical position on each chromosome. Four genes (*PvDREB1*, *PvDREB2A*, *PvDREB5* and *PvDREB6B*) from different groups were selected based on orthologs previously characterized for *Arabidopsis* and soybean. Gene expression profiles (qPCR) were generated for each gene under water deficit (PEG 10%) treatment. The coding region of these genes was resequenced in a set of genotypes contrasting for origin and tolerance to drought stress and SNP polymorphisms were found by alignment and SNP detection tools.

RESULTS AND DISCUSSION: In total, 54 putative *DREB* genes were categorized and all were distributed in sub-groups A-1 to A-6 (Figure 1.A). All common bean chromosomes present at least one *DREB* and many genes are located in clusters as checked by *in silico* mapping approaches. The expression profiles of the four transcripts selected (*PvDREB1*, *PvDREB2A*, *PvDREB5* and *PvDREB6B*) presented temporal and spatial variation and also differed among genotypes (Figure 1.B and 1.C). In general, *PvDREB1* and *PvDREB5* were strongly induced by PEG 10% in all tissues and in most times and genotypes, however *PvDREB2A* was more induced in stems and *PvDREB6B* in leaves. In Jalo EEP558 transcripts for the *PvDREB6B* gene were not detected. Resequencing results showed various SNP polymorphic sites between Andean and Mesoamerican genotypes, including non-synonymous substitutions for this locus (Figure 1.D). Other *loci* also showed SNP polymorphisms or INDEL. Further studies are needed to determine whether these polymorphisms have a functional role for these genes or if this variation is

associated with drought tolerance traits. SNPs associated with specific traits can then be converted to markers and used for MAS purposes.

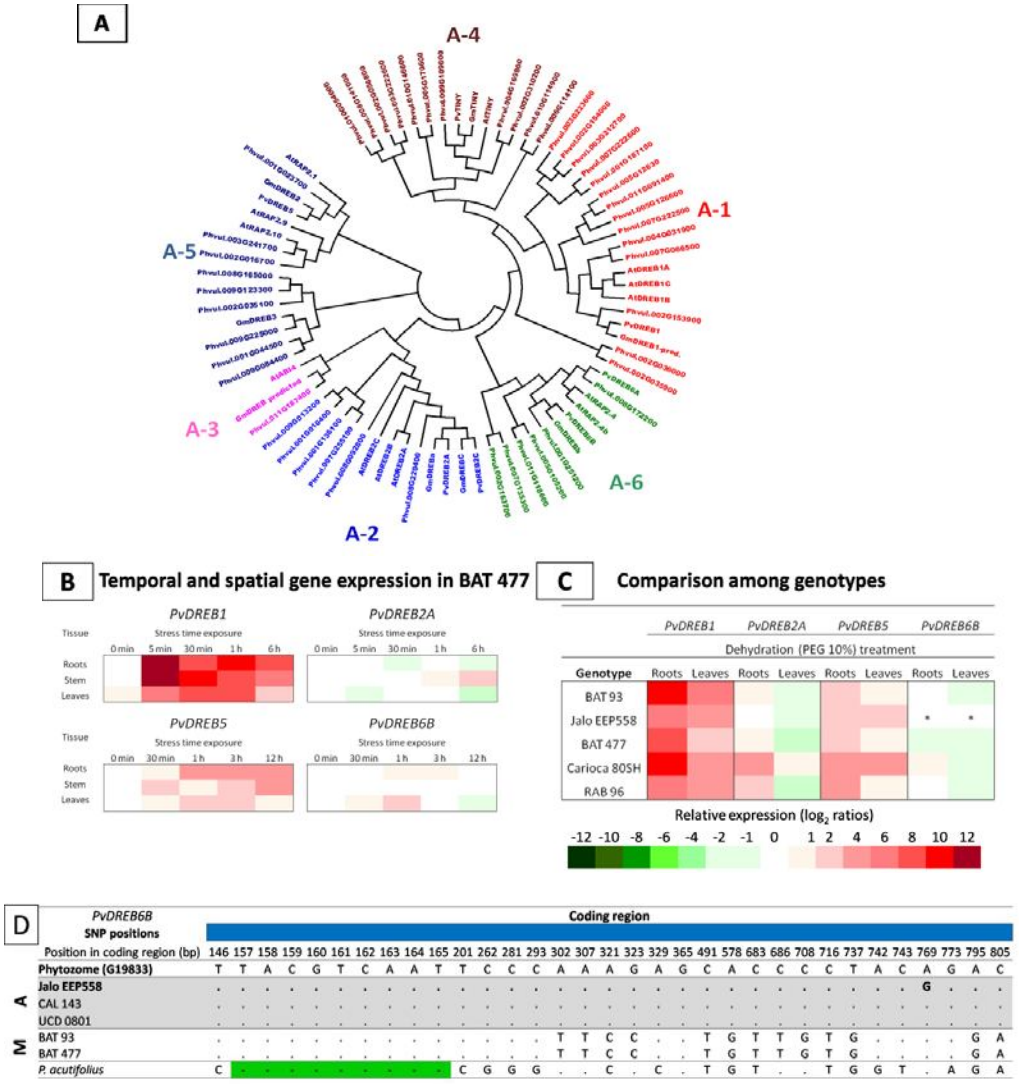

Figure 1 - (A) Phylogenetic tree showing the categorization of all putative DREB sequences for common bean, based on Phytozome and NCBI databases. (B) Temporal gene expression profile under water deficit induction (PEG 10%) at different time points in the genotype BAT 477. (C) Comparison of expression profiles among five common bean genotypes under water deficit (PEG 10%) after three hours of exposure. **: no transcripts were detected and this may be explained by many polymorphism sites (SNPs) which were detected between the Andean Jalo EEP558 and the other Mesoamerican genotypes. (D) SNP positions for the *PvDREB6B* gene of some common bean genotypes. A: Andean; M: Mesoamerican. The green color indicates an INDEL.

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POPULATION DEVELOPMENT TO INVESTIGATE DROUGHT ADAPTATION WITHIN THE MESOAMERICAN GENE POOL OF COMMON BEAN

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INTRODUCTION

Drought is one of the major constraints to common bean productivity. To improve the efficiency of breeding programs, it is imperative to understand the genetic basis of traits, especially for polygenic traits such as drought tolerance. Genetic improvement for drought adaptation within the domesticated germplasm has been relatively effective, but mostly derived from a reduced pool in race Durango. The variation among races has not been thoroughly studied and recombined. Variation within the wild ancestor remains largely untapped because of evaluation difficulties due to deleterious linkage drag when crossed to elite breeding lines. Nevertheless, wild beans have been thriving on an evolutionary time scale and are adapted to both water-limited and non-limited ecosystems; they could potentially harbor beneficial alleles not yet integrated into the domesticated gene pool.

MATERIAL AND METHODS

Population development

First set: Two sets of backcross recombinant inbred lines were developed between a domesticated cultivar (SEA5) and two wild accessions (PI319441 and PI343950). The wild accessions represent extremes within the Mesoamerican distribution of the wild species regarding precipitation. PI319441 was collected in the state of Durango, Mexico, in a site with an annual precipitation of 519 mm. PI343950 comes from Guatemala, with an annual precipitation of 1,915 mm. Both accessions are small-seeded, photoperiod-sensitive, have dehiscent pods and a climbing, indeterminate growth habit. SEA 5 (PI613166) was developed at CIAT from an interracial cross within the Mesoamerican domesticated gene pool and was selected for high productivity under drought; it has a type III indeterminate prostrate growth habit, is photoperiod neutral, has cream-colored seeds and is resistant to *Fusarium* root rot, ashy stem blight and Bean Common Mosaic Virus (Singh et al., 2001). Recombinant inbred line populations are being developed with a single backcross to SEA 5 and three subsequent generations of selfing, using the domesticated parent as the female in the last cross to control the cytoplasmic origin (Figure 1).

Second set: Eight genotypes from different market classes within the Mesoamerican gene pool were selected to maximize genetic diversity, area of adaptation, and high productivity under drought conditions, among other traits (SEA 5, Pinto San Rafael, Flor de Mayo Eugenia, SER 118, Matterhorn, UCD 9634, L88-63, and Victor). The genotypes are photoperiod-insensitive and have a non-climbing indeterminate growth habit. The crossing scheme was designed to achieve 960 recombinant lines of 8-way funnel crosses through a modified conical design that combines the nuclear genome while having equal cytoplasm presence of the parents (Figure 2a). The recombinant lines will be advanced to the F_5 generation by single-seed-descent for genotypic and phenotypic analyses.

Field evaluation of photosynthetic capacity among parental lines:

Photosynthetic productivity and stomatal conductance were measured with a LICOR 6400 gas exchange system (400 ppm of CO_2, 2000 PAR) in the eight parents under field conditions. The measurements were taken in fully irrigated conditions at the first fully expanded leaf stage, just before flowering. Measurements were taken throughout the day to explore their behavior under

different vapor deficit pressures. Stomatal conductance and photosynthetic capacity were normalized by vapor pressure deficit and stomatal conductance, respectively.

RESULTS AND DISCUSSION

First set: Two-hundred and fifty BC_1S_2 individuals of each domesticate by wild backcross population have been recovered to self pollinate for one more generation and further seed increase for field experiments in 2014. All individuals that were photoperiod sensitive and had highly dehiscent pods were discarded. We recovered a wide variation in seed color and seed size, with lines having seeds as large as the domesticated parent (Figure 1A)

Second set: Forty 4-way cross individuals were employed to randomly pollinate 3 plants of the complementary set to develop around 130 8-way crosses per cytoplasm origin set. A wide variation of seed shape, size and color were recovered among the recombinants (Figure 1B).

There were significant genotypic differences ($p < 0.001$) in both photosynthetic capacity and stomatal conductance among the parental lines (Figure 1C). Matterhorn had high levels of both photosynthetic capacity and stomatal conductance, which would be advantageous in non- water-limited environments. UCD 9634 had relatively high levels of photosynthesis and low stomatal conductance, which would be advantageous under terminal drought.

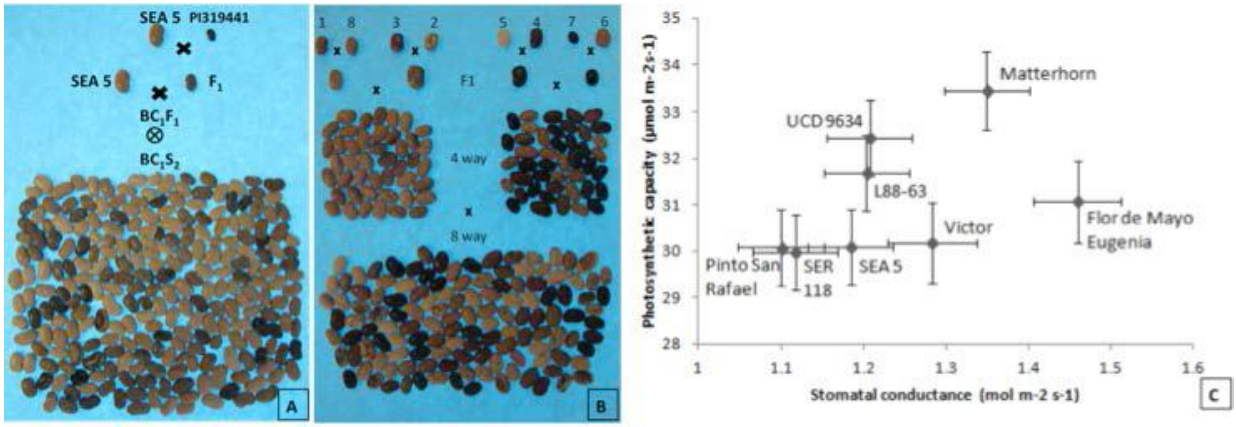

Figure 1. (A) Crossing scheme and seed variation of the population of PI319441 backcrossed to SEA 5, (B) Example of one of the 8 sets of 8-way crosses, (C) Photosynthetic capacity and stomatal conductance of the 8 parental lines.

CONCLUSIONS

A total of 250 backcross RILs of each wild by domesticate cross and 130 8-way multiparent RILs of each cytoplasm donor are being developed. This new resources will allow sampling of a wide genetic diversity within the Mesoamerican gene pool, and increase the mapping resolution in future QTL analysis. There are phenotypic differences in photosynthetic capacity and stomatal conductance among the parental lines in the multiparent population that will allow the dissection of the inheritance of the mechanisms that control these and other characteristics are segregating in this population, including the root system and photosynthate partitioning.

ESTIMATES OF GENETIC PARAMETERS IN COMMON BEAN (*Phaseolus vulgaris* L.) UNDER DROUGHT STRESS

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INTRODUCTION

The water deficit is considered the second leading cause of global agricultural productivity reduction, and in the case of common bean it is surpassed only by the occurrence of diseases (Acosta-Gallegos and Kelly 2012). In this context, the present study aimed to evaluate the effect of drought on genetic parameters estimates of common bean yield components and grain yield using the parental Perola and LP 97-28 and generations F_1, F_2, BC_1 and BC_2.

MATERIAL AND METHODS

This research was carried out under greenhouse conditions at Núcleo de Pesquisa Aplicada à Agricultura (Nupagri), Universidade Estadual de Maringá. Water deficit was induced by interrupting irrigation at three stages: vegetative, V_3; reproductive R_6 and R_8. Each period of drought lasted four complete days (Figure 1). After each period of drought, the plants were watered normally during a resting period of 20 full days.

Figure 1 - Morphological changes in plants of *Phaseolus vulgaris* L. under drought deficit.

When plants reached physiological maturity (stage R_9), the parental Perola (P_1, drought tolerant), LP 97-28 (P_2, low tolerance), F_1, F_2 , BC_1 ($P_1 \times F_1$) and BC_2 ($P_2 \times F_1$) were assessed for the following characters: number of pods per plant (NPP), number of seeds per pod (NSP) , average weight of 100 seeds (M100s; g.plant^{-1}) and gain production (PROD; g.plant^{-1}). The genetic-statistical analysis were performed to estimate the mean and variance for each of generations. We calculated the additive and dominance effects of the phenotypic data from each

generation utilizing the software Genes (Cruz, 2006). Broad-sense and narrow-sense heritabilities estimates were also obtained through this data.

RESULTS AND DISCUSSION

The results of yield data analysis showed a predominance of additive gene effects when compared with dominance effects (Table 1). It was observed that the characters PROD, M100s and NSP presented heritability narrow-sense estimates of 94.58, 70.52 and 79.74%, respectively. In addition, the generation derived from Perola × LP 97-28 provided satisfactory transmission of drought tolerance traits in progenies.

Table 1 displays the data of genetic gains, which magnitudes were of 3.15, 6.64, 2.91 and 1.54 for PROD, M100s, NPP and NSP, respectively. These results revealed satisfactory genetic gains conferred by additive genetic effects. Furthermore, selection 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, the average degree of dominance, number of genes and prediction of selection gain in segregant populations of common bean

Pérola × LP97-28	PROD	M100s	NPP	NSP
Parameters				
Phenotypic variance F_2 (σ_f^2)	4.18	55.10	11.52	2.13
Environmental variance (σ_e^2)	0.17	12.51	2.66	0.38
Genotypic variance (σ_g^2)	4.01	42.60	8.86	1.75
Additive variance (σ_a^2)	3.95	38.86	6.07	1.70
Dominance variance (σ_d^2)	0.06	3.74	2.79	0.05
Heritability broad (H^2 %)	96.04	77.30	76.94	82.25
Heritability narrow (h^2 %)	94.58	70.52	52.72	79.74
Average degree of dominance (*gmd*)	0.18	0.44	0.96	0.25
Number of genes (*n*)	4.73	3.34	5.95	3.11
Prediction of selection gains				
Gain from selection (*gs*)	3.15	6.64	2.91	1.54
Average cycle 1 (μ_{c1})	4.96	20.17	6.50	4.21

CONCLUSION

In conclusion, the results of this work show the predominance of additive genetic effects for grain yield and yield components in plants submitted to drought stress. Knowledge of the predominant type of gene action is of fundamental importance to direct a breeding program, since these results allow obtaining superior individuals.

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GENETIC ANALYSIS AND YIELD GAIN IN COMMON BEAN SEGREGANT POPULATIONS UNDER DROUGHT STRESS

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INTRODUCTION

Drought is one of the major abiotic stresses that affects all living organisms, including humans, in terms of health, food and agriculture (Akinci and Losel, 2012). The frequent and severe common bean production losses demands the development of new researches aiming to obtain drought tolerant plants. Basic researches may hold the solution for this issue, once they are of low investment and short length (Beebe et al. 2013). Therefore, this work had as objective to estimate genetic parameters of grain yield and its primary components in F_1, F_2, BC_1 and BC_2 and parental plants from IPR-Uirapuru (drought tolerant) × LP 97-28 (low tolerance) under water-stress conditions.

MATERIAL AND METHODS

The experiment was conducted under greenhouse conditions at the Common Bean Breeding and Molecular Biology Laboratory of Núcleo de Pesquisa Aplicada à Agricultura (Nupagri). Water-stress 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-stress lasted 96 hours (Figure 1). After each period of induced water-stress, plants were then irrigated normally for a period of 20 days.

Figure 1. Morphological response to water-stress in common bean.

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, 2006). The broad and narrow-sense heritabilities, the average degree of dominance, number of genes, and selection gain prediction were estimated.

RESULTS AND DISCUSSION

Variance components estimates showed 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. Similar results in common bean were obtained by Silva et al. (2004), once these authors reported the occurrence of additive genetic effects on the NSP trait.

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

LP 97-28 x IPR-Uirapuru	PROD	M100s	NPP	NSP
Phenotypic F_2 variance (σ_f^2)	8. 88	11.12	8.08	1.54
Environmental variance (σ_e^2)	2.97	2.55	2.33	0.56
Genotypic variance (σ_g^2)	5.91	8.57	5.75	0.98
Additive variance (σ_a^2)	5.75	7.66	4.73	0.76
Dominance variance (σ_d^2)	0.16	0.91	1.02	0.22
Heritability broad ($H^2_{\%}$)	66.4	77.0	71.1	63.6
Heritability narrow ($h^2_{\%}$)	64.7	68.9	58.5	49.2
Average degree of dominance (*gmd*)	0.22	0.48	0.65	0.76
Number of genes (*n*)	4.7	4.4	8.6	5.5
Gain from selection (*gs*)	43.5	15.7	34.1	16.1
Average cycle 1 (μ_{c1})	9.83	24.63	9.42	5.34

The results showed predictable genetic gains of 43.5%, 15.7%, 34.1% and 16.1% for grain yield production, average weight 100 seeds, number of pods per plant and number of seeds per pod, respectively. Also, studies conducted by Vallejo-Ramirez and Kelly (1998) also indicated high selection gain percentages for the traits of PROD, NPP and NSP. number of pods per plant (NPP), number of seeds per pod (NSP), average weight 100 seeds (M100s, g.plant^{-1}), and grain yield production

CONCLUSION

Additive genetic effects showed to be the main contributor for genetic gain in grain yield and its primary components when plants underwent water-stress conditions. This positive result may assist the obtaining of superior individuals in segregating generations.

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IDENTIFICATION OF QTL FOR DROUGHT TOLERANCE AND CHARACTERIZATION OF EXTREME PHENOTYPES IN THE BUSTER X ROZA MAPPING POPULATION

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INTRODUCTION: Terminal and intermittent drought limits dry bean production worldwide. The Buster/Roza mapping population (140 F7:9 RILs) has been screened for drought tolerance across multiple years/locations. In 2011 and 2012 the RILs were tested for terminal drought response at two locations: Othello, WA and Scottsbluff, NE. The population segregated for yield response at both locations under low to moderate drought stress (data not shown). This population was sent to Dr. Perry Cregan, USDA-ARS, for genotyping (Illumina GoldenGate) and the resulting SNP data combined with 9 location-years of phenotypic data was used to search for QTL associated with drought and multiple stresses including drought. QTL have been identified for yield response under stress and further characterization of the differential response to drought among RILs representing phenotypic extremes has been initiated.

MATERIALS AND METHODS: *Mapping and QTL analysis:* Replicated yield data collected under stress and non-stress conditions was combined with genotypic data obtained from a 6,000 SNP array. A genetic linkage map was created using JoinMap v.4.0 set to Haldane's mapping function, and default settings. Groups were selected based on an LOD >5. Linkage groups and marker order were verified using physical map data provided by Dr. Cregan. QTL analysis was conducted with QGene 4.0; 1000 permutations were used to set a significant QTL threshold at $P < 0.01$.

Extensive Phenotyping: Extreme phenotypes were selected based on: 1) QTL (Pv01 and Pv02) – presence versus absence and 2) yield response (geometric mean, drought severity index (DSI) high versus low). Lines were planted in Prosser, WA and Scottsbluff, NE. In Prosser, traits measured include: relative water content, canopy temperature, shoot biomass, flower maturity, stomatal conductance, SPAD, emergence, harvest maturity, 100 seed weight, and yield. In addition, roots were characterized under well-watered conditions including: basal root angle (minimum and maximum), basal root number, basal root whorl number, number of adventitious roots, disease rating, nodule rating, and tertiary branching score. Flower maturity, harvest maturity, yield, and root traits were measured in Nebraska. Data was analyzed using SAS 9.3 (Proc Mixed).

RESULTS: *QTL Discovery:* Of the 5,398 SNPs, 1603 were polymorphic and used to construct the linkage map. QTL on Pv1 (R^2 = 7 to 34%) and Pv2 (R^2 = 7 to 33%) were specific to yield under drought stress across years/locations (Figure 1). A search for potential candidate genes in the QTL regions resulted in several drought related hits for both QTL (data not shown) and a comparative sequence of parents for candidate gene bZip transcription factor for QTL on Pv1 is shown in Figure 1. Though six SNPs were found in this region between the parents, there was no change in the amino acid sequence.

Extensive Phenotyping: Relative water content, SPAD, canopy temperature, and pod wall ratio indicate no significant differences between Buster and Roza under drought stress, drip irrigation

(data not shown). Under multiple stress conditions (intermittent drought, compaction, root rot, low fertility) Buster and Roza significantly differed in yield (1400 kg/ha-1 and 4500 kg/ha-1) and pod wall ratio (0.29 and 0.23), respectively, Prosser, WA, 2013. In addition, there was a clear separation between drought tolerant and drought susceptible lines for pod wall ratio in a multiple stress environment (data not shown). Nine out of 10 genotypes with the lowest pod wall ratio have the Roza allele, and 8 out of 10 genotypes with highest ratio have the Buster allele for significant QTL found on Pv1 and Pv2 (data not shown).

SUMMARY

Significant QTL for yield under multiple and drought stress were found on Pv1 and Pv2. Although the bZip transcription factor was found near the QTL on Pv1, it is an unlikely candidate gene due to no change in the amino acid sequence. The first year of extensive phenotyping under drip irrigation resulted in no significant differences between drought tolerant and susceptible groups for several traits, including yield. Significant differences between drought resistant and susceptible groups were detected under multiple stress for pod wall ratio, yield, and other traits. Further research includes additional candidate gene exploration (RNAseq, RT-PCR) in addition to field studies under multiple stress.

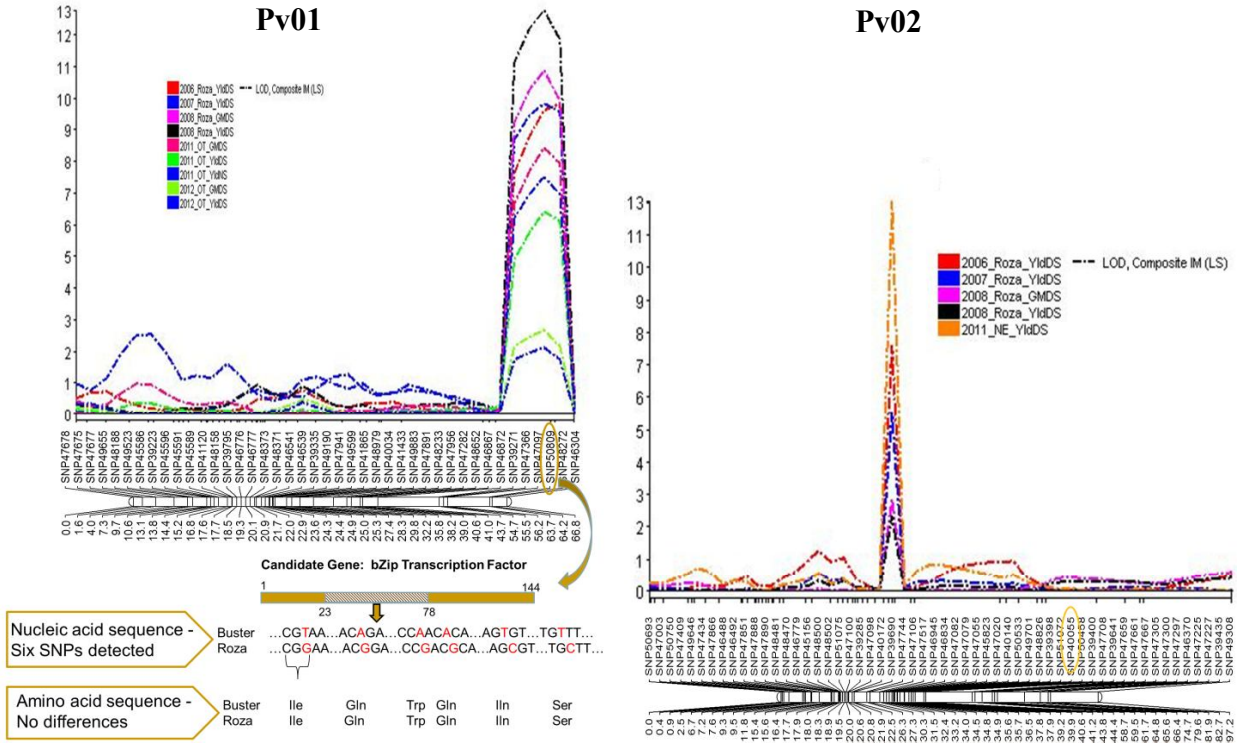

Figure 1. Significant QTL detected on Pv01 and Pv02 for yield under drought stress across 9 location-years and a potential candidate gene, bZip transcription factor, near the significant SNP on Pv01.

PERFORMANCE OF ANDEAN COMMON BEAN UNDER LOW FERTILITY STRESS IN TANZANIA

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INTRODUCTION: Low soil fertility is a limiting factor for bean production in East Africa. In Tanzania low available N and P soils are widespread. Average bean yields in Tanzania are around 500 kg/ha although the potential yield under reliable rain-fed conditions is 1500–3000 kg/ha, using improved varieties and proper crop and land husbandry. A major reason for poor performance of the local landraces grown by many smallholders is susceptibility to biotic and abiotic stresses. Biotic constraints include diseases, insect pests and weeds. Abiotic constraints include drought and high temperatures and low fertility soils (Hillock, *et al*., 2006). Bean production on degraded soils is increasing. An affordable means for small scale farmers to improve bean productivity may be the use of low fertility stress tolerant varieties (Lunze, *et al*., 2007). Our goal was to identify Andean lines for use in breeding new cultivars with low fertility stress tolerance for smallholder farmers in East Africa.

Table 1. Sixteen high yielding Andean common bean lines across two locations in Tanzania in 2013.

ADP-ID	Sub-ID	Name	Yield in Kg/ha	
			Morogoro	Mbeya
ADP-106	AF-11	Zawadi	3052	2800
ADP-277	G 13778	G 13778	2957	3288
ADP-102	AF-7	Jesca	2944	2754
ADP-651	BC 247	K 59	2795	3203
ADP-4	TZ-4	Kilombero	2585	2767
ADP-687	BC-398	Pink Panther	2403	3057
ADP-667	BC-277	VA-19	2400	3505
ADP-99	AF-4	Bwana Shamba	2337	3362
ADP-672	BC-359	CDRK	2321	2991
ADP-664	BC-274	Silver Cloud	2288	2730
ADP-666	BC-276	USWK-6	2272	2729
ADP-303	G 17913	G 17913	2248	3542
ADP-468	PI527538	IZ114	2213	2745
ADP-480	PI209804	Kikaa	2058	2814
ADP-247	G 9975	G 9975	2052	2809
ADP-345	G 22147	G 22147	2037	3616
Experiment mean			1323.9	2063.7
CV%			33.2	19.58
LSD			799.42	867.94

MATERIALS AND METHODS: There were 268 Andean lines (142 bush and 126 vine types) plus two non-nodulating checks evaluated for field performance under low Phosphorus (70.28 and 53.32 mg P/kg of soil) and medium Nitrogen (10.81 and 10.78 mg/kg NO3-N in soil) in Mbeya and Morogoro respectively. The experimental design was a randomized complete block with two replicates and two locations: Morogoro (Sokoine University of Agriculture) and Mbeya (Uyole-Agriculture Research Institute). Sowing was done at a spacing of 0.5 m between rows and 0.2 m within rows. A plot consisted of a single 8 m row which gave a plot size of 4m². There was no irrigation as the trial was conducted during the rainy season and no fertilizers were applied. Weeding and insecticide applications were used to promote good growth. The following data were collected: days to 50%

Table 2. Correlations for different variables with yield

Correlation	Morogoro	Mbeya
Flowering maturity d	-0.47**	-0.45**
Harvesting maturity	-0.53**	-0.37**
Lodging (1-9)	-0.33**	0.01
Desirability (1-9)	-0.76**	-0.54**
Number of nodules	-0.03	0.15**
Nodule dry weight g	-0.05	0.17**
Shoot dry weight g	0.05	0.30**
Root dry weight g	0.25**	0.25**
Plant stand no.	0.46**	0.45**
Pod harvest index	0.43**	0.36**
Number of pods/plant	0.62**	0.31**
Number of seeds/pod	0.19**	0.13**
Seed weight g	0.51**	0.47**

flowering, days to 85% maturity, number of nodules at flowering, shoot dry weight (g), plant stand at harvest, pod harvest index, number of pods/plant, number of seeds/pod, 100 seed weight (g) , yield kg ha^{-1} and N content in shoots at flowering – pending analysis. The collected data were subjected to analysis of variance using PROC GLM in SAS. LS Means were generated, yield was adjusted with plant stand at harvest using covariance analysis, and also correlation studies were performed. Trait means will be used for Association Mapping.

RESULTS AND DISCUSSION: The ADP lines varied significantly in their grain yield under low soil fertility stresses. Yield varied from 609 to 3705 kg/ha and 194 to 3672 kg/ha in Mbeya and Morogoro, respectively. The mean yield was 2064 kg/ha in Mbeya and 1324 kg/ha in Morogoro. One of the high yielding lines Pink Panther exhibited high numbers of nodules/plant 106 and 173 at both test sites. Interestingly the lowest yielding lines in Mbeya were the two non-nod checks with yield of 768 and 609 kg ha-1, respectively, indicating the N fertility status of the trial site was low. The bush type beans as a group out yielded the vine types at both locations 2324 vs 1764 at Mbeya and 1626 vs 961 at Morogoro, respectively. Comparing the 40 highest yielding lines from both locations revealed 16 lines with high yield across both locations. Interestingly 15 were bush type with only one vine type ADP-247. These lines represent candidates for improving low fertility stress tolerance of common bean grown in Tanzania (Table 1).

Yield was significantly correlated with flowering maturity, harvesting maturity, lodging, desirability, root dry weight, plant stand, pod harvest index, number of pods/plant and seed weight in Morogoro. Also with flowering maturity, harvesting maturity, desirability, number of nodules, nodule dry weight, shoot dry weight, root dry weight, plant stand, nod harvest index, number of pods/plant, number of seeds/pod and seed weight in Mbeya (Table 2).

Correlations indicate that early maturity, good plant stand, number of pods per plant and seed weight affects yield. These are preliminary results; more evaluations are needed to confirm lines with tolerance to low fertility stress for use in breeding programme

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ASSOCIATION OF COMMON BEAN ENDORNAVIRUSES WITH INCREASE IN SEED GERMINATION, GRAIN YIELD, AND INTERACTION WITH OTHER PLANT VIRUSES

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INTRODUCTION

Endornaviruses are persistent viruses that infect plants without causing visible symptoms. Although endornaviruses are very common and have been reported in many economically important plant species, little is known about the effect they have on their hosts. Endornaviruses have been reported in crops, such as avocado, barley, common bean, melon, pepper, and rice (1). These viruses replicate in the host cells, and viral dsRNA accumulate to levels higher than those normally encountered in plants infected by disease causing RNA viruses (Valverde, R. A. unpublished). Recently, Sela *et al*. (4) reported the isolation of small RNAs from bell pepper infected with *Bell pepper endornavirus* (BPEV). This suggests that endornaviruses can activate the RNA silencing machinery of the plant without causing symptoms. After testing over 30 cultivars of bell pepper for BPEV, the PI and collaborators found 100% infection rate (2, 6). This could be an indication that the presence of endornaviruses in bell pepper cultivars is beneficial.

Two endornaviruses, *Phaseolus vulgaris* endornavirus 1 (PvEV-1) and *Phaseolus vulgaris* endornavirus 2 (PvEV-2), have been reported infecting many common bean (*Phaseolus vulgaris*) cultivars (3). We have identified two lines of the cultivar Black Turtle Soup, one double infected with PvEV-1 and PvEV-2 and the other virus-free. We conducted comparative studies between the two lines to evaluate the effect that common bean endornaviruses may have to their host and their interaction with some acute viruses.

MATERIALS AND METHODS

The endornavirus-infected (BTS +) and the endornavirus-free (BTS -) Black Turtle Soup lines were used in all comparative experiments aimed to determine the effect these viruses may have on their host. These included germination, grain yield, and reaction to mechanical inoculation with the following acute viruses: *Bean common mosaic virus* (BCMV), *Cucumber mosaic virus* (CMV), and *Tobacco ringspot virus* (TRSV). Gel electrophoresis of dsRNA (5) and RT-PCR were used to confirm the presence of the viruses and to evaluate dsRNA relative yields. Evaluation of the grain yield of the two lines was conducted using 70 plants of each line grown in the field. Seed weight comparisons were conducted.

RESULTS AND DISCUSSION

In general, endornavirus-infected and endornavirus-free BTS plants exhibited similar phenotype. Seeds of the virus-infected line germinated faster and plants matured earlier when compared with those of the virus-free line. Furthermore, grain yield of the endornavirus-infected line was higher than yield of the endornavirus-free line. Relative dsRNA yield of PvEV-1 and PvEV-2 in mixed infections with CMV were higher than infections of PvEV-1 and PeEV-2 alone.

When inoculated with TRSV, BTS - reacted with necrotic local lesions but not systemic infection whereas BTS + reacted with chlorotic lesions, stunting, systemic necrosis, and in some cases, plant death. Two other acute viruses, BCMV and CMV, caused similar symptoms in both BTS lines. These results suggest that co-infection of endornaviruses with TRSV in BTS may overcome a host resistant to systemic infection by TRSV.

Endornaviruses of common bean do not appear to affect the plant phenotype, but they may interact with other plant viruses and could be harmful to common bean. Endornaviruses may be a new type of endophytes, but the interactions of these viruses with the plant host remain to be elucidated.

Future progress will depend on the development of an inoculation method for these viruses and/or the development of virus-free and virus-infected isogenic lines.

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BIOCONTROL OF *Rhizoctonia solani* AGAINST *Trichoderma* spp. MEMBRANES AND DUAL CULTURE

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INTRODUCTION

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. The main pathogen control methods are mycoparasitism, antibiotic synthesis and direct competition.

MATERIALS AND METHODS

It is used 25 isolates *Trichoderma* spp. and one of *R solani* originating in the production area of the IGP of " Alubia de La Bañeza – León " that they have not undergone any genetic manipulation.

It is evaluated the ability of biocontrol isolates of two tests. The first test, it is covered the entire surface of PDA medium with a cellophane membrane. It is put over them mycelium disks 6 mm in diameter of the different strains of *Trichoderma* spp. and it is incubated at 25 ° C for 48 h. Then, it is removed them and it is put over Petri dishes with PDA mycelium disks *R. solani* having the same diameter, and it is incubated at 25 ° C. Finally, it is measured the diameter of the mycelium disks every 24 h for 3 consecutive days by calculating the percent inhibition. In the second test, it is put over Petri disk with PDA medium some mycelium disks of 6 mm in diameter of the different strains of *Trichoderma* spp against to pathogen, to 5,5 cm apart and they are incubated for 5 days at 25 ° C. After it is measured the diameter of the pathogen (r1 and r2). (Figure 1) calculating the percent inhibition by the formula % I = ((r1-r2) / r1) x100. (Royse, 1978), (Ruano-Rosa, del Moral-Navarrete & Lopez-Herrera, 2010)

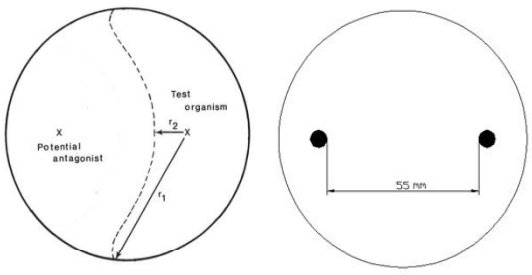

Figure 1: Dual culture test.

Percent inhibition data are subjected to analysis of variance (ANOVA) using the software SAS (SAS Institute Inc., 1990). Statistical significance of the results is determined by performing a LSD´s test ($p < 0,05$).

RESULTS AND DISCUSSION

In the membranes test it is obtained percentages above 80% in T35, T11, T32, T22, T23, T25, T39, T41. Between 60 and 80% they are T21, T37, T31, T27, T40, T38, T29, T24 and T59. Below 60% they are T26, T20, T33, T42, T34, T36, T30 and T28. In the dual culture test, the highest percent inhibition is occurred at T40, being 60.83%. The rest varies between 47.13% of T23 and 16.24% of T28.

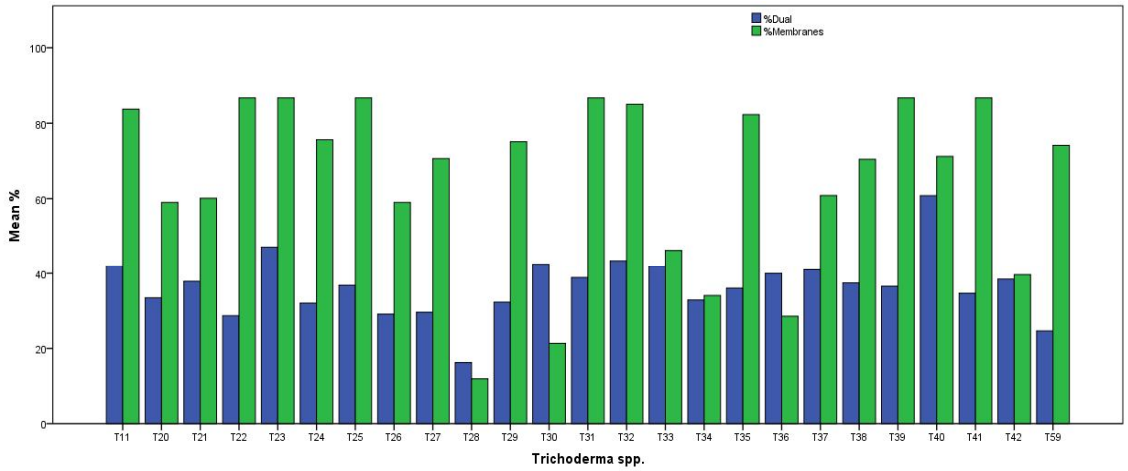

Figure 2: Percent inhibition of membranes and dual tests to 5 days.

CONCLUSIONS

It has been shown to isolates of *Trichoderma* are capable of controlling, *in vitro*, although there are differences in the percentages of inhibition, the isolation of *R. solani*. *In vitro* antagonistic activity of *Trichoderma* is an initial indication of its biological activity against phytopathogenic fungi.

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INFLUENCE OF THE ISOLATION *Rhizoctonia solani* AND *Trichoderma* spp. ON GERMINATION OF BEAN

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INTRODUCTION

The physical interaction between *Trichoderma* and the plant is limited to the first cell layers of the epidermis and cortex of the root (Yedidia et al., 1999). *Trichoderma* activates the expression of genes involved in the defense response and it promote plant growth, the root system and nutrient availability (Yedidia et al. 2003; Hanson and Howell, 2004; Harman et al., 2004)

Rhizoctonia solani J.G. Kühn. (Teleomorph: *Thanatephorus cucumeris* (AB Frank) Donk) is responsible for the "Rhizoctonia disease". It is a pathogen that it is more aggressive with temperatures between 15 and 18 º C and with moist soil, further accentuated in the fortnight after the bean planting.

MATERIALS AND METHODS

It is used 16 isolates *Trichoderma* spp. and one of *R solani* originating in the production area of the IGP of " Alubia de La Bañeza – León " that they have not undergone any genetic manipulation.

It is prepared the pots with the substrate, which it is inoculated with 50 ml of the pathogen and it is grown in a growth chamber for 8 days at 25 ° C and 45% RH day and 16 ° C and 60% RH overnight in the dark. The pots are irrigated with 250 ml of water before inoculation.

After the seeds are coated with a spore suspension of *Trichoderma* spp. with a concentration of $2 \cdot 10^7$spores/ml. It is sown 2 disinfected seeds of bean per pot. They stay with photoperiod of 16 hours light, 25 ° C / 16 ° C (day / night), 60% RH (day / night) and luminosity of 3,500 lux. It is applied two weekly irrigations. On 2nd-4th week it is applied Rigaud and Puppo nutrient solution. (Rigaud & Puppo, 1975)

Germination is assessed on 12, 17, 24 days after placement of the seeds.

RESULTS AND DISCUSSION

There is a greater germination of the bean when *Trichoderma* is present in the middle being significant with respect to whether *R. solani*, resulting in a decrease of the same. In the case of *Trichoderma* T27, when it is present with the pathogen (R27) or not (C27), germination is not significant differences from control (CC).

CONCLUSIONS

The presence of *Trichoderma* in the soil doesn´t produce negative interactions on the germination of the bean. In the case of *Trichoderma* T27, it reduces negative effects of *R. solani* germination.

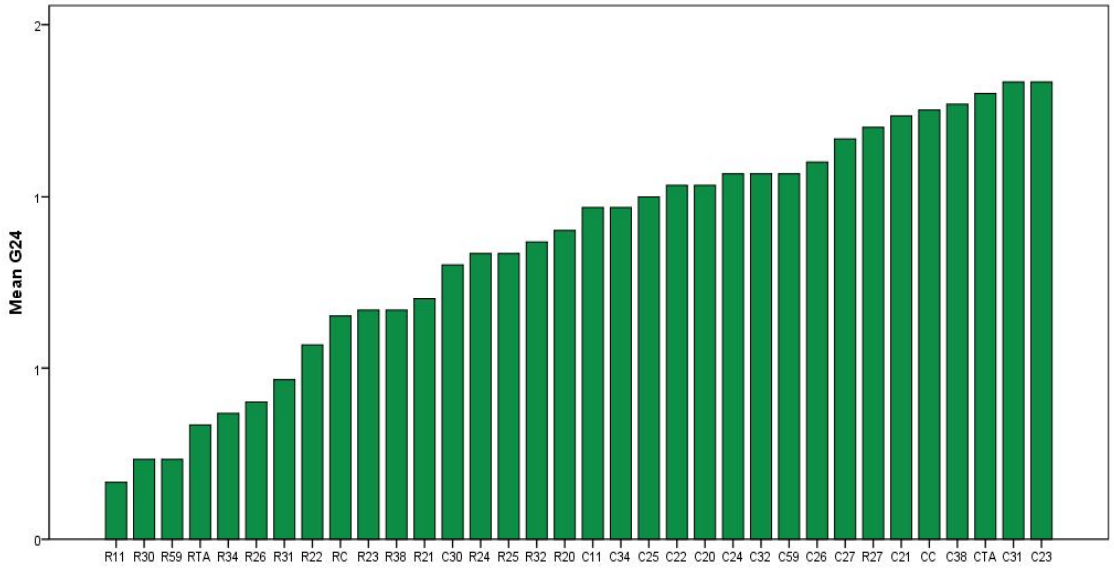

Figure 3: Germination to 24 days.

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TOLERANCE TO TEMPERATURE AND SALINITY OF *RHIZOBIUM* ISOLATES OBTAINED FROM DIFFERENT COMMON BEAN GENOTYPES

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INTRODUCTION

Bean plant develops symbiotic association in roots with bacteria *Rhizobium tropici*. When this bacteria is present in the soil, naturally or by inoculation, it recognizes and infects the roots of the host plant, resulting in nodules formation, where N_2 fixation takes place (HUNGRIA et al., 1997). Like plants, the symbiotic diazotrophic bacteria exhibit great variation in salinity tolerance. For bacteria of the genera *Rhizobium* and *Bradyrhizobium*, the detrimental effects of salts are more evident, particularly in relation to the effects of specific ion concentration than the osmotic effect (ELSHEIKH, 1998), varying according to the present ionic form and the strain tolerance. In this context, NaCl has been considered good indicator of bacterial tolerance to salts (ABDELMOUMEN et al., 1999). On the other hand, high temperatures are another limiting factor for the rhizobia where the nodule size parameter appears to be the most sensitive to high temperatures than the number of nodules in legumes studies conducted by CASTRO et al. (1993).

MATERIAL AND METHODS

Aiming to evaluate the tolerance to salinity and temperature of *Rhizobium* isolates obtained from 11 genotypes of common bean (Figure 1) cultivated in soil collected in the states of Goiás, Minas Gerais and Paraná, 114 rhizobia isolates and the three pattern strains SEMIA 4077, SEMIA SEMIA 4080 and SEMIA 4088 were tested under different temperature and salinity conditions on the laboratory of Soil Microbiology of Embrapa Rice and Beans. The 114 isolates were transferred to small glasses with liquid medium and placed to stir at 28 °C and 120 rpm for 48 hours in horizontal shaker. Solid medium was prepared with salt (NaCl) concentration of 0%, 1%, 2%, 4% and 6%. 200 µL of cell suspension were pipetted to a 96 well plate and transferred to the solid medium using a replica plate. Plates with different salt concentration were taken to BODs at different temperatures: 28 ºC, 33 ºC, 38 ºC, 43 ºC and 48 ºC for 48 hours. After this period was performed a reading to verify bacteria which grew on the different interactions of salinity and temperature and it were divided into groups based on genotypes (Table 1).

RESULTS AND DISCUSSION

Bacteria which grew under conditions of higher temperature and salinity conditions also grew under smaller amount of salt and heat applied. The isolates stemmed from genotypes 2 and 8 presented more bacteria which have grown under conditions of highest temperature and salinity (Table 1), and can be more carefully studied in order to find interactions of genotypes with potential for efficient BFN with good strains and tolerant to adverse environmental conditions. At the temperature of 48 °C and 2% of NaCl (Group 2) had shown a higher amount of bacteria grown between groups. Genotype 2 was that amounted more bacteria among genotypes. SEMIA 4080 was classified in group 2, and the SEMIA 4077 and 4088 were classified in group 5. It shows that exist some bacterias more tolerant than the pattern strains.

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Figure 1. Common bean genotypes used for the extraction of *Rhizobium* isolates.

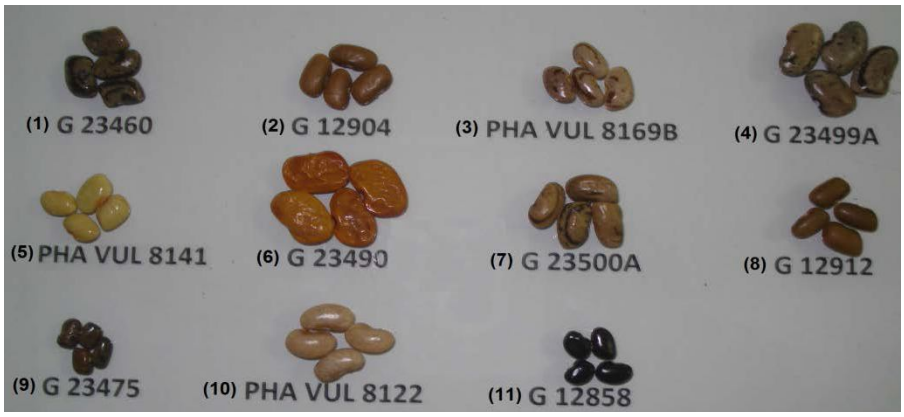

Table 1. Tolerance of *Rhizobium* isolates to salinity and temperature separated by groups* according to common bean genotypes.

Genotypes	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Sum
1	2 (9%)	2 (8%)	0 (0%)	3 (20%)	1 (11%)	1 (9%)	3 (17%)	12 (11%)
2	4 (17%)	5 (20%)	0 (0%)	0 (0%)	3 (33%)	3(27%)	1 (6%)	16 (14%)
3	3 (13%)	5 (20%)	1 (8%)	2 (13%)	0 (0%)	0 (0%)	3 (17%)	14 (12%)
4	0 (0%)	2 (8%)	1 (8%)	3 (20%)	1 (11%)	4 (36%)	1 (6%)	12 (11%)
5	2 (9%)	3 (12%)	0 (0%)	0 (0%)	0 (0%)	1 (9%)	1 (6%)	7 (6%)
6	3 (13%)	0 (0%)	3 (23%)	2 (13%)	2 (22%)	0 (0%)	1 (6%)	11 (10%)
7	2 (9%)	2 (8%)	3 (23%)	1(7%)	1 (11%)	1 (9%)	3 (17%)	13 (11%)
8	4 (17%)	3 (12%)	4 (31%)	1(7%)	1 (11%)	0 (0%)	1 (6%)	14 (12%)
9	0 (0%)	0 (0%)	0 (0%)	1(7%)	0 (0%)	0 (0%)	2 (11%)	3 (3%)
10	2 (9%)	2 (8%)	0 (0%)	1(7%)	0 (0%)	0 (0%)	1 (6%)	6 (5%)
11	1 (4%)	1 (4%)	1 (8%)	1(7%)	0 (0%)	1 (9%)	1 (6%)	6 (5%)
Sum	23	25	13	15	9	11	18	114

*Group 1 - isolates grew at 4% NaCl up to 48ºC; Group 2 – isolates grew at 2% NaCl up to 48ºC; Group 3 – isolates grew at 1% NaCl up to 48ºC; Group 4 - isolates grew at 4% NaCl up to 38ºC; Group 5 - isolates grew at 2% NaCl up to 38ºC, Group 6 – isolates grew at 1% NaCl up to 38ºC; Group 7 – isolates grew at 1% NaCl up to 33 º C.

ANALYSIS OF THE SYMBIOTIC SYSTEM BEAN-RHIZOBIA UNDER WATER STRESS CONDITIONS IN GREENHOUSE

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INTRODUCTION

The symbiotic system common bean-rhizobia is often influenced by the availability of water in the soil. Water stress supposes a reduction of the biomass of the plant, of P absorption, of nitrogen fixation and nodulation (Muñoz-Perea et al., 2007). The sensitivity of nitrogen fixation to drought in legumes is variable, and depends mainly on the type of nitrogen compound synthesized in the nodules. The common bean responds with an accumulation of ureides with a negative effect on the nodules and nitrogen fixation. The objective of this work was to analyze how the water stress affects the plant-rhizobia symbiotic relationship and its effect on yield traits.

MATERIAL AND METHODS

In this work we have used 10 common bean landraces and breeding lines, some of them tolerant to drought, that were inoculated with 10 strains of *Rhizobium* (eight local and two reference ones, *R. tropici* CIAT899 and *R. etli* CFN42) in controlled greenhouse in a factorial trial under two conditions: irrigated and water stress. The landraces, belonging to the germplasm collection at the MBG-CSIC, were selected from a previous experiment to see their performance under drought conditions. The breeding lines were provided by several institutions. Aerial and root dry matter of the plant, and number and dry matter of nodules were evaluated and the variation detected in these characters was analyzed by ANOVA.

RESULTS AND DISCUSSION

They were significant differences between the different genotypes for all variables. No significant differences in treatment-strain interaction and treatment-strain-genotype interaction were observed, due to the specificity of binding strain-genotype. Thus, they are strains that increase the yield of certain genotypes, but do not have the same effect on other genotypes (Rodiño et al., 2011). In the non-water stress trial and within each genotype, the values of aerial dry matter vary significantly between different strains used, highlighting the landraces PHA-0155, PHA-0483 and the breeding line PMB-0222 and the local strain APAFI. This specificity strain-genotype is important to find the strain that optimize the yield of improved landraces (Hungria et al. 2006). The water stress suppose generally a 32% of aerial dry matter reduction, but PHA-0471 and PMB-0220 increase their aerial dry matter in this conditions, when were inoculated with strain *R. tropici* CIAT 899. The root dry matter is similar to that reported in other works and varies from 0.26 to 4.24 g plant^{-1} under irrigated trial. The landraces with high root dry matter are those with shallow roots and many adventitious roots. The root dry matter in general suffers a significant reduction in water stress conditions. The percentage that suppose the root biomass in relation with the aerial biomass varied according the interaction strains-genotypes, and it is possible to group into three groups: 1) with a low root system, 2) with a middle root size and 3) with a large system root, which would guarantee a better performance under water stress. The nodule number and nodule dry matter showed high variability and PMB-0285 and PMB-0286 were the genotypes with high nodule number. They are strain-genotype combinations that have a high number of nodules, multiplying for six the average values of the

variety. The nodular dry matter vary depending on the genotype, the inoculated strain and the environment, and can be observed different groups: 1) plants with large nodules and no correlation with aerial biomass; 2 and 3) (with different nodulation performance) when the nodular and aerial biomass is correlated and 4) plants with low nodulation and no correlated with the aerial biomass (Figure 1). The water stress causes in the plants a reduction in the number of root hairs, thereby reducing the number of nodules per plant. In this work, the reduction in the number of nodules was 57%. The combination strain EPOB-PHA-0471 achieved the maximal nodulation in these conditions. The nodule dry matter increases significantly under conditions of water stress. The genotypes with a high nodular biomass were PHA-0683 and PHA-0483 and the local strains that induce a greater nodule biomass were EF and EPOB. PHA-0683 presents a great uniformity in the caliber of your nodules, regardless of the nodule number and with a significant correlation with aerial biomass (Figure 1).

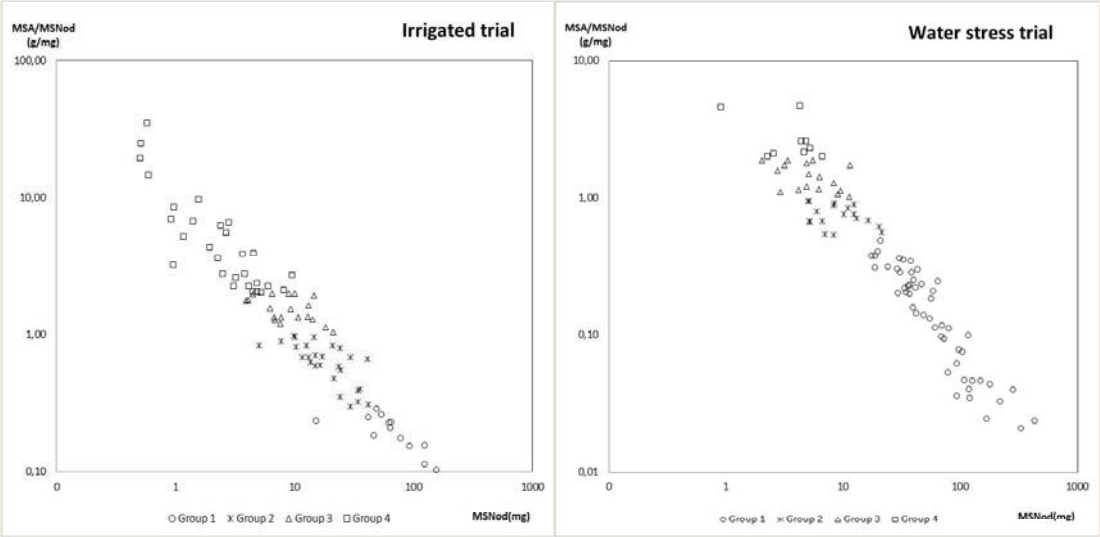

Figure 1. Nodule dry matter in relation to the aerial dry matter under irrigated and drought stress conditions

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QTL ANALYSIS OF BIOLOGICAL NITROGEN FIXATION AND AGRONOMIC TRAITS IN THE PUEBLA/ZORRO RIL POPULATION

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ABSTRACT: Improved Biological Nitrogen Fixation (BNF) in dry beans would help to improve yields, increase the area where dry beans can be grown, and reduce dependence on N fertilizer inputs. A black bean recombinant inbred line (RIL) population resulting from a cross of the landrace selection Puebla 152 and the commercial cultivar Zorro was developed to study the genetics of BNF and transfer the enhanced BNF ability of Puebla 152 (Type III growth habit) into the efficient Zorro (Type II). RILs were genotyped using single nucleotide polymorphism (SNP) markers from the BARCBean6K_3 Beadchip developed as part of the BeanCAP project. Phenotypic data was recorded in the field, greenhouse, and lab. QTL for traits such as root biomass and distribution, biomass, and percent N derived from the atmosphere, which are associated with BNF were identified. These QTL may serve as markers for further breeding of genotypes with enhanced BNF.

RESULTS:

The majority of the 125 RILs were intermediate between Puebla 152 and Zorro for harvest index and maturity (data not shown). Nitrogen derived from the atmosphere (%ndfa) was also intermediate, with several lines showing enhanced %ndfa (Figure 1.). It is interesting to note that Puebla 152 is noted as a high N-fixing genotype in the published literature, yet the commercial cultivar Zorro had a higher %ndfa than Puebla in our studies.

Several QTL for agronomic traits colocalized with traits associated with BNF on Pv01 and Pv08. QTL for plant architecture traits, such as plant height, biomass, and root to shoot ratio grouped between 32 cM and 62 cM or Pv01. QTL for harvest index, biomass, and N in biomass were found in the same interval on Pv01. QTL for %ndfa and N yield in seed colocalized with QTL for yield and harvest index on Pv08. A second region on Pv08, beginning at 90 cM possessed QTL for biomass, percent N in shoot, and N yield in seed.

There appears to be a relationship between the way resources are partitioned to the seed and N fixed, given the association of N QTL and QTL for traits which contribute to partitioning. Seed yield is the ultimate goal in breeding programs and genotypes which more efficiently remobilize resources from vegetative tissues to seed offer an advantage. Selection for agronomic traits may indirectly help to improve the BNF characteristics of dry bean when choosing those genotypes which move resources efficiently into the seed.

There may be a feedback response contributing to enhanced BNF in those lines with high harvest index, as N fixed in the nodules must be translocated away from the root system to either vegetative tissue or seeds. Plants not efficiently moving N into biomass or seed may accumulate N in the root system, which in turn reduces the fixation of N in the nodules. This may be supported by the finding that QTL for biomass, percent N in the shoot and N yield in seed appear to be associated with a linear movement of N from roots to biomass to seed.

Figure 1. Distribution of percent nitrogen derived from the atmosphere (%ndfa) for Puebla 152, Zorro and 125 RILs grown in a low N field in East Lansing, MI in 2011 and 2012.

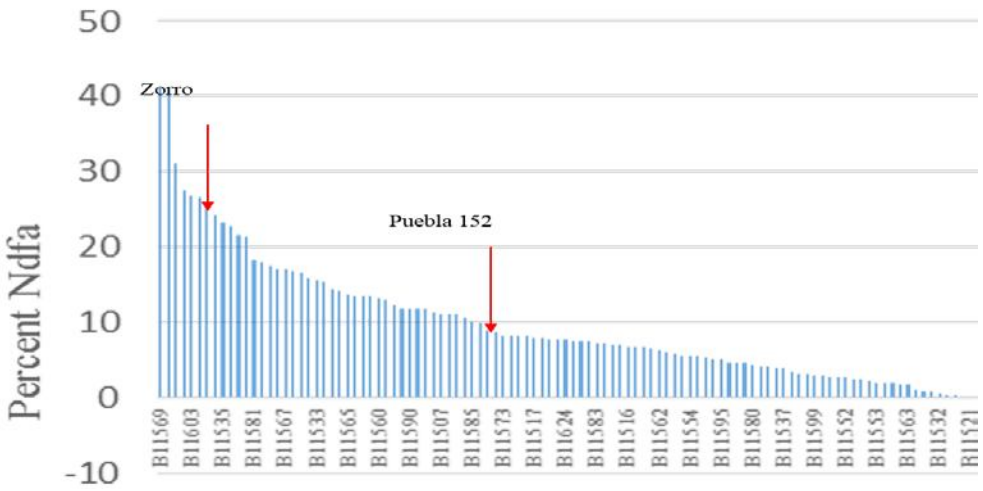

Table 1. Select BNF and agronomic QTL identified in the Puebla 152/ Zorro RIL population grown in the field in East Lansing, Michigan, in 2011 and 2012 and in the greenhouse at MSU.

Trait	Chromosome	Left Marker	Right Marker	LOD	PVE(%)	Add
Vigor 2011 & 2012	8	ss715646531	ss715650658	44.6	91.4	-22.9
Biomass 2011 & 2012	8	ss715646531	ss715650658	38.2	86.8	-22.5
Days to Flower 2011 & 2012	8	ss715646531	ss715650658	16.4	61.8	-31.2
Root Ndfa rep 1 GH	3	ss715648280	ss715644967	10.7	52.6	-0.2
N in Biomass 2011	6	ss715645782	ss715645785	11.4	39.7	0.0
Ndfa in Biomass 2011	3	ss715645144	ss715649523	3.1	34.2	0.1
N Yield, Seed 2011	8	ss715648543	ss715640265	10.3	33.5	-0.1
Percent N in Seed 2011	8	ss715648543	ss715640265	10.3	33.5	-12.6
Percent Shoot N rep 1 GH	8	ss715650658	ss715648408	3.6	30.4	0.0
Harvest Index 2011 & 2012	1	ss715645273	ss715650565	6.3	26.4	-5.4
Percent N in Seed 2011	3	ss715647551	ss715646462	5.0	26.0	0.0
Yield (CWT) 2011	8	ss715648543	ss715640265	6.7	25.4	-2.3
Yield (CWT) 2012	7	ss715645234	ss715650389	4.3	24.6	-1.9
Biomass 2012	1	ss715645273	ss715650565	4.4	23.6	0.6
Shoot Weight Greenhouse	11	ss715649816	ss715648870	4.9	23.5	1.6
Ndfa in Seed 2011	8	ss715648543	ss715640265	7.6	23.4	-0.1
N in Bioamass 2011	6	ss715646404	ss715645793	7.0	23.4	0.0
Percent N in Seed 2011	8	ss715646111	ss715646115	3.9	23.0	0.0
Total N Yield 2012	7	ss715645234	ss715650389	4.6	23.0	-0.1
Ndfa in Biomass 2011	10	ss715645522	ss715649823	4.5	16.5	0.0
Total N Yield 2012	1	ss715645304	ss715645288	2.6	15.7	-0.1
Percent N in Seed 2011	8	ss715646115	ss715646089	6.0	15.5	10.3
N Yield, Seed 2011	8	ss715646115	ss715646089	6.0	15.5	0.1
Percent N in Biomass 2011	6	ss715645785	ss715645033	3.4	15.1	0.0
N in Bioamass 2011	1	ss715645273	ss715650565	2.7	13.4	0.0
N Yield, Seed 2012	7	ss715648299	ss715644972	3.2	13.3	-0.1
Total N Yield 2012	3	ss715646624	ss715639509	3.0	13.1	-0.1

MAINTENANCE OF BIOLOGICAL NITROGEN FIXATION IN COMMON BEAN GENOTYPES WITH DIFFERENT GROWING CYCLES

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INTRODUCTION

The main biochemical reactions that occur in plants involve the presence of nitrogen (N), a constituent of various plant compounds such as aminoacids, nucleic acids, and chlorophyll. The Biological Nitrogen Fixation (BNF) has an important function for the N supply in agricultural systems. It has been estimated that, around the world, this process contributes 32 Tg year $^{-1}$ of N in cultivable areas (FILOSO et al., 2006). An important feature of common beans is their ability to establish symbiosis with certain bacteria, such as *Rhizobium* bacteria, which can fix N_2 from the atmosphere in root nodules (PINTO; HUNGRIA; MERCANTE, 2007). The different proportion of fixed N in each symbiosis can be affected by plant genotype, duration of the crop cycle, delayed nodulation and early senescence of nodules (ALCÂNTARA, 2009). About the restrictions on BFN in common beans, another limiting factor is proven that the host plant does not only affect nitrogenase activity, but also the speed of nodule senescence.

MATERIAL E METHODS

Aiming to evaluate the nodule activity of common bean genotypes with different growing cycles a field experiment was conducted at Embrapa Rice and Beans, located at the municipality of Santo Antônio de Goiás, Goiás, Brazil. It were evaluated common bean cultivars with early cycle, half-early cycle, normal cycle and late cycle, in a total of 22 cultivars. The seeds were inoculated, one day before planting, with commercial inoculant composed by three *Rhizobium tropici* strains (SEMIA 4077, SEMIA 4080 and SEMIA 4088). The experimental design was a randomized complete block. At different phenological stages (V4, R5, R6, R7 and R8) two plants were collected from each plot using a straight shovel. It was removed a 20 cm x 20 cm block of soil with the plants. The samples were washed carefully to remove any soil and the roots were separated from aerial parts. For each plant was determined leaf area (LA), and nodules activity (NA) in each of the phases. For determining the activity of nodules, all nodules were removed from each root and ten of these were randomly chosen and cut in half to check color. And then the percentage was calculated.

RESULTS E DISCUSSION

According to **Figure 1**, the cultivars showed peak of NA in phase R5, with subsequent decrease until complete senescence. The values of leaf area were increasing throughout the phenological stages, being highest in cultivars with normal cycle and late cycle. It was also observed that the NA reached zero earlier in cultivars with normal cycle and late cycle. In a study of the development of nitrogen fixation by bean, PEÑA CABRIALES et al. (1993) also observed that the highest rate of nitrogen assimilation occurred during the reproductive phase.

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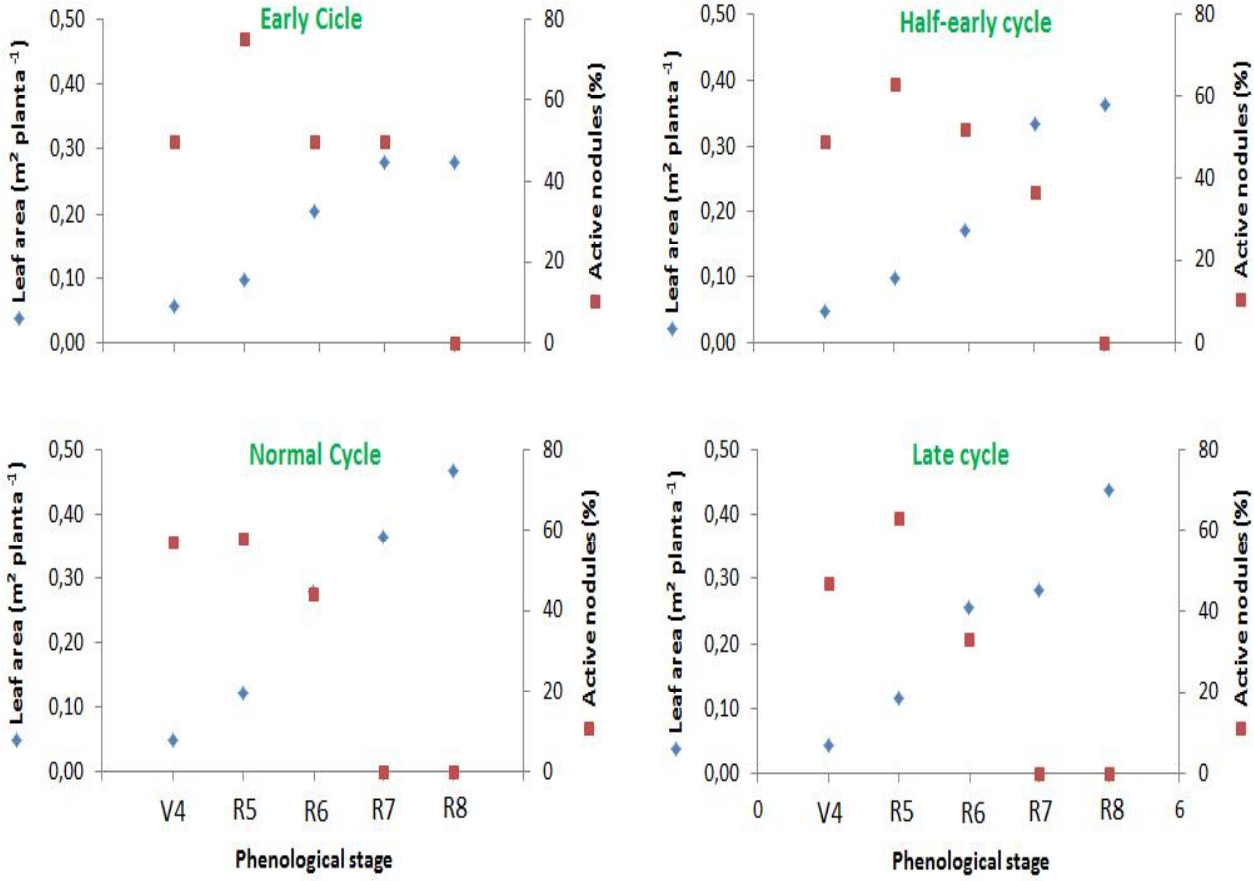

Figure 1. Relationship of leaf area and active nodules of common bean plants with different growth cycles in five phenological stages.

NODULATION OF COMMON BEAN GENOTYPES WITH NORMAL AND LATE GROWTH CYCLES

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INTRODUCTION

The common bean *(Phaseolus vulgaris L.)* is one of the main crops grown in the world. Its importance goes beyond the economic aspect, for its relevance as a factor of food security and nutrition and its importance in the cultural cuisine of different countries and cultures (POSSE et al., 2010). Nitrogen (N) is an abundant chemical element in the atmosphere, where it is found in its most stable form (N_2) and therefore not available for most organisms, animals and plants. Only a few species of bacteria that form a group of unicellular prokaryotic organisms that possess the enzyme nitrogenase are capable of reducing the N_2 to NH^{3+}. This biological process is termed biological nitrogen fixation (BNF) (ALCÂNTARA et al, 2009). Basically, the sources of N available to the crop of common beans are nitrogen fertilizers and BNF held by *Rhizobium* bacteria (BARBOSA & GONZAGA, 2012). However, some factors limit the efficiency of nitrogen fixation in this culture, as climate, soil characteristics and factors related to plants, especially the crop cycle. The low symbiotic performance of the common bean has been attributed to the characteristics of lower N fixation and limitation of modern cultivars to establish effective nodules under the environmental conditions in which it is grown (VARGAS et al., 2004).

MATERIAL AND METHODS

In order to evaluate nodulation of common bean cultivars with normal and late growth cycles, a pot experiment was conducted in the experimental area of the School of Agronomy, Federal University of Goiás, Goiânia, Goiás, Brazil. Seeds of common bean with normal and late cycles were inoculated with commercial inoculant composed by three type strains of *Rhizobium tropici* (SEMIA 4077, SEMIA SEMIA 4080 and 4088) and sown in pots with a capacity of 5 kg filled with a red oxisoil. Fertilization was supplied with absence of nitrogen. The experiment was conducted in randomized blocks. Plants were collected In five distinct phenological stages (V4, R5, R6, R7 and R8) and determined the number of nodules (NN), nodules activity (NA) and nodules dry mass (NDM).

RESULTS AND DISCUSSION

The Mean data of all phenological stages, revealed that the cultivars Pérola (normal cycle) and BRS Vereda (late cycle) showed number of nodules (NN) higher than BRS Estilo and BRS Executivo (Table 1). The evaluated common bean cultivars did not show significant difference for nodules activity (NA) and nodules dry mass (NDM). Within each phenological stage greater NN and NA were onbseerved for V4, R5 and R6 as compared to R7 and R8 stages, although the greatest NDM value has been observed for the R7 stage. These results suggest a genetic variability among bean cultivars regarding the number of nodules and, Pérola and BRS Vereda cultivars can be used as parental by the breeding program of common bean of Embrapa Rice and Beans.

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Table 1. Number of nodules (NN), nodules activity (NA) and nodules dry mass (NDM) of common bean cultivars with normal and late growing cycles.

Cultivar	Cycle	NN (n^o plant^{-1})	NA (%)	NDM (mg plant $^{-1}$)
BRS ESTILO	N*	25.42 b	11,11 a	20.07 a
BRS EXECUTIVO	N	20.30 b	20,00 a	40.86 a
BRS PITANGA	N	51.69 ab	16,67 a	76.58 a
BRS PONTAL	N	39.10 ab	17,33 a	46.21 a
BRS TIMBÓ	N	47.07 ab	18,00 a	79.32 a
BRS VALENTE	N	50.92 ab	33,33 a	73.24 a
IPR TANGARÁ	N	44.72 ab	21,33 a	47.91 a
PÉROLA	N	59.27 a	32,00 a	70.26 a
BRS GRAFITE	L	28.73 ab	15,33 a	38.25 a
BRS MG TESOURO	L	33.03 ab	7,33 a	47.96 a
BRS VEREDA	L	65.50 a	18,67 a	62.17 a
Phenological stage (average of all cultivars)				
V4		58.75 a	34.24 a	45.59 b
R5		58.70 a	36.27 a	63.13 b
R6		51.53 a	23.33 a	123.72 a
R7		29.18 b	2.12 b	30.08 bc
R8		13.53 b	0.00 b	11.63 c

*N – normal cycle, L – late cycle. Means within the same column followed by the same letter are not significantly different by Tukey´s test ($p<0.05$).

PHENOTYPING ROOT AND SHOOT TRAITS OF ZORRO AND PUEBLA 152 COMMON BEAN (*Phaseolus vulgaris* L.) LINES

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INTRODUCTION: Root-system traits were phenotyped to compare Zorro (commercial cultivar) and Puebla 152 (landrace selection) common bean lines, which were grown up to pod setting under glasshouse conditions. In common bean, drought tolerance has been associated with depth of rooting, and root architecture is an important factor for the acquisition of underground resources (Lynch, 1995). The objective of this study was to assess the phenotypic variation between two black-seeded common bean lines for root and shoot traits comparing plants at three growth stages including pod setting.

MATERIALS AND METHODS: A split plot design with four replications was used to evaluate Zorro (Type II) and Puebla 152 (Type III) common beans. Three root and shoot sampling times were assigned to the main plot and lines Zorro and Puebla 152 to the subplots. Seeds from both lines were germinated in Petri dishes on May 9, 2013. Seven days later, seedlings of similar growth were transplanted into 8.5 kg of dry silica sand in polyethylene tube bags sleeved into polyvinyl chloride (PVC) tubes, 80 cm long and 10 cm in diameter. Each bag was brought to water-holding capacity for three consecutive days for sand settlement using half-strength Hoaghland's solution provided in the glasshouse. Plants were harvested on three different days: namely, 35 (pre-flowering), 47 (flowering) and 62 days (pod setting) after planting. Roots were gently washed by hand with tap water. Number of basal lateral roots including maximum root length were counted and measured. Then, the whole root system was cut into two parts, the roots developed between 0-30 cm (shallow roots) and those below 30 cm (deep roots). Shallow root biomass, deep root biomass, total root biomass and shoot biomass were determined after placement in a forced-air drier for 24 h at 80^{o}C. Statistical analyses (ANOVA) of phenotypic data (root and shoot traits) were performed by Statistix 8 (Version 2.0).

RESULTS: Root-system traits of both parental lines, Zorro and Puebla 152, were sampled across three stages of growth and development, pre-flowering (35 days after germination), flowering (47 days) and pod setting (62 days). Statistical analyses indicated significant differences among the three sampling times and between both bean lines, Zorro and Puebla-152, for number of basal lateral roots (NRL), length of longest root (RL), shallow root biomass (SRB), deep root biomass (DRB), total root biomass (TRB), shoot biomass (SB) and total plant biomass (TPB). Interaction between the two factors was significant only for NRL, SB and PRB traits.

Results indicated that the number of basal lateral roots (NLR) was similar between the first two sample times, with an average of 8.9 and 8.4 lateral roots, respectively, with a tendency to significantly decline as plants continued to grow and develop to reach pod setting (7.1). As expected, bean plants developed deeper roots over time, length of the longest root was not

different between 35 and 47 days after germination, respectively; however, by pod setting plants developed significantly deeper roots reaching 86.4 cm deep (Table 1).

Table 1. Mean values of number of lateral roots (NLR, no.), length of longest root (RL, cm), shoot biomass (SB, g), total plant biomass (TPB, g), shallow root biomass (SRB, g), deep root biomass (DRB, g), total root biomass (TRB, g) and root biomass as percent of total plant biomass (PRB, %) sampled at three stages of growth of two bean lines (Zorro and Puebla 152) grown in a sand-tube experiment in 2013 season. All measurements are per plant basis.

Sampling[&]	NLR	RL	SB	TPB	SRB	DRB	TRB	PRB
Pre-flowering	8.92 A*	64.75 B	8.05 C	12.52 C	3.28 C	1.18 C	4.46 C	34.57 A
Flowering	8.43 AB	67.29 B	15.16 B	21.98 B	4.80 B	2.03 B	6.83 B	29.43 B
Pod setting	7.12 B	86.38 A	31.34 A	43.77 A	5.91 A	6.53 A	12.43 A	30.29 AB
Mean	8.12	72.29	18.18	26.09	4.66	3.25	7.91	31.43

* Means followed by the same letter are not significantly different at 0.05 probability level according to LSD test.
[&], Pre flowering = 35 days; Flowering = 47 days; Pod setting = 62 days after germination.

Puebla 152 significantly developed more roots (10.46) than Zorro (5.64). A similar trend was found for shallow root biomass (6.01 vs. 3.32 g plant^{-1}), deep root biomass (4.29 vs. 2.20 g plant^{-1}) and total root biomass (10.30 vs. 5.51 g). Considering above ground plant biomass, similar results were found as with the root data; Zorro developed less shoot biomass (11.25 g plant^{-1}) than Puebla 152 (25.12 g plant^{-1}); as well as total plant biomass, less biomass in Zorro compared to Puebla 152 (35.42 g plant^{-1}). On the contrary, both cultivars developed similar root length (>72 cm). Interestingly, root biomass as percent total plant biomass in Puebla 152 (30.5%) was statistically lower than in Zorro (32.4%), indicating that Zorro allocated a greater percentage of plant total dry matter for root production than Puebla 152 (Table 2).

Table 2. Mean values of number of lateral roots (NLR, no.), length of longest root (RL, cm), shoot biomass (SB, g), total plant biomass (TPB, g), shallow root biomass (SRB, g), deep root biomass (DRB, g), total root biomass (TRB, g) and root biomass as percent of total plant biomass (PRB, %) sampled at three stages of growth of Zorro and Puebla 152 bean lines grown in a sand-tube experiment in 2013 season. All measurements are per plant basis.

	Plant traits							
Cultivar	**NLR**	**RL**	**SB**	**TPB**	**SRB**	**DRB**	**TRB**	**PRB**
Zorro	5.64 B*	73.75 A	11.25 B	16.76 B	3.32 B	2.20 B	5.51 B	32.37 A
Puebla 152	10.46 A	72.27 A	25.12 A	35.42 A	6.01 A	4.29 A	10.30 A	30.50 B
Mean	8.12	72.29	18.18	26.09	4.66	3.25	7.91	31.43

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

CONCLUSIONS: Zorro and Puebla 152 were genetically different for root and shoot biomass across different stages of growth and development. These two lines followed different patterns of growth. While Puebla 152 developed a higher number of lateral roots, these were found at the same root depth as Zorro, but roots were differently distributed across the medium profile. Even though Zorro had less total plant and total root biomass than Puebla 152, the percentage of plant total biomass allocated for root production was greater in the former than in the latter line. A QTL analysis of these traits will be conducted using SNP markers.

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POPULATION STRUCTURE AND GENETIC DIVERSITY OF COMMON BEAN LANDRACES (*Phaseolus vulgaris* L.) FROM BRAZIL

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INTRODUCTION: The diversity of environments and crop management systems in Brazil made possible planting a wide diversity of Andean and Mesoamerican common bean landraces (Burle et al., 2010). These landraces grown in several regions of Brazil are valuable sources of genes for bean breeding programs and for evolutionary studies. The purpose of this study was to evaluate the genetic diversity and population structure of 109 common beans landraces from the South and Midwest regions of Brazil using 18 microsatellites markers.

MATERIALS AND METHODS: This research was conducted under greenhouse conditions at the Common Bean Breeding and Molecular Biology Laboratory of Núcleo de Pesquisa Aplicada à Agricultura (Nupagri). Total DNA was obtained from 109 Landraces from Nupagri's Common Bean Germplasm Bank of the Universidade Estadual de Maringá (UEM). Polymerase chain reaction (PCR) was performed using 18 microsatellites markers from all linkage groups of the common bean. Population structure were performed using Structure 2.3.4 (Pritchard et al., 2000). The genetic diversity parameters were estimated using PowerMarker 3.25 (Liu and Muse, 2005) and GenAIEx 6.5 (Peakall and Smouse, 2006). The C.S. Chord distance matrix (Cavalli-Sforza and Edwards, 1967) was used to create the Neighbor Joining Tree in MEGA 5 (Tamura et al., 2011).

RESULTS AND DISCUSSION: The genetic diversity results using 18 microsatellites markers revealed high polymorphism among the 109 common bean landraces used in this study. Model-based structure analysis showed the presence of two major populations, one Andean and another Mesoamerican, according to the Delta k = 2. Admixtures were observed between these two groups (Figure 1). The 109 landraces from Brazil were separated into five subpopulations, two Andean and three Mesoamerican (Figure 1 and 2) with high differentiation between them (Wright's F statistic = 0.66, $P = 0.001$). As expected the genetic diversity in the Mesoamerican group was greater than in the Andean group. Although the South and Midwest regions of Brazil are closer to the Andean than to the Mesoamerican region, most common bean cultivars grown in Brazil are Mesoamerican. The diversity found in 109 Andean and Mesoamerican landraces from Brazil used in this study suggest that these are potentially an important source of genes for common bean breeding programs.

ACKNOWLEDGEMENTS: This research was financially supported by National Counsel of Technological and Scientific Development (CNPq) and Coordination for the Improvement of Higher Education Personnel (Capes).

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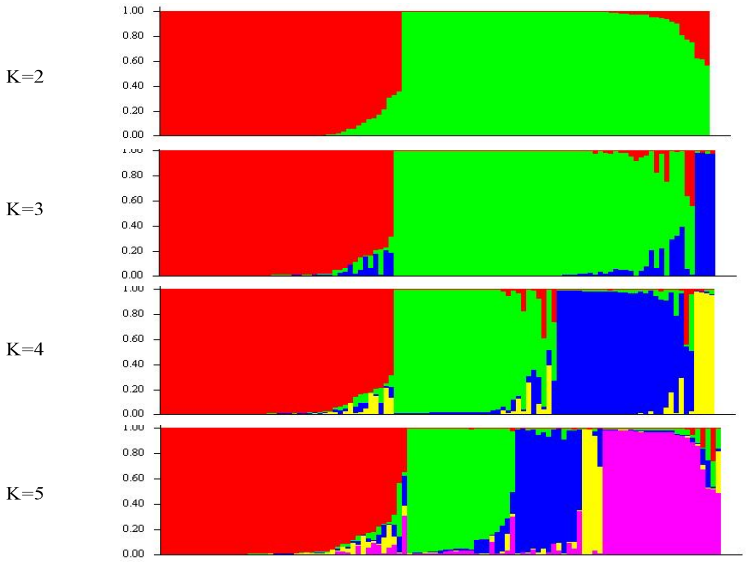

Figure 1. Population structure inferred for 109 common bean landraces using 18 microsatellites markers, assuming K = 2 to 5.

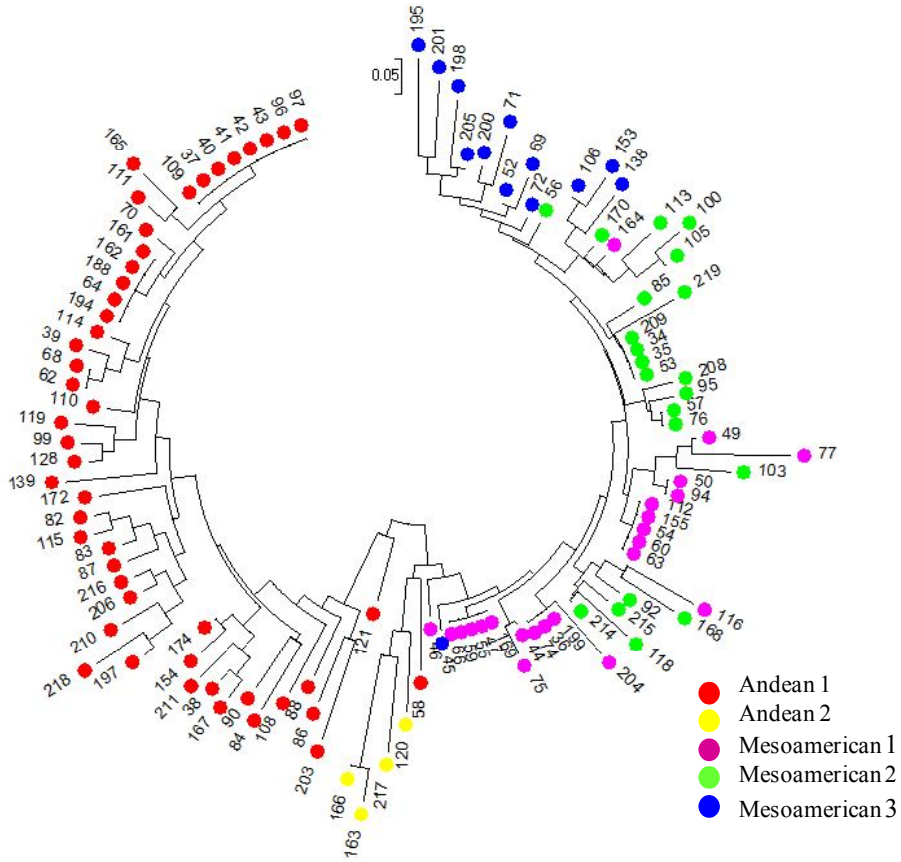

Figure 2. Neighbor joining tree for 109 common bean landraces using 18 microsatellites markers, with subpopulations determined based on population structure analysis simulating for k=5.

POTENTIAL GAIN FROM SELECTION IN HEIRLOOM DRY BEANS

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INTRODUCTION: The U.S. Midwest is a major dry bean-producing region, comprising nearly fifty percent of national production (USDA ERS, 2011). Initial market research indicates that there is interest from small producers and retailers for local, organic dry beans (Regional Sustainable Development Partnerships, unpublished data). In particular, there is an expressed demand for heirloom varieties. Also known as landrace or heritage types, heirloom varieties are loosely defined as "old varieties still maintained by gardeners or famers, particularly in isolated or ethic communities" (Whealy, 1990; Camacho Villa, 2006). Heirloom dry bean cultivars are assumed to be heterogenous mixtures of homozygous plants, which suggests that there is room for crop improvement. The pedigree and selection history of these cultivars, however, is often absent or anecdotal. In order to better understand their population structures and evaluate the potential of heirloom cultivars in breeding programs, levels of variation within these cultivars must first be established. We hypothesize that heirloom dry bean cultivars exhibit adequate levels of genetic variation to allow for the evaluation and selection within cultivars. To explore this research hypothesis, our project consists of two main objectives: estimating phenotypic variation within heirloom cultivars and selecting superior lines from within heirloom cultivars.

MATERIALS AND METHODS: Fourteen heirloom cultivars were selected to evaluate within-line variability (see Figure 1). Heirloom seed was obtained from commercial seed sources and specialty seed companies, including Seed Savers Exchange, Purcell Mountain, and Vermont Bean Company. These fourteen cultivars were selected for plant row evaluation based on bush-type architecture, maturity, and adequate yield performance.

Figure 1. Heirloom cultivars selected for plant row evaluation. Cultivar names are listed below their respective picture.

To estimate a cultivar's inherent genetic variation, sixty single plants from each cultivar were randomly chosen from 2012 field plots for pure-line trialing. Seed from randomly selected plants were grown as plant rows (i.e. 60 plant rows/cultivar) on the University of Minnesota's Student Organic Farm during the 2013 growing season. Sampling for eight morphological traits (days to flowering, total nodes, canopy height (cm), pods/plant, pods in the upper two-thirds of the plant (%), total seed yield (g), 100-seed weight (g), and days to maturity) was performed

within plant rows. F-tests comparing among- and within-row variance were used to uncover significant variation within a cultivar. Provided significant levels of variation exist within heirloom cultivars, 8-10% of plant rows will be selected as superior lines. Gain from selection, a function of selection differential, genetic, and phenotypic variation, will be estimated during 2014 observational trials with selected material.

RESULTS AND DISCUSSION: Preliminary stability analyses from 2013 yield trial data indicate that heirloom dry beans are generally poor performers (< 1,550 kg/ha) and do not demonstrate adaptability to multiple locations. Outliers, however, suggest that some heirloom cultivars may be both stable and higher yielding than others. F-tests identified significant variation within cultivars for all eight measured traits in at least one cultivar (Table 1). Variation in canopy height and 100-seed weight was significant across all cultivars.

Table 1. F-value and Coefficient of Variation (CV) within representative heirloom cultivars as measured between plant rows. Asterisks indicate level of significance for the F-tests: *p*-value(<0.001) = '***', *p*-value(<0.01) = '**', *p*-value(<0.05) = '*'.

	Nodes	Canopy Height (cm)	Pods/Plant	Upper Pods	Yield/Plant (g)	100-Seed Weight (g)	Days to Flower	Days to Maturity
Jacob's Cattle Gold								
F-value	2.551***	2.064**	1.486	1.949**	1.828**	3.211***	-	-
C.V.	18.9 %	9.7 %	24.7 %	14.0 %	28.9 %	9.9 %	3.3 %	2.2 %
Lina Sisco's Bird Egg								
F-value	1.193	2.473***	1.307	3.427***	1.241	2.344***	-	-
C.V.	10.6 %	11.0 %	24.4 %	14.5 %	30.8 %	10.4 %	3.0 %	2.8 %
Tiger's Eye								
F-value	2.348**	1.628*	1.995**	1.108	1.888*	2.529***	-	-
C.V.	12.0 %	14.3 %	29.1 %	16.9 %	34.8 %	8.0 %	4.0 %	3.6 %

Existing variation will be used to select superior lines within each heirloom cultivar and gain from selection will be estimated during the 2014 season. Ultimately, this research will result in improved pure lines of heirloom dry beans.

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THE NEW GRAIN LEGUMES WORKING GROUP (GLWG) IN THE EUROPEAN ASSOCIATION FOR RESEARCH ON PLANT BREEDING (EUCARPIA)

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Founded in 1956 in Wageningen (The Netherlands), EUCARPIA (www.eucarpia.org) is a non profit organization aiming at the promotion of scientific and technical co-operation in all fields of plant breeding in order to foster its further development. The 1500 EUCARPIA members come from 39 European countries and from 40 countries from North and South America, Africa, Australia and Asia. EUCARPIA initiates relationships and co-operates with other national and international organizations in plant breeding. EUCARPIA has 11 Sections (table 1), and several Working Groups focusing on particular crop species. Each Section is engaged in a certain field of research, or in certain groups of crops. EUCARPIA encourages its members to take part in several Sections. New methods and techniques can therefore be rapidly integrated into the wider field of plant breeding.

Table 1. EUCARPIA crop-specific and thematic Sections

Crop-specific Sections	Thematic Sections
Potatoes	Biometrics in Plant Breeding
Cereals	Genetic Resources
Fodder Crops and Amenity Grasses	Organic and Low-Input Agriculture
Maize and Shorgum	
Vegetables	
Fruit	
Ornamentals	
Oil and Protein Crops	

Every four years, the General Congress is held together with the general assembly. These congresses are an open forum for all EUCARPIA members to discuss subjects of a wider interest. They provide a forum for presentation of the problems and challenges facing plant breeding both for today and in the future. (The last General Congress was: “Plant Breeding for Future Generations”, XIXth EUCARPIA General Congress 21-24 May 2012, Budapest, Hungary).

EUCARPIA is one of the few scientific societies in the field of agriculture which is growing. The Association hopes to maintain its attractiveness to the members by continuing to offer high quality scientific meetings, but also by fostering the virtual dialogue with and among the members by creating a dedicated area for them on its homepage, with platforms for different topics.

The countries with representatives in the EUCARPIA Board are: Austria, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Lithuania, Netherlands, Norway, Poland, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

The last Meetings of the Oil and Protein Crops Section were at Pontevedra (Spain, 1998) and Budapest (Hungary, 2008); the next one will be probably in Turkey. Within this Section it was recently created the **Grain Legumes Working Group (GLWG)** focused to food and feed legumes. Special attention will be paid to the common bean, as the major protein crop for direct human consumption. The GLWG overall goal is to interact with other Grain Legumes organizations, researchers and forums to promote shared research and perform shared meetings. The first Meeting of the GLWG will be held in Spain in 2015.

LIMA BEAN QTL AND MARKER DISCOVERY TO SUPPORT BREEDING FOR NEMATODE AND LYGUS RESISTANCE

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California is the leading producer of dry lima beans in the United States. In 2013 an estimated 13,500 acres were planted in California, which account for over 99% of the total lima bean acreage across the United States (USDA 2013). California growers are largely concerned with economic loss due to yield reduction and seed damage from insects (*Lygus*) and nematodes (*Meloidogyne incognita*). Lygus insects can cause the abortion of flowers and young pods or the damage and discoloration of seeds depending on the time of attack. Nematodes infect roots, choking the plant off from vital nutrients and allowing other soil pathogens to enter through damaged tissue. Both pests cause considerable economic loss to growers. Yield reduction due to root knot nematodes can be as high as 45-90% (UC IPM 2008).

UC-Davis is developing improved pest resistant varieties of lima beans for California growers while simultaneously creating tools for future research. We are doing this by making Recombinant Inbred Lines (RILs) for Quantitative Trait Loci (QTLs) mapping and Back Cross populations for California's lima bean breeding program. We are also attempting to add novel genetic sources of pest resistance from non-Californian lima bean varieties.

Using next-generation DNA sequencing we developed a Single Nucleotide Polymorphism (SNP) map for *P. lunatus*, based on two parents, one from each of the major domestication gene pools. These parents are both adapted to California agriculture. The Andean variety, UC 92 is a bush type large lima with resistance to nematodes. The Mesoamerican variety, UC Haskell, is a vine type baby lima bean which has shown lygus tolerance. We were able to create a physical SNP map building from known homologies and synteny to common bean (*P. vulgaris*). The lima bean map currently has ~50,000 SNPs spread across all 11 chromosomes with at least 5X coverage (Fig. 1).

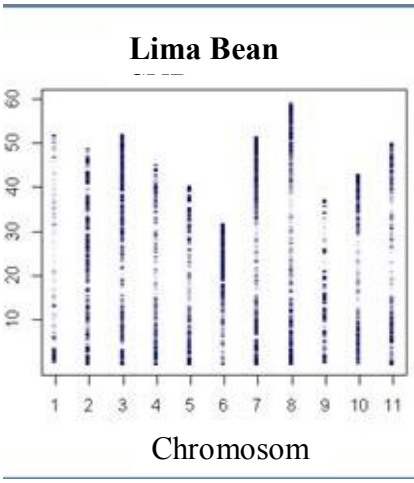

Figure 1. Lima bean SNPs. SNPs called between conspecific lima bean types UC Haskell and UC 92. Putative location on linkage group is based on synteny with *Phaseolus vulgaris.*

To develop this SNP map, genomic DNA was extracted from leaf tissue of both parents. Reduced representation libraries were prepared using NlaII enzyme to linearize the DNA and AmPure bead purification to size select fragments ~500bp in length for Illumina HiSeq paired end sequencing. Reduced representation generates more in depth coverage over a subset of the genome to increase confidence when calling SNPs. Both parents were barcoded with eight different barcodes each to prevent incorrect clustering during sequencing of simple sequence at read ends, then combined and run in a single Illumina flow cell lane.

Sequencing reads were cleaned for quality and sorted by barcode. Reads were then aligned on the common bean genome. SNPs were called based on polymorphisms at single locations. The data presented here shows SNPs, which had greater than 5X coverage at the given locus and were more than 99% consistent within genotype.

As expected there are generally fewer SNPs located near the centromeres. Linkage group one shows relatively fewer SNPs than the other linkage groups. This may be because of more genetic conservation between the two conspecific lima bean gene pools or because of less synteny between lima bean and common bean in this region.

To find the genetic loci associated with both nematode and lygus resistance we have created recombinant inbred populations from crosses between the two parents which we used for creating the SNP map, large lima bean (UC92) with resistance to nematode and a baby lima bean (UC Haskell) with tolerance to lygus. Crosses were made using the stigma-hook method without emasculation. Polymorphic Simple Sequence Repeat (SSR) marker Pv_ctt001 was used to verify F1 heterozygotes. The PCR amplicons were run on 2% agarose gel. We have approximately 300 Recombinant Inbred Lines (RILs) from reciprocal crosses, which have been brought through single seed descent to F4 and F5 generations with an estimated homozygosity of about 95%. These lines are currently undergoing seed increase at USDA-ARS in Isabela, Puerto Rico by Dr. Timothy Porch. Possibly due to the separate domestication events of the conspecific parents, UC Haskell and UC 92, 10-20% of the F2 generation did not produce mature seeds. By summer 2014 these populations will be ready for pest resistance phenotyping. Multiples seasons of phenotype data will be collected on these RILs.

Along with creating the RILs we are making backcross populations to introgress resistance genes into California market varieties. We are adding novel sources of pest resistance and genetic variation from 26 varieties of lima beans from throughout the Americas, which have shown some adaption to California growing conditions. These 26 varieties are a subset of ~250 varieties from the CIAT (International Center for Tropical Agriculture) core germplasm collection.

Once the RILs are phenotyped we will do QTL mapping for regions of the genome associated with pest resistance, which is of immediate value to California growers. The large number of genetic differences in these RILs can also be used to map many other traits including, growth habit, seed weight, stomatal conductance and flowering time.

USDA. Pacific Region Field Crop Review (August 16, 2013) National Agricultural Statistics Service
http://www.nass.usda.gov/Statistics_by_State/California/Publications/Field_Crops/201308fldrv.pdf

UC IPM University of California Integrative Pest Management Guidelines –Dry Beans (December 2008) www.ipm.ucdavis.edu/PMG/selectnewpest.beans.html

ROGUES FOR POD TRAITS IN SNAP BEAN (*PHASEOLUS VULGARIS*)

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INTRODUCTION

Varietal maintenance is a fact of life for seed companies. Thousands of dollars are spent each year to search for and remove "rogues" from seed fields. In snap bean, the rogues or off-types with greatest economic consequence are those that affect pod characteristics. For snap beans that are processed, pods with round cross-section shape are necessary for accurate grading into different sieve sizes. Oval or flat pods will tend to group with round pods of younger physiological age in the cannery thereby reducing the quality of the particular grade of pods. All contemporary snap beans for processing lack fiber in the suture walls of the pod. When an off-type for this trait occurs (reversion from stringlessness to stringy pods), the change is readily visible and reduces the visual and sensory quality of the product.

In some cases, these traits may be the result of a mechanical seed mixture, or due to a chance outcross, but in most cases, oval pod shape and strings occur spontaneously in a seed lot where there has been no opportunity for an outcross or a mix. The rate of occurrence varies among cultivars, with anecdotal evidence that the rate is higher in the Bush Blue Lake type snap beans. In general the reversion frequency for strings and ovals is several orders of magnitude higher than the normal mutation rate.

Understanding the cause of off-types may lead to strategies to reduce their occurrence and economic impact. We report preliminary results from studies on stringy and oval off-types recovered from existing cultivars and breeding lines. The purpose of this study is to find an association between pod fiber and oval pod off-types and the probable mutation occurring in a molecular level that have caused homozygous varieties to revert back to a stringy pod.

MATERIALS AND METHODS

In the summer of 2012, we identified 107 off-types in the field. These were designated the M0 generation. These were found in 11 commercially released cultivars and breeding lines that had been selfed for at least six generations. Normally, these lines produce pods without strings and with round cross-section whereas the off-types had stringy and/or oval pods. In the fall greenhouse, we grew 16 plants (designated M1 generation) from each off-type plant, and individual plants were phenotyped for pod cross section shape and suture strings. Seed was harvested from individual single plants and used to plant the subsequent generation. The off-types that showed heritable rogue traits were selfed for one additional generation (M2) with phenotypic data taken again to track inheritance of the traits of interest and their segregation ratios.

To identify strings, the beak or apex of the pod was broken pulled to see if it detached freely or if fiber in the adaxial suture was present. To identify oval shaped pods, they were rolled between the forefinger and thumb, and if it did not feel symmetrical it was examined closely to determine whether it was heart-shaped or truly oval in cross section. Only those with oval shape were retained for further study. Pod fiber was assessed in dry pods using a 1-4 rating scale where 1 = smooth pods (with high fiber) and 4 = wrinkled and constricted pods (with low fiber).

RESULTS

In about half of the M0 selections, we determined that the off-type was transmitted to the M1 progeny. In the remainder, the variation observed in the M0 generation was not heritable. Of the lines grown, 46 selections continued to segregate for one or both of the rogue traits even after three generations of selfing. Stringlessness conditioned by *St* is dominant to presence of strings (Prakken, 1934), so that any stringy progeny would be expected to be homozygous *st st* and should not continue to segregate. Thus, the continued segregation in some lines over several generations suggests that this genetic model is either wrong, or the trait is controlled by a gene other than *St*. Oval pod shape was usually associated with high fiber whereas strings were rarely associated with higher fiber levels. There were exceptions in that some pods with both traits were low fiber. Oval pods that are high in fiber may be under different genetic control from oval pods with low fiber. In the former case, the presence of fiber causes the oval pod shape, in the latter case, differences in rate of cell growth may affect pod shape. Two polymeric genes, *Ea Eb* for "flat" pods vs. *ea eb* for round pod was described (Lamprecht 1932, 1947; Tschermak 1916). A second set of polymeric genes, *Ia Ib* for parchmented vs. *ia ib* for tender pod producing an oval cross-section vs. round pod have also been described (Lamprecht 1932, 1947, 1961). The former set appears not to have been affected by fiber content whereas the latter pair are directly associated with pod wall fiber. Three possible heritable causes for off-types are spontaneous mutation, excision of a controlling element, and DNA methylation. There is a need for molecular analysis to determine what causes rogues and how their effects might be mitigated.

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A SNP-BASED LINKAGE MAP OF SNAP BEAN (*Phaseolus vulgaris*).

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INTRODUCTION: Bush Blue Lake (BBL) snap beans are grown in the Willamette Valley of Oregon for canning and freezing. While they have desirable processing (good color, low fiber, good texture and flavor) and production traits (early season cold tolerance, high yields) compared to other snap beans, plant architecture, seed production traits, and white mold resistance need improvement (Myers, et al., 2004a). Previous breeding work at OSU demonstrated that it is very difficult to recombine Oregon BBL materials with other snap beans (Myers & Baggett, 1999). Using molecular markers Myers and Davis (2002) showed that Oregon BBL snap beans are of the Mesoamerican center of origin whereas most other North American snap beans are of the Andean center of origin, which could explain the lack of useful recombinants between these types. The main objectives of the OSU breeding program are to improve plant architecture and white mold resistance while maintaining the desirable BBL characteristics. This SNP-based map should facilitate the use of marker-assisted selection to monitor the introgression of new traits while maintaining the BBL traits in future germplasm and cultivar development.

MATERIALS AND METHODS: A recombinant inbred (RI) population of 80 lines from the cross 'Minuette' × 'OSU 5630' was developed using F2:6 single seed descent. The RI population and parents were grown in an observation trial as described in Myers, et al. (2004a) to record data on plant growth habit. Pods harvested at processing maturity were graded into sieve sizes 1-6, and evaluated for pod length, cross section, straightness, smoothness, color, and for the presence of suture strings. Samples from two sieve sizes with the greatest weight were blanched and frozen, then evaluated for fiber content, Brix, and color. DNA was isolated from 77 RI lines and the population was genotyped using the Illumina 10,000 SNP BARCBEAN6K_3 Beadchip. In addition, 34 SSR and two phenotypic traits from previous mapping work were included in the analysis. A linkage map was generated with JoinMap 4.0, and QTL interval mapping performed using MapQTL 6.0. MapChart was used to produce the map figure and QTL loci were indicated by stem and box plots with lower thresholds of LOD=2.0 and LOD=3.0 for stem and box, respectively (Figure 1).

RESULTS AND DISCUSSION: The framework map consisted of 720 SNP's, 34 PCR-based markers (SSR, STS, CAPS), and 2 morphological traits in eleven chromosomes covering 1,140 cM. Table 1 lists the mapped QTL loci with their associated LOD and % explanation of variance values. QTL's were observed for two architecture traits, plant height and stem diameter, as well as for several pod traits. Many of the pod traits were found in clusters of various combinations. The largest of these clusters, Pv06 (Figure 1), also had the highest % explanation of variance values for the traits pod width, pod height, %1-4 sieve, and mean sieve distribution. Also located

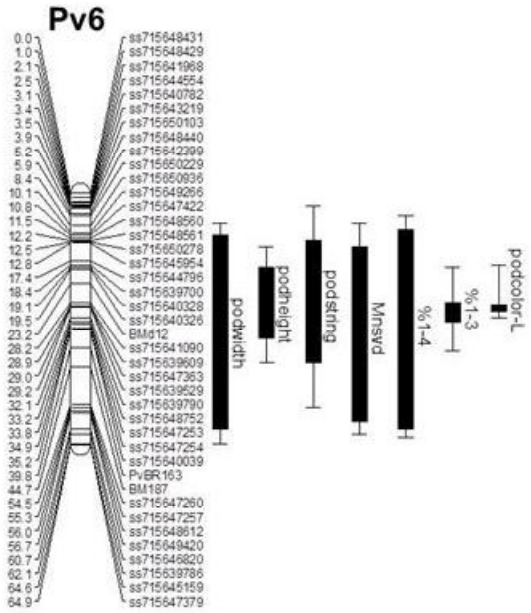

Figure 1. Pv6 linkage map with pod trait QTL's.

Table 1. QTL's for plant architecture and pod characteristics.

Trait	Chromosome	Marker Loci	LOD	% Expl. Var.
Plant height (cm)	Pv4	ss715649810	3.2	18.0
	Pv7	ss715647734	4.4	23.6
Stem diameter (cm)	Pv7	ss715639546	2.9	16.4
	Pv7	ss715647088	2.5	14.4
Pod length (mm)	Pv2	ss715646142	4.2	22.9
	Pv4	ss715649179	2.3	13.1
	Pv5	BM138	2.2	12.6
Pod straightness[1]	Pv3	ss715647446	2.9	16.7
	Pv5	BM175	2.3	13.4
	Pv8	ss715646866	2.9	16.6
Pod width (mm)	Pv6	ss715639529	10.4	47.6
	Pv10	ss715641800	2.5	14.3
Pod height (mm)	Pv6	ss715647363	5.9	30.6
Pod string[1]	Pv6	ss715641313	4.7	25.3
%1-4 sieve[2]	Pv6	ss715639529	14.7	60.4
	Pv8	ss715647143	2.7	15.7
	Pv10	ss715639891	3.6	20.2
%1-3 sieve[2]	Pv6	ss715639529	3.7	21.0
	Pv7	Phs	4.7	25.4
	Pv8	ss715647143	2.4	13.8
	Pv10	ss715645076	3.2	18.2
Mean sieve distribution[3]	Pv6	ss715639529	9.2	43.9
	Pv7	Phs	3.2	18.3
	PV8	ss715647143	3.1	18.0
	Pv10	ss715640711	3.2	18.5
Color L[4]	Pv6	ss715641091	3.1	18.9
Color b[4]	Pv7	ss715648692	2.2	14.0
	Pv7	ss715646463	2.3	14.2

on Pv06 is the QTL for pod string. The *st* gene controlling pod suture string, was previously mapped to Pv02 on the consensus map (Koinange et al., 1996), therefore our QTL probably represents a different gene affecting pod fiber. While both OSU 5630 and Minuette have round pod section and are stringless, transgressive segregation was observed in the RI lines, suggesting that different genes control these traits in the two parents (Myers, et al., 2004a). The amount of phenotypic variation and transgressive segregation observed in these RIL lines suggests that OSU 5630 has a different complex of genes for the snap bean phenotype compared to Minuette, which may relate to the difference in centers of origin of the two parents (Myers, et al., 2004b). The utility of a molecular marker map may facilitate the introgression of these and other traits of interest through marker-assisted selection methods and by developing a better understanding of the origins and contributions of desired traits from parental material.

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SNP MARKER DEVELOPMENT TO IMPROVE MARKER ASSISTED SELECTION AT CIAT BEAN BREEDING PROGRAM

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INTRODUCTION: Common bean is an important food legume particularly for smallholder farmers in developing countries, where it is an essential due to its high protein and micronutrient contents. Breeding at CIAT aims to develop improved varieties with superior resistance to abiotic and biotic stresses and higher micronutrient levels. Marker assisted selection (MAS) is utilized to accelerate the breeding process and to increase efficiency. SNP markers are developed to be analyzed in a gel-free system replacing SSR and SCAR markers. Marker conversions have been achieved for markers for BCMV, CBB, ALS, and bruchid resistance. A protocol for seed DNA extraction is being improved to make possible the genotyping of plants before bringing them to the field. As genotyping is getting even cheaper and easier while field phenotyping becomes the most resource limited activity, seed DNA extraction has a big potential to improve breeding in future.

MATERIAL AND METHODS: Part of the coat of bean seeds was first removed. Avoiding the damage of the embryo, 3 to 10 mg of seed powder was obtained using a drill and then collected. DNA was extracted according to the extraction method described by Xin et al., 2003 as modified by the Bean Molecular Genetics Team (Table 1).

Regions associated with disease resistance were amplified and sequenced in genotypes that showed, phenotypically, high resistance to different diseases such as BCMV, CBB, ALS and bruchid. For each SNP detected, two forward allele-specific primers with the 3′ base of each primer matching one of the SNP allele bases, and a reverse common primer were designed. To each of the two allele-specific primers, GC tails of different lengths were then added. The long 14-bp GC tail has the sequence 5′- GCGGGCAGGGCGGC-3′ and the short 6-bp GC tail has the sequence 5′-GCGGGC-3′ (Figure 1).

RESULTS AND DISCUSSION: The alkaline extraction method developed in this study allows the extraction of DNA from seeds, without impairing seed viability for its later germination. In this context, time and resources can be saved by selecting genotypes through molecular markers. SNP markers were designed based on the sequencing of PCR amplicons from different regions associated to resistance genes and QTLs to BCMV, CBB, ALS and bruchids in different genotypes contrasting in their resistance responses to those diseases. These markers have been tested in several genotypes with known response to the disease finding a good phenotype-genotype correlation (Figure 2).

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Table 1. DNA extraction Protocol

Reagent	Seed DNA extraction procedure
Buffer A 50 mM NaOH 1 % Tween 20	Add 200 µl of the Buffer, mix by vortexing for 1 minute, and incubate at 95°C for 10 minutes.
Buffer B (pH= 7.3) 100 mM Tris Hcl 1.7 mM EDTA	Add 120 µl of Buffer and mix by vortexing for 1 minute.
___	Centrifuge at 3.000 rpm at room temperature for 15 minutes.
___	Make a 1:10 dilution of the extract in a 96 well plate and a final volume of 100 µl.

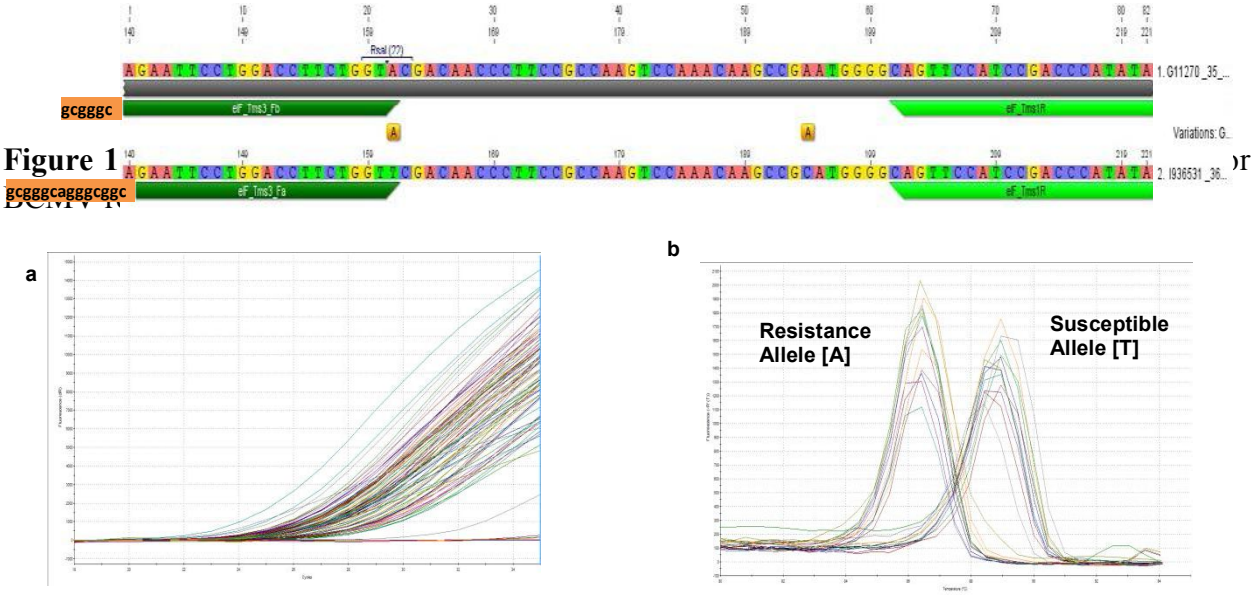

Figure 1 or BCMV-N

Figure 2. Tm-shift SNP genotyping. **a.** Amplification plot of seed DNA, **b.** Method validation and melting curve analysis showing one SNP that were genotyped by Tm shift assay.

USING GROWING DEGREE-DAYS TO ESTIMATE PHENOLOGY AND DEVELOPMENT IN COMMON BEAN

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INTRODUCTION

Plant phenology and development can be measured in terms of heat accumulation or growing degree-days (GDD). Depending under which climate or latitude the plant is grown, it will require more or less days for each developmental stage. The GDD measurements allow predicting more accurately crop developmental stages compared to calendar days, as temperature varies across years and locations. The GDD are mostly used to rate the maturity of corn production in order to maximize yield and quality, but little has been reported about common bean requirements. In this study, the GDD of two common bean (*Phaseolus vulgaris* L.) recombinant inbred lines populations were compared.

MATERIALS AND METHODS

Field trials were conducted from 2011 to 2013 at Hatton and the Prosper Research Site located ~ 99 and 30 km NW of Fargo, North Dakota, respectively. The populations were derived from inter-pool crosses [Negro Jamapa x ICA-Calima (RIJC) and Stampede x Redhawk (RISR)]. Phenological stages (Modified from Gepts et al., 1986) from emergence to flowering were recorded twice a week on six flagged plants over 180 plots replicated three times in a resolvable row-column design. Daily minimum and maximum temperatures (NDSU-NDAWN, 2013), and the base temperature below which plant growth stops, were used in the basic equation to calculate the GDD = $[(T_{max} + T_{min})/2]-T_{base}$ (McMaster and Wilhelm, 1997). Analysis of variances was performed using the PROC MIXED procedure (SAS Institute, 2008). Locations were considered as random and genotypes as fixed effects.

RESULTS AND CONCLUSIONS

Considering different phenologycal stages, the RISR population showed late vegetative development but early flowering time compared to the RIJC population (Table 1). Contrastingly, genotypic coefficients for emergence-flowering interval time, which is the photo-thermal ratio including GDD and daylight hours, suggest earliness for the RISR population (47.1 vs. 52.8). The RIJC population took longer accumulating 80 GDD more than RISR from emergence to reach flowering time. Within both populations, lines with indeterminate growth habit always showed greater GDD than the determinate types for all the variables with the exception of emergence. Calendar days can mislead the estimation of plant growth. Cool days can delay emergence and early vegetative stages while warm days can advance physiologic maturity and harvest time. Our data suggest that GDD values allowed detecting smaller differences for first trifoliate (V_1) and flowering time (R_1). In contrast, using the number of days after planting (DAP), no significance can be detected for the same variables. Primary leaves (V_0) followed the same trend and either DAP or GDD have similar significance level.

The GDD can be useful to predict common bean phenology and development in absence of adverse climatic conditions such as drought, flooding or frost.

Table 1. Phenologycal stage comparison RIJC vs. RISR

Stage	Population	Mean	Std. Dev.†	Min.‡	Max. §	DAP¶
		----------GDD----------				(day)
	RIJC	216.6[a]	15.8	188.2	288.2	17.0[a]
V_0	RISR	257.7[b]	17.0	227.1	312.2	21.0[b]
	RIJC	333.9[a]	20.4	292.9	419.1	24.0[a]
V_1	RISR	366.1[b]	20.8	296.0	433.8	27.0[a]
	RIJC	897.3[a]	110.7	639.0	1228.8	57.0[a]
R_1	RISR	860.7[b]	122.3	582.5	1120.5	54.0[a]

Different letters indicate statistical differences LSD (P<0.05)
†Standard deviation
‡Minimum value
§Maximum value
¶Days after planting

GDD are more accurate than DAP and the number of day's difference can be important for crop management practice. More accurate predictions of specific crop stage can be obtained by crop models. Traits correlated to economic yield can be integrated into genetic improvement strategies and ideotype design for specific environment. Crop model can separate yield gain into components and quantify yield reductions. Through modeling approaches components integration of various agro-ecosystems at multi-location can allow understanding genotype by environment interactions. Gene-based crop model including dynamic QTL are promising tools for predictions. Our preliminary data will be combined across four more locations (Puerto Rico, Florida and 2 in Colombia) to develop such model in the near future.

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CHARACTERIZATION OF A COMMON BEAN (*PHASEOLUS VULGARIS* L.) HOMOLOG OF AN ANTI-YIELD GENE

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INTRODUCTION

AT1G74730 is a member of a large gene family, coding for a protein of unknown function (DUF1118) in Arabidopsis. Previous research has found that in canola (*Brassica napus*) microspore cultures, microspores are induced to form haploid embryos by a mild heat stress treatment, this gene AT1G74730 is expressed at a significantly higher level in embryogenic cells than in nonresponsive cells (Pauls and Chan, 2006). *B. napus* homologues of AT1G74730 have been isolated and named BnMicEmUp. Bioinformatic analyses of the structure of the protein encoded by BnMicEmUp suggests that it is a leucine zipper type transcription factor and transgenic Arabidopsis plants which over-express AT1G74730 or BnMicEmUp had lower seed yield than nontransformed control plants and transgenics which had reduced levels of gene expression had increased yield. The current study was initiated to characterize the AT1G74730 homolog from common bean (*P. vulgaris* L.), and determine its function as it relates to seed yield.

MATERIALS AND METHODS

Twelve white navy bean (*P. vulgaris* L.) varieties with different yield potentials were grown using a randomized complete block design with four replications at the University of Guelph, Elora Research Station and Woodstock Research Station during the summer of 2013.

A homolog of AT1G74730 was amplified from *P. vulgaris* L. genomic DNA isolated from leaf tissue by PCR (Polymerase Chain Reaction) with the following primers: Forward:5′-CTGCAACATCCTCCGCTT-3′ and Reverse:5′-AGCCAACGAAGAGCCCAA-3′. The resulting fragment was cloned to a PCR™2.1-TOPO® vector (Invitrogen, Burlington, ON) and moved into competent *E. coli* cells. Total RNA was isolated from young trifoliate leaves collected from each of the plots and reverse transcribed to cDNA. Gene expression analysis was carried out by real-time (RT)-PCR using iQ SYBR Green Supermix (Bio-Rad, Mississauga, Ontario). Differences in RNA expression levels were calculated using threshold cycles by the ΔΔCt method.

RESULTS

A BLAST search of Arabidopsis AT1G74730 against the Phytozome *P. vulgaris* L. sequence database identified a gene that has 74.3% (179 of 241) homology to the Arabidopsis AT1G74730 sequence. It is annotated as Phvul.009G190100, and located on *P. vulgaris* chromosome 9 at 28,180,739–28,181,741bp. A search of the protein database yielded a single match to Pfam: 06549; a protein of unknown function (DUF1118).

Analysis of the TOPO clone of the PCR fragment showed that the insert in the *E.coli* plasmid perfectly matched the sequence expected from the Phytozome *P. vulgaris* L. database, indicating that the candidate gene was successfully isolated and cloned.

Real-time PCR analysis of Phvul.009G190100 expression indicated that levels varied among the twelve navy bean varieties, with Midland showing the highest level of expression. The expression levels of Phvul.009G190100 were negatively correlated with total yield (r = -0.7245, P = 0.0076) (Fig.1A) and the average gained yield (r = -0.7233 P = 0.0079) in the trial of the twelve navy bean varieties (Fig.1B)

DISCUSSION

A putative translation of the *P. vulgaris* sequence homologous to AT1G74730 and BnMicEmUp shows that it has a leucine zipper structure similar to the Arabidopsis and *B. napus* sequences, and may function as a transcription factor (Jakoby et al. 2002). Similar to these genes, the observation that the Phaseolus gene expression is negatively correlated with yield, suggests that it can be characterized as an anti-yield gene.

Future work will test the ability of the gene from *P. vulgaris* to complement AT1G74730 mutants.

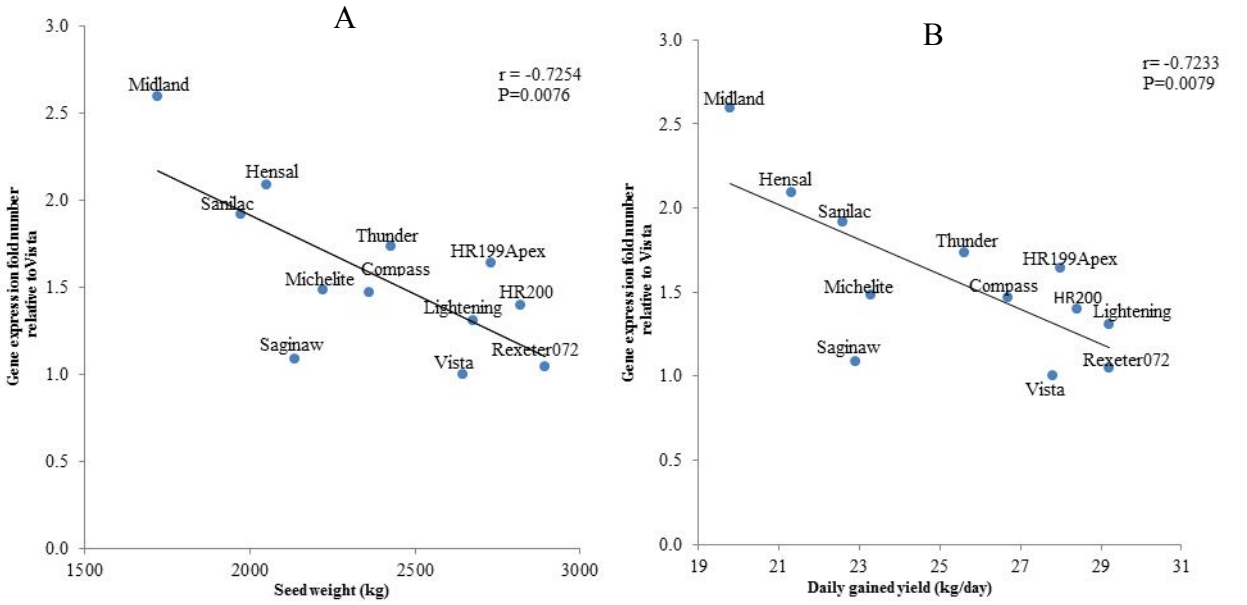

Figure 1. Correlation between gene expression fold numbers relative to Vista (the check variety) and (A) daily gained yield (kg/day), (B) seed weight (kg/hector). Fold number of expression was calculated using the comparative Ct value method. Each point stands for quadruplicate samples means.

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PURSUIT (Imazethapyr) TOLERANCE IN POST EMERGENT DRY BEANS

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INTRODUCTION

Imazethapyr is used extensively in the production of soybeans and dry beans in Ontario. This product is used both in pre plant incorporated and pre emergent applications in soybeans and dry beans, although at a lower rate in dry beans. Post emergent applications are limited to soybeans (OMAFRA Publication 75, 2012-2013), as dry beans are not tolerant at this growth stage. Pursuit can also be tank mixed with other post emergent herbicides such as Basagran and Basagran Forte (Bentazon) when applied to soybeans. Dry beans are also tolerant to Basagran in post emergent herbicide applications. Post emergent Pursuit applications alone or in tank mix combinations could be simplified for growers if dry beans tolerant to this herbicide were available. This study examines the response of dry bean genotypes of various seed classes to the post- emergent application of Pursuit (Imazethapyr). This study also screens dry bean breeding materials from different market classes for tolerance to post-emergent applications of Pursuit.

MATERIALS AND METHODS

Two separate experiments were conducted, one in the growth room and one in the field:

Growth room treatments grown in three replications included 5 dry bean genotypes: Zorro black bean, three other black bean lines, and one navy bean; combined with eight levels of Pursuit spray concentrations, sprayed at the unifoliate stage. The eight Pursuit combinations included one control spray containing only the adjuvant Agral 90 along with UAN and water. The 7 treatment combinations included the lower recommended soybean field rate of 312 ml/ha (75 g ai/ha) of Pursuit, three lower (50, 25 and 12.5 g ai/ha), and three higher (150, 300 and 600 g ai/ha) concentrations. The sprayed plants were scored 8, 15, and 21 days after application for percent damage to above ground plant parts.

The field experiment was a more simplified exercise designed more to find tolerant plants rather than to score and rate damage. 59 rows, six metres in length were grown at The Elora Research Station in 2013. Four rows contained the soybean varieties Acora, Lakeview, Champion, and Kent as tolerant checks. Fifty-two rows contained a composite dry bean population, with seed sourced from each selected single plant in the F5 segregating populations (two seeds each) in 2012, and from all entries in the preliminary and advanced yield trials, representing the overall genetic variation in the breeding program. Zorro and two other entries from the previous growth room experiment were included. The plots were sprayed at the second trifoliate stage using the higher recommended soybean rate of 420 ml/ha. Tolerant plants were tagged for harvest at the 2-3 trifoliate stage. Seed from tolerant plants will be planted inside during the winter season and plants screened to determine whether the observed lack of symptoms was due to herbicide escape or true tolerance. Crossing will be initiated on true tolerant plants. The tolerant plants will be retested in the field in 2014.

RESULTS AND DISCUSSION

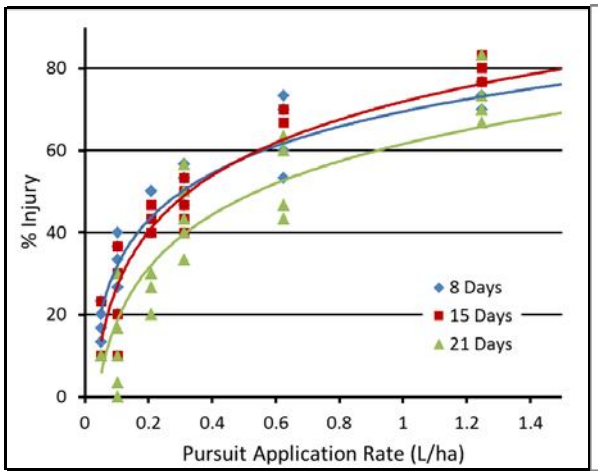

Figure 1. Injury curves (logarithmic (base e)) at 3 times after treatment at different pursuit application rates. Analysis retained application rate of 2.496 L/ha, but graph is truncated for display purposes.

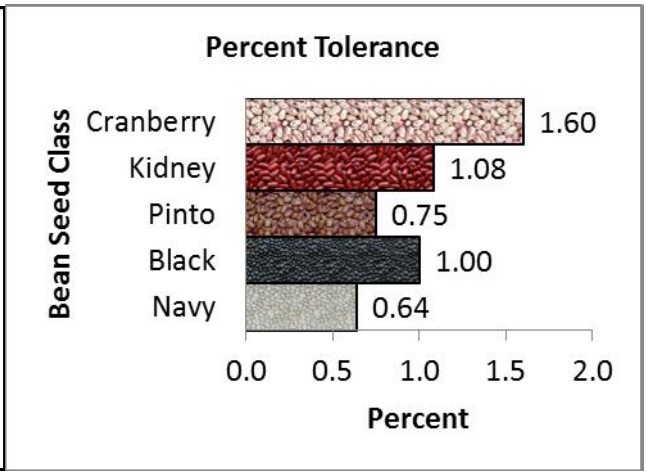

Figure 2. 90 tolerant plants were identified from a total of 10,600 plants screened, a 0.85% tolerance rate. Seed classes from the Andean gene pool had a 1.32% tolerance rate, while Mesoamerican had a tolerance rate of 0.73%.

In the growth room experiment Percent Injury was scored judging yellowing of younger leaves, interveinal chlorosis, and nodal shortening (Blackshaw and Saindon 2009) as well as growing point damage. Improvement of symptoms occurred after the first or 8 daa scoring for rates at or below the recommended rate. Above the recommended rate symptoms worsened at 15 daa, while all symptoms were improving at 21 daa. Significant differences between varieties were seen at 8 daa for rates $\leq$ 2/3x (0.208), but there was not a clear winning genotype (varies by rate) and none were "low injury". Significant differences between varieties were seen at 21 daa for rates $\leq$ 2x (0.624). Also significant differences in 'rate of recovery' ((Injury @ 8 daa) – (Injury @ 21 daa)) were seen between genotypes at rates $\leq$ 2x (0.624), but genotype order wasn't consistent between rates (i.e. no single genotype was the best at 'recovery').

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GENETIC IMPROVEMENT OF PROTEIN QUALITY IN EDIBLE BEANS WITH ADAPTATION TO MANITOBA

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INTRODUCTION: Protein quality in beans is limited by the suboptimal levels of sulphur-containing amino acids, methionine (Met) and cysteine (Cys). The germplasm line SMARC1N-PN1 lacks major seed storage polypeptides (Osborn et al. 2003 Crop Sci. 43, 1570). This leads to increased total Cys (up to 70%) and Met content (about 10%) and decreased levels of *S*-methylcysteine as compared with the corresponding wild-type line, SARC1 and the parental cultivar, Sanilac (Taylor et al. 2008; House and Marsolais, unpublished results). A cross was made between SMARC1N-PN1 (S) and the navy bean cultivar Morden003 (M) to generate an $F_{2:8}$ population of 185 recombinant inbred lines (RIL). Sulphur amino acids were quantified in a subset of lines.

MATERIALS AND METHODS: Soluble proteins were extracted from mature seed and separated by SDS-PAGE on a gradient gel. Total amino acids were quantified from mature seed, after acid hydrolysis of seed tissue, by HPLC after derivatization with phenylisothiocyanate (PITC), at the Advanced Protein Technology Centre, Hospital for Sick Children, Toronto, ON. Cys was determined separately as cysteic acid after oxidation with performic acid.

RESULTS AND DISCUSSION: Protein profiles classified the lines into four groups according to genetic inheritance at the phaseolin and APA loci (Fig. 1). Among those with MS profile, two lines had increased legumin concentration (major band directly above phaseolin), including F08-18-01-112, over two successive generations. Met levels were remarkably high in Morden003, higher than in SARC1 or Sanilac (Fig. 2). On average, Cys levels were high in lines having the SS protein profile. However their Met levels were lower than Morden003. *S*-Methylcysteine levels were reduced in parallel with increased Cys. Looking at the sum of Met and Cys, line 75 (SS) had 60 nmol per mg, as compared with 51 nmol per mg for Morden003 and 46 nmol per mg for SMARC1N-PN1. Among lines with SS protein profile, number 76 stands out due to its high *S*-methylcysteine concentration. The lines are being examined for their protein composition, amino acid profile and agronomic characteristics at different locations.

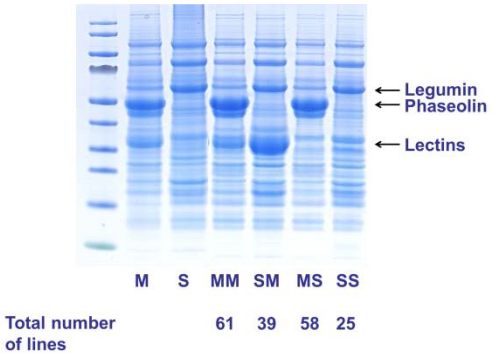
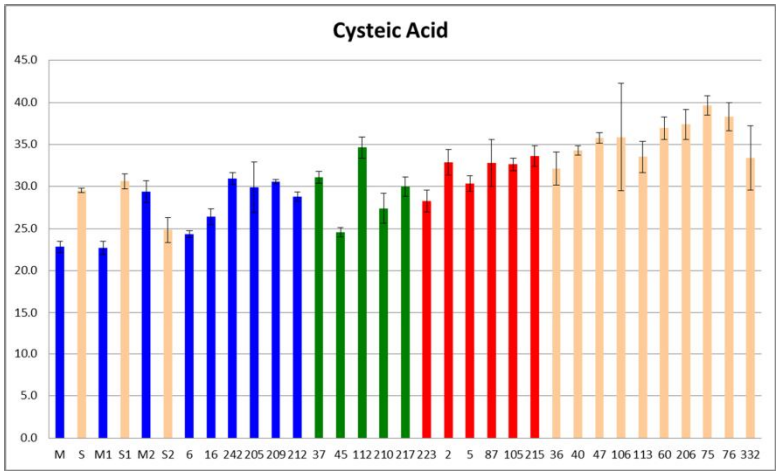
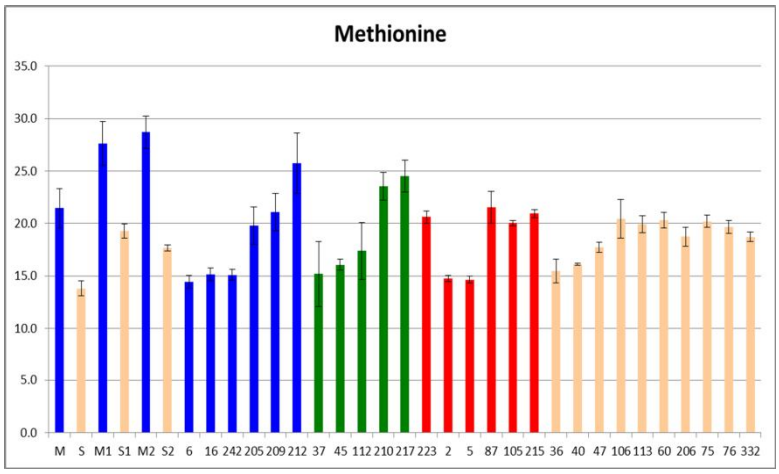


Fig. 1. Representative protein profiles from parental genotypes and RILS. The number of lines observed for each storage protein group is indicated.





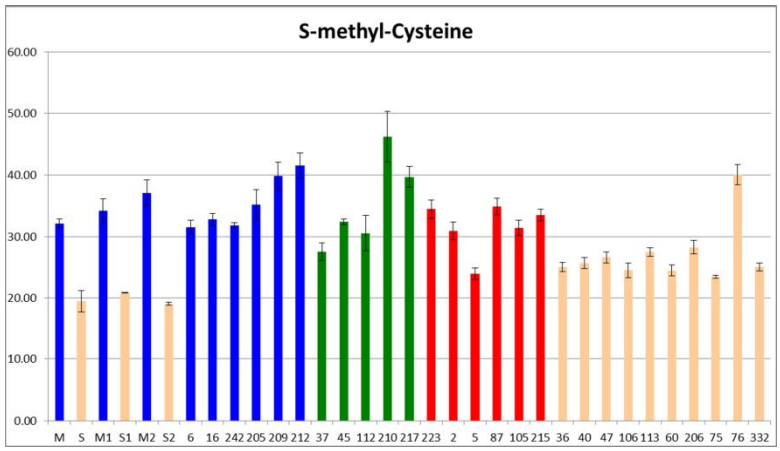


Fig. 2. Cysteic acid, Met and *S*-methylcysteine profiles of RILs. Values are expressed in nmol per mg. M/M1 and S/S1 are technical replicates, and M2/S2 are biological replicates. Lines are ordered according to protein profile: MM, blue; MS, green; SM, red; and SS, beige; n = 4; error bars represent s. d.

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ASSOCIATION OF SLOW DARKENING GENE 'SD' WITH GRAIN QUALITY TRAITS IN CARIOCA BEAN AND NEW CANDIDATE MARKER

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INTRODUCTION: The carioca dry bean market class is preferred by most consumers and accounts for 70% of the Brazilian consumer market (Del Peloso & Melo, 2005). Recently, characteristics related to the commercial grain quality such as grain color, cooking time and darkening period have become more important due to an increasingly demanding consumer market (Carbonell et al., 2010). The seed darkens during storage becoming less acceptable to consumers and depreciation of the economic value. Cultivars with delayed grain darkening associated with reduced cooking time after storage will be advantageous for the farmer and seed dealers. Grain storage for longer periods allows flexibility, i.e., the producer can await better prices on the market. The cultivar BRSMG Madrepérola is a slow darkening carioca cultivar with recessive gene inheritance for the trait (Silva et al., 2008).

MATERIALS AND METHODS: Two carioca recombinant inbred populations - BRSMG Madrepérola / BRS Estilo (56 $F_{5:7}$ RILs) and BRSMG Madrepérola / BRS 9435 Cometa (57 $F_{5:7}$ RILs) were planted at three locations in Goiás – Brazil in 2012 using random complete block designs with three replications. BRS Estilo and BRS 9435 Cometa are regular carioca cultivars that rapidly darken during storage. Yield (kg ha^{-1}), plant architecture (scale 1 to 6), weight 100 seeds (g), lodging 1 to 9 score and cooking time (90 and 180 days after harvest – Mattson cooker) were the agronomic traits studied. The populations were exposed to ultraviolet light for 72 hours (Junk-Knievel et al., 2007) and visual dark measurement was obtained at three months post-harvest using the scale 1 (slow dark) to 6 (most dark) (Silva et al., 2008). Means for the traits were obtained using GLM SAS. The molecular marker SSR 1158 (Felicetti et al., 2012) was identified as linked to the *sd* gene and a CAPs (Cleaved amplified polymorphic sequences – digested by enzyme BsiHKAY) marker within a candidate gene were tested across the RILs (Table 1).

Table 1. Primer set used for amplification of CAPs marker.

Seq Name	Seq 5' to 3'
Pv_bHLH-F8	ACTGAGAGAGACACATCATATGTGA
Pv_bHLH-R8	GGATAATCTCATTGTTTGTTGATTC

RESULTS AND DISCUSSION: All investigated traits varied significantly ($P<0.05$) across the carioca bean RILs. SSR 1158 is a co-dominant marker and able to distinguish homozygous and heterozygous RILs in carioca beans (Figure 1). The RILs were separated in two groups – slow and regular – and means of the traits for each group is below (Table 2). Positive correlation between the scale for darkening and the molecular marker SSR 1158 (0.88), the SSR 1158 and UVC light test (0.82) and between darkening scale and UVC light test (0.79) were obtained. Positive correlations were observed between darkening scale (0.84) and UVC light test (0.76) with the CAPs marker. There were no correlations between cooking time, plant architecture and yield with grain darkening.

Table 2. Traits means in the two RIL populations separated by slow and regular darkening.

UV test	nº	Yield[1]	Arch[2]	Lodg[3]	W100S[4]	Scale[5]	CT[6]180	CT[6]90
	BRSMG Madrepérola x BRS 9435 Cometa							
Sd	24	3183	4.9	4.9	24.9	2.0	25.6	21.0
Rd	33	3422	4.8	4.7	25.0	3.7	24.7	19.6
	BRSMG Madrepérola x BRS Estilo							
Sd	15	3614	5.1	5.1	24.8	1.9	28.4	21.0
Rd	41	3639	4.8	4.7	26.2	3.3	28.6	21.4
	For both populations							
Sd	38	3336	5.0	5.0	24.9	1.9	26.7	20.9
Rd	74	3542	4.8	4.7	25.7	3.5	26.8	20.6

[1] kg ha^{-1}, [2]plant architecture, [3]lodging, [4]g 100 seeds, [5]scale post-harvest, [6]cooking time (min.)

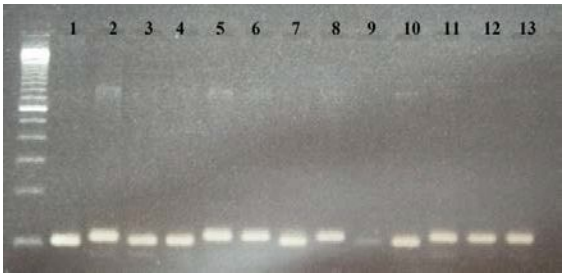

Figure 1. Co-dominant marker SSR 1158 assayed across a set of slow dark (lower band) and dark (upper band) genotypes: 1 SDP, 2 Stampede, 3 BRSMG Madrepérola, 4 CNFC 16688, 5 CNFC 16689, 6 BRS Estilo, 7 CNFC 16697, 8 BRS Cometa, 9 CNFC 16702, 10 CNFC 16709, 11CNFC 16714, 12 CNFC 16718 and 13 CNFC 16736.

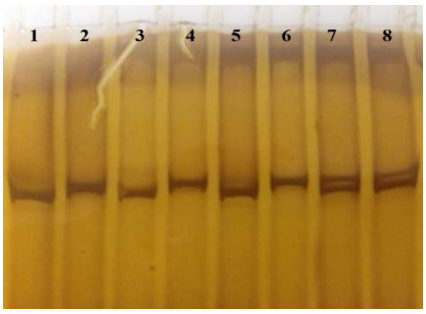

Figure 2. CAPs marker run on a 8% non-denaturing acrylamide gel and visualized with silver staining (1 – SDP, 2 – Stampede, 3 – BRSMG Madrepérola, 4 – BRS Estilo, 5 to 8 - RILs).

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GENOTYPIC ASSOCIATION OF PARAMETERS COMMONLY USED TO PREDICT CANNING QUALITY OF DRY BEANS (*Phaseolus vulgaris* L.)

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INTRODUCTION

Canning quality of dry beans determines the acceptability of new dry bean varieties by the canning industry as well as by consumers, and is known to be greatly influenced by the growing environment (Balasubramanian et al., 1999) and processing procedures (Jackson and Wiese, 1993). The bean genotype and its interaction with environment and processing factors determine the final canning quality (Hosfield and Ubersax, 1980). Bean breeding programs often evaluate their breeding materials for a series of phenotypic parameters, by which they can predict the final canning quality of genotypes. The objectives of this study were to evaluate the effects of genotype and environment on dry bean canning quality parameters, and investigate the associations among canning quality parameters in a multi-location-year dataset of canning quality parameters in Ontario.

MATERIALS AND NETHODS

The dataset consists of canning quality evaluation parameters of 96 navy and 62 large-seeded elite bean genotypes from bean breeding program in Ontario from 2004 to 2009. The dataset was balanced in each year with same set of genotypes tested in all locations. However, it was unbalanced across years, with the set of varieties being different, with some common genotypes, from year to year. Samples were processed and evaluated for cooking and canning quality and seed composition parameters in the food laboratory. Yearly data were analysed with the PROC GLM procedure in SAS 9.3 (SAS Institute, 2010) to compute the estimates of sums of squares due to genotype, location, and genotype × location interaction. Multi-year analysis of the unbalanced data was performed using the PROC MIXED procedure in SAS 9.3 as explained by Piepho and Möhring (2006). Genotype × Trait (GT) biplot analysis was implemented to display the genotype × trait data in biplots.

RESULTS

Yearly analyses

The genotypic main effect was significant for all traits except degree of packing and can yield in navy beans and degree of packing, hydration coefficient, and can yield in large-seeded beans. For composition traits in both navy and large-seeded beans, genotype × location appeared to be the least important factor, with genotype often being the largest contributor in total variation.

Yearly genotype × trait biplot analyses

The yearly biplot analyses of the navy beans indicated a positive association between hydration coefficient and can yield. Moreover, the texture parameters were negatively associated with percent washed-drained solids. Protein content was negatively associated with starch, whereas free sugar was positively associated with raffinose, stachyose, and sucrose. For the large-seeded beans, positive associations were also observed between can yield and hydration coefficient. Free

sugar was positively associated with sucrose and negatively associated with protein. In addition, protein was negatively associated with starch whereas it was positively associated with other composition traits. The observed associations of quality parameters were often repeated across years, even though the strength of individual associations varied from year to year.

Multi-year analyses

In genotype by trait biplot analyses using standardized genotypic BLUP values, PC1 and PC2 together accounted for 53.38 and 51.27 % of the variation for navy and large-seeded beans, respectively (Fig. 1). For navy beans, the biplot indicated a significant negative association between protein and starch, whereas significant positive association between sucrose and free sugar. Hydration coefficient was strongly and positively associated with can yield. The texture parameters were significantly negatively correlated with percent washed-drained solids. For large-seeded beans, the biplot indicated a positive association between hydration coefficient and can yield, and degree of packing and washed-drained solids. The texture parameters, plateau force and firmness, were negatively associated with washed-drained solids. As in the navy beans, protein content was negatively associated with starch, sucrose and free sugar.

In summary, the effect of environment was more important for physical and texture parameters than for composition parameters**.** The repeatable negative association between texture parameters and washed-drained solids and between protein and starch contents can be highlighted as the most obvious and repeatable associations among quality parameters in this study. The associations between different canning quality parameters suggest that there are tradeoffs that must be made when selecting genotypes with superior canning quality (Walters et al., 1997) and these associations may guide further selection efforts in bean breeding programs.

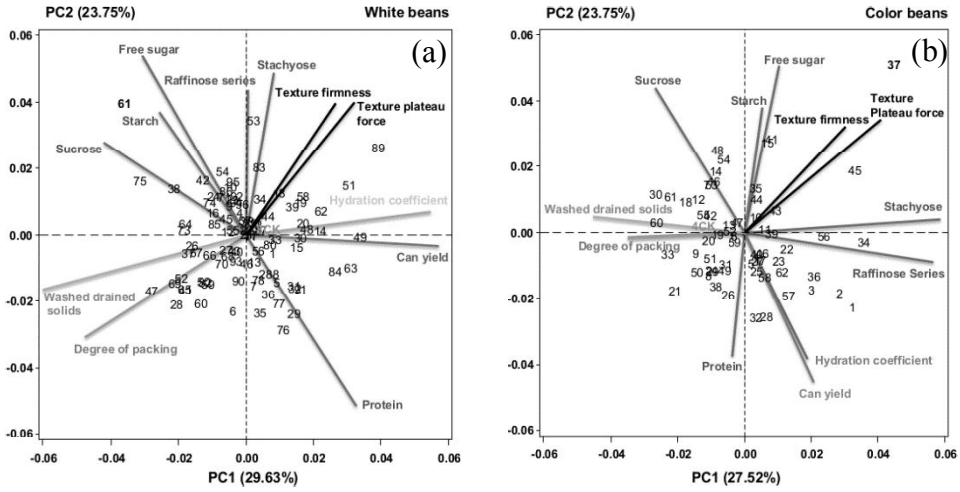

Fig. 1 Genotype × trait biplot of 12 canning quality parameters of (a) 96 navy bean genotypes and (b) 62 large-seeded bean genotypes evaluated in different locations in Ontario, Canada from 2004 to 2009.

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TRANSCRIPTOMIC ANALYSIS OF IRON AND ZINC TRANSPORTER RELATED GENES FROM DEVELOPING BEAN PODS AND POTENTIAL APPLICATIONS FOR PLANT BREEDING

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INTRODUCTION

Zinc is an essential nutrient in human diets. However, it is deficient in the diets of many people in both industrialized and non-industrialized countries. Low zinc status in children has been associated with retarded growth and low immune response. Common bean *P. vulgaris*, is an alternative to reduce deficiencies for minerals specially zinc. Its content is one of the highest among vegetable sources (on average 30 ppm), representing 15% the daily allowance. Strategies of plant breeding for increments of nutritional quality require an understanding of the genes and mechanisms of mineral accumulation. Hence, the goal in this research was obtain candidate genes for zinc accumulation with an important phenotypic effect through characterization of transcriptome of developing bean pods. Additionally, *in silico* mapping and co-location to QTLs of members of gene families associated with iron and zinc transport and accumulation.

MATERIALS AND METHODS

The two common bean genotypes used for this study were Albion and Voyager small white seeded beans from the Mesoamerican gene pool. Voyager has higher levels of seed zinc and Albion exhibits foliar Zn deficiency symptoms in low zinc and/or calcareous soils. Seeds were planted in green house and fertilized with one half strength modified Hoagland solution. At 12 days after anthesis individual pods were removed from plants and flash frozen in liquid nitrogen. Total RNA was extracted from the samples using RNA easy Plant kit (Qiagen). Three replications of cDNA of developing pods of Albion and Voyager were sequenced at the Michigan State University Research Technology Support Facility (RTSF) using an Illumina Genome Analyzer II (GA II). The sequencing was done as 75-bp paired-end reads. The data was filtered based on quality using scripts from FASTX-Toolkit. Reads from Albion and Voyager were separately processed and aligned to The *P. vulgaris* reference genome sequence v 1.0 (DOE-JGI and USDA-NIFA http://www.phytozome.net). The transcript profile and abundance estimation was carried out using Tophat (version 1.4.1) and Cufflinks (version 1.3.0) with default parameters. Their estimated abundance and test for differential expression between the tissue samples were performed using the program Cuffdiff. The unit of measurement was Fragment per Kilobase of Exon per Million fragments mapped (FPKM).

RESULTS AND DISCUSSION

Developing pod tissue is the stage prior to seed filling where nutrient transfer processes are carried out. Phaseolin and LTP3 lipid transfer genes were the most expressed. During this time period these genes are described as the main storage protein in beans and the transient storage proteins respectively. PRXR1 Peroxidase was highly expressed as well and may be involved in the metal transport. Seven out of 381 differentially expressed genes belong to the gene families related to iron and zinc transportation. In the SNP discovery analysis non synonymous amino acids change were detected on genes PvFer5, PvHMA1, PvHMA6, PvYSL1, PvYSL8, PvZIF6, PvZIF10. Transporter genes identified in the transcriptome were *in silico* mapped to bean reference map. They were distributed along of the eleven chromosomes and coincide with QTLs for zinc on chromosomes 1, 2, 3, 4, 6, 7, 8, 9, and 11. On the other hand, integration from different QTL studies was carried out. Congruency of QTL locations was found using Biomercator v 2.1 of the following population: Dor364 x G19833 (Blair et al., 2009), AND969 × G19839 (Cichy et al., 2009), G21242 × G21248 (Blair et al., 2011), Bat93 x Jalo EEP53 (unpublished). Consensus QTL analysis conducted for zinc classified 14 QTLs into 3 consensus QTL on chromosome 2, 6 and 11.

In this work, we identify members of mineral transporter gene families expressed during bean pod development. The comparative analysis of two closely related bean genotypes with different levels of seed Zn, indicates which genes are differentially expressed. This information is useful to identify candidate genes for seed mineral biofortification. *in silico* mapping of zinc transporter genes and their alignment to QTLs may be another approach to select candidate genes for QTLs responsible of the expression and inheritance of the zinc content in dry bean seed.

ACKNOWLEDGMENTS

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INFLUENCE OF ELEVATED FE, ZN, AND CD ON UPTAKE AND TRANSLOCATION OF MINERAL ELEMENTS IN COMMON BEAN

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INTRODUCTION: Common bean is an important crop plant and source of human health related macro- and micronutrients. Common bean uptake these nutrients from the soil environment and transport them to various storage tissues using proteins and genes located in different tissues (Phan-Thein et al. 2010). However, along with essential elements plants also uptake and translocate toxic metals from soil environment. At leaf senescence period, minerals from other parts remobilize and accumulate into seed (Rossato et al. 2001). The uptake and translocation of these elements depends on the chemical properties of the elements, their binding specificities with the ligands as well as tissue types (Ghandilyan et al. 2009). Our objectives in this study were to understand the influence of elevated Fe, Zn, and Cd on different mineral elements concentration and translocation through tissues of common bean.

MATERIALS AND METHODS: At Mayville State University, we conducted replicated (three) trials using three common bean genotypes [(A55, G122, and Dorado (Dor)] with controls (non-treated). We planted the seeds in 8″x11″ pots filled with "Sunshine Mix" and soaked sunshine mix with water till seed germination. At two-leaf stage, we applied 200 mgL^{-1} Fe (iron), 100 mgL^{-1} Zn (zinc), and 50 mgL^{-1} Cd (cadmium) and kept the sun-shine mix moisten with mineral/metal solution till harvesting. During pod filling stage, root, stem, and head and after harvesting seeds were analyzed for Aluminum (Al), Boron (B), Beryllium (Be), Calcium (Ca), Cadmium (Cd), Chromium (Cr), Copper (Cu), Iron (Fe), Potassium (K), Lithium (Li), Magnesium (Mg), Manganese (Mn), Molybdenum (Mo), Sodium (Na), Phosphorus (P), Sulfur (S), Silicon (Si), Strontium (Sr), Titanium (Ti), and Zinc (Zn) concentration on Spectra Genesis ICP-OES using Smart Analyzer Vision software (v. 3.013.0752). Mean concentration of 20 mineral elements influenced by different treatments [control (co), elevated Fe, Zn, and Cd] were analyzed by Duncan Multiple Range Test (DMRT) using SAS 9.3 and presented in table 1.

RESULTS AND DISCUSSIONS: In seeds, Fe, Zn, and Cd elevation increased the concentration of these mineral and metal elements respectively. In the tissues like root, stem, and pod, Cd concentration varied for different treatments but including seed its concentration significantly increased for Cd elevation but influence of Fe and Zn elevation did not observe the translocation Cd into seed. Fe concentration increased significantly in seed for Fe elevation but reduced for Zn and Cd elevation. The effect of Fe, Zn, and Cd elevation on Fe uptake and translocation from root to stem to pod varied significantly. After stem, Fe translocation reduced in pod but increased in seed. Similar trends of translocations were also observed for Zn. The significant reduction of Fe and Zn concentration in pod and increased in seed for respective treatments showed that the Fe and Zn elevation respectively enhanced the translocation of these minerals to leaf and others plant parts and subsequently influenced the remobilization of these two minerals into seed. The influence of Fe, Zn, and Cd elevation varied for other elements during uptake and translocation. Such as, the seed contents of Al, Mn, and Si increased for Fe and Zn elevation; Cu, Li, and Ti increased for the elevation of all three mineral and metal

elements; Ca and Na increased for Fe and Zn elevation but reduced for Cd elevation however the elevation effect of these three elements varied while uptaking and translocating elements through other tissues.

Table. 1. Comparison of means of 20 mineral concentrations among different tissues derived from Duncan Multiple Range Test (DMRT). Means with same letter are not significantly different.

									Root											
Treatment	Al	B	Be	Ca	Cd	Cr	Cu	Fe	K	Li	Mg	Mn	Mo	Na	P	S	Si	Sr	Ti	Zn
	μg g^{-1}	μg g^{-1}	μg g^{-1}	μg g^{-1}	μg g^{-1}	μg g^{-1}	μg g^{-1}	μg g^{-1}	μg g^{-1}	μg g^{-1}	μg g^{-1}	μg g^{-1}	μg g^{-1}	μg g^{-1}	μg g^{-1}	μg g^{-1}	μg g^{-1}	μg g^{-1}	μg g^{-1}	μg g^{-1}
Co	155.92^{B}	4.90^{D}	2.77^{C}	7130.47^{C}	0.268^{B}	0.647^{B}	7.06^{C}	141.05^{C}	10825.88^{A}	1.53^{D}	2920.06^{D}	10.10^{B}	6.29^{B}	3872.63^{B}	1336.76^{C}	3443.48^{A}	145.18^{D}	14.3^{D}	1.14^{C}	263.24^{C}
Fe	585.58^{A}	9.18^{A}	12.19^{A}	7799.04^{B}	0.153^{B}	0.499^{C}	8.73^{B}	709.2^{A}	9762.69^{D}	2.65^{C}	4262.81^{B}	8.56^{D}	0.67^{C}	2067.31^{D}	1263.96^{D}	1369.27^{D}	223.1^{A}	16.10^{A}	2.68^{A}	75.76^{D}
Zn	90.58^{C}	7.80^{B}	3.88^{B}	7161.79^{C}	1.00^{B}	0.510^{C}	4.97^{D}	60.15^{D}	9962.53^{C}	4.98^{A}	3557.27^{C}	8.94^{C}	1.21^{C}	2681.8^{C}	2132.04^{A}	1748.48^{C}	152.64^{C}	15.40^{C}	1.43^{B}	555.2^{A}
Cd	132.58^{B}	6.11^{C}	2.82^{C}	8022.55^{A}	38.90^{A}	0.892^{A}	13.99^{A}	210.25^{B}	10141.06^{B}	3.40^{B}	5198.16^{A}	14.14^{A}	15.51^{A}	4547.22^{A}	2011.22^{B}	2879.68^{B}	155.31^{B}	15.51^{B}	0.917^{D}	411.26^{B}
									Stem											
Co	76.53^{B}	6.68^{C}	3.6738^{C}	9089.8^{D}	0.211^{B}	0.449^{A}	2.67^{C}	52.07^{C}	16241.9^{B}	0.69^{C}	4352^{A}	19.46^{B}	43.86^{A}	70.47^{D}	1758.31^{C}	2979.26^{A}	132.26^{C}	18.12^{D}	0.41^{C}	456.14^{C}
Fe	380.57^{A}	11.12^{B}	9.1616^{A}	13896.8^{A}	0.152^{B}	0.444^{A}	8.01^{A}	201.67^{A}	11007^{D}	4.27^{A}	4920^{A}	8.84^{D}	0.82^{C}	467.17^{A}	1346.57^{D}	1348.34^{C}	463.24^{A}	24.75^{A}	5.20^{A}	114.63^{D}
Zn	55.30^{B}	13.16^{A}	7.1923^{B}	12244.4^{B}	0.2326^{B}	0.319^{C}	5.4^{B}	47.87^{C}	18349.7^{A}	1.95^{B}	5775^{A}	18.62^{C}	1.65^{C}	127.09^{C}	1909.89^{B}	1370.74^{C}	196.63^{B}	24.35^{B}	0.97^{B}	1283.77^{A}
Cd	65.33^{B}	6.00^{D}	3.4316^{C}	10415.2^{C}	40.95^{A}	0.374^{B}	5.06^{B}	106.64^{B}	12864.8^{C}	0.69^{C}	15397^{A}	29.07^{A}	12.40^{B}	337.4^{B}	2678.39^{A}	2536.02^{B}	179.08^{B}	20.06^{C}	0.83^{B}	803.98^{B}
									Pod											
Co	61.90^{B}	10.75^{B}	2.22^{C}	5901.77^{B}	0.37B	0.567^{B}	3.04^{B}	107.25^{A}	20403.8^{C}	0.387^{C}	2852.14^{A}	39.40^{A}	15.09^{A}	156.36^{B}	3844.01^{A}	1657.99^{B}	304.12^{B}	6.75^{B}	0.353^{D}	307.77^{B}
Fe	96.14^{A}	12.54^{A}	3.092^{A}	3859.87^{C}	0.155^{A}	0.291^{C}	2.62^{C}	75.43^{C}	21085.69^{B}	0.513^{A}	2174.8^{B}	15.80^{B}	1.87^{C}	28.63^{C}	2697.89^{C}	1349.01^{D}	225.62^{C}	4.94^{C}	1.23^{B}	49.10^{D}
Zn	46.28^{C}	8.02^{D}	1.87^{D}	3402.79^{D}	0.21^{B}	0.277^{C}	4.57^{A}	48.53^{D}	21595.67^{A}	0.402^{B}	2171.19^{B}	12.59^{C}	2.29^{C}	26.02^{C}	3444.86^{B}	1483.66^{C}	213.77^{D}	4.63^{D}	1.39^{A}	160.91^{C}
Cd	43.68^{C}	8.55^{C}	2.52^{B}	6336.28^{A}	6.42^{A}	0.897^{A}	2.11^{C}	98.43^{B}	17848.41^{D}	0.276^{C}	2839.14^{A}	38.45^{A}	3.66^{B}	266.48^{A}	3422.13^{B}	1742.35^{A}	389.81^{A}	7.26^{A}	0.779^{C}	342.88^{A}
									Seed											
Co	7.51^{B}	9.63^{A}	0	1318.5^{C}	0	0.172^{C}	3.56^{C}	72.16^{B}	29711^{A}	0^{B}	5355^{AB}	12.32^{B}	21.49^{A}	5.41^{C}	8577^{A}	2151.11^{B}	22.63^{C}	1.71^{B}	0^{C}	39.81^{C}
Fe	26.84^{B}	8.00^{A}	0	2192^{A}	0	0.145^{C}	3.82^{B}	81.26^{A}	30540^{A}	0.170^{A}	6928^{A}	17.23^{A}	2.39^{C}	7.11^{B}	8532^{A}	2236.73^{B}	46.88^{B}	2.74^{A}	0.329^{B}	42.54^{BC}
Zn	27.26^{A}	7.61^{B}	0	1715.4^{B}	0	0.235^{A}	5.54^{A}	45.54^{C}	19854A	0.129^{A}	2017^{BC}	16.12^{A}	3.87^{C}	20.39^{A}	11714^{A}	2417.71^{A}	69.48^{A}	2.41^{A}	0.782^{A}	111.28^{A}
Cd	5.84^{B}	7.37^{B}	0	859.6^{D}	2.28^{A}	0.120^{B}	3.89^{B}	50.01^{C}	13688^{A}	0.127^{A}	1774^{C}	11.58^{B}	8.82^{B}	1.98^{D}	4021^{A}	2083.94^{B}	25.61^{C}	1.86^{B}	0.254^{B}	44.50^{B}

The synergistic and antagonistic interactions (Nan et al. 2002) as well as competitiveness among elements with similar chemical properties (Ghandilyan et al. 2009) influence the uptake and translocation of elements. Elements like Fe, Zn, Cd, Cu, Mn, Cr, and Ti in our study are belong to transitional group, however we observed the influence of elevated Fe, Zn, and Cd on uptake and translocation of other elements across the group which could be due to the nonspecific and unstable ligand binding among elements (Berg and Shi 1996).

CONCLUSION: We have collected root, stem, and pod samples from treated and non-treated genotypes. Analyses of RNA sequences of these samples could help identifying mineral element specific transporter genes in common bean.

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VARIABILITY IN SEED MINERAL AND PROTEIN CONCENTRATION IN AN ANDEAN COMMON BEAN DIVERSITY PANEL

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INTRODUCTION

Iron and zinc deficiency are the most prevalent micronutrient deficiencies in the world (Pfeiffer and McClafferty, 2007). Micronutrient malnutrition particularly iron (Fe) and zinc (Zn) afflicts millions of people in the global south. Biofortification has a potential to address micronutrient malnutrition in developing countries where plant based staples are widely grown and consumed (Bouis and Welch, 2010). Common bean (*Phaseolus vulgaris* L.) is an important source of micronutrients and can supply over 50 µg g^{-1} and 30 µg g^{-1} Fe and Zn respectively. Additionally, protein supply of common bean is of primary importance to people living in rural areas.

Objective: Our study was aimed at characterizing mineral and protein concentrations in cooked common bean seed of the Andean gene-pool. The sub-objective was to determine the effect of region of origin, seed weight, and seed type on mineral and protein concentrations.

MATERIALS AND METHODS

Plant materials: A total of 271 Andean common bean genotypes were grown at Montcalm, MI in the summer of 2012. The genotypes were assembled from 28 countries representing six continents. The genotypes are comprised of landraces, breeder's lines, and cultivars and belong to nine seed types/ market classes.

Mineral and protein analyses: Fe, Zn, and N were measured on cooked common bean seed powder using the Inductively Coupled Plasma Emission Spectroscopy (ICP-ES). Percentage crude protein was estimated by multiplying N content with a factor of 6.25.

RESULTS AND DISCUSSION

There was a high significant positive correlation between zinc and protein concentrations within the panel (Table 1). This can allow for selection of these nutritional quality traits together in common bean breeding populations. Also, our results showed a wide range for Fe concentration (48-100 µg g^{-1}) and Zn (21-45 µg g^{-1}), demonstrating the quantitative nature of these mineral traits (Fig.1). Protein concentration and seed weight varied from 19-29% and 7-21 g per 30 seeds respectively (Fig. 1). Light red and dark red kidneys, and whites had higher seed weight, zinc, and protein concentrations (Fig. 2).

CONCLUSIONS

- This Andean diversity panel contains genotypes that have higher iron and zinc levels compared to the HarvestPlus breeding targets of 80 and 40 µg g^{-1} respectively. This is especially encouraging because our values are from cooked samples which are more representative of what people would consume.
- Breeding for increased zinc levels in common bean may not have an effect on yield since zinc and seed weight were moderately correlated. Also, zinc was highly correlated to iron and protein concentration.

- Light red and dark red kidneys and white colored beans were associated with high yield, zinc, and protein concentrations.

Table 1. Correlations among the four response variables measured on the Andean diversity panel

Variable	Seed wt.	Zinc conc.	Iron conc.	Protein conc.
Seed wt.		0.655	0.124	0.484
Zinc conc.	0.655		0.666	0.780**
Iron conc.	0.124	0.666		0.559
Protein conc.	0.484	0.780**	0.559	

** Values are significant at α=0.05

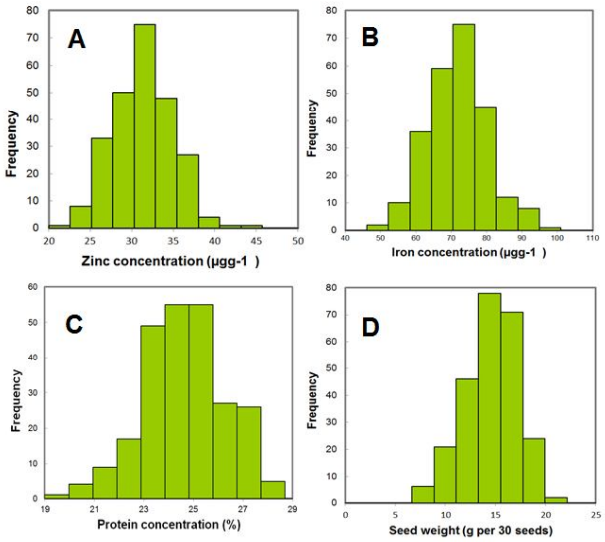

Fig.1: Frequency distribution of (A) Zinc (B) Iron, and (C) Protein concentrations and (D) seed weight among the 271 Andean diversity panel genotypes

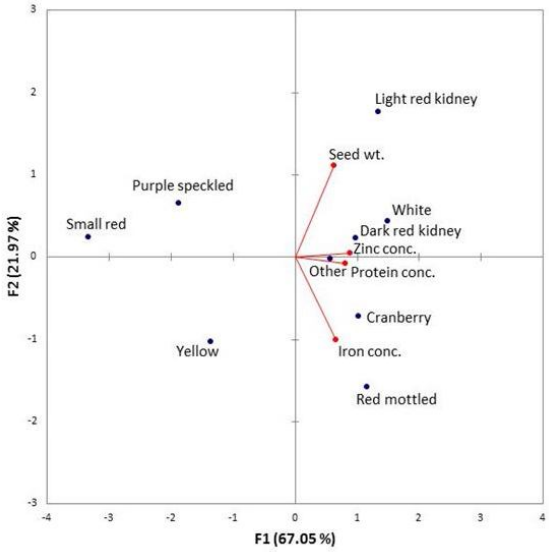

Fig. 2: Principal component analysis showing interaction among seed types and the response variables

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INVESTIGATING GENOTYPE BY ENVIRONMENT (GXE) INTERACTIONS IN NAVY BEAN PERFORMANCE TRIALS IN ONTARIO

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INTRODUCTION

Interactions of genotype by environment (GxE) profoundly complicate the identification of superior varieties. The effect of individual environments (locations and/or years), in terms of the way they influence the relative performance of genotypes, can be characterized in order to deal with GxE interactions. Among the statistical models that have been suggested for the interpretation of GxE, the site regression (SREG) analysis and shifted multiplicative model (SHMM) cluster analysis are known to be capable of distinguishing between significant genotypic rank change (crossover interaction; COI) and no genotypic rank change.

MATERIALS AND METHODS

Data from 23 years of the variety registration and performance trials in Ontario, conducted by the Ontario Pulse Crop Committee (OPCC), comprised of 17 locations and 257 genotypes, with 2 to 8 locations in any given year was used. Environmental data was collected from the Historical Climate Archive of Environment Canada accessed at http://climate.weather.gc.ca/. The daily precipitation and temperature data was collected from the weather station closest to the test site, with distances ranging from 0 to 36 km. Cumulative thermal units (CTU base 5.05, the sum of (daily mean temperature - 5.05°C)) and precipitation (PPT) were calculated from 24 May to 30 Sept for each location and year. Thirty year averages of each measure were also calculated for each location. SREG (Yan et al. 2000) was performed on single years, and the repeatability of 7 locations co-occurring in SREG groupings was analyzed. SHMM (Trethowan et al. 2001) was used on a subset of the data, comprised of 5 genotypes and 56 environments (sites nested within year). All analyses were performed in SAS 9.3 (SAS Institute Inc., 2010).

RESULTS AND DISCUSSION

I) Is GxE a concern in the OPCC variety registration and performance trials?

The average yield results of 23 years of data from the OPCC variety registration and performance trials were analyzed and show that dry bean breeding and agronomic research has resulted in an average yield gain of over 38 kg/ha per year (Fig.1). However, large year to year variation can be seen. The complication of GxE and especially COI presents a significant obstacle to determining superior varieties. In the 23 years analyzed, 12 years had a significant GxE variance component. Interestingly, and perhaps significantly, 10 of those 12 years with significant GxE interactions have occurred since the year 2000.

II) Are SREG groupings repeatable?

The 23 years of SREG analysis groupings were analyzed and clustered based on the pairwise frequency of locations co-occurring in SREG groups omitting groups with zero-value denominators, yielding 7 locations upon which clustering analysis was performed to form three groups explaining approximately 75% of the total variance, but representing only weak repeatability within the clusters. This weak grouping is evidence that there are no mega-environments for dry bean production in Ontario.

III) How well does a repeated site predict future GxE effect at the same site?
A sub-set of the data, comprised of 5 genotypes tested in 56 environments, was used in a mixed model analysis to determine that site nested within year has, by far, the largest effect size (Fig. 2). SHMM analysis, which groups environments with low or changes of yield rank, determined that no SHMM clusters strongly represent a single location or multiple locations in a single year; however, Kippen clusters repeatedly with itself, suggesting it is somewhat more likely than other sites to produce repeatable GxE patterns across years.

IV) Can environmental data explain GxE clustering?
The historical CTU and PPT data were applied to the results of the SHMM analysis. Yield and PPT are significantly different between the group site/years (at the fourth fission level), but CTU is not. Historical environmental data does not sufficiently explain the observed GxE patterns; however, it is likely too coarse to be used effectively: important parameters such as timing of precipitation and drought in relation to physiological stage are not included.

Conclusions:
- No major mega-environments were determined in the Ontario dry bean testing sites.
- Year has a large influence on genotype x site interaction.
- Historical environmental data offers little explanatory power to the observed GxE interactions.
- Effective variety recommendations will take GxE and yield stability into consideration when significant GxE effects are present.

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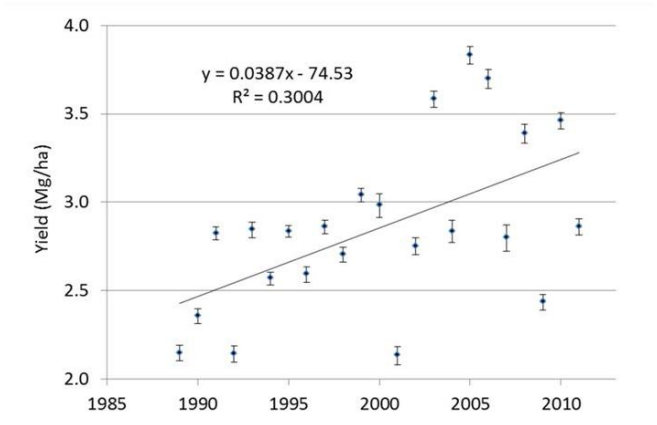

Figure 1. Average yield over 23 years of OPC trials.

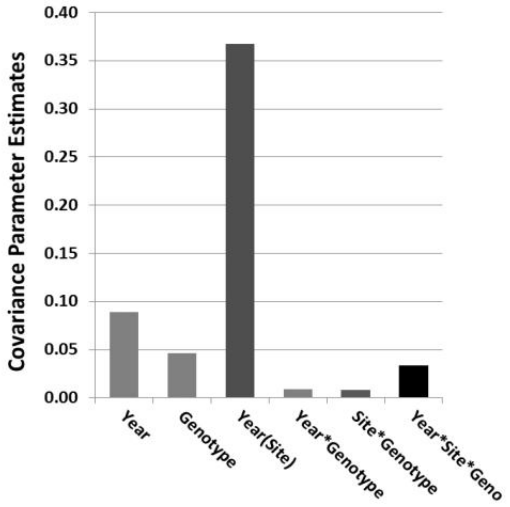

Figure 2. Relative contribution of model effects on grain yield using 5 genotypes in 56 environments over 14 years.

ANALYSIS OF DIVERSE *Colletotrichum lindemuthianum* ISOLATES OF COMMON BEAN (*Phaseolus vulgaris* L.) FROM MATO GROSSO STATE, BRAZIL

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INTRODUCTION

The occurrence of physiological races of *Colletotrichum lindemuthianum*, causal agent of common bean's anthracnose, in several cultivation regions, should be monitored as a measure to facilitate the using of genetic resistant to control this disease. However, a factor that complicates the control of this disease is wide pathogenic variability. About 247 races of *C. lindemuthianum* have been identified worldwide and more than 57 in Brazil (Balardin et al., 1997; Thomazella et al., 2002; Mahuku and Riascos 2004; Damasceno and Silva 2007; Nunes et al., 2013). Thus, the present study aimed to characterize 30 isolates of *C. lindemuthianum* collected in common bean from Mato Grosso, Brazil.

MATERIAL AND METHODS

Virulence phenotype of each monosporic isolate was confirmed by inoculation of the 12 differential cultivars of *C. lindemuthianum*, resulting in a total of 10 plants for each cultivar. For that, trays containing the 12 differential cultivars with complete developed first trifoliate leaf (approximately 15 days after cultivation) were transferred to mist chamber with temperature of 20 ± 2ºC. Inoculation was carried out with suspensions of each pathotype individually, avoiding contaminations. This process was conducted according to methods established by Cárdenas et al. (1964), using a pressure atomizer (De Vilbiss, no. 15) with a reservoir for the yeast suspension. Abaxial and adaxial leaf surfaces were sprayed. After inoculation, the plants were kept in an environment with high humidity (> 95%) for 72 h at 20 ± 2 º C. The symptoms were evaluated ten days after inoculation using the severity scale proposed by Pastor-Corrales et al. (1995), with values ranged from 1 to 9.

RESULTS AND DISCUSSION

A total of 30 isolates of *C. lindemuthianum* collected in 14 common bean producer regions in Mato Grosso state demonstrated different virulence patterns, when inoculated in the 12 differential cultivars, resulting in identification of 10 distinguished physiological races (Table 1). The race 64 presented the highest frequency of occurrence (20%), then the races 65 and 81 (17 and 13%, respectively). This was the first report of races 1, 8, 9, 10, 24, 64, 72 and 73 in the state of Mato Grosso, it is worth mentioning that this is the first report of race 24 in the world.

Races 64, 65, 72 and 73 presented reactions compatibility only with cultivars of Mesoamerican origin. On the other hand, the races 10, 24 and 81 showed compatibility reactions with both Andean and Mesoamerican cultivars (Table 1). These results are in agreement with those obtained by Thomazella et al. (2002), which revealed that races 65, 69, 73, 81 and 89, also occur at a high frequency in Parana State.

All isolates were incompatible with the cultivars Perry Marrow, Kaboon, PI 207262, TO, TU, AB 136 and G 2333, thus becoming important sources of resistance for use in common bean breeding programs aimed at the control of anthracnose in the Mato Grosso state.

Table 1. Reaction of differential cultivars to isolates of *C. lindemuthianum* collected in the Mato Grosso, Brazil

Isolates	Counties	Differential Cultivars												Races
		A	B	C	D	E	F	G	H	I	J	K	L	
1	Nossa Sra Livramento	S	R	R	R	S	R	S	R	R	R	R	R	81
2	Nova Mutum	S	R	R	R	S	R	S	R	R	R	R	R	81
3	Sto Antônio Leverger	R	R	R	R	R	R	S	R	R	R	R	R	64
4	Sinop	R	R	R	R	R	R	S	R	R	R	R	R	64
5	Sinop	R	R	R	R	R	R	S	R	R	R	R	R	64
6	Pontes e Lacerda	R	R	R	S	S	R	R	R	R	R	R	R	24
7	Pontes e Lacerda	R	R	R	S	S	R	R	R	R	R	R	R	24
8	Pontes e Lacerda	R	R	R	S	S	R	R	R	R	R	R	R	24
9	Primavera do Leste	S	R	R	R	R	R	S	R	R	R	R	R	65
10	Sorriso	R	R	R	S	R	R	S	R	R	R	R	R	72
11	Nossa Sra Livramento	R	R	R	S	R	R	S	R	R	R	R	R	72
12	Nossa Sra Livramento	R	R	R	S	R	R	S	R	R	R	R	R	72
13	Pontes e Lacerda	R	R	R	R	R	R	S	R	R	R	R	R	64
14	Primavera do Leste	S	R	R	S	R	R	S	R	R	R	R	R	73
15	Primavera do Leste	S	R	R	R	R	R	R	R	R	R	R	R	1
16	Sinop	S	R	R	S	R	R	S	R	R	R	R	R	73
17	Nova Mutum	R	R	R	R	R	R	S	R	R	R	R	R	64
18	Campo Verde	S	R	R	R	R	R	R	R	R	R	R	R	1
19	Paranatinga	R	R	R	S	R	R	R	R	R	R	R	R	8
20	Nobres	R	R	R	S	R	R	R	R	R	R	R	R	8
21	Barão de Melgaço	S	R	R	S	R	R	S	R	R	R	R	R	73
22	Campo Verde	S	R	R	R	R	R	S	R	R	R	R	R	65
23	Campo Verde	S	R	R	R	R	R	S	R	R	R	R	R	65
24	Tangará da Serra	S	R	R	R	R	R	S	R	R	R	R	R	65
25	Barão de Melgaço	R	S	R	S	R	R	R	R	R	R	R	R	10
26	Campo Verde	S	R	R	R	S	R	S	R	R	R	R	R	81
27	Pontes e Lacerda	S	R	R	S	R	R	R	R	R	R	R	R	9
28	Nobres	R	R	R	R	R	R	S	R	R	R	R	R	64
29	Primavera do Leste	S	R	R	R	R	R	S	R	R	R	R	R	65
30	Primavera do Leste	S	R	R	R	S	R	S	R	R	R	R	R	81

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.

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MOLECULAR CHARACTERIZATION OF COMMON BACTERIAL BLIGHT PATHOGEN ISOLATES SHOWING DIFFERENTIAL PATHOGENICITY

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INTRODUCTION: Common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*), is a damaging disease of common bean (*Phaseolus vulgaris* L.) throughout the world. Previous reports (Mkandawire et al. 2004; Mutlu et al. 2008) as well as our research indicate that different isolates of CBB pathogens may have different levels of pathogenicity on bean genotypes. The objectives of this study were to conduct molecular characterization of different isolates of the CBB pathogen with differential pathogenicity; and to carry out genomic comparisons of CBB pathogens with *Xanthomonas axonopodis* pv. *citri 306* (*Xac*) complete genome.

MATERIALS AND METHODS: Ten to twelve single colonies were purified from each of the four locally collected CBB pathogen isolates, including two non-fuscans isolates i.e., #18 and #98, and two fuscans isolates i.e., #12 and #118 (Yu et al. 2000). All single colonies were characterized with molecular marker X4c-X4e, which was reported to be diagnostic for both fuscans and non-fuscans isolates (Audy et al. 1994). Two plants (four unifoliolates) of susceptible cultivar Nautica were inoculated with each of 42 colonies in a growth room using a multiple needle approach. Disease severity was estimated visually following a 0 to 5 scale at 5, 7, and 10 days after inoculation (Yu et al. 2000). The area under the disease progress curve (AUDPC) was calculated based on three consecutive CBB ratings. Sequencing of four CBB pathogen colonies showing differential pathogenicity was carried out using the illumina Hiseq platform at the Center for Applied Genomics, Toronto, Ontario. Sequencing reads were assembled, aligned, and annotated using various software packages including SOAPDenovo2, MUMmer3.23, Artemis (ACT), RAST and the CLC genomic workbench on a LINUX operating system. Sequences of the four CBB pathogen colonies were compared to the *Xac* complete genome sequence. Thirty-eight known effector genes that were related to virulence on different plant species (Hajri et al 2009; Boureau et al. 2013) were BLASTed against the assembled *Xap* sequences. Statistical analyses were performed using SAS (version 9.2; SAS Institute Inc).

RESULTS: From the 10-12 colonies that were tested from each isolate, 100%, 8%, 30%, and 100% were pathogenic for isolates #18, #98, #12, and #118, respectively, indicating that non-pathogenic Xanthomonads existed in CBB pathogen populations (Fig. 1). The X4c-X4e marker, a diagnostic tool for pathogenic CBB pathogens reported by Audy et al. (1994), was present in all colonies of non-fuscans isolates (#18 and #98), including the non-pathogenic colonies. In contrast, X4c-X4e marker was absent in pathogenic colonies of fuscans isolates (#12 and #118). Bacteria from single colonies from isolate #18 showed different levels of pathogenicity, while colonies in isolate 118 had same level of pathogenicity (Fig. 1). One single colony from each of the four isolates was selected for sequencing. Those four colonies had differential pathogenicities (Table 1, Fig. 1).

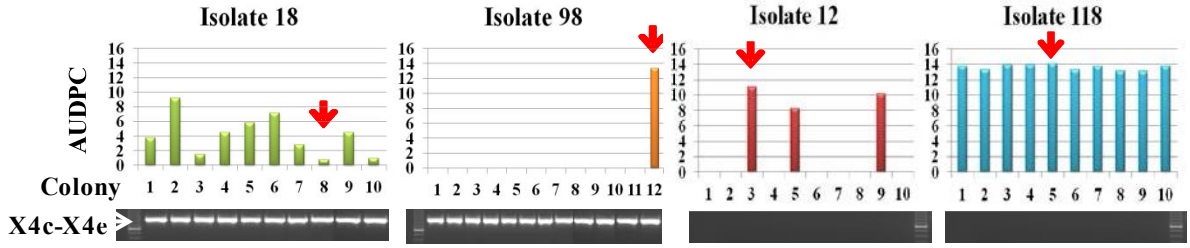


Fig. 1. CBB test and X4c-X4e marker PCR amplification for 10 to 12 single colonies purified from non-fuscans isolates (#18, #98) and fuscans isolates (#12, #118), respectively. Red arrows indicate the colonies selected for sequencing; AUDPC, Area Under Disease Progress Curve.

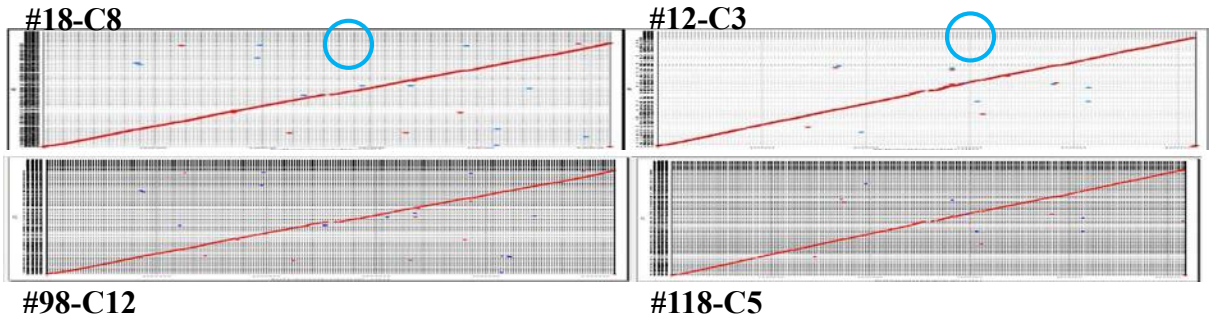
Table 1. Four colonies selected for sequencing

	Colony	Species	AUDPC
#18	C8	Non-fuscans	0.8 a
#98	C12	Non-fuscans	13.3 c
#12	C3	Fuscans	11.1 b
#118	C5	Fuscans	14.1 c

Sequencing reads of four *Xap* colonies were assembled into 544, 1681, 1239, and 1499 contigs respectively. Genome sizes ranged from 5.3-5.4 Mbp with 64.7% G+C content. The number of coding sequences ranged from 4672 to 4747, which was slightly higher than what was reported for the reference genome of *Xac* (4562). Genomic comparisons against the *Xac* complete genome revealed that there is high conservation between the *Xap* colonies and *Xac*. Breaks in the middle of each alignment indicated that the *Xap* sequences have unique sequence regions (Fig. 2).

Missing segments (86 bp) in the X4c-X4e marker sequences were found in fuscans colonies #12-C3 and #118-C5, explaining the lack of X4c-X4e marker PCR amplification for these fuscans isolates. Our results indicate that the presence of the X4c-X4e marker is not always an indication of pathogenicity of Xanthomonas strains on common bean.

Fig. 2. Mummer sequence alignment plots showing high conservation of the sequence alignments between the two non-fuscans (left) and the two fuscans (right) *Xap* colonies with the reference genome of *Xanthomonas axonopodis pv. citri.* str. 306.The circles identify breaks in the alignments.



A BLAST search of the 38 known effector genes revealed that 16 effector genes had matches to at least one of the four *Xap* clone sequences. There were amino acid differences predicted between *Xac* and the four *Xap* colonies, and between fuscans and no-funscans colonies for some effector genes. But, there were no differences within the two fuscans or non-funscans colonies for those 38 effector genes. Assembly of contigs into whole genomes and further searches for unique genes, that might cause differential levels of pathogenicity, are underway.

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RACE STRUCTURE OF *PSEUDOMONAS SYRINGAE* PV. *PHASEOLICOL*A FROM DRY BEAN FIELDS ON THE CANADIAN PRAIRIES

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INTRODUCTION: The major bacterial diseases that impact production of dry bean in the Prairie Provinces are common bacterial blight (CBB) and halo blight (HB) (Table 1). CBB and HB are treated with routine preventative foliar sprays of copper-containing bactericides. Other bacterial diseases, such as brown spot and bacterial wilt, are also common in dry bean fields, and all four bacterial diseases are difficult to distinguish. In order to develop cultivars with resistance to both CBB and HB, the races of the halo blight pathogen present on the Canadian Prairies need to be determined. However, because of the difficulty in identifying bacteria, a molecular diagnostic technique is required to identify and differentiate pathogenic bacteria collected from the field. Several species-specific primers have already been published (Audy et al. 1996; Sorensen et al. 1998; Stevens et al. 1998; Tegli et al. 2002; Toth et al. 1998; Tsiamis et al. 2000), allowing for development of a multiplex PCR assay for efficient screening of field samples for common bacterial pathogens. The objectives of this research were to: 1) Assess incidence of multiple bacterial diseases on dry beans in southern Alberta by field survey, and 2) Determine the prevalent races of the halo blight pathogen from dry bean fields in the Prairie Provinces.

MATERIALS AND METHODS: *Disease surveys:* Dry bean fields in southern Alberta were surveyed bi-weekly for bacterial diseases from July – August in 2012 and 2013. The incidence of blights in each field was calculated as percentage of infected plants, and severity was rated on a scale of 1 (healthy) to 5 (dead). Leaves from new growth were collected from 10-15 fields at each sampling date, except on August 30 when pods from only 3 fields were sampled.

Pathogen identification: One gram of plant tissue, collected from composite samples/fields, was crushed in sterile distilled water, and the supernatant collected. These crude plant extracts were used in a multiplex PCR (Qiagen Multiplex PCR Kit) for detection of pathogenic bacteria, using previously published species-specific primers. The plant extract was also streaked onto Milk-Tween (Goszczynska and Serfontein 1998) agar for isolation of single bacteria colonies. Single colonies were identified according to reaction on this media, and by multiplex PCR.

Race testing: Pph (halo blight) isolates were also obtained from surveys conducted previously in Alberta (2010 and 2011) and Manitoba (2005, 2008 and 2011). *Pph* isolates were categorized into race groupings using PCR primers specific to known avirulence genes and placed in a race-grouping based on presence of Avr*Pph*E (all races), Avr*Pph*F (races 1, 5, 7 and 9), or Avr*Pph*B (races 3 and 4). Presence of *Pph*E, and absence of *Pph*F and *Pph*B indicated isolate belongs to either race 2 or 6 (Stevens et al. 1998; Tsiamis et al. 2000). Their reaction response was then confirmed on detached leaves from the set of 8 bean differential lines (Taylor et al. 1996).

RESULTS AND DISCUSSION: In 2012, *Pph* (halo blight) was the most common pathogen in Alberta bean fields, followed by *Pss* (brown spot) (Table 1). Both were present over the survey period from June to the end of August. *Xanthomonas* spp. were recovered at a high percentage

from plant samples, but PCR assays failed to identify isolates as pathogenic species. Multiple pathogens were detected in 15% of collected samples, and fields with the highest incidence and severity ratings were often infected with multiple pathogens. Bacterial blights were not present in any of the surveyed dry bean fields in 2013. Hot, dry weather during most of August 2013 resulted in conditions that were not favourable for foliar disease development. Multiplex PCR was a successful tool in identifying pathogenic bacteria directly from crude plant extracts, and was invaluable in identifying samples with multiple infections. However, results did not always agree with bacterial isolations onto selective media, indicating that a combination of both methods is required for successful determination of pathogen composition. All *Pph* isolates collected from Alberta and Manitoba in 2005-2012 were either race 2 or 6 based on PCR and screening of the 8 bean differential lines (Table 2). However, screening of all the collected isolates is still ongoing.

Table 1. Bacterial diseases, frequency of isolation from dry bean samples collected in Alberta in 2012, and band size used for PCR identification using species-specific primers.

Disease	**Pathogen**	**Percent isolation**	**PCR band size (bp)**
Common bacterial blight	*Xanthomonas axonopodis* pv. *phaseoli (Xap)*	0	750
	Xanthomonas fuscans var. *fuscans (Xff)*	2	450
Halo blight	*Pseudomonas syringae* pv. *phaseolicola (Pph)*	42	1450
Brown spot	*P. syringae* pv. *syringae (Pss)*	29	790
Bacterial wilt	*Curtobacterium flaccumfaciens* pv. *flaccumfaciens (Cff)*	7	300
N/A	Fluorescent pseudomonads (Pf)	40	980
N/A	*Xanthomonas* spp.	53	

Table 2. Total number of *Pph* isolates recovered from bean fields in each year, belonging to either race 2 or 6, as determined by inoculations onto the bean differential set

Year	2000-2010			2011			2012			2013
	Total #	Race 2	Race 6	Total #	Race 2	Race 6	Total #	Race 2	Race 6	Total #
Alberta	5	5	0	0			30	29	1[a]	10[b]
Manitoba	6	4	2	11[b]	2	3	11[b]	ND		2[b]

[a]Isolate was collected from a red-rooted pigweed plant with halo blight symptoms
[b]Race screening not yet completed for these *Pph* isolates

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ENHANCEMENT OF DRY BEAN PRODUCTION BY SOIL RIPPING AND IRRIGATION INTERVAL

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ABSTRACT: A two-year study compared the performance of pinto bean varieties with different growth habits when inter-row ripped during early vegetative growth to enhance root vigor, production efficiency and return to growers. Type II Croissant and Stampede were compared to Type III Montrose grown as 2 lines (15 cm apart) per 75 cm wide bed at 207564 plants per hectare under furrow irrigated conditions in Colorado. Yields averaged more than 3800 and 2600 kg per hectare with a 200 seed weight of 80 and 67 g in 2012 and 2013, respectively. Soil ripping at planting reduced the AUCC in 2012 but not in 2013, due to less compaction at that research site. The irrigation interval post-flowering demonstrated that the more frequent interval increased the yield of these pinto varieties. Future research will repeat portions of this study, with an emphasis upon measuring the effects of moisture availability post-flowering upon bean plant development and yield responses.

INTRODUCTION

A two-year study was conducted at the Colorado State University (CSU) research farm north of Fort Collins, CO. The objective was to evaluate the performance of pinto bean varieties with more upright growth habits when plants are exposed to stresses such as soil compaction and moisture deficiency such as drought or limited water post-flowering. Upright type II cultivars Croissant (CSU release) and Stampede (North Dakota State University release) were compared to the prostrate type III cultivar Montrose (CSU release).

MATERIALS AND METHODS

A 4-bed Mechanical Transplanter System planted 2 lines per 30-in wide bed at a desired plant population of 207564 seeds / Ha at the CSU research site near Fort Collins in 2012 and 2013; with 4 reps of each 4-bed wide plot by 8 m in length. Standard grower practices were applied for fertilizer, disease, weed and insect management; no fungicide or insecticide treatments were required. Data included plant emergence, node height, biomass, yield as kg / Ha and seed size as 200 seed weight (g). Soil compaction (AUCC = area under the compaction curve) was measured in 2.54 cm increments from the surface to the 45-cm depth with a Field Scout SC 900 Meter; data shown as the mean cumulative psi recorded from 2 sites on top of the middle bed in each plot. All data were analyzed statistically with PC SAS combined over years and locations, as well as for individual location and year effects.

Entries – pinto varieties adapted to northern Colorado growing conditions**:**
'Montrose' – type III vine; 'Croissant' – type II upright vine; 'Stampede' – type II upright semi-vine

Agronomic Treatments:
Inter-row Ripping at Planting to 25 cm depth (2012, 2013) –
YES vs NO

Furrow Irrigation Interval post-flowering to maturity (2013 only) -
7 days (6 weekly applications) vs 14 days (3 biweekly applications)

RESULTS AND DISCUSSION

The 2012 growing season was more favorable than 2013 for bean plant development with trace infection by plant pathogens or insect pests either year. The 2013 conditions were cool during the vegetative and pod fill periods, and apparently contributed to the lower than expected yields. Furrow irrigation (approximately 5 cm per application) and fertility levels were adequate for overall plant development, flowering and pod set stages of growth. Average yields (combined treatments) of the 3 entries averaged 3840 and 2710 kg/Ha with a 200 seed weight of 80 and 67 g in 2012 and 2013, respectively. Soil ripping at planting reduced the AUCC (cumulative area under the compaction curve to a 45 cm depth) in 2012 but not in 2013 with less compaction stress overall at this field location. The 2012 ripping treatment did reduce AUCC by 20% or more; but had no consistent effect on yield response.

The irrigation interval post-flowering demonstrated that the more frequent application increased the yield of Croissant, Montrose and Stampede by 12, 39 and 45 %, respectively; suggesting that varietal response to post-flowering moisture stress can vary significantly. Future research plans will repeat portions of this study, with an emphasis upon measuring the effects of moisture availability post-flowering upon bean plant development and yield responses. We gratefully acknowledge funding for this research provided by the Colorado Dry Bean Administrative Committee, Colorado State University, and edible bean producers and handlers in Colorado.

Table 1. Soil compaction and pinto bean yield during 2012 and 2013 at Fort Collins, Colorado.

		AUCC (45 cm depth)		Yield (kg / Hectare)	
Entry	**Ripped**	2012	2013	2012	2013
Croissant	yes	1894	2153	3684	2479
	no	2592	2290	3571	2371
Stampede	yes	2107	2177	3487	2827
	no	2664	2095	4160	2962
Montrose	yes	1834	2257	4089	2479
	no	2255	2109	4059	2371
	LSD (P = 0.05)	276		non significant	

Table 2. Influence of irrigation interval post-flowering on yield during 2013.

		200 Seed Wt. (g)		Yield (kg / Hectare)	
Entry	**Ripped**	7-day	14-day	7-day	14-day
Croissant	yes	67.4	64.1	2620	2337
	no	65.9	64.9	2305	2237
Stampede	yes	70.3	62.7	3359	2295
	no	70.9	61.4	3488	2437
Montrose	yes	68.9	67.1	3288	2372
	no	69.4	65.9	3247	2342
	LSD (P = 0.05)	3.1		507	

EFFECT OF FOUR PVTFL1Y HAPLOTYPES CONFERRING DETERMINATE GROWTH HABIT ON PHENOLOGY, ARCHITECTURE AND YIELD IN *PHASEOLUS VULGARIS*

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INTRODUCTION: Domestication of *Phaseolus vulgaris* resulted in selection of plants with determinant growth habits that develop a terminal inflorescence, flower earlier, and have a markedly different architecture compared to determinate wild types that develop phytameric units continuously until senescence. A QTL for growth habit, mapped in our lab, was found on chromosome 1 and was found to co-segregate with the phenotypic locus[1], *fin*, known to be associated with growth habit. At that locus a candidate gene, PvTFL1y, was shown to have similar structure and function as *A. thaliana* TFL1, known to be responsible for maintenance of totipotency in the central zone of the apical meristem[3]. Moreover, amongst 349 wild and domesticated common beans surveyed for their TFL1y haplotype, 70% of determinate types have a 4.1 Kb retrotransposon in the fourth exon, which alters expression of the gene[2,3]. To better understand the effect of the four most frequently observed PvTFL1y haplotypes on growth habit, phenology, architecture and yield, we back crossed determinate types to indeterminate types from the same gene pool and conducted a field trial during summer 2013.

MATERIALS AND METHODS: To create backcross populations for field evaluation, determinate Andean and Mesoamerican types with four different PvTFL1y haplotypes, Midas, G04627, G00750 and G00705, were used as donor parents in crosses to indeterminate parents, Bat93 or JaloEEP558, from the same gene pool. Before each cross, plants were screened using PCR primers specific to the PvTFL1y locus to confirm their haplotype (work done by Shelby Repinski). After backcrossing to the recurrent parents three times, plants were selfed twice to create homogeneity in the populations.

In a field trial, conducted during summer 2013 at UC Davis, donor, recurrent and backcrossed plants were grown in single row 20 ft plots (*see* Table 1, Figure 2). A randomized complete block design using three replicates per line was used to statistically quantify the effect of the four different PvTFL1y haplotypes on days to first flower, flowering duration, length of first internode, length of fifth internode, internode ratio, number of internodes, yield, and 100 seed mass. For first internode, fifth internode and internode number, five plants per plot were sampled at random and measured. Statistical analysis was performed using a one-way ANOVA in SAS with alpha = 0.05. Means comparisons were done using Tukey tests with alpha 0.05 (*see* Figure 1).

RESULTS: Days to first flower and flowering duration were significantly less between determinate types Midas and JaloEEP558xMidas BC_3F_2 plants compared to the indeterminate, recurrent parent, JaloEEP558. The other determinate backcross lines did not display mean flowering times that were significantly different from their recurrent parent lines, suggesting the Midas haplotype does decrease days to first flower and flowering duration. These results also suggest a possible explanation for why the Midas haplotype (4.1 Kb retrotransposon insertion) is present in 70% of domesticated accessions previously analyzed[2].

Comparing the backcross lines to their determinate or indeterminate parents for first and fifth internode lengths, the backcross lines had mean first and fifth internode lengths closer to their

indeterminate, recurrent parents. This is consistent with the assumption that the majority of the genome in BC_3F_2 lines is like that of the recurrent parents, and the QTL for architecture would be more like that of the indeterminate lines.

Table 1 – Donor, recurrent, and backcross lines evaluated in summer 2013 field trial at UC Davis.

	Line	Mutant PvTFL1y haplotype
Donor Parents (Indeterminate)	JaloEEP558	
	Bat93	
Recurrent Parents (Determinate)	Midas	4.1 Kb retrotransposon insertion
	G00750	T to A at +435 at end of exon 2 (putative splice site mutation)
	G04627	2 bp insertion at +21 in first exon and A to C @ +439
	G00705	whole gene deletion
Backcross Lines (Determinate)	BC3F2 JaloEEP558 x Midas	4.1 Kb retrotransposon insertion
	BC3F2 Bat93 x G00750	T to A at +435 at end of exon 2 (putative splice site mutation)
	BC3F2 JaloEEP558 x G04627	2 bp insertion at +21 in first exon and A to C @ +439
	BC3F2 JaloEEP558 x G00705	whole gene deletion

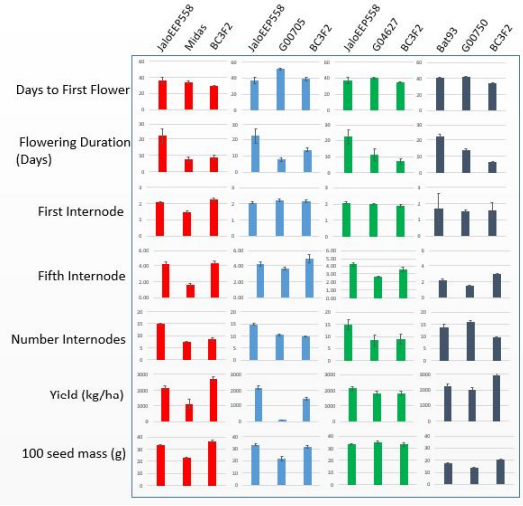

Figure 1 - Results of measurements for phenology and architecture between donor, recurrent, and backcross lines.

Figure 2 - Recurrent, donor and backcross lines planted in the summer 2013 field trial.

The determinate donor parents, Midas, G00750 and G04627, had significantly shorter internodes and fewer internodes, compared to the BC_3F_2 and indeterminate lines; however their yield was not significantly higher compared to lines with greater vegetative branching. This suggests different mutant PvTFL1y haplotypes do not increase their allocation of carbon to reproductive structures concurrent to the decrease in energy they spend on shoot development.

Indeterminate types, as well as the determinate type G00750, had significantly more internodes compared to the other determinate parents and the BC_3F_2 lines. This is consistent with the finding that loss or alteration of function of PvTFL1y results in the termination of the axillary meristem in a terminal inflorescence.

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ANALYSES OF COMPLEX MENDELIAN SEGREGATIONS FOR ANTHRACNOSE RESISTANCE IN THE RIL POPULATION Xana/Cornell 49242

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Anthracnose in common bean is caused by the fungus *Colletotrichum lindemuthianum* (Sacc.&Magnus) Lambs-Scrib. In this work, response against five *C. lindemuthianum* isolates classified as races 3, 6, 19, 73, and 449 was analyzed in a RIL population derived from the cross XanaxCornell49242 (XC RIL population).

MATERIALS AND METHODS: A population of 120 $F_{2:6}$ recombinant inbreed lines (RIL) developed from the cross Xana/Cornell 49242 by single seed descent method was used. Resistance tests were carried out in accordance with standard methods (see Ferreira et al. 2013). A genetic linkage map developed in this population (Pérez-Vega et al. 2010) was used as support for the identification of genes or chromosome regions involved in the resistance. When the segregation ratio observed suggested the presence of one resistance gene, it was directly mapped. For resistances controlled by more than one gene, contingency chi-square analyses corresponding to the joint segregation for each resistance with markers included on the linkage map were analyzed. To confirm this, segregation of resistance was analyzed within subpopulations established from the total RIL population.

RESULTS AND DISCUSSION: Xana was resistant to races 3, 19, 73, and 449, and susceptible to race 6. Cornell49242 was resistant to races 3, 6, 19, and 449, and susceptible to race 73. A monogenic segregation was observed for resistance to races 6 (47 Resistant:38 Susceptible, $\chi^2_{1:1}$= 0.95, p>0.05) and 73 (37R:40S, $\chi^2_{1:1}$= 0.12, p>0.05). These genes were directly mapped on linkage groups (LGs) Pv11 and Pv01, respectively (see Figure 1). A segregation ratio corresponding to a 5R:3S (expected for three independent genes, two of them with a complementary mode of action) was observed for resistance to races 3 (67R:35S, $\chi^2_{5:3}$= 0.44, p>0.05), 19 (71R:35S, $\chi^2_{5:3}$= 0.91, p>0.05) and 449 (64R:39S, $\chi^2_{5:3}$= 0.01, p>0.05). Contingency chi-square analyses revealed a significant deviation with markers tagging anthracnose resistance clusters Co-2 (on LG Pv11) and Co-3 (on LG Pv04), and with markers of LG Pv02 that tag the physical position of 40Mb, deduced from the position of Indel markers (Table 1). For resistance to race 3, subpopulation analyses revealed changes in the segregation within subpopulations involving LG Pv02 (Table 2). This situation can be explained if one complementary resistance gene from Xana is located at this position of LG Pv02, being this gene fixed in the X-Pv02 subpopulation while the opposite C-Pv02 subpopulation lacks it. Similarly, segregation ratios observed within the Co-3 subpopulations can be explained if one complementary gene from Xana is located at this position. In the C-Co-2 subpopulation almost all lines were resistant to race 3 (except one susceptible line, probably due to recombination). A good fit to a 1 R: 3 S ratio, expected for two complementary genes, was observed in the opposite X-Co2 subpopulation. This result can be explained if the resistance gene without complementary mode of action derived from Cornell 49242 and was located at the Co-2 cluster. The same scenario deduced for resistance to race 3 can be concluded for races 19 and 449 (Figure 1). Results shown herein reveal a complex and specific interaction between bean and fungus genotypes leading to anthracnose resistance.

Table 1. Contingency-chi square tests corresponding to the joint segregation of races 3, 19 and 449 with six molecular markers which tag three different chromosome regions. LG= linkage group; ns= not significant, *= 0.05>p>0.01, and **= 0.01>p.

			Race 3		Race 19		Race 449	
Marker	LG	Resistance cluster	Cont. χ^2	p	Cont. χ^2	p	Cont. χ^2	p
NDSU_IND_2.403966	Pv02	-	4.24	*	3.56	ns	3.85	*
NDSU_IND_2.404411	Pv02	-	4.51	*	3.79	*	4.23	*
254-G15F$_{550}$	Pv04	Co-3	15.62	**	14.11	**	11.85	**
SW12	Pv04	Co-3	17.99	**	16.58	**	13.91	**
SQ4	Pv11	Co-2	32.54	**	38.09	**	35.56	**
SCAreoli	Pv11	Co-2	35.24	**	41.90	**	38.89	**

Table 2. Observed segregations for resistance to races 3, 19 and 449 within six subpopulations. X-Pv02 and C-Pv02: subpopulations formed by RILs showing the Xana and Cornell49242 allele, respectively, for IND_2_403966 and IND_2_404411 markers; X-Co-3 and C-Co-3: subpopulations formed by RILs showing the Xana and Cornell49242 allele, respectively, for 254-G15F550 and SW12 markers tagging the Co-3 anthracnose resistance cluster; X-Co-2 and C-Co-2: subpopulations formed by RILs showing the Xana and Cornell49242 allele, respectively, for SQ4 and SCAReoli markers tagging the Co-2 anthracnose resistance cluster. ns, not significant.

	Subpopulations considered within the XC RIL population																							
	X-Pv02					C-Pv02					X-Co-3					C-Co-3					X-Co-2					C-Co-2				
Race	Ob freq (R:S)	Ratio	χ^2	p		Ob freq (R:S)	Ratio	χ^2	p		Ob freq (R:S)	Ratio	χ^2	p		Ob freq (R:S)	Ratio	χ^2	p		Ob freq (R:S)	Ratio	χ^2	p		Ob freq (R:S)	Ratio	χ^2	p	
3	39:13	3:1	0.00	ns		25:21	1:1	0.35	ns		32:4	3:1	3.70	ns		14:20	1:1	1.06	ns		16:26	1:3	3.84	ns		34:1	-	-	-	
19	42:14	3:1	0.00	ns		26:20	1:1	0.78	ns		33:4	3:1	3.97	ns		17:20	1:1	0.24	ns		16:26	1:3	3.84	ns		38:0	-	-	-	
449	37:15	3:1	0.41	ns		24:23	1:1	0.02	ns		30:7	3:1	0.73	ns		13:21	1:1	3.18	ns		13:29	1:3	0.79	ns		34:2	-	-	-	

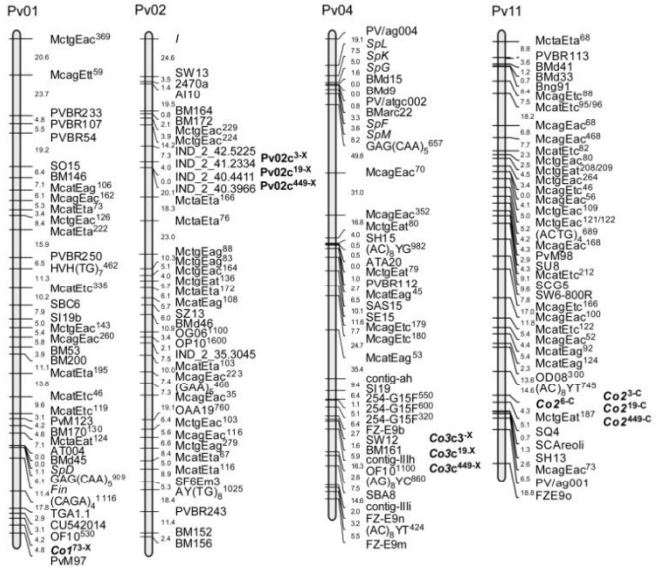

Figure 1. Linkage groups Pv01, Pv02, Pv04 and Pv11 in which anthracnose resistance genes were directly or indirectly located in the XC RIL population. Resistance genes are named by using its location at anthracnose resistance clusters previously described (Co-cluster) or linkage group, "c" for genes showing a complementary mode of action, name of the isolate or race (in superscript), followed by the bean genotype in which the resistance gene was identified in superscript (X, Xana, C,Cornell49242).

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BLACK DRY BEAN GERMPLASM RESOURCES RICH IN RESISTANCE TO ANTHRACNOSE

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INTRODUCTION: Dry bean is prone to diseases. One of the most serious diseases is anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cav. Anthracnose resistance has been reported in various bean market classes including in black bean. Using artificial inoculation and molecular markers, Dongfang et al. (2008) revealed the presence of *Co-1*, *Co-2*, and *Co-3* genes in the twenty selected cultivars grown in Manitoba, Canada. However, not one of the black beans evaluated had resistance to either race 73 or 105, the major anthracnose races in the region. The black bean cultivars currently grown in the region are generally susceptible to anthracnose. Chemical control of anthracnose has been the common practice in commercial production, but it is costly. Use of resistant cultivars is considered the most effective management strategy for control of anthracnose in dry bean. This study was conducted to evaluate black bean germplasm for resistance to races 73 and 105, and provide much needed publicly available breeding materials for dry bean cultivar development in Canada.

MATERIALS AND METHODS: One hundred and fifty-seven dry bean (*Phaseolus vulgaris*) accessions were introduced in 2008 from the *Phaseolus* Germplasm Collection, USDA-ARS National Plant Germplasm System, Pullman, Washington, USA. The accessions were increased in the greenhouse during the summer of 2008 at the Morden Research Station. Spore suspensions of *C. lindemuthianum* were prepared from 2-4 week old colonies on PDA medium cultured at 22ºC following the procedures of Conner et al. (2009). Inoculation and screening were conducted following Dongfang et al. (2008). Plants were rated using a scale of 0-9 as described by Tu and McNaughton (1980) with 0 = no infection symptoms on plants and 9 = plants with more than 90% leaf veins with symptoms or the leaves were dead. Disease severity (DS) was determined as $DS = \Sigma(nr)/t$, where n = number of plants, r = plant rating (scale 0-9), and t = total number of plants rated. Accessions that had an average anthracnose disease severity above 3 in any of the tests were considered susceptible (Dongfang et al. 2008).

RESULTS AND DISCUSSION: Black bean is one of the most widely grown dry bean market classes in the world, especially in the Americas. The 157 accessions originated in twenty countries with most from Mexico and Guatemala, the region considered to be the centre of origin for Mesoamerica black beans (Table 1). While screening of these materials at Morden, 35 accessions did not flower and were considered photoperiod-sensitive. Seeds of these lines were reproduced under short day (14 h) conditions.

The reactions of the black bean accessions to both races 73 and 105 of *C. lindemuthianum* were determined. Resistance to anthracnose race 73 was identified in 41 accessions with a disease severity equal to or less than 3.0. Of these, 39 were also resistant to race 105. Many lines had an intermediate disease index, indicating that selection had not previously been made for anthracnose resistance in these accessions. The majority (36) of the accessions resistant to race 73 and/or 105 were from Mexico and Guatemala and 24 of these accessions were photoperiod-sensitive. Resistance to anthracnose was also identified in accessions from Costa Rica,

Honduras, Japan, Ethiopia and Turkey. Not one of the 13 accessions originating from the USA had resistance to either race 73 or race 105. While the genetic controls of the anthracnose resistance in the germplasm are subjected to further study, the materials provide much-needed genetic diversity and resistance source for dry bean improvement in Canada.

Table 1. Origin, number, photo-period sensitivity and anthracnose resistance to races 73 and 105 of black bean accession.

Origin	Number of collection	Number of accessions photo-period sensitive	Number of accession resistant to race 73	Number of accessions resistant to race 105
Afghanistan	1	1	0	0
Brazil	2	0	0	0
Chile	6	0	0	1
China	2	1	0	0
Costa Rica	4	0	1	1
El Salvador	1	0	0	0
Ethiopia	1	0	1	0
France	1	0	0	0
Guatemala	28	10	10	11
Honduras	5	3	3	3
India	1	0	0	0
Japan	1	0	1	1
Mexico	81	17	24	21
Puerto Rico	1	0	0	0
Turkey	1	1	1	1
United States	13	0	0	0
Venezuela	1	0	0	0
Zimbabwe	2	0	0	0

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GENETIC ANALYSIS OF THE REACTION TO NINE *Colletotrichum lindemuthianum* RACES IN THE RIL POPULATION AB136xMDRK

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In this work, the inheritance of resistance against nine *Colletotrichum lindemuthianum* races, was analyzed in a RIL population derived from the cross AB136xMDRK. Both bean genotypes are included in the set of twelve differential cultivars used in the characterization of pathogenic variants of this fungus.

Materials and Methods

A population of 132 $F_{2:6}$ recombinant inbreed lines developed from the cross AB136xMDRK by single seed descent method was used. Races 3, 7, 19, 38, 39, 65, 102, 449, and 1545 were used in the resistance tests, conducted according to standardized methods. A genetic linkage map of 120 loci, including markers that tag the main anthracnose resistance clusters identified in common bean, was previously developed in this population. Genetic analyses were conducted using contingency chi-square tests corresponding to the joint segregation for the response to each race and the molecular markers that tag the main anthracnose resistance clusters. A significant deviation of the chi-square value from the expected random segregation suggests that the chromosome region labeled with the marker locus can be involved in the resistance response.

Results and Discussion

Segregation for resistance to races 3, 38, 449, and 1545 showed a good fit to a 3 resistant (R): 1 susceptible (S) ratio, expected for two independent genes (Table 1). Segregation for resistance to races 7, 19, 39, 65, and 102 showed a good fit to a 7 R: 1 S ratio expected for three independent genes (Table 1).

	Parental phenotypes		RIL population						
			Observed		Expected				
Race	AB136	MDRK	R	S	R	S	Ratio (R:S)	$\chi 2$	*p*
3	R	S	75	19	70.5	23.5	3:1	1.15	0.28
38	R	S	76	19	71.3	23.8	3:1	1.27	0.26
449	R	R	66	32	73.5	24.5	3:1	3.06	0.08
1545	S	R	74	23	72.8	24.3	3:1	0.09	0.77
19	R	S	79	11	78.8	11.3	7:1	0.01	0.94
39	R	S	90	15	91.9	13.1	7:1	0.31	0.58
102	R	S	87	8	83.1	11.9	7:1	1.45	0.23
65	R	R	79	8	76.1	10.9	7:1	0.87	0.35
7	R	S	84	3	31.5	4.5	7:1	3.11	0.08

Table 1. Observed segregations for resistance to nine *C. lindemuthianum* races in the ABM population

Table 2 shows contingency-chi square test values for the reaction to each race and markers that tag anthracnose resistance clusters Co-1, Co-2, Co-3 and Co-5. Resistance to races 3 and 38, controlled by two independent genes from AB136, was significantly associated with markers that tag the Co-5 and Co-2 clusters, indicating that resistance genes against both races were located at these positions (Figure 1). Resistance to race 449, controlled by two genes, was significantly associated with markers that tag the clusters Co-3 and Co-5. Considering the genotype (AB136 & MDRK) in the RIL population for markers tagging both clusters was possible deduce that the gene located at Co-5 derived from AB136 and the gene located at Co-3 derived from MDRK.

Resistance to race 1545 was controlled by two independent genes from MDRK. Contingency tests revealed significant association with markers tagging clusters Co-1 and Co-3. Resistance to races 19, 39, and 102, controlled by three independent genes in AB136, was significantly associated with markers located at cluster Co-5 and Co-2. The location of the third gene was not revealed by contingency tests. Resistance to race 65, controlled by three independent genes, was associated at Co-1, Co-3 and Co-5 clusters. Genotype of these markers suggested that the resistance gene located at Co-5 derived from AB136, and the genes located at Co-1 and Co-3 derived from MDRK. Resistance to race 7 was controlled by three genes from AB136, one of them associated to the Co-5 cluster. Mapping saturation and genetic dissection of these resistance genes are currently being developed in order to verify these preliminary analyses. In any case, results revealed the complexity of anthracnose resistance in the differential cultivars AB136 and MDRK.

Table 2. Contingency-chi square tests corresponding to the joint segregation of races 3, 7, 19, 38, 39, 65, 102, 449, and 1545 with eleven markers that tag chromosome regions including anthracnose resistance loci. LG= linkage group; ns= not significant, *= 0.05>*p*>0.01, and **= 0.01>*p*

			Race 3		Race 38		Race 449		Race 1545		Race 19		Race 39		Race 102		Race 65		Race 7	
	LG	*Co* locus	Con. χ^2	p	Con. χ^2	p	Con. χ^2	p	Con. χ^2	p	Con. χ^2	p	Con. χ^2	p	Con. χ^2	p	Con. χ^2	p	Con. χ^2	p
CV542014	Pv01	*Co-1*	0.87	ns	1.84	ns	0.00	ns	37.97	**	0.10	ns	3.90	ns	0.05	ns	9.45	**	1.80	ns
TGA1.1	Pv01	*Co-1*	0.63	ns	0.90	ns	0.06	ns	33.86	**	0.05	ns	1.90	ns	0.04	ns	13.16	**	2.14	ns
SAH18	Pv04	*Co-3*	1.40	ns	0.01	ns	3.48	ns	7.64	**	2.82	ns	0.74	ns	0.50	ns	2.25	ns	1.71	ns
Pvctt001	Pv04	*Co-3*	0.23	ns	0.78	ns	26.69	**	11.31	**	3.00	ns	1.32	ns	1.41	ns	4.93	*	0.02	ns
SF10	Pv04	*Co-3*	0.09	ns	1.20	ns	8.28	**	5.42	*	0.34	ns	1.35	ns	0.36	ns	0.84	ns	1.55	ns
Phs	Pv07	*Co-5*	5.88	*	7.71	**	9.68	**	0.83	ns	7.96	**	6.64	**	13.02	**	0.41	ns	0.25	ns
Pv-atcc003	Pv07	*Co-5*	11.47	**	14.33	**	18.55	**	0.16	ns	12.32	**	12.70	**	5.08	*	1.86	ns	0.88	ns
SZ4b	Pv07	*Co-5*	24.11	**	30.20	**	31.60	**	0.15	ns	14.21	**	13.47	**	3.69	ns	4.87	*	4.57	**
SCARAZ20	Pv07	*Co-5*	17.06	**	18.15	**	15.38	**	0.04	ns	8.97	**	5.58	*	0.03	ns	1.90	ns	2.54	ns
SH13b	Pv11	*Co-2*	7.03	**	4.79	*	2.02	ns	0.47	ns	6.08	*	7.10	**	9.90	**	0.55	ns	0.35	ns
PVag001	Pv11	*Co-2*	7.64	**	7.25	**	3.12	ns	0.20	ns	10.07	**	8.49	**	14.03	**	0.84	ns	2.67	ns

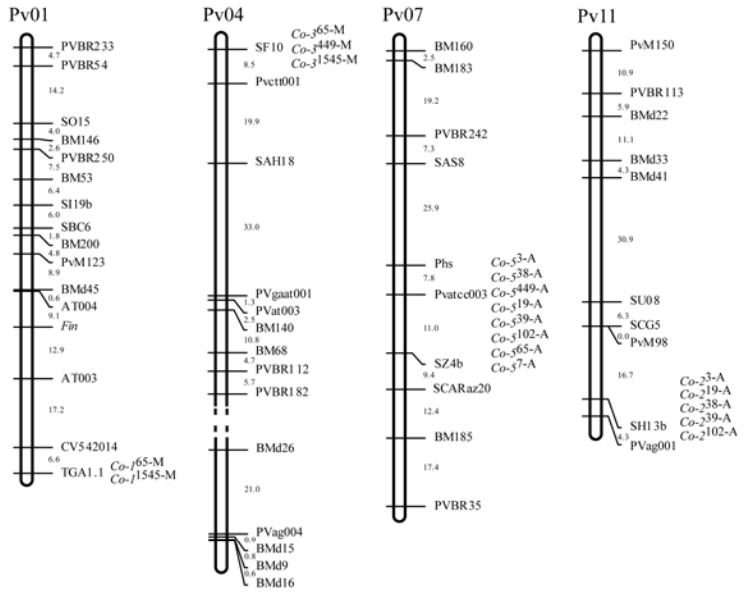

Figure 1. Linkage groups Pv01, Pv04, Pv07 and Pv11 obtained from the RIL population AB136/MDRK showing the tentative positions of the anthracnose resistance genes. Resistance genes are named by using its location at anthracnose resistance clusters previously described (Co-cluster), name of the isolate or race (in superscript), followed by the bean genotype in which the resistance gene was identified in superscript (A, AB136; M, MDRK).

IDENTIFICATION OF ANTHRACNOSE RESISTANCE SOURCES OF COMMON BEAN (*Phaseolus vulgaris* L.)

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INTRODUCTION

Anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cav., is one of the major diseases of common bean (Singh and Schwartz, 2010). One of the most practical and economic method of managing and controlling common bean anthracnose is genetic resistance (Silva et al., 2007). Therefore, the objective of this work was to evaluate the reaction of common bean accessions inoculated with races 2, 65 and 2047 of *C. lindemuthianum.*

MATERIAL AND METHODS

A total of 103 common bean accessions from Núcleo de Pesquisa Aplicada à Agricultura (Nupagri) Germplasm Bank were evaluated with races 2, 65 and 2047. Ten plants of each accession were inoculated separately with races. The pathogen spores were obtained according to the methodology proposed by Cárdenas et al. (1964) and adjusted to a final concentration of 1.2×10^6 conidia/ml. After inoculation, plants were maintained at >95% relative humidity at 21-23^{o}C and 16-h day length (light intensity of 300 micromoles m^{-2} s^{-1} at 1 m height) in a mist chamber for 3 days. After this period, the plants were removed from the mist chamber and transferred to benches in a greenhouse with suitable environment at 22^{o}C with artificial light (12-h day length) for seven days. Anthracnose disease reactions were rated visually using a scale from 1 to 9 (Pastor-Corrales et al., 1995). Plants with disease reaction scores between 1 and 3 were considered resistant, whereas plants that rated 4-9 were considered susceptible.

RESULTS AND DISCUSSION

The resistance index, which measures the genotype response average across all the distinct Andean and Mesoamerican races of *C. lindemuthianum*, ranged from 0% to 100%. High levels of resistance were observed in accessions of the Andean origin with 11 (10.7 %) of the resistant accessions to all *C. lindemuthianum* races against 4 (3.9 %) of Mesoamerican origin. Differential response was observed between the races. The pathogenicity index in this study ranged from 39% to 77%. The Mesoamerican races 65 were the most virulent, infecting 61 % and 77 % of Andean and Mesoamerican accessions, respectively (Table 1).

Of the 103 accessions evaluated only 15 accessions (Jalo Pintado II, Rosinha Opaco, Carnaval 1 SC, Carnaval 2 SC, Preto SC, Porto Real, Amendoim Cavalo, Jalo de Listras Vermelha, Jalo Vermelho, Carnaval 1 PR, Bolinha 1 PR, Jalo A, Jalo EEP558, Feijão Moro and Fogo na Serra) were resistant to all races of *C. lindemuthianum* and can be considered as valuable sources of resistant in future bean breeding programs.

Table 1. Disease reaction of Mesoamerican and Andean accessions to races 2, 65 and 2047 of *C. lindemuthianum*

Reation	Races						Total
	2		65		2047		
	A	MA	A	MA	A	MA	
Resistant	28 (27.2%)	23 (22.3%)	18 (17.4%)	13 (12.6%)	21 (20.4%)	27 (26.2%)	15 (14.6%)
Susceptible	18 (17.5%)	34 (33.0%)	28 (27.2%)	44 (42.7%)	25 (24.3%)	30 (29.1%)	26 (25.2%)

A: Andean Cultivars; MA: Mesoamerican Cultivars
(%) Represent the percentage of accession that was resistant or susceptible within the Andean and Mesoamerican.

CONCLUSION

The results show that both Andean and Mesoamerican bean accessions evaluated in this study are genetically highly variable in response to different races of *C. lindemuthianum*. Some of these accessions would be valuable in future bean breeding programs as new sources of resistance to anthracnose.

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MOLECULAR CHARACTERIZATION OF ANTHRACNOSE RESISTANCE TO RACE 73 IN THE NAVY BEAN VARIETY BOLT

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INTRODUCTION: Anthracnose of common bean *(Phaseolus vulgaris* L.), caused by the fungus *Colletotricum lindemuthianum*, is a destructive seed-borne disease of dry beans worldwide (Barcelos *et al*., 2011), which may cause yield losses as high as 100% (Schwartz *et al*., 2005). Anthracnose can infect all plant parts including stems, leaves, pods, and seeds (Agrios, 2005). Most commercial navy bean varieties grown in Central Canada are susceptible to the prevalent race of *C. lindemuthianum*, race 73, which was first reported in Ontario in 2003.

MATERIAL AND METHODS: 126 $F_{4:6}$ recombinant inbred lines (RILs) of a cross between Bolt (resistant) and the susceptible genotype H4784A-29844, the two parental lines, 12 differential lines with known anthracnose resistance genes (Dongfang et al., 2008), and 4 check cultivars were evaluated for resistance to anthracnose and genotyped with genome-wide single nucleotide polymorphic (SNP) markers. Field trials were planted at the University of Guelph Elora Research Station and Huron Research Station in 2012 and 2013. The experiment in each site was grown in a two-replication 12 × 12 unbalanced square lattice design, in which each genotype in each site/replication was one experimental unit. Plots were inoculated by growing spreader rows around the experimental units (at all sites) and by inoculation with conidial suspension at flowering stage (except at Elora in 2012). Anthracnose severities were rated using a 0 to 10 visual scale, 4 times during the growing season starting when the susceptible check was 60% infected and repeated every 7 days until maturity. The Area Under the Disease Progress Curve (AUDPC) was estimated based on four ratings. DNA was extracted from young leaves harvested from each RIL following Yu et al. (1999). RILs were genotyped with a panel of 786 SNP markers, from which 86 were found polymorphic between the parental lines. Linkage and physical maps were formed using JoinMap 4 (Van Ooijen JW, 2006) and MapChart 2.2 (Voorrips, 2002). Single marker QTL analysis was performed using PROC GLM in SAS 9.3(SAS Institute, 2010.) Four candidate genes, all from the kinase gene family were identified in the region defined by significant SNP markers (Phaseolus reference genome sequence; http://www.phytozome.net). Primers were designed to amplify the coding region of the candidate genes in the resistant and susceptible parental lines. PCR products were sequenced, assembled, and compared with the sequence of the genes in the reference genome. Amino acid sequence of the amplified sequences were predicted and compared with that of the reference genome.

RESULTS AND DISCUSSION: Anthracnose severity in Elora Research Station was low in 2012 and high in 2013, due to an unusually dry and wet season, respectively. In Huron Research Station in 2012 and 2013, anthracnose severity of susceptible lines reached rating of 10. The frequency distribution of AUDPC in all tests followed a bi-modal distribution with high frequency of lines in the two ends of the disease scale (Figure 1). Linkage and physical maps were constructed for the polymorphic markers and single marker QTL analysis was performed to identify genomic regions associated with resistance to Anthracnose in Bolt. Two SNP markers in a 3.2 cM region on Pv01, corresponding with a 300 kbp region on the physical map, were found to be significant QTL for anthracnose AUDPC and accounted for up to 85% of the

variation (Table 1). Four candidate genes were identified in the region defined by the significant SNP markers (Figure 2). Analysis of the candidate gene sequence Phvul.001G243500 identified a frame shift mutation caused by a 10 bp insertion in the susceptible genotype that leads to premature stop codon and a shortened protein. Of the previously identified anthracnose resistance genes, only Co-1, from the Andean gene-pool, has been reported on chromosome 1, a susceptible response of Bolt to race 105 points to *Co-1*^{2} as the most likely allele of the gene in Bolt.

Table1. Summary of the results of single marker QTL analysis for anthracnose AUDPC of the RILs in anthracnose nurseries in Huron and Elora Research Stations in 2012 and 2013

				Huron 2012		Huron 2013		Elora 2012		Elora 2013	
SNP Marker	Chr	cM	Mbp	R^2_p	Additive Effect	R^2_p	Additive Effect	R^2_p	Additive Effect	R^2_p	Additive Effect
Pv09sc00008p 1574781	1	61.3	50.5	0.85	87.52	0.62	46.03	0.54	23.77	0.57	45.03
Pv09sc00008p 1922017	1	58.1	50.2	0.84	87.22	0.62	46.04	0.54	23.78	0.57	45.02

cM represents the position on the linkage map developed using the RIL population in this study, while Mbp refers to the physical position of the SNP markers in the reference genome. R^2_p is the proportion of variance accounted for by the marker in a linear model.

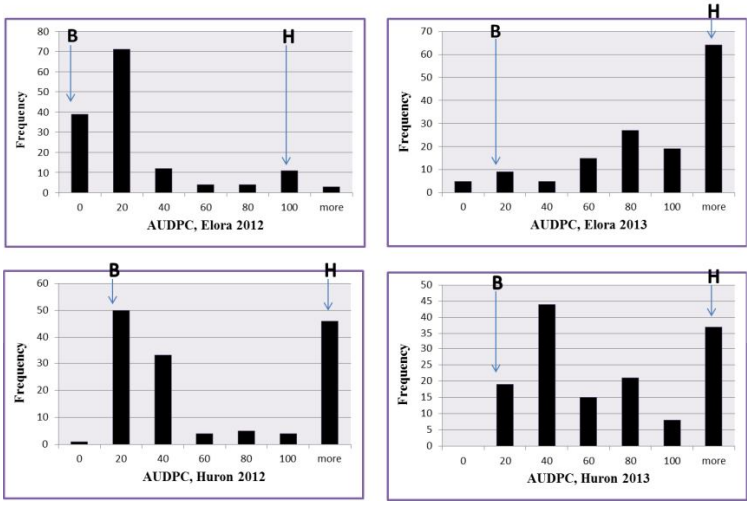

Fig 1. Frequency distribution of AUDPC for the RIL population in 2012 and 2013 at the Elora and Huron Research Stations. Scores of the parental lines are indicated on each graph by B (Bolt) and H (H4784A-29844)

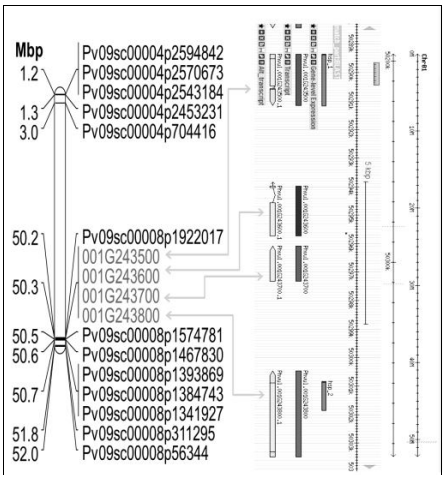

Fig 2. Four anthracnose candidate kinase genes in chromosome 1.

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INHERITANCE AND ALLELIC RELATIONSHIPS OF ANTHRACNOSE RESISTANCE IN COMMON BEAN PALOMA CULTIVAR

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INTRODUCTION

Anthracnose, caused by *Colletotrichum lindemuthianum* L., is one of the most important fungal diseases of common bean (*Phaseolus vulgaris,* L) (Pastor-Corrales and Tu 1989). Genetic resistance is the most effective method for this disease control. So far, twenty one resistance genes have been already characterized, and among them, only eight are Andean and due to it, the search for Andean resistance sources is really necessary. This way, the work aimed to characterize the genetic resistance of Andean cultivar Paloma.

MATERIAL AND METHODS

Genetic characterization of Paloma cultivar, was carried out through inheritance and allelism tests. Inheritance test was conducted in F_2 population from the cross between Paloma (resistant) and Cornell 49-242 (susceptible) cultivars, by using race 2047 of *C. lindemuthianum.*

Allelism tests were conducted in 16 F_2 populations derived from crosses between Paloma cultivar (R) and the other resistant cultivars that have genes previously characterized. Pathogen races used in these tests were: 65, 73 and 2047. The parental, F_1 and F_2 populations derived from each cross of Paloma with the differential cultivars and other sources of resistance to anthracnose were inoculated with a spore suspension of *C. lindemuthianum* at 1.2×10^6 esporos.mL^{-1} concentration from each race. Visual evaluation of symptoms was conducted ten days after inoculation. Anthracnose disease reactions were rated visually using a scale from 1 to 9 (Pastor-Corrales et al., 1995). Plants with disease reaction scores between 1 and 3 were considered resistant, whereas plants that rated 4-9 were considered susceptible. The genetics analysis of F_2 populations were performed through Chi- Square Test (χ^2) using Genes Software (Cruz, 2006).

RESULTS AND DISCUSSION

The results of inheritance and allelism tests are disposed in Table 1. The inheritance studies demonstrated a 3R:1S ratio in the F_2 population from the cross between Paloma and Cornell 49-242 ($p = 0.77$), indicating the presence of single dominant gene in Paloma, conferring resistance to race 73. The allelism tests fitted to 15:1 R/S ratio in 16 F_2 populations from the crosses (R × R) involving Paloma and the cultivars Michigan Dark Red Kidney, Cornell 49-242, PI 207262, TO, TU, AB 136, G 2333, Ouro Negro, Jalo Vermelho, Jalo Listras Pretas, Pitanga, Corinthiano, Crioulo 159, Jalo Pintado 2, Perla and Amendoim Cavalo (Table 1). These results indicating the action of two dominant resistance genes, one of them present in the Paloma cultivar and the another in the remaining cultivars. Moreover, allelism tests revealed that the gene present in Paloma is independent from those previously characterized.

Table 1. Disease reaction in F_2 populations from R × S and R × R crosses for the genetic characterization anthracnose resistance in Paloma

Crosses	Race	Resistance Gene	Observed Ratio		Expected Ratio	χ^2	*p* value
			R[a]	S[b]	R:S		
Paloma (A) × Cornell 49-242 (MA)	2047	*Co-2*	73	26	3:1	0.084	0.77
Paloma × Cornell 49-242 (MA)	65	*Co-2*	84	6	15:1	0.027	0.87
Paloma × TO (MA)	65	*Co-4*	83	6	15:1	0.037	0.85
Paloma × PI 207262 (MA)	65	*Co-4*^{3}	70	6	15:1	0.351	0.55
Paloma × TU (MA)	65	*Co-5*	109	6	15:1	0.209	0.65
Paloma × AB 136 (MA)	65	*Co-6*	88	6	15:1	0.003	0.96
Paloma × Jalo Vermelho (A)	65	*Co-12*	47	3	15:1	0.005	0.94
Paloma × JLP[d] (A)	65	*Co-13*	92	6	15:1	0.003	0.96
Paloma × Perla (A)	65	*	111	19	15:1	0.320	0.57
Paloma × MDRK[c] (A)	73	*Co-1*	92	8	15:1	0.523	0.47
Paloma × Pitanga (A)	73	*Co-14*	56	4	15:1	0.018	0.89
Paloma × Ouro Negro (MA)	73	*Co-3*^{4}	118	9	15:1	0.152	0.70
Paloma × G 2333 (MA)	2047	*Co-4*^{2}	155	13	15:1	0.635	0.43
Paloma × Corinthiano (A)	2047	*Co-15*	94	6	15:1	0.011	0.92
Paloma × Crioulo 159 (A)	2047	*Co-16*	94	6	15:1	0.011	0.92
Paloma × Amendoim Cavalo (A)	2047	*	109	7	15:1	0.009	0.92
Paloma × Jalo Pintado 2 (A)	2047	*	90	6	15:1	0.001	0.99

* = gene not identified yet; [a]R = resistant; [b]S = susceptible; [c]MDRK= Michigan Dark Red Kidney; [d]JLP= Jalo Listras Pretas; A= Andean gene pool; MA= Mesoamerican gene pool.

CONCLUSION

The inheritance tests indicated the presence of one dominant resistant gene in Paloma. The allelism tests demonstrated that the gene present in Paloma is independent from those genes previously characterized. Paloma has been shown to be an important source of resistance to anthracnose and possesses a new Andean gene that supports its use in common bean breeding programs.

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INHERITANCE AND ALLELIC RELATIONSHIPS OF ANTHRACNOSE RESISTANCE IN COMMON BEAN AMENDOIM CAVALO

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INTRODUCTION

Anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. and Magnus) Briosi and Cavara, is one of the most important fungal disease of common bean (Gonçalves-Vidigal et al., 2009). An efficient method to control this disease is the use of resistant cultivars. Thus, a continuous search of new resistance sources is necessary. Previously studies conducted in our laboratory revealed that Andean cultivar Amendoim Cavalo is resistant to races to races 2, 7, 65, 73, 89 and 2047 of the anthracnose pathogen used in this study. The objectives of this work were to conduct allelism tests and evaluate anthracnose resistance inheritance in Amendoim Cavalo cultivar.

MATERIAL AND METHODS

This research was conducted in greenhouse and in the Common Bean Breeding and Molecular Biology Laboratory of Núcleo de Pesquisa Aplicada à Agricultura (Nupagri). The inheritance studies were conducted in two F_2 populations derived from the cross of Amendoim Cavalo (AC) × Mexico 222 (susceptible to race 73). Allelism tests were conducted in F_2 populations from the crosses (R × R). Seedlings of the parents, F_1 and F_2 populations from each cross, were inoculated with races 2, 65, 73 and 2047 of the *C. lindemuthianum* (Table 1). The spore concentration was adjusted to 1.2×10^6 spores mL^{-1} for each race. After the inoculation, plants were maintained at high relative humidity (>95%) for 48h at 21-23°C. Each seedlings were evaluated for their disease reaction using a 9 class scale proposed by Pastor-Corrales et al. (1995). Plants with no visible symptoms or with only a few, or very small lesions mostly on primary leaf veins were recorded as resistant (scored 1-3), whereas plants with numerous small or enlarged lesions, or with sunken cankers on both sides of leaves and the seedling stem, were recorded as susceptible (scale 4-9). The genetics analysis of F_2 populations were performed through Chi- Square Test (χ^2) using Genes Software (Cruz, 2006).

RESULTS AND DISCUSSION

The inheritance tests fit to 3R:1S ratio in the F2 population derived from Amendoim Cavalo and Mexico 222 cross, inoculated with race 73. This result indicates the presence of one resistant gene in Andean cultivar Amendoim Cavalo. According to Table 1, allelism tests in the crosses involving Amendoim Cavalo with Michelite, Michigan Dark Red Kidney, Cornell 49-242, PI 207262, TO, TU, AB 136, G 2333, Jalo Vermelho, Jalo Listras Pretas, Corinthiano and Crioulo 159 cultivars fitted a 15R:1S ratio. These results support the independence of the gene present in Amendoim Cavalo from the other Andean and Mesoamerican resistance genes previously described.

Table 1. Inheritance and allelism tests for genetic characterization of anthracnose resistance in Amendoim Cavalo. Reaction of F_2 populations observed and expected ratios of resistant (R) and susceptible (S) plants to inoculation with different races of *C. lindemuthianum*

Crosses	Race	Resistance Gene	Observed Ratio		Expected Ratio	χ^2	*P* Value
			R	S	R:S		
AC* x Mexico 222	73	*Co-3*	80	18	3:1	2.299	0.129
AC x Michelite	2	*Co-11*	102	7	15:1	0.005	0.941
AC x Cornell 49-242	65	*Co-2*	81	5	15:1	0.028	0.867
AC x TO	65	*Co-4*	65	6	15:1	0.587	0.444
AC x PI 207262	65	*Co-4³*	105	7	15:1	0	1
AC x TU	65	*Co-5*	73	5	15:1	0.003	0.953
AC x AB 136	65	*Co-6*	106	5	15:1	0.557	0.447
AC x Jalo Vermelho	65	*Co-12*	92	6	15:1	0.003	0.958
AC x MDRK**	73	*Co-1*	53	3	15:1	0.761	0.783
AC x Ouro Negro	73	*Co-3⁴*	81	5	15:1	0.028	0.867
AC x Jalo Listras Pretas	73	*Co-13*	81	5	15:1	0.028	0.867
AC x G 2333	2047	*Co-4²*	237	17	15:1	0.085	0.771
AC x Pitanga	2047	*Co-14*	103	4	15:1	1.152	0.283
AC x Corinthiano	2047	*Co-15*	77	5	15:1	0.003	0.955
AC x Crioulo 159	2047	*Co-16*	89	7	15:1	0.178	0.673
AC x Paloma	2047	NI	109	7	15:1	0.009	0.924
AC x Perla	2047	NI	73	6	15:1	0.244	0.621
AC x Jalo Pintado 2	2047	NI	94	6	15:1	0.011	0.918

*Amendoim Cavalo, ** Michigan Dark Red Kidney, NI: Gene not identified yet

CONCLUSION

The results indicates that the cultivar Amendoim Cavalo has one dominant resistant gene to *C. lindemuthianum*. The allelism tests demonstrated that the dominant gene present in Amendoim Cavalo is independent from the genes previously characterized. The identification of the Andean cultivar Amendoim Cavalo as a new source of resistance is very important for the breeding programs aimed for anthracnose disease resistance.

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GENETIC ANALYSIS OF ANTHRACNOSE RESISTANCE IN JALO PINTADO 2 DRY BEAN CULTIVAR

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INTRODUCTION

Anthracnose, caused by the fungus *Colletotrichum lindemuthianum* (Sacc. and Magnus) Briosi and Cavara, is one of the most widespread and economically important diseases of common bean (*Phaseolus vulgaris* L.) worldwide (Pastor-Corrales and Tu, 1989). Search for new sources of resistance have been the objective of many breeding programs, since genetic resistance is the most effective and environmentally friendly management strategy for the control of anthracnose disease in common bean. Therefore, this study aimed to investigate the genetic resistance to anthracnose in the Andean cultivar Jalo Pintado 2.

MATERIAL AND METHODS

The experiment was conducted under greenhouse conditions at the Common Bean Breeding and Molecular Biology Laboratory of Núcleo de Pesquisa Aplicada à Agricultura (Nupagri). Previous studies carried out in our laboratory and by Vidigal Filho et al. (2007) demonstrated that the Andean cultivar Jalo Pintado 2 confers resistance to races 2, 7, 9, 31, 65, 73, 95, 453 and 2047. The inheritance test was evaluated on F_2 populations derivate from the cross of Jalo Pintado 2 and Cornell 49-242 (susceptible to race 73). Additionally, allelism tests were conducted on F_2 populations from the crosses (R × R) between Jalo Pintado 2 (JP2) and the cultivars Michigan Dark Red Kidney, Cornell 49-242, Mexico 222, PI 207262, TO, TU, AB 136, G 2333, Ouro Negro, Michelite, Jalo Vermelho, Jalo Listras Pretas, Pitanga, Corinthiano, Crioulo 159, Amendoim Cavalo, Paloma and Perla. The parents, F_1 and F_2 populations from each cross were inoculated with races 2, 65, 73 and 2047 of the *C. lindemuthianum* (Table 1). The spore concentration was adjusted to 1.2 x 10^6 spores.mL^{-1} for each race. After inoculation, plants were maintained in high relative humidity (>95%) for 72h at 20±2°C. After this period, seedlings were evaluated for their disease reaction using a scale of 1 to 9 (Pastor-Corrales et al., 1995) 7 days after inoculations. Plants with disease reaction scores of 1-3 were considered resistant, whereas plants that were rated 4-9 were considered susceptible. The genetics analysis on F_2 populations was performed through Chi- Square Test (χ^2) using Genes Software (Cruz, 2006).

RESULTS AND DISCUSSION

Segregation analysis in the F_2 population of the cross between the cultivars Jalo Pintado 2 (R) and Cornell 49-242 (S) fitted to 3R: 1S ratio (Table 1), indicating the action of one dominant gene present in the cultivar Jalo Pintado 2. Considering that the Mesoamerican cultivar Cornell 49-242 possess the *Co-2* gene, which does not confer resistance to race 73 of *C. lindemuthianum*. The allelism tests involving crosses (R × R) between Jalo Pintado 2 and the cultivars Michigan Dark Red Kidney, Cornell 49-242, Mexico 222, PI 207262, TO, TU, Ouro Negro, Jalo Vermelho, Jalo Listras Pretas, Corinthiano and Crioulo 159 fitted to 15:1 R/S ratio (Table 1). These results suggesting the action of two dominant resistance genes, one of them present in the JP2 cultivar and the another in the remaining cultivars. Segregation in the F_2 population derived

from the cross between JP2 and cultivar Mexico 222 fit a ratio of 63:1 R/S, indicating that there are three dominant genes segregating for resistance to race 2 of *C. lindemuthianum*. The two dominant loci in Mexico 222 confers resistance to race 2.

Table 1. Inheritance and allelism tests for genetic characterization of anthracnose resistance in Jalo Pintado 2. Reaction of F_2 populations observed and expected ratios of resistant (R) and susceptible (S) plants to inoculation with different races of *C. lindemuthianum*

Crosses	Race	Resistance Gene	Observed Ratio		Expected Ratio		*P* Value
			R	S	R:S		
JP 2*× Cornell 49-242	73	*Co-2*	73	27	3:1	0.213	0.644
JP 2 × Mexico 222	2	*Co-3*	98	2	63:1	0.124	0.724
JP 2 × Michelite	2	*Co-11*	89	6	15:1	0.001	0.978
JP 2 × Cornell 49-242	65	*Co-2*	61	4	15:1	0.001	0.974
JP 2 × TO	65	*Co-4*	105	7	15:1	0	1
JP 2 × PI 207262	65	*Co-4*^{3}	97	6	15:1	0.031	0.858
JP 2 × TU	65	*Co-5*	94	5	15:1	0.243	0.621
JP 2 × AB 136	65	*Co-6*	108	7	15:1	0.005	0.942
JP 2 × Jalo Vermelho	65	*Co-12*	94	5	15:1	0.010	0.917
JP 2 × MDRK**	73	*Co-1*	91	6	15:1	0.001	0.979
JP 2 × Ouro Negro	73	*Co-3*^{4}	94	6	15:1	0.010	0.917
JP 2 × Jalo Listras Pretas	73	*Co-13*	80	5	15:1	0.019	0.888
JP 2 × G2333	2047	*Co-4*^{2}	147	10	15:1	0.003	0.950
JP 2 × Pitanga	2047	*Co-14*	111	7	15:1	0.020	0.886
JP 2 × Corinthiano	2047	*Co- 15*	87	6	15:1	0.006	0.936
JP 2 × Crioulo 159	2047	*Co-16*	93	5	15:1	0.220	0.638
JP 2 × A. Cavalo***	2047	NI	94	6	15:1	0.010	0.917
JP 2 × Paloma	2047	NI	90	6	15:1	0	1
JP 2 × Perla	2047	NI	97	6	15:1	0.031	0.858

* Jalo Pintado 2, ** Michigan Dark Red Kidney, ***Amendoim Cavalo, NI: Gene not identified yet

CONCLUSION

These results demonstrated that the Jalo Pintado 2 cultivar possesses one dominant gene segregating independently from those previously characterized: *Co-1, Co-2, Co-3, Co-3*^{4} *, Co- 4, Co-4*^{2}*, Co-4*^{3}*, Co-5, Co-6, Co-11, Co-12, Co-13, Co-14, Co-15* and *Co-16*. This new gene is a valuable source of resistance to anthracnose which can be transferred to commercial cultivars to enhance the effectiveness of resistance gene pyramiding in bean breeding programmes.

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COMMON BEAN WHITE MOLD RESISTANCE SOURCES IDENTIFIED BY GREENHOUSE SCREENING IN BRAZIL

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INTRODUCTION

White mold (WM) caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is a major disease problem for the common bean crop worldwide (Schwartz and Steadman, 1989). WM can cause severe yield and seed quality losses, which dramatically reduce farmer incomes. In Brazil, *S. sclerotiorum* is widely distributed in the majority of agricultural areas and can cause total yield losses under favorable environmental conditions and without chemical control (Oliveira, 2005). The main goal of the present work was to evaluate the reaction of 39 common bean lines to WM in a greenhouse screening. These bean genotypes include Brazilian cultivars and advanced lines from different market classes, in addition to resistance sources previously reported by CIAT (Cali, Colombia). The effective identification of resistance sources and superior genotypes is a basic and continuous step of breeding efforts aiming to develop/select resistant cultivars to be used in the integrated control of WM.

MATERIAL AND METHODS

Common bean genotypes were grown in a completely randomized design with six replications composed by one plant each. Plants were inoculated at the fourth/fifth node, about 35 days after seedling emergence (R5 stage), using the *S. sclerotiorum* isolate SS 1370, the most virulent one maintained at Embrapa Rice and Beans (Santo Antônio de Goiás, Brazil). Inoculation was accomplished based on the straw test method initially reported by Petzoldt and Dickson (1996), but with modifications. The inoculum (mycelial plugs) was grown for 72 h on PDA medium at 21 ± 1ºC. Plants were inoculated with mycelial plugs using micropipette tips of 200 μL with filter. After inoculation, plants were kept under greenhouse condition (28 ± 1ºC and relative humidity > 85%). WM severity was scored at eight days after the inoculation using a 1-to-9 scale, where 1= no symptoms and 9= dead plants. All six plants of the same genotype were evaluated and the mean scores of disease severity were calculated. Variance analysis was performed followed by the Scott-Knott test.

RESULTS AND DISCUSSION

The results showed significant genetic variation for WM reaction among the 39 screened common bean lines (ANOVA, F test at 1% probability), which were grouped on five resistance levels according to Skott-Knott test at 5% probability. Cultivar BRS Cometa and the advanced line CNFC 9500, both "carioca" seeded genotypes, in addition to the CIAT variety K0407, formed the group with lower severity scores, showing to be potential WM resistance sources to Brazil (Table 1). The "carioca" seeded cultivars BRS Estilo, Pérola, BRS Pontal, BRSMG Madrepérola, and BRS Requinte, in addition to the local variety Bola Cheia, formed the genotype group with high mean severity scores, which range from 6.50 to 8.17 (Table 1). The obtained results are agreed with previous reports from field screening realized in Brazil. The

identified resistance sources are being also evaluated for WM resistance in field trials and will be used as parents in crosses aiming to develop breeding lines resistant to WM.

Table 1. Reaction of common bean lines, including Brazilian cultivars and advanced lines from different market classes, to white mold (*Sclerotinia sclerotiorum*) expressed as mean scores of disease severity.

Genotype	Disease Severity[a]		Genotype	Disease Severity	
BRS Cometa	2.67	a	IAC Alvorada	4.67	c
K0407	2.83	a	CNFC 10429	4.83	c
CNFC 9500	3.17	a	CNFP 10104	4.83	c
AND 277	3.50	b	IPR Colibri	4.83	c
IPR Eldorado	3.67	b	BRS Pitanga	5.00	c
PI204717	3.83	b	BRS Radiante	5.17	c
CNFC 15873	4.00	c	BRS Executivo	5.17	c
BRS Embaixador	4.17	c	BRS Ametista	5.33	d
CNFC 10729	4.17	c	BRS Esplendor	5.33	d
BRS Notável	4.17	c	BRS Supremo	5.33	d
CNFC 15874	4.33	c	BRS Campeiro	5.67	d
Rudá	4.33	c	BRSMG Realce	5.67	d
IPR Juriti	4.33	c	BRSMG Majestoso	6.17	d
CNFP 10794	4.33	c	BRS Estilo	6.50	e
Jalo Precoce	4.50	c	Pérola	6.67	e
IPR Uirapuru	4.50	c	Bola Cheia	6.83	e
K059	4.50	c	BRS Pontal	7.00	e
CNFC 10762	4.67	c	BRSMG Madrepérola	7.33	e
BRS Agreste	4.67	c	BRS Requinte	8.17	e
CNFC 15875	4.67	c			
CV (%): 9.88					
Average: 4.91					

[a]Mean scores of disease severity based on a 1-to-9 scale, where 1= no symptoms and 9= dead plants. Scores followed by the same letter do not differ from each other according to Skott-Knott test at 5% probability. The genotype grouping was done using transformed data ($y=\sqrt{x}$).

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INHERITANCE OF WHITE MOLD RESISTANCE IN OTHELLO/A 195 AND A 195/G 122 COMMON BEAN CROSSES

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INTRODUCTION

White mold caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is a severe disease of common bean (*Phaseolus vularis* L.) on the American continents causing seed yield losses up to 100%. Use of cultivars with physiological resistance or simply resistance is crucial for control of severe white mold outbreaks. Qualitative and quantitative resistance occurs in the primary and secondary gene pools. Thirty-five resistance QTL coalesced into 21 distinct regions across nine linkage groups (Soule et al., 2011; also see Miklas et al., 2013). Genchev and Kiryakov (2002) reported a single recessive gene controlling resistance in the greenhouse and a dominant allele controlled in the field test in A 195/'Lime Light' common bean population. The objectives were to determine (1) the inheritance of resistance in A 195 and (2) its relationship with G 122.

MATERIALS AND METHODS

Susceptible pinto 'Othello' was crossed with an Andean A 195 and A 195 was crossed with another Andean G 122. Both Andean genotypes possess high levels of resistance. Parents and F_1 were inoculated with an aggressive pathogen isolate ND710 at the 5th internode on the main stem, using the cut-stem method (Terán et al., 2006) in the greenhouse in Idaho in 2011. Disease severity was recorded at 35 days post inoculation, using a 1 to 9 scale (1= no fungal infection, and 9= white mold passed the second node from inoculation). Only F_2 seed from resistant (scores ≤ 4) F_1 plants was harvested. Parents and F_2 were inoculated first with a less aggressive isolate ARS12D at the 5th internode. Only resistant plants were re-inoculated one week later with ND710 and evaluated at 35 days. The F_3 progeny test was carried in 2012 in a similar manner.

RESULTS AND DISCUSSION

Othello with a mean score of 9 was susceptible to both isolates (Table 1). A 195 and G 122 were variable to both isolates, but both had lower mean scores in response to ARS12D. Similarly, both F_1 hybrids were variable in response to ND710 (Table 1). Thus, only three resistant plants in Othello/A 195 and five in A 195/G122 were harvested for the F_2 progeny test. The Othello/A195 F_2 segregated into 39 resistant to 23 susceptible in response to ARS12D, giving a good fit to a 9 resistant to 7 susceptible ratio (χ^2=1.04; $P \geq 0.05$). Thus, resistance in A 195 is controlled by two independent complementary dominant genes. But, there was an excess of susceptible plants against ND710, not fitting the 9 resistant: 7 susceptible ratio (χ^2=5.31; $P \leq 0.05$). The F_3 seed from resistant F_2 plants (presumed double heterozygotes) again segregated into 9 resistant: 7 susceptible ratio in response to ARS12D. Furthermore, a segregation ratio of 73 resistant to 31 susceptible (3 resistant: 1 susceptible) was also noted with presumed heterozygotes at one locus. The F_3 from susceptible F_2 plants were all susceptible to ND710 (Table 1). These results considered together support two independent complementary dominant genes controlling white mold resistance in A 195. These results are contradictory to those of Genchev and Kiryakov

(2002) who reported a single recessive resistance gene in A 195/Lime Light population in the greenhouse test. Only one plant was susceptible in the F_2 in response to ARS12D in A195/G122 (Table 1). But, the F_2 segregated into 75 resistant to 29 susceptible (3 resistant: 1 susceptible χ^2=0.46; $P \geq 0.05$) against ND710 suggesting the difference between A 195 and G 122 was controlled by a single dominant gene. The F_3 from resistant F_2 plants also segregated into 3 resistant: 1 susceptible ratio against ND710 (Table 1).

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Table 1. White mold response of Othello, A 195, G 122, and their F_1, F_2 and F_3 against ND710 and ARS12D pathogen isolates in the greenhouses at Kimberly, Idaho in 2011 and 2012.

				ND710		
Genotype	N0. plants	Range	Mean	Ratio	χ^2	*P*
Othello	27	9	9.0	-	-	-
A 195	27	4-9	5.0	-	-	-
G 122	27	4-8	5.3	-	-	-
Othello/A 195						
F_1	17	4-9	5.6	-	-	-
F_2	62	4-9	6.2	26R:36S (9R:7S)	5.31	≤0.05
F_3	47	4-9	5.9	20R:27S (9R:7S)	3.09	≥0.05
	89	6-9	8.0	0R:89S	-	-
A 195/G 122						
F_1	10	4-7	4.9	-	-	-
F_2	104	2-9	4.8	75R:29S (3R:1S)	0.46	≥0.05
F_3	132	4-9	4.9	98R:34S (3R:1S)	0.04	≥0.05
				ARS12D		
Othello	27	9	9.0	-	-	-
A 195	27	2-9	4.3	-	-	-
G 122	27	2-7	4.0	-	-	-
Othello/ A 195						
F_2	62	4-9	5.5	39R:23S (9R:7S)	1.04	≥0.05
F_3	61	3-9	5.7	35R:26S (9R:7S)	0.07	≥0.05
	104	4-9	4.9	73R:31S (3R:1S)	1.28	≥0.05
A 195/G 122						
F_2	104	1-5	3.5	103R:1S (3R:1S)	32.05	≤0.05

Scored on a 1 to 9; 1= no symptoms, and 9= fungus invasion passed the second node.

ASSOCIATION MAPPING OF WHITE MOLD RESISTANCE IN A PANEL OF NORTH AMERICAN BREEDING LINES AND CULTIVARS REPRESENTING THE MIDDLE AMERICAN GENE POOL

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INTRODUCTION: Host resistance and avoidance can limit losses due to white mold (WM) disease in dry beans. Previous studies to understand the genetic mechanisms underlying WM resistance used bi-parental mapping populations and an assortment of markers to identify QTL for WM. Our objective was to use a genome-wide association approach based on a 6K SNP array to examine marker-trait associations.

MATERIALS AND METHODS: A Panel of 266 Middle American lines was phenotyped for reaction to white mold in the greenhouse and field. The panel was previously genotyped by BeanCAP AFRI project using the new >6000 SNP Infinium chip (BeanCAP NIFA Project #2009-01929). Straw test was carried out for six replications 30 d after planting and evaluation for disease severity was at 7 and 11 d after inoculation with disease severity ratings based on a 1 to 9 scale (Petzoldt and Dickson, 1996).

Field trial was conducted in the summer of 2013 at the USDA research farm in Paterson, WA, and consisted of three row plots with two replications in a RCBD. Multiple agronomic traits including white mold severity score (1 to 9), lodging (1 to 9), canopy porosity (1 to 9), flowering date, harvest maturity, and plant stand was recorded. Frequent applications of water delivered by overhead sprinkler promoted the white mold epidemic. Disease was otherwise based on natural infection.

The panel was examined in STRUCTURE 2.3.4 (Pritchard et al. 2000) using the "Admixture with allele frequencies correlated" model with a 'burn-in' of 10,000 and 50,000 MCMC replications with K set at 2 to 10, repeated 20 times. The best value of k = 10 was determined by lnP(d) (log posterior probability) and ΔK, as described by Evanno et al. (2005). Coefficient of membership (Q) values generated in 'STRUCTURE' and the kinship matrix generated in 'TASSEL v4.3.2' (Bradbury et al. 2007) were used in 'TASSEL' as covariates for determining marker-locus-trait associations using the MLM model. Monomorphic SNPs and others with minor allele frequencies (MAF) (i.e., <0.05) were filtered leaving only 3,694 SNPs for the association tests.

RESULT AND DISCUSSION: The population structure analysis assigned the 266 dry bean lines into 10 main groups with significant admixture within groups. Nine SNP showed significant associations ($p<0.004$-0.0001) with WM straw tests. These SNPs mapped on Pv02, 3, 4, 6, 7, 8, 9, 10 and 11. There were significant associations ($p<0.004$-0.0006) between six SNPs and field WM disease severity. These SNPs mapped on Pv02, 5, 8, 10 and 11. The MLM association analyses identified six SNPs that were significantly associated with lodging ($p<0.001$-0.0001) (Table 1). These SNPs mapped on Pv01, 4, 5, 7, 8 and 11. Compared with previous studies, these results confirmed the QTL on Pv08 for lodging, WM field infection and straw test. Also, the QTL on Pv06 had pleiotropic effects on WM field and green house incidence in both studies (Miklas et al 2013). However, while the QTL on Pv02, 4, 7 and 8 had

pleiotropic effects on WM field and greenhouse infection in previous studies, different QTL were identified for the straw test and field incidence in our study. Thus, AM analysis revealed new putative QTL for lodging on Pv01, 5, 7 and 11 and for both WM field and greenhouse severity on Pv10 and on Pv11 for WM field infection.

Table 1. MLM Marker Significance

Trait	**Marker**	**Locus**	**Position (cM)**	**Marker p**	**markerR2**	**Previous QTL**
Lodge	SNP46585	Chr01	111.24	0.0001	0.077	WM1.1, WM1.2
Straw test	SNP46371	Chr02	40.06	0.0001	0.077	WM2.1*
Field	SNP48920	Chr02	92.46	0.003	0.061	WM2.2*
Straw test	SNP49326	Chr03	39.12	0.004	0.048	WM3.1, WM 3.2
Straw test	SNP48320	Chr04	38.09	0.001	0.056	WM4.1*
Lodge	SNP46130	Chr04	107.57	0.0004	0.080	WM4.2
Field	SNP48245	Chr05	31.72	0.0006	0.066	WM5.3, WM5.4
Lodge	SNP45315	Chr05	117.31	0.004	0.057	
Straw test	SNP39610	Chr06	16.91	0.001	0.043	WM6.1*
Field	SNP39610	Chr06	16.91	0.004	0.035	WM6.1*
Lodge	SNP46498	Chr07	19.75	0.001	0.068	
Straw test	SNP39744	Chr07	62.21	0.002	0.040	WM7.1*
Lodge	SNP46480	Chr08	31.96	0.001	0.058	WM8.4
Straw test	SNP39719	Chr08	56.82	0.001	0.066	WM8.2
Field	SNP44978	Chr08	59.59	0.00009	0.068	WM8.1*
Straw test	SNP47627	Chr09	118.51	0.002	0.040	WM9.1[F], WM9.2[F]
Straw test	SNP50873	Chr10	29.38	0.001	0.058	
Field	SNP47382	Chr10	133.88	0.002	0.039	
Lodge	SNP42990	Chr11	98.29	0.0004	0.069	
Field	SNP47544	Chr11	100.19	0.001	0.056	
Straw test	SNP48856	Chr11	106.50	0.002	0.063	WM11.1

* Pleitropy [F]Field only

SUMMARY: These results demonstrate the utility of association mapping for detecting marker-trait associations which can potentially be used for developing marker assisted breeding strategies in dry bean.

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ANALYSIS OF VARIATION FOR WHITE MOLD RESISTANCE IN THE BEAN CAP SNAP BEAN PANEL

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INTRODUCTION

White mold disease caused by *Sclerotinia sclerotiorum* Lib. de Bary, is one of the most devastated diseases that infect snap and dry beans (Miklas *et al*. 2013). The USDA-NIFA supported Bean Coordinated Agricultural Project (CAP) has assembled and genotyped dry and a snap bean panels. The snap bean panel consists of 150 accessions (both Middle American and Andean origin). The panel was genotyped using an Illumina 10,280 SNP GeneChip. The snap bean panel was screened for resistance to white mold and was used in an association mapping study to identify trait associations with this disease.

MATERIALS AND METHODS

A subset of the snap bean panel with non-pole bean habit (134 lines) was grown in a white mold screening nursery arranged in a RCV with three replicates at the Vegetable Research Farm, Corvallis, OR. Percent stand establishment was estimated and. number of flowers per inflorescence was obtained. Canopy porosity was evaluated by observing morning percent ground cover. ICanopy height and plant vigor were rated. Plots were rated at physiological maturity for incidence and severity, and plots were rated lodging and estimated yield. The geometric mean of incidence and severity was calculated to establish the disease index. Data were analyzed in SAS using PROC GLM and PROC CORR. GWAS was conducted at NDSU to obtain Manhattan Plots and significance levels.

Table 1. Performance of a subset of the Bean CAP snap bean panel plus selected checks grown in a white mold screening trial at the Vegetable Research Farm, Corvallis, Oregon in 2012.

Entry	Incidence (%)	Severity (%)	Disease Index (%)	Est. Yield
Corbette Refugee	0.0	0.0	0.0	3.0
Unidor	0.3	0.3	0.3	1.7
G122	0.7	0.3	0.5	3.0
Selecta	0.7	0.7	0.7	1.3
Idaho Refugee	2.0	1.0	1.4	2.7
Angers	3.7	0.7	1.4	1.3
Sirio	3.3	0.7	1.5	1.3
Cadillac	5.3	1.0	2.1	1.7
US Refugee #5	16.7	0.3	2.4	2.7
OSU 5613	31.7	20.0	25.1	1.7
Hercules	36.7	18.3	25.4	1.7
Zeus	33.3	20.0	25.6	2.0
Envy	33.3	20.0	25.7	2.3
LSD 0.05	34.7	26.7	29.3	1.1

RESULTS AND DISCUSSION

Disease index ranged from 0 to 75% (table 1) with several accessions showing levels of disease similar to the partially resistant checks. Susceptible checks were located towards the

middle of the range, and a number of snap bean panel lines had significantly higher disease scores. A LSD of 29.3 suggests that disease incidence and severity was variable across the field. Some lines with low disease scores may possess avoidance traits (determinate habit, open canopy, late maturity) but others (such as Corbette Refugee and US Refugee #5) have type III growth habit and would normally be expected to have high levels of disease. Both Andean and Mesoamerican snap beans were found in the low disease score group. Lodging showed a significant positive association with disease, while canopy porosity, vigor and canopy height all showed negative associations with disease.Flower number and estimated yield showed no statistical association. Genome wide association (GWAS) study of white mold severity (Figure, 1) reveals that SNPs on chromosome 2, 9, and 10 are associated with this trait.

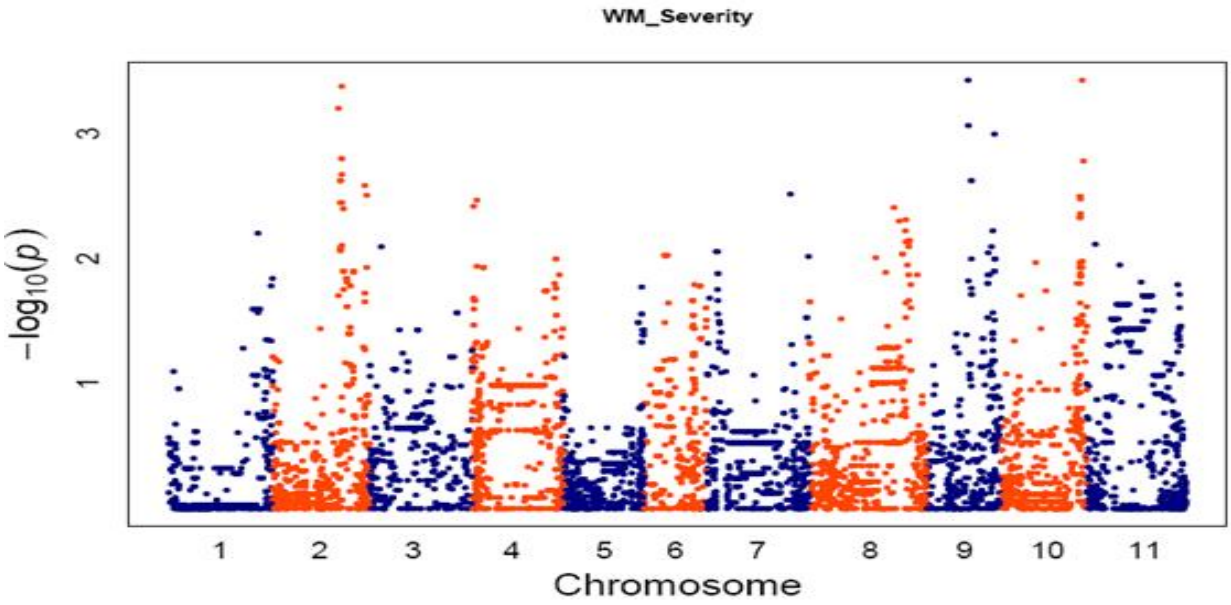

Figure 1. Genome wide association study of white mold severity from the Bean CAP grown in the field in 2012 using a 1PC EMMA model. SNPs on chromosome 2, 9, and 10 show highly significant association with white mold resistance.

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SOURCES OF PHYSIOLOGICAL RESISTANCE TO *SCLEROTINIA SCLEROTIORUM* IN *PHASEOLUS VULGARIS* LINES FROM BULGARIA

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INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary, the casual agent of white mold, is one of the serious pathogens of snap bean (*Phaseolus vulgaris* L.) in the irrigated areas in Bulgaria. Use of resistant cultivars is pivotal to any effective and economical long-term strategy to control the disease. The objective of this study was to identify snap bean genotypes with physiological resistance to white mold among advanced breeding lines from working collection of Maritza Vegetable Crops Research Institute (MVCRI) using the Straw Test.

MATERIALS AND METHODS

The experiment was conducted with a total of 42 $F_{4:6}$ snap bean lines at Dobrudza Agricultural Institute facility in 2009. The tests were set in a field plots, consisted of: one row of each entry and one row of the WM susceptible check - Bulgarian common bean cultivar Dobrudzanski ran. Ten plants out of 20 were inoculated per each entry with five plants of each side of the row as borders. Inoculation was done using the Straw method of Petzoldt and Dickson (1996) at plant stage R6 with a 3 day-old mycelia plug from a colony of *S. sclerotiorum* bean isolate SsPh-2 grown in the dark on PDA at 20°C. Disease severity was rated 7, 14 and 21 days after inoculation on the 1-9 scale of Teran et al., (2006). The following agronomic traits were recorded per plot: plant type; days to flowering; branching pattern (on the 1-5 scale, where 1 = acute upright branching, and 5 = obtuse prostrate branching); lodging (on the 1-5 scale, where 1 =100% plants standing erect, and 5= 100% plants flat on the ground); pod clearance (on the 1-5 scale as 1 = no pods on plants touching the ground; and 5 = all pods in contact with the soil surface). Observation on the plant canopy, leaf density and pod distribution were also recorded.

RESULTS AND DISCUSSIONS

The tested genotypes were separated in three groups based on the disease severity (Table 1). There were recorded four resistant (rate ≤ 3.0) and 26 moderately resistant lines (rate 4.0÷ 6.0). They were with very high homogeneity and statistically distinguished. All of the screened bean lines had type I growth habit. However, variety in plant architecture was observed: plants with erect and others with prostrate stems and branches; short and very long internodes; concentrated and prolonged pod set and distribution. In overall the susceptible lines were rated with low lodging resistance (rate ≥ 4), bad pod clearance (≥ 3.5) and poor branching patterns (≥ 4). Days to flowering varied in relation to white mold severity and yield but still they were positively associated. The results highlight six genotypes from the MVCR snap bean breeding program both with good physiological resistance and architectural characteristics that could be combined in order to reduce the disease on the field.

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Table 1. ***STRAW TEST SCREENING TO WHITE MOLD PHISIOLOGICAL RESISTANCE AND PLANT ARCHITECTURE PATTERN OF 42 ADVANCED SNAP BEAN BREEDING LINES IN BULGARIA***

Genotype	Plant type	Lodging (1-5)	Branching pattern (1-5)	Pod clearance (1-5)	Days to flowering	Minimum rate	Maximum rate	Severity	Grouping
76	I	1,5	1,5	1,0	29	1,0	3,0	**2,5**	A
75	I	2,0	2,0	1,0	29	2,0	3,0	**2,8**	A B
74	I	2,0	2,0	1,0	29	3,0	3,0	**3,0**	A B
78	I	2,0	1,5	1,0	29	1,0	7,0	**3,3**	A B
79	I	2,0	2,5	1,0	30	3,0	7,0	**3,8**	B
3	I	2,5	3,5	2,8	29	3,0	6,0	**4,0**	B
26	I	2,0	3,0	2,5	29	2,0	6,0	**5,3**	C
11	I	3,0	3,8	3,0	30	3,0	6,0	**5,5**	C
12	I	3,3	3,8	2,8	30	3,0	6,0	**5,5**	C
15	I	3,5	4,0	2,5	30	3,0	6,0	**5,5**	C
16	I	3,0	3,5	2,8	30	3,0	6,0	**5,5**	C
25	I	2,5	3,0	2,8	29	3,0	6,0	**5,5**	C
27	I	2,0	3,0	2,5	30	3,0	6,0	**5,5**	C
28	I	2,5	3,5	2,5	30	3,0	6,0	**5,5**	C
30	I	2,5	3,5	3,0	30	3,0	6,0	**5,5**	C
37	I	4,0	3,8	4,0	30	3,0	6,0	**5,5**	C
39	I	4,0	3,8	4,0	30	3,0	6,0	**5,5**	C
41	I	4,0	3,8	4,0	30	3,0	6,0	**5,5**	C
52	I	2,5	2,0	1,0	33	3,0	6,0	**5,5**	C
62	I	2,0	2,5	2,0	34	3,0	6,0	**5,5**	C
70	I	2,0	2,0	2,0	35	3,0	6,0	**5,5**	C
72	I	2,0	2,0	1,0	34	3,0	6,0	**5,5**	C
8	I	3,5	3,8	3,3	31	4,0	6,0	**5,7**	C
9	I	3,5	4,0	3,5	31	4,0	6,0	**5,7**	C
22	I	3,5	3,8	3,3	31	4,0	6,0	**5,7**	C
29	I	3,3	3,5	3,5	30	4,0	6,0	**5,7**	C
31	I	3,0	3,5	3,0	30	4,0	6,0	**5,7**	C
54	I	3,0	2,0	3,0	35	4,0	6,0	**5,7**	C
36	I	2,5	4,0	2,0	30	5,0	6,0	**5,8**	C
65	I	3,0	2,0	3,0	35	5,0	6,0	**5,8**	C
7	I	3,5	4,0	3,5	32	6,0	6,0	**6,0**	C
14	I	3,8	4,3	3,5	32	6,0	7,0	**6,2**	C
32	I	3,5	4,0	3,0	31	6,0	7,0	**6,2**	C
35	I	3,5	4,0	3,5	31	6,0	7,0	**6,2**	C
38	I	3,8	4,0	3,5	31	6,0	7,0	**6,2**	C
40	I	4,0	4,0	3,8	31	6,0	7,00	**6,2**	C
42	I	4,0	3,8	4,0	31	6,0	7,0	**6,2**	C
53	I	3,0	2,0	3,0	36	6,0	7,0	**6,2**	C
57	I	3,0	2,0	3,0	36	6,0	7,0	**6,2**	C
63	I	3,0	2,0	3,0	37	6,0	7,0	**6,2**	C
69	I	3,0	2,0	2,0	37	6,0	7,0	**6,2**	C
33	I	3,5	4,0	3,5	31	6,0	7,0	**6,3**	C
Susceptible check	I	3,0	4,3	3,0	35	7,0	9,0	**8,8**	D

Degree of significance at P≤0,05 by Duncan's Multiple Range Test (1955)

INHERITANCE OF HIGH LEVELS OF RESISTANCE TO COMMON BACTERIAL BLIGHT CAUSED BY *XANTHOMONAS AXONOPODIS* PV. *PHASEOLI* IN COMMON BEAN (*PHASEOLUS VULGARIS* L.)

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Common bacterial blight caused by the pathogen *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) is an important biotic factor limiting common bean (*Phaseolus vulgaris* L.) production (Lema-Márquez *et al*., 2007). A few interspecific bean breeding lines such as VAX 6 exhibit a high level of resistance to a wide range of *Xap* strains representing different pathogenic races (Jara *et al*., 1999). In this study, the inheritance of high levels of resistance to common bacterial blight with the pathogenic strain *Xap* UPR 3353 was evaluated in a population derived from a cross between the breeding line PR0313-58, which has moderate levels of resistance (Zapata *et al*., 2011) and VAX 6, which has high levels of resistance to common bacterial blight (Singh and Muñoz, 1999). Common bacterial blight evaluations were conducted in the field and greenhouse on F_2 plants and $F_{2:3}$ lines. Three central trifoliate leaflets of each plant were inoculated using a procedure described by Zapata (2006). Disease readings were recorded at 14, 21 and 28 days after inoculation using a 1-10 scale. The inheritance of resistance to *Xap* 3353 was studied using Chi-squared tests in the F_2 and F_3 generations. The results suggest that in this population two putative dominant complementary genes confer high levels of resistance to bacterial blight caused by strain *Xap* UPR 3353 (Tables 1-3). Park *et al.* (1998) evaluated RILs from the cross 'PC-50 x XAN-159' and estimated that one or two genes conferred leaf resistance to common bacterial blight. The QTL SCAR marker SU91 and the polymorphic candidate gene markers: SU91-CG3, CG9A, CG9B, CG10, CG11 and CG12 (Shi *et al.,* 2012) were evaluated in the F_2 and F_3 generations and showed no consistent cosegregation with plants having high levels of resistance. Therefore, there is a need to identify molecular markers that can facilitate the selection of the putative genes that confer high levels of bacterial blight resistance. White and black bean lines that combine high levels of common bacterial blight resistance, heat tolerance and resistance to BGYMV and BCMV virus were identified in this study.

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Table 1. Chi-squared test for the hypothesis that two dominant genes confer CBB resistance (15R:1S segregation in the F_2 generation) based on reactions to the common bacterial blight strain *Xap* UPR 3353.

Common blight score	Expected	Observed
Susceptible > 3	8.875	9
Resistance < 3	133.125	133

$\chi^2_{(cal)} = 0.15$, $P = 0.70^{NS}$

Table 2. Pattern of segregation in the F_3 generation in the greenhouse at Mayagüez and the field at Isabela, Puerto Rico.

Putative F_2 genotype	Common bacterial blight scores[1]		
$F_{2:3}$ line	F_2 plant score	Individual $F_{2:3}$ plant scores in the greenhouse	Individual $F_{2:3}$ plant scores in the field
R1/r1 R2/r2			
MG 203	1.7	7 plants ≤ 4.0 & 3 plants ≥ 6.0	all ≤ 2
MG 119	1.5	8 plants ≤ 4.0 & 2 plants≥ 5.0	all ≤ 3
R1/R1 R2/r2 or R1/r1 R2/R2			
MG 141	1.7	7 plants ≤ 4.0 & 3 plants > 4.0	all ≤ 2
MG 32	1.8	5 plants ≤ 4.0 & 5 plants > 4.0	all ≤ 3
MG 41	1.5	8 plants ≤ 4.0 & 2 plants > 4.0	all ≤ 3
MG 176	1.8	9 plants ≤ 4.0 & 1 plant > 4.0	all ≤ 2
Total		29 plants ≤ 4.0 & 11 plants > 4.0	
R1/R1 R2/R2			
MG 74	1.5	10 plants ≤ 4.0	all < 2
MG 133	1.5	10 plants ≤ 4.0	all < 2

[1] Based on a 1 to 10 scale where 1 = no symptoms of infection, 2 = chlorotic halos from 1-2 mm in diameter, 3 = chlorotic halos 3-5 mm in diameter, 4 = the clorotic halos of the three points of inoculation begins to coalesce, 5 = approximately ½ of the trifoliate showing symptoms, 6-10 = > ½ of the leaflet showing symptoms.

Table 3. Chi-squared test for $F_{2:3}$ lines having the putative F_2 genotypes (R1/R1 R2/r2 or R1/r1R2/R2) based on reactions to the common bacterial blight strain *Xap* UPR 3353.

Common blight score[1]	Expected	Observed
Susceptible > 4	10.0	11
Resistance < 4	30.0	29

[1] Disease pressure in the greenhouse was more severe due to high temperature and humidity. Therefore, $F_{2:3}$ plants with scores < 4 were considered resistant.

$\chi^2_{(cal)} = 1.33$, $P = 0.24^{NS}$

EVALUATION OF THE TEPARY BEAN (*PHASEOLUS ACUTIFOLIUS*) CIAT GERMPLASM COLLECTION FOR RESPONSE TO COMMON BACTERIAL BLIGHT AND BEAN COMMON MOSAIC NECROSIS VIRUS

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Aphid-transmitted Bean Common Mosaic Necrosis Virus (BCMNV) and Bean Common Mosaic Virus (BCMV) are potyvirus that cause production losses in common and tepary beans. Developing resistance to viruses, specifically BCMV, BCMNV and BGYMV, will be critical for expanding tepary bean production. Few evaluations of the international tepary collection at CIAT have been conducted. This study evaluated the response of the tepary collection to BCMNV and to common bacterial blight in controlled greenhouse evaluations and found variability in response to both pathogens. For BCMV, pathotype groups correspond with resistance genes that can be organized in two groups. One group has resistance conferred through recessive isolate-specific genes with five alleles, *bc-1, bc-1*^{2}*, bc-2, bc-2*^{2}*,* and *bc-3* at three loci that need *bc-u* for expression. The second group has resistance conferred through the dominant *I* gene. Common bacterial blight (CBB) caused by *Xanthomonas axonopodis* pv. *phaseoli* (Xap) is a serious disease in temperate and tropical production zones. The role of tepary bean as a source of high levels of resistance to this disease in common bean has been shown (Urrea et al., 1999).

Response of 207 accessions of tepary bean from the CIAT tepary collection to BCMNV was evaluated in a glass house at the University of Puerto Rico in Mayaguez, PR. The inoculum of the NL3 strain was prepared using BCMNV infected leaves in cold 0.05 M phosphate buffer, pH 7 (aprox. 1:10 w/v). One week after emergence, the surface of the primary leaves were inoculated (Morales, 1989). Cultivar 'Verano', which has the *I* gene, was used as a control. Accessions with a resistance response were tested in two additional trials. SCAR markers SBD5 and SW13 were used to determine the presence of the bc-1^2 and I genes, respectively.

Response to CBB was completed visually in a screen house in two replicated trials, at USDA-ARS-TARS in Mayaguez, PR. Two strains, 484A and 3353 (Dr. Mildred Zapata-UPR), were used. The inoculum was produced on YDCA media for 48 h at 26 °C, and diluted in sterile water to 10^7 cfu ml^{-1} (Zapata et al., 2007). Trifoliolate leaves were inoculated using the multiple needle technique. VAX 3 was used as the resistant and cultivar 'Morales' as the susceptible checks. At 14 days after inoculation, the plants were evaluated on a 1-9 scale, with 1= no symptoms and 9= systemic infection of the leaf (Van Schoonhoven and Pastor-Corrales, 1987).

Veinal necrosis and local pinpoint necrotic lesions were identified as a response to BCMNV. G40041, G40042, G40044 and G40177E1 showed consistent symptoms in three inoculations (Table 1), with veinal necrosis on the inoculated leaves suggesting the presence of a gene that functions like the *bc-1*^{2} gene, but this needs to be further verified. All accessions showed the presence of the SCAR marker SBD5, which is linked to *bc-1*^{2} in common bean, but appears to be uninformative in tepary bean. Genotype G40177E showed restricted local pinpoint necrotic lesions, which in *P. vulgaris* indicates the presence of *I* protected by the *bc-2*^{2} gene, however the SW13 SCAR marker did not function in tepary. G40001, G40078, and G40177A2 showed an inconsistent inoculated response.

A large range of response to CBB was found (Table 2), with the collection skewed for resistance. Higher disease scores were observed with the strain 3353, as is found in common

bean. Populations have been generated for the study of the genetics of BCMV and CBB response in tepary bean. BCMV resistance is also being incorporated in breeding lines.

Table 1. Characterization of a subset of the CIAT tepary bean collection showing a resistance response to NL3 (BCMNV) inoculation.

Accession	Seed color	100 sd. weight	Origin	NL3 Reaction	
				Response	Repeatibility
G40001	White	12.8	Veracruz, MX	Veinal necrosis	variable
G40041	White	18.0	South Africa	Veinal necrosis	consistent
G40042	White	19.7	Nebraska, USA	Veinal necrosis	consistent
G00044	Brown	4.0	South Africa	Veinal necrosis	consistent
G00078	Brown	2.9	Texas, USA	Veinal necrosis	variable
G40177A2	Maroon	11.0	Arizona, USA	Veinal necrosis	variable
G40177E	Brown	9.9	Arizona, USA	Pinpoint necrotic lesions	consistent
G40177E1	Brown	6.2	Arizona, USA	Veinal necrosis	consistent

Table 2. CIAT tepary collection genotypes and common bean controls with extreme reactions to inoculation with *Xanthomonas axonopodis* strains 484A and 3353.

Accession	Seed color	100 sd. weight	Origin	Xap score on 1-9 scale[1]	
				484A	3353
G40224	Maroon	3.4	Arizona, USA	1.0	1.0
G40010	White	14.1	San Salvador, SLV	1.0	1.3
G40040	Yellow	20.6	Arizona, USA	1.4	1.0
G40057	White	15.3	United Kingdom	1.2	1.4
G40150	White	17.9	Sonora, MX	1.5	1.7
G40122	Brown	15.4	Eastern, Zambia	1.8	1.9
G40006B	Black speckled	14.8	Chiapas, MX	4.0	4.8
G40089	Maroon	3.3	Durango, MX	6.8	5.8
G40233	Brown	3.2	Arizona, USA	6.9	6.9
G40196	Brown	6.1	Texas, USA	8.0	7.0
G40245	Brown mottled	3.9	Michoacan, MX	9.0	9.0
VAX 3	Red (*P. vulgaris*)		CIAT	1.0	1.0
Morales	White (*P. vulgaris*)		U. of Puerto Rico	9.0	8.0
Mean (n=200)				2.1	4.6
LSD				1.2	2.0

[1] *Xanthomonas axonopodis* pv. *phaseoli* (Xap) score on 1-9 scale, with 1 resistant and 9 susceptible.

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SCREENING THE USDA CORE COLLECTION OF COMMON BEAN FOR RESISTANCE TO HALO BLIGHT

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INTRODUCTION

Halo blight, caused by *Pseudomonas syringae* pv. *phaseolicola* (*Psp*) is an important seed-borne bacterial disease of common bean (*Phaseolus vulgaris* L.). Out of the nine races identified, race 6 of *Psp* bacterium is most prevalent worldwide including North Dakota and Minnesota. Halo blight is reported to cause 43% yield losses under experimental conditions, especially on susceptible cultivars (Fourie, 2002). A characteristic foliar symptom of the disease includes the development of chlorotic areas of yellow-green tissue that resembles a halo and may appear around necrotic lesions. Under favorable conditions, the disease symptoms are often extended to stems and pods. Pod symptoms are characterized by the presence of red or brown water-soaked lesions. Several sources of resistance to *Psp* have been identified along with five putative genes (Taylor *et al*., 1996b); however to date, no dry bean cultivars with high levels of resistance to race 6 of *Psp* are commercially available. The purpose of this study is to screen 283 plant accessions (PIs) from the United States Department of Agriculture-National Plant Germplasm System (USDA-NPGS) bean core collection for resistance to race 6 of *Psp* under greenhouse conditions.

MATERIALS AND METHODS

Eight plants of each of 283 PIs of dry bean coupled with USHBR6, as a resistant pinto line, and Pink Panther, as a susceptible light red kidney were evaluated for reaction to *Psp* in a randomized complete block design (RCBD) with four replications under greenhouse conditions. An individual unifoliate leaf from each accession was inoculated at the V-2 growth stage with a 48 h old *Psp* race 6 isolate at a density of $1x10^8$ cfu/mL using the multiple-needled florist pin frog method. The inoculated plants were maintained at 100% RH and 19°C ± 1°C for 48 h in a humidity chamber. Inoculated plants were transferred into a growth chamber maintained at 20-22°C, and rated for disease symptoms 10 days after inoculation using a disease severity scale of 1 – 9 [1-3 = resistant (R), 4-6 = intermediate (I), 7-9 = susceptible (S)] (Mills and Silbernagel, 1992). Disease severity data were analyzed using the PROC MIXED procedure (SAS Institute, 2010). Genotype and replication were considered as fixed effects and random effects, respectively.

RESULTS AND DISCUSSION

The results obtained showed a significant variation in halo blight reaction among 283 accessions (Table 1). Of these accessions, 37, 219, and 25 accessions were classified as highly resistant, moderately resistant, and susceptible respectively. Of the 37 highly resistant accessions, PI_533259, PI_319592, PI_201329, PI_310826, and PI_309810 had significantly higher levels of resistance to race 6 of *Psp* compared to the standard resistant and susceptible checks (Fig. 1), and are potential sources of resistance.

Table 1. Analysis of Variance (ANOVA) for halo blight reactions.

Source	df	Sum of Squares	Mean Squares	F-Value	Pr > F
Genotypes	282	4932.290132	17.490391	2.79	<.0001
Replication	3	441.827807	147.275936	24.93	<.0001
Error	1186	1505.566667	1.269449		

** F – test was considered at level of significance (α = 0.05).*

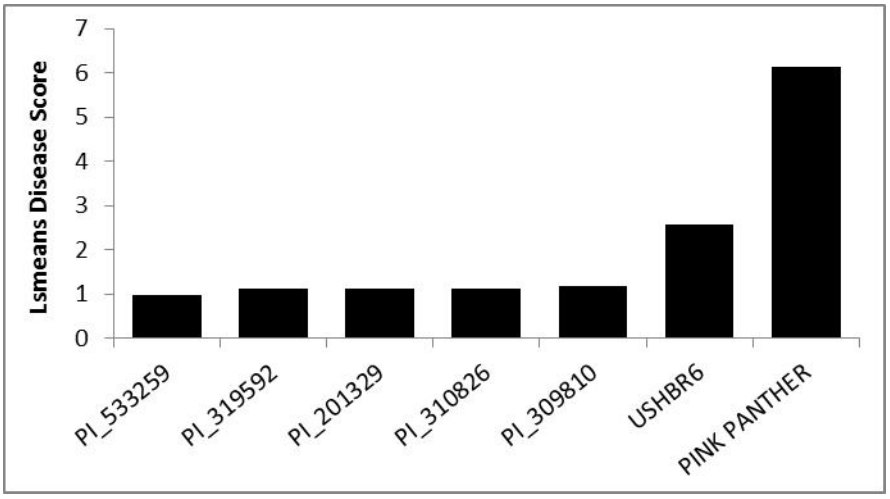

Figure 1. Adjusted means (lsmeans) for disease score of the 5 most resistant accessions to halo blight infection in the greenhouse, along with a resistant (USHBR6) and susceptible check (Pink Panther).

A selected group of accessions exhibiting resistance to both leaf and pod infection by race 6 of *Psp* will be further evaluated under both greenhouse and field conditions. Genomic regions linked to resistance in the accessions will be identified using Genome-Wide Association Mapping (GWAS).

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QTL ANALYSIS FOR FUSARIUM ROOT ROT RESISTANCE IN SNAP BEAN UNDER GREENHOUSE CONDITIONS

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INTRODUCTION

Fusarium root rot (FRR), caused by *Fusarium solani* f. sp. *phaseoli* (syn.*F. phaseoli* T. Aoki & O'Donnell, *F. cuneirostrum* O'Donnell & T. Aoki), is considered as one of the most economically important and widespread fungal diseases of common bean (1). Progress in breeding for FRR resistance has been hampered by the low efficiency of phenotypic selection. Hence, the development of fast and efficient screening methods and marker-assisted breeding tools for FFR resistance will help in transferring higher levels of resistance and shorten the time for commercial cultivar development. The objectives were to determine utility of greenhouse screening method for identification of QTL conditioning resistance to FRR in a snap bean recombinant inbred line (RIL) population using traits such as disease severity index and root and foliar biomass (dry weight), and to integrate the genomic regions associated to resistance onto the reference genome of common bean.

MATERIALS AND METHODS

An F_5 derived RIL population (RR138, n=168) from the cross between the highly resistant line RR6950 and the susceptible OSU5446 line of snap bean (2) was evaluated for FRR resistance under greenhouse conditions. Two weeks after planting a set of four plants per line, grown in perlite-filled cones (5 cm diameter x 18 cm height), was inoculated at the basal stem with 5 mL of a conidial suspension (1 x 10^6 conidia/ mL) from the local and highly virulent isolate "Roza Stampede" (3). Another set of plants of similar height and foliar area were mock-inoculated with sterile water and used as control. Two weeks after inoculation plants were uprooted, and roots washed and evaluated for reaction to FRR using a disease severity index (DSI) (1= no visible symptoms to 9= 100% of root system necrotic). In addition, the reduction in root (RDW) and foliar (FDW) biomass of inoculated plants relative to the control plants was evaluated as determined by dry weight. The experiment was arranged and data evaluated as complete randomized block design using one-way ANOVA, and means separated by Tukey's HSD test at P= 0.05. The experiment was repeated once.

The RIL population was previously genotyped on an Illumina 10K SNP BARCBEAN6K_3 Beadchip, a genetic linkage map constructed and QTL for FRR resistance mapped (2). QTL for DSI, RDW and FDW were detected with composite interval mapping (CIM) analysis using WinQTL Cartographer, and a minimum LOD of 2.5 (P= 0.05). The nucleotide sequences flanking each SNP marker was used to BLAST search the reference genome of common bean at Phytozome to determine the location of SNP significantly associated to FRR.

RESULTS AND DISCUSSION

Our results confirm the utility of the perlite-based greenhouse screening method to phenotype the disease reaction to FRR by allowing the evaluation of both hypocotyl and root damage. The parental lines RR6950 and OSU5446 consistently displayed resistance and susceptibility, respectively, over the course of the experiments in the greenhouse. The mean DSI for the RR6950 and OSU5446 for the first experiment was 3.4 and 6.0, and for the second experiment was 3.1 and 4.5, respectively.

Six genome-wide significant QTL peaks for DSI, FDW, and RDW traits associated with FFR resistance were identified in four chromosomes (1, 4, 9, 11). An important QTL mapped to a region in chromosome 9 (between 69-79 cM), corresponding to DSI and RDW (R^2 =0.06 to 0.11 (Table 1). Previous work on this RIL population identified two QTL for FFR field resistance in chromosomes 3 (3.2 cM, R^2 =0.09) and 7 (47.8 cM, R^2 =0.22) using field disease rating scale (2). Our study has found novel QTL, in chromosomes 9 and 11, for FRR resistance not previously reported in the literature. The co-localization of DSI and RDW traits suggests a reliable association between the phenotypic characterization of FRR in the greenhouse and the QTL genomic region in chromosome 9.

Several gene candidates involved in disease resistance were found in ~500 kb regions surrounding the QTL regions including a few containing leucine-rich repeat and kinase domains, and several involved in plant cell wall remodeling (hydrolase, lyase, esterase).

Table 1. Identification and location of QTL and significant SNP markers associated to FRR on the physical map of the common bean reference genome deposited at Phytozome.

Trait	Chromosome	QTL peak (cM)	LOD	R2	Genomic location of SNP (bp)	Candidate genes (± 500 kb from SNP marker)
DSI	1	38.61	3.0	0.06	50,203,547	Phvul.001G242400 Alpha/beta hydrolase related protein; Phvul.001G242700 SGNH hydrolase-type esterase superfamily protein
DSI	1	39.01	2.8	0.06	50,093,966	Phvul.001G240400 Leucine-rich repeat (LRR) family protein
FDW	4	25.21	3.4	0.07	37,469,548	Phvul.004G112200 Glycosyl hydrolase family protein
FDW	9	58.21	2.7	0.06	29,048,216	Phvul.009G195900 Leucine-rich repeat protein kinase family protein
FDW	9	59.31	2.6	0.05	28,879,788	Phvul.009G194300; Phvul.009G194400 alpha/beta-Hydrolases superfamily protein
FDW	9	59.71	2.5	0.05	28,607,538	Phvul.009G193200; MLO10; ATMLO10; Seven transmembrane MLO family protein
DSI	9	69.21	4.3	0.09	23,955,707	Phvul.009G161600 Glycosyl hydrolase family 38 protein
RDW	9	70.71	2.9	0.06	23,568,358	Phvul.009G161600 Glycosyl hydrolase family 38 protein; Phvul.009G161900 auxin response factor 2
DSI	9	72.21	3.6	0.08	22,065,465	Phvul.009G151200 Pectin lyase-like superfamily protein; Phvul.009G152700 Pectinacetylesterase family protein
RDW	9	72.21	3	0.07	22,065,465	Phvul.009G151200 Pectin lyase-like superfamily protein; Phvul.009G152700 Pectinacetylesterase family protein
DSI	9	74.31	5.1	0.11	18,663,004	Phvul.009G125800 Pectate lyase family protein
RDW	9	74.31	3.5	0.08	18,663,004	Phvul.009G125800 Pectate lyase family protein
DSI	9	78.51	3.5	0.08	16,715,203	Phvul.009G110600 Leucine-rich receptor-like protein kinase family protein; Phvul.009G112200 Protein kinase family protein with leucine-rich repeat domain
DSI	9	80.21	3.6	0.08	16,142,203	Phvul.009G107400; Phvul.009G107400 alpha/beta-hydrolases superfamily protein ; Phvul.009G108100; Phvul.009G108200 Plant invertase/pectin methylesterase inhibitor superfamily
FDW	9	113.41	2.7	0.06	4,130,641	Phvul.009G021000 alpha/beta-Hydrolases superfamily protein
FDW	9	113.61	2.7	0.06	6,038,392	None
FDW	9	115.31	2.6	0.05	2,571,923	Phvul.009G015600 Auxin responsive protein
RDW	11	0.01	2.9	0.06	3,190,094	Cluster of 13 SAUR-like auxin-responsive Phvul.011G036500 - Phvul.011G037700
RDW	11	0.71	2.6	0.05	3,200,152	Same as above

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SSR DNA MARKERS LINKED WITH BROAD-SPECTRUM RUST RESISTANCE IN COMMON BEAN DISCOVERED BY BULK SEGREGANT ANALYSIS USING A LARGE SET OF SNP MARKERS

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INTRODUCTION: Effective genetic resistance to control the rust disease of common bean is difficult to achieve due to the broad virulence diversity of the rust pathogen, comprised of a myriad of virulent strains. The success of an effective genetic solution to control rust is reliant on availability of broad spectrum resistant genes. The Mesoamerican common bean PI 310762 was evaluated in as resistant to 89 of the 90 races of the rust pathogen. Furthermore, PI 310762 was resistant to all races that rendered susceptible all ten known rust resistance genes in common bean. The broad rust resistance in PI 310762 was conferred by a single dominant gene. The objective of this study was to identify simple sequence repeat (SSR) DNA markers linked to the resistance locus in PI 310762. These markers were discovered by bulk segregant analysis (BSA) using a large set of single nucleotide polymorphism (SNP) DNA markers.

MATERIALS AND METHODS: Total genomic DNA was isolated from newly emerged first trifoliate leaflets of each F_2 plant and the two parents, Pinto114 (susceptible) and PI 310762 (resistant) using the DNeasy 96 Plant Kit (Qiagen, CA) according to manufacturer's instructions. The DNA was quantified using a 1% agarose gel (Agarose SFR, Amresco) with TBE buffer and stained with 1μg mL^{-1} ethidium bromide. Since the resistance in PI 310762 is conferred by a dominant locus, three susceptible bulks were generated for BSA so that heterozygous resistant plants were not included in the bulks. Each bulk consisted of equal amounts of DNA from nine susceptible F_2 plants. The DNAs of the two parents and the susceptible bulks were screened with 5,399 SNP DNA markers 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). All allele call data were manually checked, and positive hits for BSA were recorded when a SNP was polymorphic between Pinto114 and PI 310762 and all susceptible bulks clustered tightly with Pinto114 in the GenomeStudio output. The SNP-containing fragments were aligned to the common bean genome DNA sequence at Phytozome, DOE, JGI (http://www.phytozome.net) using standalone Megablast (Morgulis et al., 2008) at a stringency of W=50 and p=95 to determine the genome positions of the SNPs identified using the Illumina Infinium SNP analysis. The sequence scaffold to which a SNP-containing sequence aligned was then interrogated for the presence of SSRs using the Perl script "MISA" (Thiel et al., 2003) as described by Song et al. (2010). Polymerase chain reaction (PCR) primers were designed to the flanking sequence of SSRs using Primers (Rozen and Skaletsky, 2000). The PCR product lengths ranged from 113 to 300 bp. The PCR reactions were performed with 40 cycles of denaturation at 94°C for 60 s, annealing at 58°C for 30 s, extension at 72°C for 45 s, and a final extension at 72°C for 5 min with a hold at 15°C and the resulting PCR products were analyzed on a 3% agarose gel (Agarose SFR, Amresco) with TBE buffer and stained with 1μg mL^{-1} ethidium bromide. Each primer pair was used to amplify genomic DNA of the parents Pinto114 (S) and PI310762 (R). The resulting PCR products were analyzed using agarose gel electrophoresis to find polymorphic SSR markers

between Pinto 114 and PI 310762. The selected polymorphic SSR markers were used to amplify the genomic DNA of each of the 156 F_2 plants and two parents. The resulting PCR products were then analyzed using agarose gel electrophoresis. The genotype of each F_2 plant was recorded as homozygous for the same allele as the Pinto114 susceptible parent, heterozygous or homozygous for the PI 310762 parent allele. The resulting genotypic data for each F_2 plant based on the analysis with the SSR marker loci were then compared with the phenotypic data based upon rust reaction in order to determine how well the SSR marker genotype predicted the rust reaction type. Linkage between the common bean rust resistant locus in PI 310762 and SSRs in the F_2 population was determined using JoinMap 4.1 (Kyazma B.V, Netherlands) software. Default settings for the "regression mapping" method were used to define linkage order and distances (cM). Linkage distances (cM) were based on Kosambi map units.

RESULTS AND DISCUSSION: We wanted to develop the capacity for marker-assisted selection for the unique rust resistance allele in Mesoamerican common bean PI 310762. In this study, we used BSA and SNP genotyping to identify SSR DNA markers linked with a rust resistance allele in PI 310762. A total of 1759 of the 5,399 SNPs screened from the common bean SNP chip were positive for BSA; that is, 1759 SNPs were polymorphic as they produced allele calls that distinguished Pinto 114 (S) from PI 310762 (R). Ten SNPs were positive for BSA. These SNPs, identified using the Ilumina Infinium SNP analysis, produced allele calls that distinguished Pinto 114 from PI 310762. Each of the three susceptible bulks used clustered tightly with the susceptible parent Pinto 114. The SNP-containing sequences were aligned to the common bean genome DNA sequence at Phytozome, DOE, JGI, using standalone Megablast analysis to determine the genome positions of the polymorphic SNPs. This analysis determined that they were located in seven sequence scaffolds of the 20x build of the *Phaseolus vulgaris* DNA sequence. The sequence scaffolds in which the SNP-containing sequences were located were interrogated for the presence of SSRs. Twenty two SSRs were identified but only eight were polymorphic between the parents. PCR primers were designed to the eight SSRs. Each primer pair was used to amplify genomic DNA of the two parents. The resulting PCR products were analyzed using agarose gel electrophoresis to find polymorphic SSR markers between Pinto 114 and PI 310762. Genomic DNA of each of the 156 F_2 plants and the two parents were amplified with the eight selected SSR markers. The resulting PCR products were then analyzed using agarose gel electrophoresis. Four SSR markers were closely linked with the rust resistance gene in PI 310762. SSR 1167 had the tightest linkage at a distance of 0.1 cM. SSR 1170, SSR 1168, and SSR 0778 were located at 0.5 cM, 0.9 cM, and 1.4 cM from the rust resistance gene, respectively. Markers SSR 1167 and SSR 1170 are potentially useful flanking markers. These markers, closely linked to the rust resistance gene in PI 310762, will facilitate the introgression of this very important, broad-spectrum resistance allele into bean cultivars in the United States and other countries of the world.

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DEVELOPMENT OF TOOLS FOR *MACROPHOMINA PHASEOLINA* EVALUATION AND FOR GENETIC IMPROVEMENT OF COMMON BEAN

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Ashy stem blight, caused by *Macrophomina phaseolina*, causes significant yield reduction in the tropics and subtropics, often in association with drought stress, while multiple loci conditioning resistance in bean have been found (e.g., Miklas et al., 1998). In this study, a collection of *Macrophomina phaseolina* isolates was assembled in Puerto Rico for the study of pathogen diversity using sequence analysis of the ITS region. The response of the Andean Diversity Panel (ADP) to controlled inoculation was also evaluated with the goal of conducting association mapping for this trait.

M. phaseolina isolates were collected from farmer fields and Experimental Stations in the main common bean production regions of Puerto Rico (Table 1). Those confirmed to be *M. phaseolina* through sequence analysis and/or pathogenicity tests were analyzed. Pathogenicity tests used the "toothpick" method in Juana Diaz, PR on three contrasting genotypes, and evaluated 14 d after inoculation. The ITS region, amplified with the ITS1 and ITS4 primers, was sequenced and alignments were completed with additional Genbank accessions. The phylogeny analysis was completed using MEGA software. One of the isolates, Mph-55, was used to prepare inoculum for field inoculation of the ADP by homogenizing mycelial mats with abundant picinidia in a blender, followed by washing and drying at 30°C (Abawi and Pastor-Corrales, 1989). Inoculum was adjusted to OD=0.4 and applied with a backpack sprayer.

A broad range of reaction to ashy stem blight in the ADP was found in the field (Table 2). Although morphological diversity was evident (not shown), with variable rates of mycelial growth and time to pycnidia formation, a high level of sequence similarity was found in the ITS region between the isolates collected in Puerto Rico and isolates of *M. phaseolina* from other regions (Table 1, Fig. 1), thus other genomic regions and microsatellite markers are being used to continue to further investigate genetic diversity. Virulence characterization of the pathogenic *M. phaseolina* isolates is currently underway.

Table 1. Characteristics of *Macrophomina phaseolina* and control isolates collected.

Strain ID	Identity	Location of collection	Host	Pathogenicity in common bean	% identity to *M. phaseolina*
Mph-2	*M. phaseolina*	Juana Diaz, Puerto Rico	common bean	Pathogenic	100%
Mph-7	*M. phaseolina*	Aguadilla, Puerto Rico	unknown	Pathogenic	100%
Mph-13	*M. phaseolina*	Aguadilla, Puerto Rico	common bean	Pathogenic	99%
Mph-25	*M. phaseolina*	Aguada, Puerto Rico	common bean	Pathogenic	99%
Mph-34	*M. phaseolina*	Coloso, Puerto Rico	common bean	Pathogenic	99%
Mph-50	*M. phaseolina*	Juana Diaz, Puerto Rico	common bean	Not tested	100%
Mph-55	*M. phaseolina*	Isabela, Puerto Rico	common bean	Pathogenic	99%
Mph T-2	*M. phaseolina*	Ulongwe, Mozambique	common bean	Not tested	100%
Mph T-3	*M. phaseolina*	Chitedze, Malawi	common bean	Not tested	100%
Asp.1	*Aspergillus niger*	Juana Diaz, Puerto Rico	common bean	Not tested	94%
Asp.36	*Aspergillus niger*	Coloso, Puerto Rico	common bean	Not tested	94%
Las.27	*L. theobromae*	Aguada, Puerto Rico	common bean	Not tested	94%
Las.43	*L. theobromae*	Coloso, Puerto Rico	common bean	Not tested	93%

Table 2. ADP genotypes with resistant (1-3) or susceptible (7-9) responses to *M. phaseolina*.

Genotype	ADP#	Agron. Score[1], 1-9	Maturity score[1], 1-3	Macro. Score[1], 1-9
Resistant genotypes				
Rozi Koko	ADP-1	5.3	2.3	3.0
G 20554 (Tostado)	ADP-322	8.3	3.0	3.0
A193	ADP-119	6.3	2.7	3.3
NABE 4	ADP-166	5.0	2.0	3.3
G 4001 (34-P)	ADP-201	6.7	2.7	3.3
G 10060 (Aff. Berna)	ADP-248	7.7	3.0	3.3
G 21210 (Monte Oscuro)	ADP-336	7.7	3.0	3.3
G 21975 (Favinho)	ADP-343	5.0	2.0	3.3
49-2	ADP-440	5.0	2.3	3.3
INIAP 418	ADP-448	8.0	2.7	3.3
Sodan	ADP-86	7.7	3.0	3.7
Rojo	ADP-96	6.0	2.3	3.7
Badillo	ADP-128	5.0	2.0	3.7
G 4494 (Diacol Calima)	ADP-205	5.7	1.7	3.7
Susceptible genotypes				
Kabuku	ADP-55	7.0	2.7	8.0
Njano	ADP-68	8.3	1.7	8.0
OPS-RS4	ADP-113	7.0	2.3	8.0
Vazon 7	ADP-443	7.0	1.7	8.0
G 22357	ADP-349	8.3	2.0	8.3
G 4474	ADP-204	9.0	2.0	8.7
G 10167	ADP-250	8.3	2.3	8.7
G 10258	ADP-252	5.7	1.0	9.0
G 17206	ADP-298	8.7	2.0	9.0
G 23086	ADP-367	9.0	1.7	9.0
Mean			2.2	5.9

[1]Agronomic score on 1-9 scale with 1 being desirable and 9 undesirable; Maturity score on 1-3 scale with 1 being early and 3 late; Macrophomina score on 1-9 scale with 1 resistant and 9 highly susceptible.

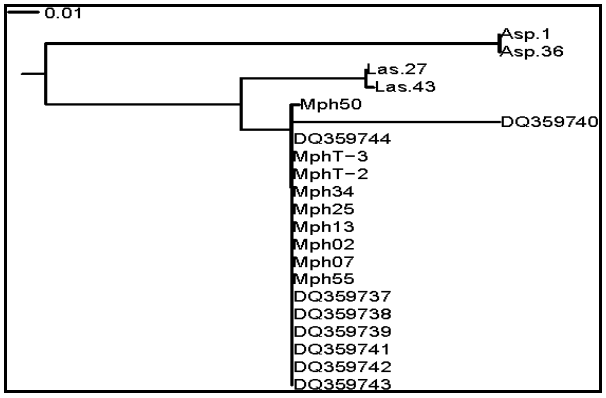

Figure 1. Phylogenetic tree constructed based on ITS sequence of isolates in Table 1 and additional sequences from Genbank.

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INTAKE OF COMMON BEAN AND ITS EFFECT ON GLUCOSE METABOLISM AND LIPID PROFILE IN DIABETIC RATS

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INTRODUCTION: Diet plays an important role in diabetic development, and the consumption of foods with known functional properties may help to control it. Diabetes is often associated with disorders on the glucose metabolism and changes in serum lipids pattern which is a major risk in the development of atherosclerosis, in addition to accelerating the risk of cardiovascular disease. Common bean is a legume regularly consumed in México; its seed has been used to control this disease due to its low glycemic index and phytochemical content (Hernández *et al*., 2012; Kaur *et al*., 2005). However, the potential of this legume to control the effects of diabetes may depend on the type of grain. The objective of the present study was to evaluate the effects of common bean on the glucose metabolism and lipid profile in diabetic rats.

MATERIALS AND METHODS: Common bean Negro Comapa (NC), Flor de Junio León (FJL) and Flor de Junio Dalia (FJD) were cooked and freeze dried. For the experiment, 40 male Wistar rats were used with an average weight of 250-270 g of body weight. After a week of adaptation, diabetes was induced by a single intraperitoneal injection of streptozotocin. Eight days later, blood glucose was measured by a reflective glucometer (Glucose Accutrend, Roche, Germany). Animals corresponding to the diabetic group were separated into a diabetic control (DC) and treatment groups (n=8), the remaining animals were the healthy control (HC) (n=8). Diabetic and healthy controls were fed with normal diet (Rodent Lab Shaw) while animals from treatment groups were fed a diet supplemented with 10 % (w/w) cooked bean. After four weeks, animal were sacrificed and blood samples were collected for biochemical analyses. Total Cholesterol, high density lipoprotein (HDL) and triglycerides were determined from serum samples under fasting conditions using commercially kits (Randox Laboratories Ltd. UK). Serum levels of insulin were quantified using a rat ELISA kit (EMD Millipore, U.S.).

RESULTS AND DISCUSSION: Blood glucose levels in rats fed with FJD, responded to treatment with significant decreases compared to diabetic control values, with 50.3%, 37.1% and 26.7% for week 1, 2 and 3 respectively; therefore, the seed of this cultivar has antihyperglycemic activity. On the other hand, NC and FJL promote serum glucose concentration in greater numbers than the diabetic control, which could be associated with a lack of an antidiabetic effect, having no extra-pancreatic actions such as the stimulation of periferial glucose utilization. The group that was fed the diet supplemented with NC had a significant decrease of insulin compared to the two controls, which is consistent with the high content of blood glucose. Moreover, in the groups supplemented with FJD and FJL, an increase of 30 and 29% respectively was observed, however, compared to the diabetic there was no significant difference (Figure 1).

No significant difference in the quantification of total cholesterol between healthy and diabetic controls nor in the comparison of these controls with the supplemented groups was found.

However, in the HDL concentration an increase of 32 % in FJD was observed compared to the diabetic control. This could suggest a cardiovascular protective effect of the intake of this cultivar, having the ability to decrease surplus cholesterol, and prevent the accumulation of LDL and its possible oxidation, thus reducing the risk of diseases such as atherosclerosis, which is one of the major complications in diabetes (Figure 1). The group that was fed with NC had significantly higher content of triglycerides, with 291.8 ± 25.5 comparing with HC and DC.

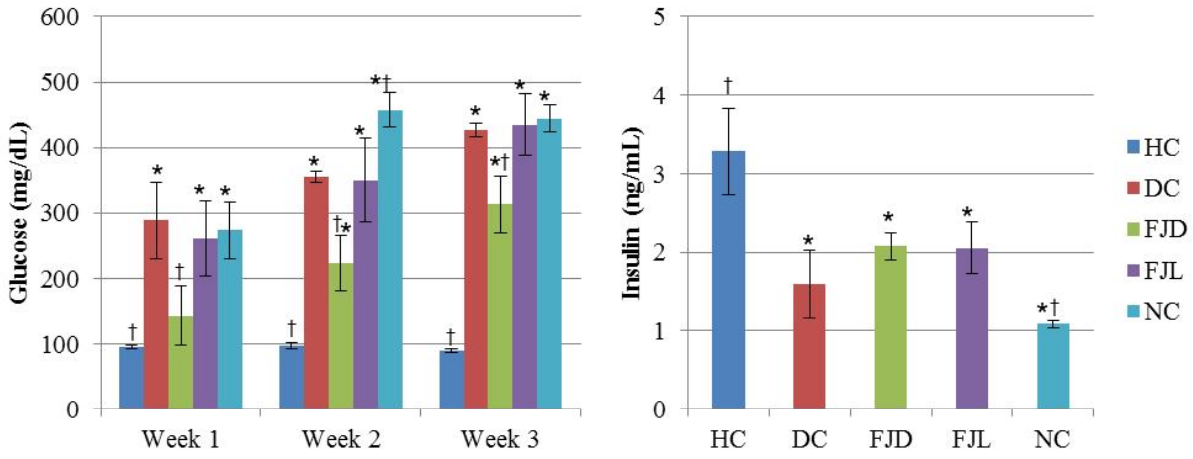

Figure 1. Serum glucose and insulin concentration in diabetic rats fed with a common bean supplemented diet.
Values are presented as mean ± SE. * Indicates statistically significant difference ($p < 0.05$) compared to healthy control and † compared to diabetic control, analyzed with Dunnet test.

Table 1. Lipid profile of diabetic rats fed with a common bean supplemented diet.

	Healthy control	Diabetic control	NC	FJD	FJL
Total Cholesterol	82.6 ± 7.1	87.2 ± 18.3	80.9 ± 6.8	73.5 ± 4.9	79.5 ± 6.6
HDL	24.1 ± 2.4†	18.7 ± 0.3*	21.6 ± 0.8	24.7 ± 0.2†	19.1 ± 1.2*
Triglycerides	131.4 ± 3.1	190.5 ± 10.4	291.8 ± 25.5*†	167.9 ± 7.8	190.7 ± 5.5*

Values are presented as mean ± SE. * Indicates statistically significant difference ($p < 0.05$) compared to healthy control and † compared to diabetic control, analyzed with Dunnet test.

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GENETIC RELATIONSHIPS AMONG BLACK-SEEDED COMMON BEAN CULTIVARS WITH DIFFERENT LEVELS OF BIOACTIVE COMPOUNDS

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INTRODUCTION: Black-seeded common bean (*Phaseolus vulgaris* L.) cultivars are considered an important component of traditional diet in Central and Southern México, due to their nutritive value and nutraceutical properties (Gutiérrez *et al*., 2006). Nutraceutical properties of common beans have been mainly associated to some opaque black-seeded cultivars. The objective of this work was to examine genetic relationships among black-seeded common bean cultivars showing different levels of bioactive compounds using SSR markers.

MATERIALS AND METHODS: In 2012 seven black-seeded common bean cultivars were planted in the Spring and Summer cropping cycles at Hualahuises, Nuevo León (24° 52' 50" N, 99° 37' 19" W and 371 m elevation). A randomized complete block design (RCBD) was used with three (Spring) and four replications (Summer) for each cultivar. Plant samples (one row 5 m in length and 1.28 m in width = 6.4 m^2) were taken in each plot for flavonols and soyasaponins quantification according to Guajardo *et al*. (2012). ANOVA was used to compare levels of bioactive compounds following a RCB design combined over environments. Post-hoc comparisons among means were computed by Tukey´s honestly significant difference ($P \leq 0.05$). Seven SSR *loci* (BM143, BM154, BM164, BM181, BM188, GATs54, GATs91) (Gill *et al*., 2011) were used to analyze genetic relationships among cultivars. PCR amplification of each SSR was performed according to Gill *et al*. (2011). The reaction volume included DNA (75 ng), 0.16 μM of each primer (sense and antisense), 2 μL of PCR buffer 10X, 1.5-2.5 mM of Mg (depending on each primer), 2 mM of dNTPs and 1 U of Taq DNA polymerase. Amplification products were separated on six percent polyacrylamide gels. Gels were documented for allele detection by using the Kodak Molecular Imaging System Ver. 4.0 (Eastman Kodak, Rochester, USA). Euclidean distances among cultivars were calculated using the NTSYS ver. 2.02e software (Rohlf, 1997) and one dendogram was constructed using the UPGMA clustering method.

RESULTS AND DISCUSSION: Significant differences ($P \leq 0.01$) among cultivars across environments were observed for contents of myricetin 3-*O*-glucoside, quercetin 4-*O*-galactoside and kaempferol 3-*O*-glucoside (Table 1). The cultivar Negro Sahuatoba showed the highest average value for myricetin, quercetin and kaempferol. Similar results for myricetin were found in Negro Pacífico, Negro Tacaná and Negro Nayarit, compared to Negro Sahuatoba. Quercetin levels in Negro Sahuatoba resulted similar to those observed in NP_{NL}, Frijozac and Negro Altiplano. Similar average values were observed among cultivars for soyasaponins such as Af (124 μg g^{-1}), V (4 μg g^{-1}), αg (43 μg g^{-1}) and βg (25 μg g^{-1}). On average, higher amounts of quercetin 4-*O*-galactoside (145 μg g^{-1}) and soyasaponin Af (124 μg g^{-1}) were accumulated in all the cultivars across environments. Results showed differences from those reported by Díaz *et al*., (2006), where quercetin 4-*O*-galactoside averaged 10.9 μg g^{-1} and kaempferol 3-*O*-glucoside 52.3 μg g^{-1}. High yielding cultivars also showing higher values for a particular bioactive compound need to be selected instead of selecting by the use of total flavonol or soyasaponin

contents. Negro Sahuatoba, Negro Pacífico and Negro Nayarit were considered as an important input for domestic cooking and food industry. Cluster analysis separated germplasm into two groups (Fig. 1), one including cultivars derived from crosses where Jamapa is the common parent. The second group included cultivars with common parents derived from improved lines developed at CIAT´s breeding program. Common parents were Porrillo Sintético, C. N. Chimaltenango and other landraces collected in Central America and Southern México. Advances obtained for field, laboratory and molecular characterization represents an important tool for systematic use of common bean diversity in breeding programs for yield, nutritive value and nutraceutical properties.

Table 1. Bioactive compounds (µg g^{-1}) in eight black-seeded common bean cultivars planted in Hualahuises, México. 2012.

Cultivar	[1]Myricetin	Quercetin	Kaempferol	[2]SS Af	SS V	SS αg	SS βg
Negro Nayarit	31.8 ^{ab}	134.2^{bc}	1.7^{b}	118.4	3.1	43.5	24.1
Negro Tacaná	48.0^{ab}	107.1^{c}	1.7^{b}	111.5	4.3	36.1	22.3
Negro Pacífico	68.6^{a}	131.5^{bc}	1.9^{b}	129.1	5.0	48.6	27.7
Negro Sahuatoba	70.7^{a}	183.5^{a}	7.2^{a}	113.8	4.5	39.5	26.9
Negro Altiplano	28.1^{b}	142.8^{abc}	3.3^{b}	125.5	3.3	45.5	26.1
Frijozac	28.6^{b}	155.0^{abc}	2.5^{b}	147.0	4.5	42.4	25.1
NP_{NL}	19.9^{b}	159.5^{ab}	2.0^{b}	119.7	3.9	46.8	25.0
[3]Negro San Luis	7±1	116±13	0.3±0.1	98±19	2.3±0	1.7±1	14±1
Average	**42**	**145**	**2.9**	**124**	**4**	**43**	**25**

[1]myricetin 3-*O*-glucoside; quercetin 4-*O*-galactoside and kaempferol 3-*O*-glucoside. [2]SS= Soysaponin; [3]Eliminated from ANOVA. [a-c]Letters in each column indicate significant differences according to Tukey's HSD ($P \leq 0.05$).

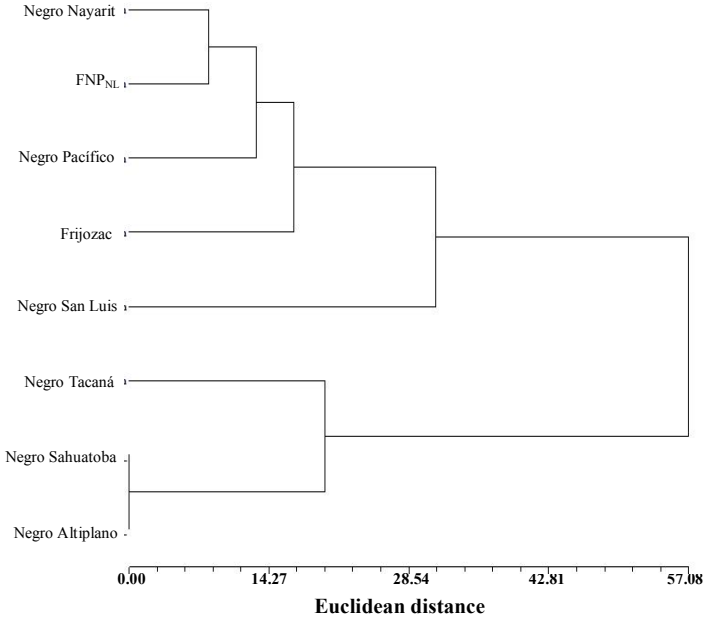

Figure 1. Dendrogram of genetic similitude (Euclidean distance) between eight common bean cultivars obtained by using the UPGMA clustering method.

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DIETARY FIBER CONTENT IN DRY EDIBLE BEAN CULTIVARS

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Dietary fiber (DF) from plant foods is an important contribution to the human diet and there is renewed interest in the effects of DF on human health. Dietary fiber is composed of a complex mixture of carbohydrate polymers with ten or more monomeric units, which are not hydrolyzed by the endogenous enzymes of the small intestine in humans (McCleary, 2010, 2011). DF has been associated with physiological effects in the human alimentary canal and has demonstrated positive health benefits; primarily increased laxation, blood cholesterol attenuation, blood glucose attenuation, and alleviation of diverticular disease. The development of an improved method to measure dietary fiber in food was approved by the American Organization of Analytical Chemists known as the "Integrated Total Dietary Fiber Method". This method was named the AOAC 2011.25 method and classifies DF into three main components, namely insoluble dietary Fiber (IDF), soluble dietary fiber (SDF) and the oligosaccharides raffinose, stachyose, and verbascose (McCleary et al., 2012). Collectively, these components are knows as total dietary fiber (TDF). Breeding efforts to alter the DF content of pulse crops has been very limited, however the goals would be to increase the SDF and IDF portions, and reduce the oligosaccharide content. Previously we reported the results of dietary fiber content for a limited set of dry bean (*Phaseolus vulgaris L.*) lines (Brick et al, 2011, Kleintop et al. 2012) and modified the AOAC 2011.25 method to adapt it for dry bean seed (Kleintop, et al. 2013). This update reports the variation in fiber and oligosaccharide content for a wider range of cultivars/lines grown at two locations.

MATERIALS AND METHODS

A set of diverse dry edible bean cultivars/lines from the Common Bean Coordinated Agricultural Project (BeanCAP) were grown during 2011 in Fort Collins CO and Fargo ND. Seed from each entry was evaluated for dietary fiber and oligosaccharide content using the AOAC 2011.25 method (McLeary et al., 2012). The samples were prepared for analysis by soaking 1 g of seed then transferred to an autoclave and cooked for 65 minutes. The cooked samples were then homogenized, and analyzed with the Integrated Total Dietary Fiber Assay using a commercial assay kit available from Megazyme, Inc. (Wicklow, Ireland) according to the manufacturer's instructions. The assay was used to quantify the percentages of IDF, SDF, TDF, raffinose, stachyose, and verbascose.

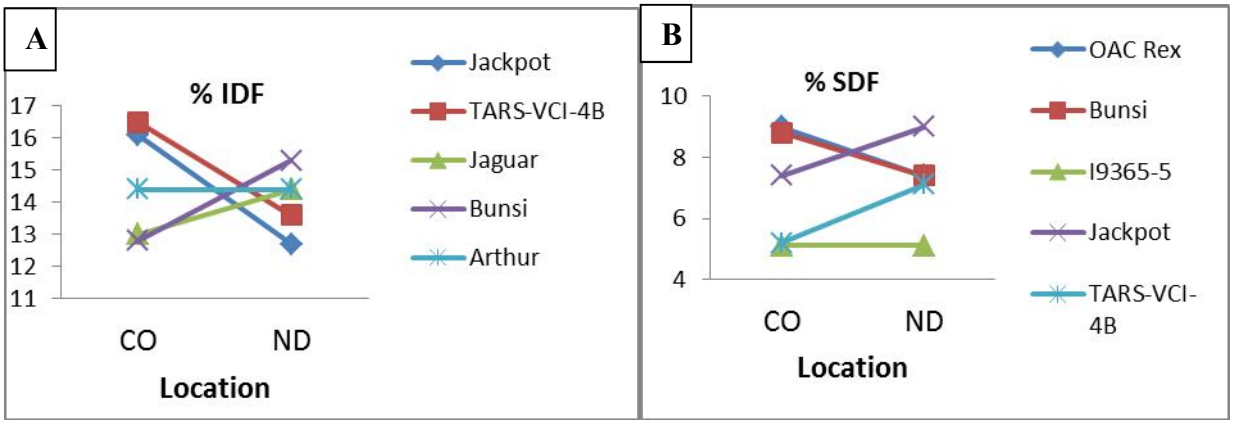
RESULTS AND CONCLUSIONS

Fiber content among 282 cultivars grown in Colorado varied for all components of dietary fiber (Table 1). Genotype by environmental (GXE) interactions were measured from seed grown in North Dakota and Colorado. GXE effects occurred for IDF, SDF and TDF (Figure 2A and 2B) but not for raffinose, stachyose, or verbascose or total oligosaccharide content. These results demonstrate that variation exists among dry bean cultivars/lines for both dietary fiber and oligosaccharide content, and that GXE occurs for some components of DF. Based on these results breeders should be able to modify content using selection, however the GXE component of variation may contribute to a slower response to selection.

Table 1. Dietary fiber (IDF, SDF, and TDF) and oligosaccharide (raffinose, stachyose, and verbascose) content among 254 entries grown in Fort Collins, CO.

Table 2. Genotype by environment interactions for IDF (A) and SDF (B) for selected bean entries grown in North Dakota and Colorado.

	% IDF	% SDF	% TDF	% Raffinose	% Stachyose	% Verbascose	Total Oligos
Range Among Entries	**11.4 – 16.9**	**5.1 - 10.3**	**22.8 - 30.2**	**0.3 – 0.9**	**3.0 - 5.2**	**0.02 – 0.23**	**3.5 – 60**
LSD $_{(0.05)}$	**1.5**	**1.0**	**1.3**	**0.3**	**1.2**	**0.15**	**1.4**



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ESTIMATES OF GENETIC AND PHENOTYPIC PARAMETERS OF COMMON BEAN COOKING TIME

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INTRODUCTION: Out of the technological characteristics of the bean grain, cooking time is, without a doubt, of greatest importance (Oliveira *et al*., 2011). Therefore, the bean improvement program aiming to develop a cultivar with commercial acceptance should consider selecting lines with reduced cooking time.

Previous studies have shown that there is variability for this character, suggesting genetic progress with selection (Ribeiro *et al.*, 2013). In this context, the objective of this study was to determine the genetic and phenotypic parameters of the cooking time, such as genetic variance, heritability and prediction of genetic gain with selection based on the mean of progenies.

MATERIALS AND METHODS: The experiment was set up in the Department of Biology at the Federal University of Lavras, (910 meters altitude 21° 14'S latitude and 45° 22'W longitude) in Minas Gerais, Brazil.

The BRSMG Madrepérola cultivar was crossed with RP-2 line, both are carioca grain type, with short and prolonged cooking time, respectively. The progenies were grown in the field to obtain the $F_{2:4}$. After maturation, the grains were harvested manually and sun-dried for three days, then stored in cold chamber at 12°C for 60 days.

A triple lattice design 17 x 17 was used, with two common checks per block represented by the parents, totaling 291 treatments. Each plot had 50 whole and healthy grains of each treatment. Thus, each group of 17 progenies and two checks (constituting the block) were placed together in electric pressure cooker for 30 minutes after pressure stabilization. Then we measured the percentage of grains cooked through the Mattson apparatus, according to the method described by Carvalho (2013).

After checking assumptions of normality of error distributions, the data were transformed using arcsine transformation. The percentage of cooking time data was subjected to analysis of variance considering all the effects as random, except the overall mean and the checks. Data were analyzed using SAS 9.3 (SAS institute, Cary, NC).

RESULTS AND DISCUSSION: The coefficient of variation (CV) and accuracy (r_{gg}) were 13.31% and 96.50%, respectively, indicating a good experimental precision. Significant differences ($P<0.01$) were detected among progenies and between parents. The average cooking time (CT) was 46.84% (table 1), varying from 9.14% to 78.98% for progenies 154 and 258, respectively (data not showed). This variability among genotypes allows selection of those with reduced cooking time.

In the $F_{2:4}$, genetic variance among progenies contains 1 σ_A^2 (additive variance) and 1/16 σ_D^2 (dominance variance) leading, in principle to the estimation of heritability in the broad sense. However, in this generation σ_D^2 is negligible because only explores 1/16 σ_D^2, and can be inferred that almost all of the genetic variance is due to σ_A^2. Thus, for practical purposes, one can consider the heritability estimates in narrow sense.

Table 1. Analysis of variance of the percentages of cooked common bean grains.

Source of variation	DF	MS
Replications	2	638.9172**
Blocks within replication (adjusted)	48	53.8507
Progenies (adjusted)	288	564.5432**
Among parents (checks)	1	4292.9810**
Parents vs progenies	1	419.4029*
Checks x blocks	100	40.7573
Error	528	38.8851
Mean		46.84
CV (%)		13.31
r_{gg} (%)		96.50
h^2 (%)(LI – LS)[x]		93.11(91.57 - 94.40)
σ_p^{2}[y]		185.5286
RS (%)		46.12

*, ** Significant at the P=0.05 and P=0.01, respectively.; [x] limits of the confidence interval of heritability (h^2);
[y] σ_p^2, r_{gg}, RS Genetic variance of progenies, accuracy and response to selection, respectively.

The magnitude of heritability is high, indicating a greater contribution of genetic factors in the control of bean cooking time, and wide genetic variation in Brazilian lines. Similar results have been reported by Elia (2003) and Elia *et al*. (1997), where they found that narrow sense heritability was 90%. The heritability of this magnitude can be related to character controlled by a few major genes. This confirms the significant gain with selection at progeny mean level. These results also open the possibility of successful use of molecular marker assisted selection (MAS), since the phenotypic selection is performed at the end of the crop cycle and it is laborious.

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GENETIC AND PHENOTYPIC PARAMETERS OF COOKING TIME FOR COMMON BEAN GRAINS

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Until a short time ago in Brazil, recommendation of new common bean cultivars was primarily made based on their agronomics traits, such as yield performance and pest and disease resistance. However, a species for human consumption, like common bean, should have the approval not only of producers, but also of consumers. For that reason, in addition to the agronomic traits of common bean, breeding programs should have a greater concern for the appearance and, above all, for the cooking time of the grains, which also brings about improvements in culinary and nutritional quality (Batista et al., 2010).

A recurrent selection program with the aim of improving yield and grain appearance in carioca type beans (beige with brown stripes) has been conducted by the Federal University of Lavras (UFLA), Brazil, since 1990 (Silva et al., 2010). Up to now, 13 selection cycles have been carried out. It is important to check if there is still variability for the traits under selection, especially for cooking time, which was not used as a selective criterion in the past, and at the same time demonstrate if simultaneous selection for yield and qualitative aspects of grains is viable.

To obtain this information, 252 $S_{0:2}$ progenies selected from cycle XIII of the recurrent selection program were used. Details of this program are presented by Silva et al. (2010). The progenies and four controls were assessed in three locations of the state of Minas Gerais, Brazil – Lavras, Patos de Minas and Lambari. Seeds were sown in November 2012 and harvested in February 2013. At around 30 days after harvest, cooking ability was evaluated, using the method proposed by Pádua et al. (2013), and grain quality was evaluated using a scoring scale from 1 to 9, 1 being very bad and 9 being very good. The shape, size and color of the grains (especially early darkening) were considered in the evaluation.

Using the mean values per location, joint analysis was realized. Based on these analyses, genetic and phenotypic parameters were estimated, using a method similar to that described by Ramalho et al. (2012). Gain for simultaneous selection for the three traits was obtained using the sum of the standardized variables (ΣZ) selection index. Since the Z_{ij} variable may assume both negative and positive values, a constant was added to avoid negative values.

A positive correlation was found between percentage of cooked beans and grain type (Table 1). However, due to the low magnitude of the correlation, it would not be possible to undertake indirect selection for cooking ability based only on one of these two traits. It may also be seen that there is no association between these traits and grain yield.

It may also be observed in table 2 that the selection carried out by the ΣZ index led to positive gains for all three traits involved. Moreover, the magnitudes of the indirect gains (correlated response) were greater than 50% of the gain expected if the selection had been carried out directly for each trait. In contrast, if the strategy applied in the program in previous cycles had been used, i.e., selection with an emphasis of grain yield, the correlated response in cooking time and grain type would not be very expressive, and would actually be negative for grain type score.

Table 1. Estimates of Pearson phenotypic correlations between the traits of grain yield, grain type score and percentage of cooked grains.

Mean value of the locations		Cooking	Grain type
	Yield	0.181	0.116**
	Cooking		0.414**

** Significant at 1% of probability by the t test

Table 2. Estimate of gain from selection (GS) considering the three traits simultaneously, based on grain yield and the Z index.

		Z Index	Yield (g/plot)	Grain type score	Cooking (%)
Response of direct selection in each trait	M_S[1]	-	513.58	06.89	53.72
	M_O[2]	-	421.77	04.74	36.46
	h^2	-	051.82	57.47	24.72
	GS	-	047.58	01.23	04.27
	GS/Mo (%)	-	011.28	26.02	11.71
Correlated response to selection using grain yield	M_S	-	513.58	04.49	40.64
	M_O	-	421.77	04.74	36.46
	h^2	-	051.82	57.47	24.72
	GS	-	047.58	-0.14	01.03
	GS/Mo (%)	-	011.28	-3.06	02.84
Correlated response to selection using the Z index	M_S	10.29*	474.36	06.47	46.95
	M_O	07.00*	421.77	04.74	36.46
	h^2	67.23*	051.82	57.47	24.72
	GS	02.21*	027.25	00.99	02.59
	GS/Mo (%)	31.62*	006.46	20.97	07.12

[1/] Mean of 5% superior progenies $S_{0:2}$, [2/] Mean considering all progenies $S_{0:2}$

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CORRELATION AMONG PRINCIPAL CHEMICAL COMPONENTS IN COMMON BEANS GENOTYPES FROM RIO GRANDE DO SUL, BRAZIL

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INTRODUCTION: The common bean has been object of breeding programs aiming the development of new cultivars adapted to varied production system and shown differentiated nutritional characteristics. Food quality and economic viability has been common objectives in breeding programs in several countries. The bean breeding program of Embrapa are looking for cultivars that have highlighted features of the nutritional point of view due to the recognized fact that inadequate intake of mineral nutrients leads to numerous disorders and metabolic abnormalities (FRANCO, 1999). Minerals are vital nutrients for life and are found naturally in soil; are passed to the plant and its grain, which are consumed by animals and humans. Pinheiro et al (2010) analyzing germplasm from Portugal, observed high degree of variability for P, Fe, Zn, Cu, Mn and Ca. The high mineral variability observed in the seeds can be useful for the selection of cultivars with higher nutrition value and for the improvement of seed nutrition quality traits. The aim of this paper was to verify the correlation between nutritional characters in breeding cultivars and landraces of bean from Rio Grande do Sul state, Brazil.

MATERIAL AND METHODS: The experiment was conducted in 2009/2010 in Experimental Station Cascata, of Embrapa Temperate Agriculture. Whole grain of 54 bean genotypes with black and no black coat including commercial cultivars and landraces from Rio Grande do Sul State were analyzed. The fertilization was made with 300 kg ha^{-1} of NPK fertilizer formulation 10-30-10, without the use of topdressing nitrogen. The soil cultivated was a planossolo with low fertility.

Were determined the follow components: potassium (K) analyzed through atomic emission mode, calcium (Ca) and magnesium (Mg) were evaluated by the method of Miyazawa et al. (1992), using atomic absorption spectrophotometry (AAS), as cited by Silva (1999). The determination of phosphorus (P) was made by UV-VIS spectrophotometry, quoted by Silva (1999). For nitrogen (N) and sulfur (S) technique was used in combustion equipment CHN elemental analyzer TruSpec-S. The oligoelements were analyzed by the following methods: copper (Cu) - using the Perkin-Elmer (1982), Miyazawa et al. (1992b), Malavolta et al. (1989); iron (Fe) - by means of the method Ohlweiler (1974) and Malavolta et al. (1989); manganese (Mn) - methodology with Perkin-Elmer (1982), Miyazawa et al. (1992b); and zinc (Zn) - method using Perkin-Elmer (1982) and Malavolta et al. (1989), by atomic absorption spectrophotometry (AAS), as stated by Silva (1999). The crude protein content was determined according to the Kjeldahl method, considering the mean of two readings per sample. The content of the antioxidant astragalina was determined using HPLC techniques according Correia et al. (2006).

RESULTS AND DISCUSSION: The correlations between various components in whole grains beans were quite low, as is seen in Table 1. In opposite Silva et al. (2012), that observed positive correlations between most nutrients, indicating the possibility of obtaining lines with higher nutritional value by selection. The potassium content correlated positively with phosphorus and copper, with a correlation coefficient of 0.57 and 0.43, respectively. Nitrogen showed high positive correlation with iron (R = 0.42) and, as expected, was correlated with the total protein

content, although it may be considered reasonable. This fact is related with the different methods used for the determination of N.

Table 1- Correlation coefficients between macro and microelements, protein content and antioxidant astragalina (AST) in whole grains of bean landraces and breeding cultivars from Rio Grande do Sul state. Brazil, 2012.

	K	Mg	P	N	S	Cu	Fe	Mn	Zn	AST
Ca	0.18	0.0009	0.23	-0.35	-0.1	0.16	0.09	0.21	0.09	0.14	-0.22
K	#	-0.04	0.57*	0.25	-0.29	0.43*	0.25	0.17	0.14	0.32	-0.23
Mg		#	0.009	0.16	0.31	0.08	0.21	0.32	0.27	0.04	-0.33
P			#	0.3	0.04	0.29	0.39	0.28	0.14	0.14	-0.35
N				#	-0.03	-0.08	0.42*	0.12	-0.05	-0.10	0.68*
S					#	-0.22	-0.16	0.03	-0.37	-0.29	0.17
Cu						#	0.18	-0.02	0.44*	0.43*	-0.48*
Fe							#	-0.21	0.64*	-0.15	-0.44*
Mn								#	-0.02	-0.02	-0.003
Zn									#	0.24	-0.49*
AST										#	-0.17

*correlation significant at 5% probability

These data show that in breeding, the lines selection for high levels of macronutrients and oligoelements in grain can be realized, thus specific nutrient such iron and calcium, not exhibit negative correlations with other important nutrients. Copper and iron showed high positive correlation with zinc (R = 0.44 and 0.64, respectively). These results are in agreement with Mesquita et al. (2007). Among all nutrients analyzed only copper showed positive correlation (R = 0.43) with the content astragaline, the other elements had no correlation with that component. As the correlation with the nutrient content of protein, only the oligoelements, copper, iron and zinc showed a high negative correlation with protein content in grain, with a correlation coefficient of -0.48, -0.44 and -0.49, respectively. This may demonstrate that increasing levels of oligoelements may negatively affect protein and antioxidants in the grain. These results disagree with Mesquita et al. (2007), in which the strains analyzed showed higher protein contents also stood out in relation to P content, however high protein was not associated with high potassium content. Fe content was positively correlated with Mn and Ca content and Zn was positively correlated with the N, P, Cu and S, fact not observed in this results.

CONCLUSION: The oligoelements Cu, Fe and Zn are negatively correlated with protein, but Fe is correlated positively with nitrogen and Cu with astragaline; Ca showed no correlation with elements analyzed.

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EFFECT OF DROUGTH ON IRON AND ZINC CONTENT IN DIFFERENT VARIETIES OF PINTO BEAN

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INTRODUCTION

Mineral nutrients play an important role in the biochemical and physiological functions of biological systems. Common bean is an important source of iron and zinc, however, the content of these minerals can be affected by environmental conditions (Beebe, 2000). The objective of this study was to determine iron and zinc content in three different varieties of Pinto bean produced under a water deficit in Celaya, Gto., México.

MATERIAL AND METHODS

Bean varieties Pinto Durango (PD), Pinto Saltillo (PS) and Pinto Rarámuri (PR) were sowed in greenhouse under three soil moisture conditions: irrigation (Ir), severe drought during the reproductive stage (SD) and a constant drought during the stages of development of the cultivar (CD). A factorial design 3 X 3 with randomized completed blocks was used. With irrigation, soil moisture remained at 70 % of field capacity, with severe drought irrigation was suspended at the beginning of flowering and with constant drought soil moisture remained at 40 % of field capacity during the developing of plants. After harvest, grain was cooked and freeze dried. The content of iron and zinc was determined by the digestion of the samples with nitric and perchloric acid (1/5 v/v) at 60 °C during 12 h. Standards and samples were filtered and absorbance was read at 238.2 and 213.8 nm for iron and zinc respectably, using an atomic absorption spectrophotometer. Statistical analysis was conducted with JMP 5.0.1.

RESULTS AND DISCUSSION

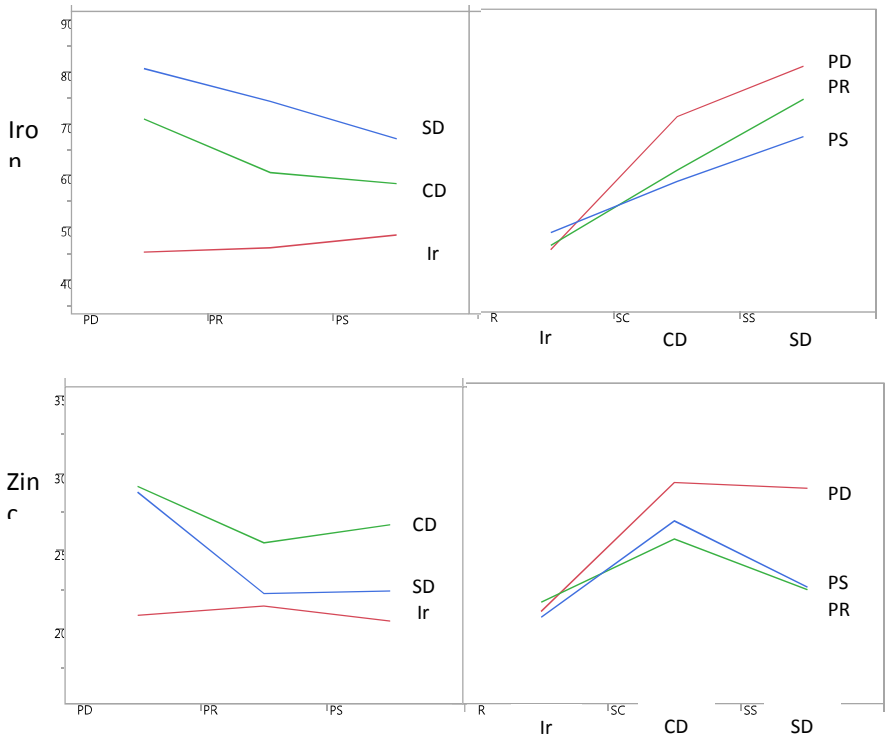
Variations were observed among the soil moisture condition and varieties (Table 1). In the iron content, an increase of 78% for PD, 64% for PR and 49% for PS grown under SD was obtained, the highest concentration were observed on PD cultivated under SD (80.6 ± 4.7 ppm) while the lowest concentration was for the same variety with Ir (45.3 ± 3.7 ppm). In the other hand, the highest amount of zinc was observed under CD, with 39%, 22% and 18% for PD, PR and PS respectably; the concentration of this mineral was significantly higher for PD grown under CD (29.17 ± 2.3) and SD (28.80 ± 3.9). In Figure 1, we can see that PS is the variety with the greatest capacity to absorb iron under irrigated conditions; however, with decreasing soil moisture, it has the lowest content of this mineral compared to PD and PR. The interaction profile plot of zinc content indicates that the increase between CD and SD is less for PD than the one observed in PR and PS. Some research indicates that in a condition of stress, accumulation of minerals is related to the redistribution between different plant organs; for this investigation, the accumulation of iron in the bean plants subjected to severe drought and zinc under constant drought was probably due to a distribution of the mineral to developing grains.

Table 1. Iron and zinc content of Pinto bean varieties cultivated under different soil moisture conditions.

Variety	Soil moisture condition	Iron content (ppm)	Zinc content (ppm)
Pinto Durango	Irrigation	45.30 ± 3.7 f	20.87 ± 1.8 b
	Severe Drought	80.60 ± 4.7 a	28.80 ± 3.9 a
	Constant Drought	70.93 ± 1.1abc	29.17 ± 2.3 a
Pinto Rarámuri	Irrigation	46.13 ± 1.0 ef	21.47 ± 2.0 b
	Severe Drought	74.27 ± 3.8 ab	22.27 ± 2.1 b
	Constant Drought	60.60 ± 1.6 cd	25.53 ± 1.2 ab
Pinto Saltillo	Irrigation	48.60 ± 3.7 def	20.50 ± 0.6 b
	Severe Drought	67.07 ± 2.4 bc	22.43 ± 2.4 b
	Constant Drought	58.47 ± 1.8 cde	24.70 ± 1.8 ab

Values are presented as mean ± SD. Means in the same row with a common letter are not significantly different ($p<0.05$, Tukey).

Figure 1. Interaction profile plots of iron and zinc content in Pinto bean cultivated under different soil moisture conditions.



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OCCURRENCE OF OXIDATIVE STRESS IN CARIOCA BEANS

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INTRODUCTION: The accumulation of Reactive Oxygen Species (ROS) in the cells of the seed coat and cotyledon of carioca bean grain can cause: (a) oxidation of compounds present, mainly, in the tegument, which culminates in the formation of compounds responsible for the color change of the grains; (b) lipid peroxidation (damage to the cell membranes) and, consequently, electrolyte leakage as Ca^{2+} and Mg^{2+} with the probable formation of insoluble pectates, in the case of seeds of legumes[1] and (c) changes in texture, which is considered one of the most important attributes of legume seeds, since it affects the palatability and, consequently, the consumer acceptability[2]. Due to the lack of information about the primary event triggering of the hardening and darkening processes of the carioca bean grains, this study evaluated the SOD (Superoxide Dismutase) activity, enzyme responsible for the production of ROS, specifically, hydrogen peroxide (H_2O_2), hydrogen peroxide content, lipid peroxidation, effect originated by accumulation of H_2O_2 and technological parameters usually evaluated by consumers, such as color and cooking time.

METHODS: Freshly harvested naturally dried grains of carioca genotypes (BRS Pontal, BRS Madrepérola, CNFC 10467, BRS Estilo) and a Pinto Bean slow-darkening line 1533-15[3] (Canadian genotype used as negative control), were cultivated at the Capivara farm of Embrapa Rice and Beans (Oct 2013). 200 g of grains were peeled using rice dehuller (TM05C, SATAKE), and the cotyledon and seed coat were separated by using sieves of 9, 14 and 16 mesh, followed by grinding and maceration with liquid nitrogen. The extracts were prepared according to Lee & Lee[4]. The supernatant (crude extract of cotyledon and seed coat) was used for determination of SOD activity[5], hydrogen peroxide content[6] and for lipid peroxidation level[7]. The specific activity of SOD was obtained on the basis of the total soluble protein content with BSA as standard[8]. Color assessments of whole grains were held at the Colorimeter Color Quest XE, Hunter Lab, using CIELAB system[9], thus obtaining the values of 10 readings for coordinates L * (lightness), a* (yellowish) and b* (reddish). The cooking time was determined in Mattson cooker[10]. Mean values were compared by Tukey test at 5% probability and analysis of Pearson correlation coefficient using Statistica 7.0 (STATSOFTINC, Tulsa, Ok, USA).

RESULTS AND DISCUSSION: In stored grains, the presence of SOD and its activity indicate that oxidative stress may occur during this period. In the tegument of grains of BRS Pontal and BRS Estilo, genotypes with known fast darkening, the SOD activity was higher compared to that found in BRSMG Madrepérola and CNFC 10467, known as slower darkening beans. The Pinto Bean line, in turn, which is considered resistant to darkening, presented SOD activity significantly reduced in tegument compared to other samples (Table 1). Regardless of the genotype, the SOD activity in the cotyledon was lower than that found in the tegument and, among the genotypes, there was no significant difference. A higher SOD activity, mainly in the carioca bean grain hull, may indicate the occurrence of oxidative process and, ultimately, the darkening process. The hydrogen peroxide content was higher in the BRS Estilo seed coat and Pinto Beans cotyledon. As a result of the generation of H_2O_2 from SOD activity, damage may occur to the constituents of membranes of bean grain (lipid peroxidation) and favor the formation of insoluble pectates. It is observed that the higher level of lipid peroxidation took place in

tegument of BRSMG Madrepérola, while in cotyledon it did not varied significantly among genotypes. However, the level of lipid peroxidation in cotyledon was greater than that observed in the tegument. This parameter seems to be highly related with the hardening than with the darkening process, therefore, more variables, such as the contents of Ca^{2+} and Mg^{2+}, need to be quantified in order to support such a hypothesis. In association with the analyses of oxidative stress level, luminosity (color) and the cooking time of the bean grains were assessed. While the color of the grains of BRS Madrepérola and CNFC 10467 remained clear, in comparison to BRS Pontal and BRS Estilo (Table 1), the cooking time was not different between genotypes, with exception of BRS Pontal which had a cooking time 3 times longer than others. The correlation analysis of data ($p \leq 0.05$) showed a moderate negative correlation between the average cooking time and the color of the beans ($r = -0.67$), which means that the higher the cooking time, the darker the grains. The activity of SOD in the tegument, and color (L) of whole grains also presented high negative correlation ($r = -0.93$), suggesting that biochemical events as SOD activity may explain, in part, the process of darkening of the carioca beans. In contrast, the evaluated parameters cannot explain the process of hardening, once there was low significant correlation ($r= -0.25$) in the cotyledon, between the index of lipid peroxidation and cooking time.

Table 1 – Oxidative stress parameters and color/cooking time of freshly harvested bean grains.

Analysis / Genotypes	**Madrepérola**	**BRS Estilo**	**CNFC 10467**	**BRS Pontal**	**Pinto Beans**
			SOD specific activity(Un SOD mg^{-1} proteína)		
Seed Coat	$193,2^{b} \pm 5,7$	$458,5^{a} \pm 2,9$	$158,0^{b} \pm 14,5$	$382,0^{a} \pm 54,4$	$45,1^{c} \pm 13,4$
Cotyledon	$55,9^{c} \pm 2,9$	$44,0^{c} \pm 4,8$	$47,2^{c} \pm 6,0$	$54,1^{c} \pm 12,3$	$57,8^{c} \pm 8,2$
			Hydrogen Peroxide (nmol g^{-1} MF)		
Seed Coat	$13,7^{c} \pm 1,3$	$60,7^{b} \pm 2,6$	$15,72^{c} \pm 0,9$	$8,5^{c} \pm 2,3$	$11,5^{b} \pm 0,1$
Cotyledon	$15,0^{c} \pm 2,5$	$33,9^{a} \pm 3,9$	$17,7^{c} \pm 0,4$	$8,4^{c} \pm 1,1$	$59,6^{b} \pm 7,4$
			MDA equivalents (nmol g^{-1} MF)		
Seed Coat	$10,2^{d} \pm 0,8$	$2,8^{e} \pm 0,4$	$4,4^{b,c} \pm 0,4$	$6,3^{b,c} \pm 0,7$	$6,7^{a,b} \pm 0,7$
Cotyledon	$12^{d,e} \pm 1,3$	$14,7^{e} \pm 3,4$	$13,2^{d,e} \pm 0,5$	$13,2^{d,e} \pm 0,5$	$14,3^{e} \pm 0,5$
Whole Grains			**Color**		
L*	$52,5^{d} \pm 1,3$	$48,9^{b} \pm 1,3$	$51,9^{d} \pm 1,3$	$47,0^{c} \pm 2,1$	$55,6^{a} \pm 2,1$
a*	$7,0^{e} \pm 0,3$	$9,7^{b} \pm 0,3$	$7,7^{d} \pm 0,4$	$10,2^{a} \pm 0,6$	$8,4^{c} \pm 0,6$
b*	$19,3^{c} \pm 0,7$	$20,0^{b} \pm 1,1$	$19,2^{c} \pm 0,9$	$20,4^{b} \pm 0,7$	$21,9^{a} \pm 1,0$
			Cooking time (min)		
	$43,6^{b} \pm 9,7$	$37,1^{b} \pm 3,7$	$46,5^{b} \pm 0,9$	$124,5^{a} \pm 35,9$	$30,8^{b} \pm 6,5$

Results are the mean of three repetitions ± SD. Within lines, means with same superscript are not significantly different by Tukey test ($p>0.05$). MDA = malonaldehyde. FM = fresh matter.

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ACCELERATED AGING EFFECTS ON THE GRAIN QUALITY OF STORED CARIOCA BEAN

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INTRODUCTION: In Brazil, the common bean production need to be stored to ensure its supply in all regions of the country, avoiding shortages in dry season and reducing the fluctuation of market prices (Brackmann et al., 2002). However, it is known that during storage, grain exposure to uncontrolled conditions such as light, moisture and temperature, causes changes in its technological quality which depreciates its commercial value. The browning process of bean tegument has been referenced by various authors to the presence of intense ultraviolet light (Junk-knievel; Vandenberg; Bett, 2007). For research purposes, the accelerated aging of bean grains is one of the options available for the optimization of lab experiments in the evaluation of quality traits. Through an artificial aging simulator chamber of radiation it is possible to accelerate the aging process that occurs naturally, to predict and control the degree of darkening. This research evaluated the effect of the solar radiation simulator chamber on the technological characteristics of carioca bean grains, such as color and cooking time.

MATERIALS AND METHODS: Carioca bean, cv. Pérola, was grown at Embrapa Rice and Beans (Feb/2011). The grains were naturally dried until they reached the humidity around 13%. It was built a chamber to accelerate the aging of grains by simulating solar radiation (80 x 45 x 25 cm (L x W x H) and features two UV lamps Philips TL 40W Actinic BL-K, 60 cm). The temperature increase within the chamber was minimized by the use of 5 hoods (Saron et al., 2000). Half of the sample (500g) was submitted to storage for two months in the dark at room temperature (control bean – CB) and the other half (artificial aged bean – AAB) was submitted to the accelerated aging on the solar radiation simulator chamber (SRSC) at the same period. Samples were evaluated every three or four days for changes in grain tegument color by the CIELab color system (L*, a*, b*), using D65/10° illuminant in a ColorQuest XE (Hunter Lab), and every month for changes in cooking time, using a Mattson cooker (Proctor; Watts, 1987). Quantitative data were evaluated by ANOVA and means were compared by Tukey test ($p<0.05$) using SAS (2003).

RESULTS AND DISCUSSION: Data revealed that the solar radiation simulator chamber was effective for aging bean grains, once their luminosity decreased and the red and yellow colors intensified (Figure 1). The L mean value was different for CB (41.73) and AAB (36.30). It was observed that the mathematical model with a quadratic effect is significant ($p<0.05$), although it had a better data adjustment ($R^2 = 0.66$) for beans submitted to accelerated aging. The mean intensity of a* was also statistically different for CB (10.45) and AAB (12.74). It seems that the mathematical model with linear effect is significant ($p<0.05$), and similarly to the luminosity, the model fits better to AAB data ($R^2 = 0.78$). The mean intensity of b* was statistically different between the two storage conditions (17.92 for CB and 18.69 for AAB), however, the mathematical model was not significant ($p>0.05$). Although the correlation coefficients obtained were low, the mathematical models generated can be used to predict the darkening of common bean cv. Pérola. The exposure of the grain to solar radiation at the SRSC was able to accelerate the darkening process of AAB at 53 days when compared to CB. Results are in agreement with literature, showing that the darkening should be due to oxidation of phenolic compounds present in the seed coat, where solar radiation acts as a powerful catalyst in the reaction (Beninger et al., 2005; Junk-knievel; Vandenberg; Bett, 2007). Regarding cooking time, the sample stored under ambient conditions for 34 days was the most difficult to cook, requiring 51.76 min, although it did not differ from the CB stored for 62 days or from sample stored in SRSC for 34 days. The samples submitted to SRSC for the longest period (62 days) had the lowest cooking time (35.76 min), equivalent to the freshly harvested grain. This shows that UV light did not affect directly the hardening process in cv. Pérola. Differently,

prolonged storage (30 and 60 days) of black beans under adverse conditions (41 °C / 75% RH) has shown to accelerate the aging process (Ribeiro; Prudencio-Ferreira; Miyagui, 2005), and to make grains harder and more resistant to cook. It is important to notice that the darkening process is not necessarily associated to the higher grain hardness, as the CB samples had the lower luminosity and the highest cooking time and the AAB were darker and less resistant to cook (Table 1).

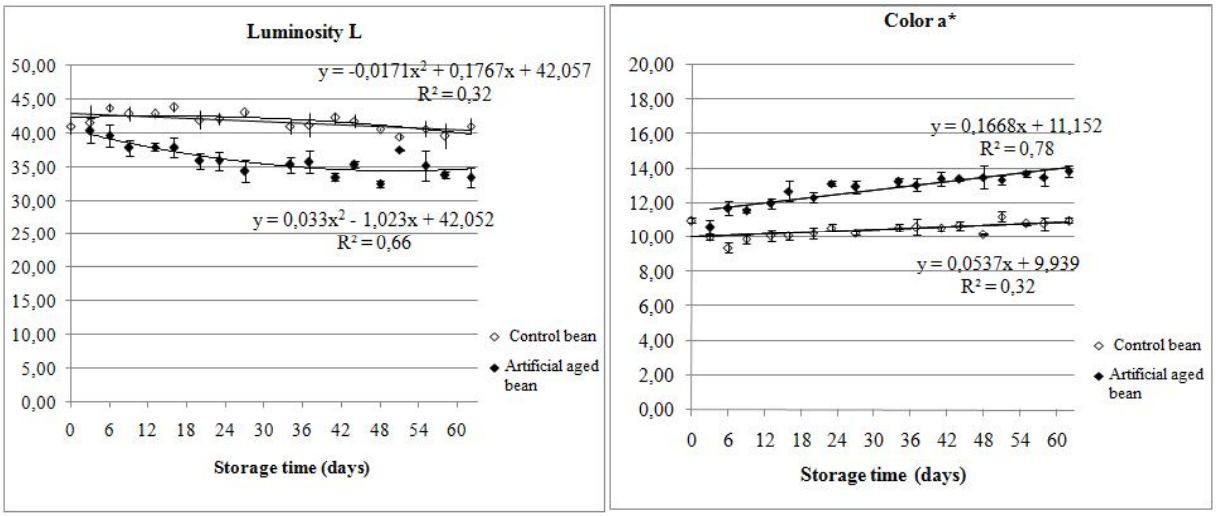

Figure 1 – Luminosity (L) and chroma a* of carioca bean (cv. Pérola) during storage under ambient conditions (control) and in the solar radiation simulator chamber (artificial aged beans).

Table 1 – Cooking time of carioca bean (cv. Pérola) stored under different conditions.

Storage time (days)	Storage condition	Cooking time (min)
0	-	$40.17^{bc} \pm 3.06$
34	Control	$51.76^{a} \pm 3.53$
	Aging Chamber	$47.51^{ab} \pm 0.04$
62	Control	$49.22^{ab} \pm 1.71$
	Aging Chamber	$35.76^{c} \pm 1.82$

Means ± SD (n = 3). Values (within columns) with same letter are not significantly different (p>0.05).

CONCLUSION: The aging chamber was efficient to accelerate the carioca bean darkening, except hardening. Thus, it should be applied to distinguish slowly from fast darkening beans. A deep investigation is still necessary to elucidate UV light effects on hardening or the real relationship between darkening and hardening in carioca beans.

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NUTRIENT CONTENT AND VIGOR CORRELATION IN WHOLE COMMON BEAN SEEDS IN TWO ENVIRONMENTS

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INTRODUCTION

The knowledge of the factors that can influence the quality of seed is crucial for the selection of materials to be used both for cultivation and breeding programs. One of the characters of relevance to examine the seed is the concentration of the main macro minerals and micronutrients, as some may provide higher germination percentage, seedling length and storability (Carvalho; Nakagawa, 1988). Genotypes that have greater capacity translocate and store nutrients in the seed has the greatest potential to produce seeds with high germination and seedling vigor under adverse conditions of biotic and abiotic stress (Marcos Filho, 2005). This trait is influenced by genetic factors and the large variability provides genotypes with various levels of macro and micronutrients in their seeds. This study was to evaluate the correlation between the concentration of nutrients of whole bean seed and physiological quality and vigor in two crop environments.

MATERIAL AND METHODS

Seeds of 15 varieties produced in São Luiz Gonzaga, in Rio Grande do Sul, Brazil, harvests in 2011 and 2012. The samples comprised the seeds produced in the competition assay of bean cultivars installed by Embrapa Temperate Climate.

The seeds obtained were subjected to follow tests: germination, accelerated aging, cold test, electrical conductivity, shoot and root length. In addition to the data from these tests were analyzed in whole seeds the follow nutrients: phosphorus, potassium, iron, magnesium, zinc, calcium and manganese, according to the methodology de Silva (1999).

Data were submitted to analysis of variance and correlation, using Pearson correlation coefficient between nutrients content and seed vigor.

RESULTS AND DISCUSSION

The analysis showed that some characteristics of seed quality correlate with the nutrient contained in its constitution (Table 1). The germination and shoot length were the features that stood out in this behavior. This fact was reported by Adams et al . (1993), which noted that the high percentage of germination of peanut seeds was related to the calcium content of the seed itself. In various functions in plants, manganese and zinc are determinants members of various processes such as protein synthesis, membrane permeability, ion absorption, respiration, synthesis of starch and hormonal control. Thus, there is a chance that both nutrients are involved in seed quality (Teixeira et al., 2005). While this work is no correlation of quality tests with zinc has been observed, manganese correlated for germination, accelerated aging and seedling length corroborating this hypothesis. The fact vigor tests, the seeds of the 2011 season, did not present a higher correlation between quality and nutrient content compared to the 2012 harvest, can be attributed to the difference of these levels for a crop to another. This result indicates that besides the presence of the element, its concentration influence the quality and availability of seeds and

were changed as the year of cultivation, a fact that according to Lemos et al. (2004), the nutritional characteristics are influenced by both the genotype and the environmental conditions on plant development and seed.

Table 1- Correlation coefficient between nutrients content and seed quality in two crops environment in São Luiz Gonzaga, RS, season 2011 and 2012.

Nutrient	Year	G	AA	CT	EC	RL	SL
P	2011	-0.1^{ns}	-0.2^{ns}	-0.18^{ns}	-0.19^{ns}	0.09^{ns}	-0.02^{ns}
	2012	0.32*	0.05^{ns}	-0.14^{ns}	0.60**	0.42**	0.59**
Ca	2011	0.01^{ns}	-0.18^{ns}	-0.36*	-0.09^{ns}	0.09^{ns}	-0.01^{ns}
	2012	0.44**	0.30^{ns}	0.15^{ns}	0.03^{ns}	0.21^{ns}	0.30^{ns}
K	2011	-0.16^{ns}	-0.19^{ns}	-0.18^{ns}	0.23^{ns}	0.20^{ns}	0.18^{ns}
	2012	0.15^{ns}	0.22^{ns}	-0.23^{ns}	0.31*	0.09^{ns}	0.37*
Mg	2011	-0.17^{ns}	0.21^{ns}	0.32*	0.42**	-0.10^{ns}	0.38**
	2012	0.40**	0.31*	0.17^{ns}	-0.06^{ns}	0.25^{ns}	0.23^{ns}
Fe	2011	0.12^{ns}	-0.15^{ns}	0.49**	-0.20^{ns}	0.25^{ns}	-0.29^{ns}
	2012	0.43**	0.13^{ns}	-0.23^{ns}	0.08^{ns}	0.38*	0.39**
Zn	2011	-0.12^{ns}	-0.18^{ns}	-0.14^{ns}	0.01^{ns}	0.28^{ns}	-0.09^{ns}
	2012	0.05^{ns}	0.13^{ns}	-0.14^{ns}	-0.12^{ns}	0.13^{ns}	0.13^{ns}
Mn	2011	-0.07^{ns}	-0.13^{ns}	-0.35*	-0.19^{ns}	0.29^{ns}	-0.07^{ns}
	2012	0.40**	0.55**	0.25^{ns}	-0.18 ^{ns}	0.36*	0.32*

*significant at 5%; ** significant at 1%; nutrients: phosphorus (P), calcium (Ca), potash (K), magnesium (Mg), iron (Fe), zinc (Zn) and manganese (Mn); vigor tests: germination (G), accelerated aging (AA), cold test (CT), eletrical conductivity (EC), root length (RL) e shoot length (SL).

The 2011 season, showed correlation negative, although not significant, between some nutrients, markedly P and Mn, with physiological quality and seed vigor. This fact can be connected with the climate conditions occurred, with abundant rainfall.

CONCLUSION

The mineral composition showed a pronounced effect on the vigor of seeds in the year 2012, which was not observed in 2011. The phosphorus and manganese were the nutrients that showed higher correlation with seed vigor, but only for the year 2012.

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BIOSTIMULANTS IN SEED GERMINATION AND VIGOR OF COMMON BEAN SEEDLINGS.

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INTRODUCTION

Due to the socio economic importance of common bean, the research activities was designed to develop cultivars with high productivity associated with low cost of production. In this context, the use of biostimulants has shown great potential even if its utilization is not a routine practice in crops did not reach high level of technology. According to Castro & Viera (2001) plants bio-regulators are substances synthesized, applied externally and have similar action like known plant hormone. The influence of plant hormones on the seed germination, growth and development of seedlings have been the objective of various researches but still it is necessary the study of different new products with common objective. The present work have the objective of evaluating the effect of different level of biostimulants Stimulate®, Booster® ZnMo and Acadian® on seed germination and seedling vigor in common bean.

MATERIALS AND METHODS

The experiment was conducted the seed laboratory of UFLA, Minas Gerais, Brazil. The experiment was laid in the laboratory and seed bed conditions. In both situations completely randomized design (CRD) with factorial of (5 x 3) +1 was used to establish the experiments. Where, 5 doses of the commercial recommendation of each product for common bean (33%, 66%, 100%, 133% and 166%) and three products used for seed treatments (Stimulate®, Booster® ZnMo and Acadian®) and one additional treatment that constituted the seed immersion in distilled water. In the seed bed treatment the experimental units used were 50 seeds with 4 replications and in the laboratory were used 8 replications with 25 seeds in each experimental unit. In the laboratory was evaluated germination at the end of 9 days, by counting the normal and abnormal seedlings where this counting was realized according to the Standard of Seed Analysis (Brasil, 2009) and expressed in percentage. In the seed bed evaluated the index of velocity of seed emergence, using the formula proposed by Maguire (1962) and emergency (seedlings that emerge after 14 days of seeding), root length, areal part, fresh and dry weight of root and areal parts. The data was submitted for normality and homogeneity of variance test and when necessary the data is transformed using $\sqrt{x}$ (index of germination velocity), Arc sine $\sqrt{x}$ (emergency) and Arc sine $\sqrt{x}/100$ (germination). After these procedures the data was subjected for analysis of variance using the statistical software SISVAR.

RESULTS AND DISCUSSION

In the laboratory, significant effect was not observed among the products -P and doses -D tested. Observed significant P x D interaction but the partitioning of the interaction effect of doses within the product was not significant. Similar result was found by Arruda et al. (2010) when evaluated the seed germination of two common bean cultivars Carioca Precoce and IAC Apuã in different time of application of biostimulant Stimulate®. For the experiment realized in the seed bed all the variable analyzed also did not have the effect of all the factors evaluated and the interaction among them, and also not observed among the factors and the check (Table 1).

Table 1- The summary of analysis of variance for the characteristics evaluated in the seed bed (length of the areal part and root, fresh and dry weight of areal part and the root, index of velocity of emergence)

FV	GL	LAR (cm)^{ns}	RL (cm)^{ns}	FWAP (g)^{ns}	FWR (g)^{ns}	DWAP (g)^{ns}	DWR (g)^{ns}	IVE^{ns}	E (%)^{ns}
Treatments	15	0.8778	2.8177	2.5180	2.6058	0.0601	0.0888	0.0025	0.0138
Products (P)	2	1.0589	2.9355	4.6737	1.6859	0.0252	0.1052	0.0003	0.0247
Doses (D)	4	1.2083	4.3831	0.9351	5.2355	0.0138	0.1552	0.0054	0.0084
P x D	8	0.4260	1.9161	2.5571	1.8453	0.0979	0.0590	0.0016	5.0167
Treat. x Adic.	1	2.8080	3.5332	4.2241	0.0109	0.0135	0.0286	0.0022	0.0021
Error	48	1.9428	4.4656	2.1095	2.1668	0.0518	0.0678	0.0032	0.0107
CV (%)	-	6.92	16.35	8.80	32.17	11.35	35.08	1.85	7.21

LAR= length of areal part; RL= root length; FWAP= fresh weight of areal part; FWR= fresh weigh of root; DWAP= dry weight of areal part; DWR= dry weight of root; IVE= index of velocity of emergency; E= emergency. ^{ns}: not significant for the test at 5% probability.

In relation to the length of areal part of the seedlings (cm) (Table 2), similar results were obtained by Albuquerque et al. (2004) that did not observed significant difference for variable in different time of soaking and doses of biostimulant Stimulate® in seeds of castor bean. Such results are not consistent with the affirmation of Taiz & Zeiger (2009), that reported seedling growth in height occurred due to gibberellin, component of biostimulant Stimulate®, that improve the cellular division and elongation. The difference in response can be related to divers aspects such as species and concentration used, seed origin among others. It is believed that bio-stimulates can be differ in its function of its composition, concentration and proportion of substances, increase growth and development of plants by stimulating the cell division and also increase water and nutrient absorption of the plants

Table 2- The mean value of length of areal part and root, fresh weigh to areal part and root, index of velocity of emergency and emergency of seedlings as a result of bio-stimulates.

Products	LAR (cm)	RL (cm)	FWAP (g)	FWR (g)	DWAP (g)	DWR (g)	IVE	E (%)
ACADIAN	20.07	15.60	16.76	4.33	2.03	0.71	9.37	96.90
BOOSTER®	20.46	13.31	16.92	4.90	2.03	0.83	9.36	96.90
STIMULATE®	20.06	12.70	16.02	4.51	1.97	0.70	9.41	97.70
TESTEMUNHA	19.33	13.84	15.51	4.53	1.95	0.66	9.53	96.5

LAR= length of areal part; RL= root length; FWAP= fresh weight of areal part; FWR= fresh weight of root; DWAP= dry weight of areal part; DWR= dry weight of root; IVE= index of velocity of emergency; E= emergency

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SOURCES OF RESISTANCE TO ANGULAR LEAF SPOT IN A *PHASEOLUS VULGARIS* GERMPLASM COLLECTION IN BRAZIL

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INTRODUCTION

Angular leaf spot disease is one of the most important diseases of common bean, causing economic losses in Brazil and worldwide. The Germplasm Collection from Universidade Federal de Lavras, Brazil, comprises landraces from producers and cultivars or inbreeding lines from breeding programs that were conducted mainly to increase grains yield. Greenhouse evaluations of these accesses can provide information of potential sources of resistance to introduce on current breeding programs. New methodologies of inoculation and evaluation of symptoms using seedlings offer advantages to the process of resistance evaluation (Pereira et al. 2011; Librelon, 2013). Therefore, our aim is to evaluate the reaction common bean accesses of the Germplasm Collection to the most frequent *Pseudocercospora griseola* race 63-63 (Damasceno-Silva et al. 2008, Balbi et al. 2009, Pereira et al. 2013) and obtain useful information to breeding programs.

MATERIAL AND METHODS

A sample of one hundred and forty eight common bean accesses maintained at the common bean Germplasm Collection from the Universidade Federal de Lavras (UFLA) – Minas Gerais, Brazil were evaluated for their reaction to a *P. griseola* strain provided by the culture collection from the Department of Biology, UFLA. Fungal colonies were inoculated in leaf-dextrose-agar medium and incubated at 24ºC for seven days in the dark to produce spores. Spore solutions were made by harvesting in sterile water and the concentration adjusted to $2x10^4$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 Ouro Negro 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. The scale from 1 to 9 developed by Librelon (2013) was used to evaluate plant symptoms. The average scores were calculated and scores below 3 were considered as resistant, whereas plants scoring more than 3 were susceptible.

RESULTS AND DISCUSSION

The figure 1 shows that 20.3% of the accesses were resistant and 79.7% were susceptible to race 63-63. Between the resistant accesses 60% were obtained most recently by breeding programs. The type of seed Carioca was the most frequent among the resistant acesses (73.3%). The leaf spot disease resistance is being associated with other commercially favorable traits in order to identify potential sources or resistance to include in current breeding programs. The resistant lines ESAL 657, ESAL 512, BP-24, BP-28, MAV-1-7 and MAV-3-36 have the Carioca type of seed and are also resistant to at least one race of *C. lindemuthianum* evaluated in another study (Barcelos et al., 2013). The cultivar ESAL 683 (Carioca type of seed) is resistant to three *C. lindemuthianum* races. The lines H-4-18, CV-55 and OPNS/VC3-117 also have the Carioca type

of seed and resistance to two *C. lindemuthianum* races (Barcelos et al. 2013). The majority of resistant lines were developed by recurrent selection breeding program to angular leaf spot disease of the Universidade Federal de Lavras.

The methodologies of inoculation in primary leaves (Pereira et al. 2011) and evaluation of symptoms using an scale developed to this stage (Librelon, 2013) were efficient to evaluate plant symptoms and provided advantages compared with previous methodologies such as the need of a smaller amount of inoculum and shorter period of time of the plants in the greenhouse decreasing the contamination by other pathogens and reducing the total time to obtain results.

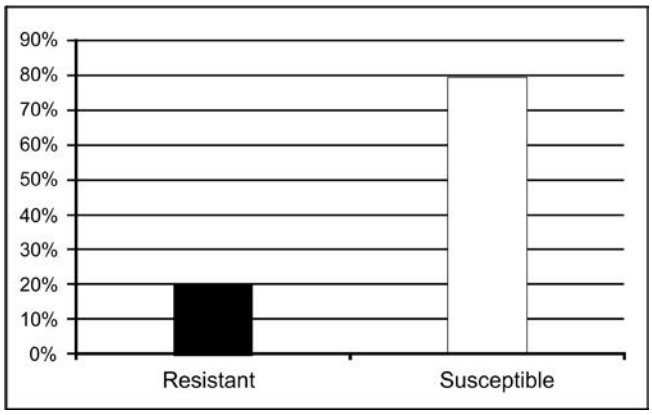

Figure 1. Percentages of resistant and susceptible accesses to race 63-63 of *P. griseola*.

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RESISTANCE OF COMMON BEAN BREEDING LINES TO *PHAEOISARIOPSIS GRISEOLA* ISOLATES FROM HONDURAS

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INTRODUCTION

Angular leaf spot (ALS) disease caused by *Phaeoisariopsis griseola* Sacc. Ferraris, is currently one of the most important factors limiting bean productivity in Central America. More than 60% of the beans in this region are produced during the second planting season ("postrera"). Weather conditions are characterized by moderate temperatures and the occurrence of high relative humidity alternated with short periods of low relative humidity which favors the dissemination of ALS disease. High incidence of ALS can reduce yield significantly (>50%) in susceptible lines. The development of breeding lines which combine resistance to ALS and *Bean Golden Yellow Mosaic Virus* (BGYMV) and tolerance to drought are being developed by breeding programs in Central America. Results from screening for ALS resistance of a group of advanced breeding lines are presented in this article.

MATERIALS AND METHODS

The study was conducted in a greenhouse at Zamorano, Honduras using 15 cm diameter pots containing 4.5 kg of a 2:1 mixture of soil: compost substrate, previously sterilized with steam. Three *P. griseola* isolates from plant samples collected at Zamorano, F. Morazan (Pg-ZII), Ocotal, El Paraíso (Pg-O) and Zacapa, Santa Barbara (Pg-PRR), were characterized using the ALS differential nursery. Plants were inoculated with the isolates grown in a V8 medium using a concentration of 2×10^4 conidias/ml. The inoculum was applied with a Bilviss atomizer 14 days after planting, followed by 48 h incubation period using micro-spray irrigation. ALS disease reactions were observed at 14 days after inoculation using a 1 to 9 scale, where plants with a 1-3 rating were classified as resistant and those with a 4-9 rating as susceptible.

After the pathogenic characterization of the ALS isolates, 19 breeding lines and three checks, Tío Canela 75 (susceptible), G06727 and G10474 (resistant), were evaluated with these isolates using four replications and previously described inoculation and evaluation procedures. The breeding lines included in the trial were derived from crosses between BGYMV, resistant commercial cultivars and ALS resistant parents, and were previously selected for agronomic adaptation, BGYMV and ALS resistance in the field.

RESULTS AND DISCUSSION

The *P. griseola* isolates were characterized as pathotypes 31-63 (isolate Pg-ZII), 62-33 (isolate Pg-0) and 63-63 (isolate Pg-PRR), and can be considered as virulent as other pathotypes that have been characterized in previous studies in Honduras (Zeledón 2003; Rodriguez 2013). The reactions of the 22 bean lines after inoculation with these three

pathotypes varied from resistant to susceptible (Table 1). The breeding lines ALS 0531-97, ALS 0532-6, ALS 532-38 and ALS 546-97 were resistant to all three pathotypes. These lines were developed from crosses made to recombine BGYMV and ALS resistance with desirable commercial small red and black seed types from breeding lines derived from the resistant parents, G06727 (ALS 9951-42 and -62 lines) and G10474 (MR13697-2-5 line), and can be used as cultivars in bean production areas where these two diseases are simultaneously present, which frequently occurs during the "postrera" seasons in Central America. These resistant lines can also be used as breeding parents.

Table 1. Reaction of 22 bean breeding lines and check cultivars to inoculation with three highly- virulent *Phaeoisariopsis griseola* pathotypes from Honduras. Zamorano, 2013.

Line	Pedigree	*P. griseola* pathotypes		
		31-63	62-33	63-63
ALS 0531-41	Amadeus 77//ALS 9951-42/MR 13697-2-5	1 [z]	1	5
ALS 0531-97	Amadeus 77//ALS 9951-42/MR 13697-2-5	2	1	3
ALS 0532-4	Cardenal//ALS 9951-62/MR 13697-2-5	1	2	4
ALS 0532-6	Cardenal//ALS 9951-62/MR 13697-2-5	2	1	3
ALS 0532-38	Cardenal//ALS 9951-62/MR 13697-2-5	1	2	3
ALS 0546-60	Aifi Wuriti//ALS 9951-62/MR 13697-2-5	1	1	5
ALS 0546-78	Aifi Wuriti//ALS 9951-62/ MR 13697-2-5	3	1	4
ALS 0546-97	Aifi Wuriti//ALS 9951-62/MR 13697-2-5	2	2	2
ALS 0626-35	Cardenal//ALS 9951-62/MR 13697-2-5	5	3	5
SX 14816-71	/S/B122/PRF9653-16B-1/F1/S/B125/MC-16P-	4	5	5
MER 2221-20	Tío Canela 75/ Orgulloso	5	7	5
AMFF 1-12-16-2	Amadeus 77/FF1	4	6	5
AMFF 1-12-1-17-1	Amadeus 77/FF1	7	4	4
MHR 314-18	DEORHO//MH4-9/MH5-14	7	1	5
ALS 9951-101-R1	Tío Canela 75//Tío Canela 75/ G06727	6	7	6
SEQ 341-99	L88-63// L88-63/Milenio	6	3	6
SEQ 342-87	L88-63//L88-63/Carrizalito	5	1	5
SJC 730-70	Negro Vaina Blanca/BCN 20-02-94	7	4	5
SJC 730-40	Negro Vaina Blanca/BCN 20-02-94	7	6	6
Tio Canela 75	Small red (susceptible check)	8	9	7
G10474	Guatemala 889 (resistant check)	2	1	3
G06727	Guarzo Popayan (resistant check)	1	2	3

[z] Resistant (1-3); susceptible (4-9).

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ANTHRACNOSE RESISTANCE SOURCES TO BE EXPLORED BY THE COMMON BEAN BREEDING PROGRAMS IN BRAZIL

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The attack of pests and pathogens is one of the main causes of yield and quality losses in the common bean (*Phaseolus vulgaris* L.) crop worldwide. This is especially true for small farmers with low-technology inputs in Brazil. Among the most destructive diseases that attack the crop we find anthracnose (ANT), caused by the fungus *Colletotrichum lindemuthianum*. The pyramiding of different race-specific resistance (R) alleles could be used as a strategy for developing broad and durable resistance to a large number of pathogen races (Ragagnin *et al.* 2009). However, it is necessary to check what R alleles are still effective and, for this reason, really useful to the breeding programs. Thus, the main goal of the present work was to evaluate common bean anthracnose resistance sources for disease reaction under greenhouse and field conditions. In addition, the tested genotypes were also screened with SCAR markers linked to R genes presented as useful for the breeding programs in Brazil.

Artificial inoculations were carried out according to Pastor-Corrales *et al.* (1995), using three *C. lindemuthianum* pathotypes selected based on the criteria prevalence (races 73 and 81) and virulence (race 2047). At least 16 plants per genotype were screened with each one of the pathotypes. The field trial was conducted during the growing season of winter/2013, in a disease endemic area (ANT screening site) located at Embrapa Rice and Bean Experimental Station (Santo Antônio de Goiás, GO, Brazil), using a randomized complete block (RCB) design with three replications. Each plot consisted of two rows each 3.0 m long, spaced by 0.5 m, with 15 plants per meter. Pathogen inoculation was done by natural infection. At both greenhouse and field screening, ANT severity was scored using a 1-to-9 scale, where 1= no symptoms and 9= dead plants. The molecular marker screening followed standard methods, using available SCAR markers previously identified (http://www.css.msu.edu/bic/PDF/SCAR_Markers_2010.pdf).

'G 2333' (*Co-4*^{2}, *Co-5* and *Co*-7) was the only genotype resistant in the field and to all tested *C. lindemuthianum* pathotypes. 'MDRK' (*Co-1*), 'Kaboon' (*Co-1*^{2}), 'Perry Marrow' (*Co-1*^{3}), 'AND 277' (*Co-1*^{4}), 'TO' (*Co-4*), 'PI 207262' (*Co-4*^{3} and *Co-9*), 'TU' (*Co-5*) and 'AB 136' (*Co-6* and *co-8*) were resistant in the field and in the greenhouse screening with the pathotypes 73 and 81. 'SEL 1308' (*Co-4*^{2}) showed to be resistant to the pathotypes 73 and 2047, being also resistant in the field (Table 1). The results demonstrated that the R alleles *Co-3*, *Co-3*^{3}, *Co-7*, *Co-10*, *Co-11* and *Co-13* were not effective to the tested pathotypes, although *Co-10* conferred resistance in the field screening (Table 1). *Co-4*^{2} ('SEL 1308') was the only allele conferring resistance to the pathotype 2047, although it was supplanted by the pathotype 81, what can be controlled by other alleles, e.g. *Co-5* ('G 2333' and 'TU') and *Co-6* ('AB 136'). For this reason, the combination of *Co-4*^{2} with *Co-5* or *Co-6* should be useful for the common bean breeding programs in Brazil. As shown in Table 1, in addition to race-specific reactions, the pyramiding of the mentioned R alleles can be also supported in some way by available SCAR markers linked to these target alleles.

Table 1. Reaction of common bean lines to anthracnose (*Colletotrichum lindemuthianum*) at greenhouse and field testes, and molecular screening of those lines with SCAR markers linked to resistance genes.

Genotype	Resistance (R) gene	*C. lindemuthianum* pathotype[a]			Field[a]	SCAR marker[b]			
		73	81	2047		SY20/ *Co-4*	SH18/ *Co-4*^{2}	SAB3/ *Co-5*	SAZ20/ *Co-6*
MDRK	*Co-1*	1.0	1.0	6.0	1.6	0	0	0	0
Kaboon	*Co-1*^{2}	1.0	1.0	5.0	1.6	0	0	0	0
Perry Marrow	*Co-1*^{3}	1.0	1.0	5.0	1.3	0	0	0	0
AND 277	*Co-1*^{4}	3.0	3.0	4.0	2.6	0	0	0	0
Widusa	*Co-1*^{5}	1.0	9.0	6.0	1.6	0	0	0	0
Cornell 49-242	*Co-2*	9.0	1.0	9.0	5.6	0	0	0	0
Mexico 222	*Co-3*	9.0	9.0	6.0	3.3	0	0	0	0
BAT 93	*Co-3*^{3}	9.0	5.0	6.0	8.6	0	0	0	0
TO	*Co-4*	1.0	1.0	5.0	1.6	1	0	0	0
SEL 1308	*Co-4*^{2}	1.0	5.5	2.0	1.0	1	1	0	0
PI 207262	*Co-4*^{3} and *Co-9*	1.0	1.0	6.0	1.6	1	0	0	0
G 2333	*Co-4*^{2}, *Co-5* and *Co-7*	1.0	1.0	2.0	1.3	1	0	1	0
TU	*Co-5*	1.0	1.0	5.0	3.0	0	0	1	0
SEL 1360	*Co-5*^{2}	1.5	6.0	3.5	-	0	0	1	0
AB 136	*Co-6* and *co-8*	1.0	1.0	5.0	1.0	0	0	0	1
H1	*Co-7*	9.0	4.5	5.0	-	0	0	0	0
Ouro Negro	*Co-10*	3.5	4.0	6.5	1.0	0	0	0	0
Michelite	*Co-11*	9.0	9.0	9.0	8.3	0	0	0	0
Jalo Vermelho	*Co-12*	1.5	8.5	3.5	-	0	0	0	0
Jalo Listras Pretas	*Co-13*	6.5	7.0	8.0	-	0	0	0	0
Rosinha G2[c]	-	8.0	9.0	6.5	8.6	0	0	0	0

[a] Mean scores of disease severity based on a 1-to-9 scale, where resistance reaction= 1-to-3 (1= no symptoms and 9= dead plants).
[b] Presence (1) or absence (0) of SCAR marker.
[c] Susceptible cultivar control.

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MOLECULAR DETECTION OF *COLLETOTRICHUM LINDEMUTHIANUM* IN DRY EDIBLE BEAN SEED

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INTRODUCTION: Anthracnose of common bean (*Phaseolus vulgaris*), caused by the fungal pathogen *Colletotrichum lindemuthianum,* can cause large yield reductions and reduced seed quality (Markell et al, 2012). The introduction of *C. lindemuthianum* into a field most commonly occurs through planting infected seed; therefore, the most important management practice is the use of certified seed (Dillard et al, 1993; Markell et al, 2012; Tu, 1983). Early infections caused by seed to seedling transmission can be devastating and have been proven to be the most important factor in disease development. Seed to seedling transmission has been reported to be as high as 15% in symptomless seed and increases with increasing symptom severity (Tu, 1983). Anthracnose has traditionally been diagnosed by visualizing the pathogen on infected tissues; however, this method may not be sensitive enough to detect symptomless seed infections. The purpose of this work was to develop a real-time PCR assay for the quantification of *C. lindemuthianum* in infected host tissue, regardless of the presence of disease symptoms.

MATERIALS AND METHODS: Eleven primer pairs were developed from NCBI sequence data using Primer3. These primer pairs were chosen based on their essential gene function within the pathogen and appropriate product sizes. Initial optimization was completed using a temperature gradient of 52°C to 60°C, SsoAdvanced Universal SYBR Green Supermix (Bio-Rad), and six, ten-fold serial dilutions of *C. lindemuthianum* DNA. Assay specificity was evaluated using DNA extracted from 10 *Colletotrichum* species and common bacterial pathogens of dry bean.

RESULTS AND DISCUSSION: Primer pair ClF1527/ClR1609 was chosen for further optimization based on reaction efficiency and sensitivity (Fig. 1). ClF1527/ClR1609 produced an amplification product from *C. lindemuthianum* but not from any of the other *Colletotrichum* species or bacterial bean pathogens evaluated (Table 1). Fungal pathogens of dry bean are yet to be tested. This assay was also able to detect *C. lindemuthianum* in 46% of symptomless seeds produced from infected pods compared to a 4% detection rate using traditional methods. Further evaluations of pathogen detection in symptomless seed are underway. This assay will be utilized to quantify seed to seedling transmission of the pathogen in stem tissue generated in greenhouse and field trials.

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Figure 1. Amplification and standard curves of primer pair ClF1527/ClR1609 in a 10 µl reaction of six, ten-fold serial dilutions of *C. lindemuthianum* DNA.

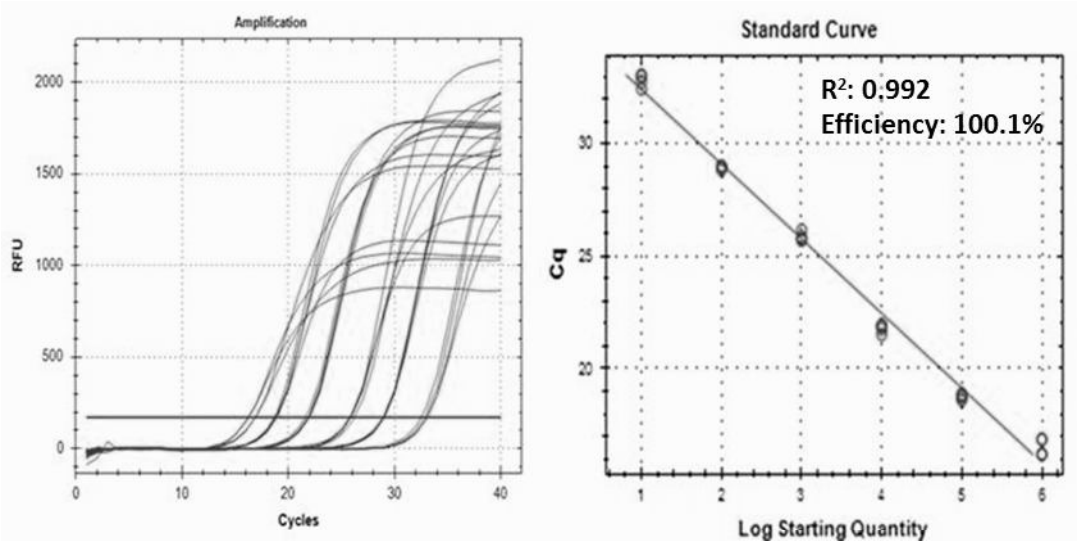

Table 1. List of *Colletotrichum* species and other bean pathogens used in the evaluation of ClF1527/ClR1609 real-time PCR assay specificity.

Species Tested	Host	Result (+/-)
C. lindemuthianum	Dry bean	+
C. truncatum	Lentil	-
C. truncatum	Lima bean	-
C. truncatum	Soybean	-
C. truncatum	Scentless chamomile	-
C. dematium	Field eryngo	-
C. linicola	Flax	-
C. higginsianum	Bok choy	-
C. destructivum	Red clover	-
C. acutatum	Strawberry	-
C. acutatum	Apple	-
C. acutatum	Almond	-
C. cereale	Orchardgrass	-
C. cereale	Rye	-
C. sublineolum	Sorghum	-
C. sublineolum	Wheat	-
C. graminicola	Corn	-
Curtobacterium flaccumfaciens pv. *flaccumfaciens*	Dry bean	-
Pseudomonas syringae pv. *syringae*	Dry bean	-
Pseudomonas syringae pv. *phaseolicola*	Dry bean	-
Xanthomonas spp.	Dry bean	-
Xanthomonas campestris pv. *phaseoli*	Dry bean	-
Phaseolus vulgaris (dry bean)	--	-

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PARENTAL GENETIC REPRESENTATIVENESS IN BLACK SEEDED COMMON BEAN PROGENIES FROM THE EMBRAPA RECURRENT SELECTION PROGRAM FOR TOLERANCE TO BGMV

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Bean golden mosaic virus (BGMV) can seriously limit common bean (*Phaseolus vulgaris* L.) yield in some of the main production areas in Brazil. Unfortunately, up to now, no cultivars or elite lines have been developed with satisfactory levels of resistance or tolerance to the virus by conventional breeding methods. There is no consensus in regard to genetic control of the common bean reaction to BCMV, but there is evidence that it is a polygenic trait (Pessoni *et al.*, 1997). For this reason, the breeding program conducted by Embrapa is using recurrent selection (RS) as a strategy to develop carioca and black seeded elite lines tolerant to BGMV. Thus, the main goal of the present work was to identify progenies with greater parental genetic representativeness among the black seeded $C_2S_{1:4}$ progenies selected in this RS cycle as highest yielding and tolerant to the virus.

The initial population (C_0S_0) was formed from crosses among seven parents selected as tolerant to BGMV in field conditions ('Pinto 114', 'A775', 'A429', 'IAPAR 57', 'LM 21306-0', 'Ônix' and 'RGLC'). This population was advanced, evaluated and subjected to selection regarding to BGMV reaction throughout its generations. During the RS program for black seeded beans, 27 $C_2S_{1:4}$ progenies were developed and selected as the highest yielding and tolerant to the virus. These progenies would be recombined to form the base population in the next RS cycle (C_3). In the present work, these 27 progenies were then evaluated regarding to genetic representativeness of the seven parents. For this purpose, the presence of private alleles identified in the parents by microsatellite markers was used as criterion. The DNA extractions of the progenies and parents were carried out using samples composed of leaf tissue from 10 plants collected in bulk. Twelve fluorescent microsatellite markers were used. PCR reactions, electrophoresis and genotyping of the markers were done as described by Valdisser *et al.* (2013).

A total of 70 alleles were detected in the seven parents using the 12 microsatellite loci. Out of these 70 alleles, 34 were identified as private in at least one of the parents and thus used in the present work. Private alleles are useful in RS programs as they assist in estimating the real contribution of each parent in the genetic composition of the genotypes and progenies generated and selected for the subsequent steps of recombination (Brondani *et al.*, 2004). The parent that showed the greatest number of private alleles was 'RGLC' (10 alleles). In contrast, the parent 'LM21306-0' showed only two private alleles. However, 'LM21306-0' and 'A775' showed greater genetic representativeness in progeny formation, exhibiting at least one private allele in 21 and 25 progenies, respectively. Among the 27 $C_2S_{1:4}$ evaluated progenies, 10 were selected for the next recombination cycle (C_3) because they exhibited greater parental allele representativeness. This is a strategy aiming to maximize the genetic diversity of the base population in the next RS cycles and, thereby, also maximize the opportunity to get genetic gain for different traits during the future selection steps of the breeding program.

Table 1. Parental genetic representativeness in $C_2S_{1:4}$ black seeded progenies from the Embrapa recurrent selection program for tolerance to BGMV.

Progeny (Pro)	PA/Pro[a]	Parent (Par) - PA/Par[b]							GR (%)[c]
		Par1 - 5	Par2 - 5	Par3 - 5	Par4 - 3	Par5 - 2	Par6 - 4	Par7 - 10	
Pro1	3	1(33.3)	-	-	1(33.3)	1(33.3)	-		3/7 (42.9)
Pro2	8	2(25.0)	3(37.5)	2(25.0)	-	-	-	1(12.5)	4/7 (57.1)
Pro3	7	1(14.3)	3(42.8)	2(28.6)	-	-	-	1(14.3)	4/7 (57.1)
Pro4	8	3(37.5)	3(37.5)	-	1(12.5)	-	-	1(12.5)	4/7 (57.1)
Pro5	7	3(42.8)	2(28.6)	-	-	1(14.3)	-	1(14.3)	4/7 (57.1)
Pro6	6	3(50.1)	1(16.7)	-	-	1(16.7)	-	1(16.7)	4/7 (57.1)
Pro7	8	2(25.0)	3(37.5)	-	1(12.5)	1(12.5)	-	1(12.5)	5/7 (71.4)
Pro8	7	2(28.6)	3(42.8)	-	-	1(14.3)	-	1(14.3)	4/7 (57.1)
Pro9	6	2(33.3)	-	1(16.7)	2(33.3)	1(16.7)	-	-	4/7 (57.1)
Pro10	6	1(16.7)	1(16.7)	1(16.7)	1(16.7)	2(33.3)	-	-	5/7 (71.4)
Pro11	6	1(16.7)	2(33.3)	1(16.7)	1(16.7)	1(16.7)	-	-	5/7 (71.4)
Pro12	6	1(16.7)	2(33.3)	1(16.7)	1(16.7)	1(16.7)	-	-	5/7 (71.4)
Pro13	6	1(16.7)	1(16.7)	-	1(16.7)	1(16.7)	-	2(33.3)	5/7 (71.4)
Pro14	6	1(16.7)	1(16.7)	1(16.7)	1(16.7)	-	-	2(33.3)	5/7 (71.4)
Pro15	6	1(16.7)	2(33.3)	-	2(33.3)	1(16.7)	-	-	4/7 (57.1)
Pro16	4	-	1(25.0)	-	-	1(25.0)	-	2(50.0)	3/7(42.9)
Pro17	7	-	2(28.6)	-	2(28.6)	1(14.3)	-	2(28.6)	4/7 (57.1)
Pro18	4	-	2(50.0)	-	2(50.0)	-	-	-	2/7 (28.6)
Pro19	7	-	2(28.6)	-	2(28.6)	1(14.3)	-	2(28.6)	4/7 (57.1)
Pro20	5	-	2(40.0)	1(20.0)	1(20.0)	1(20.0)	-	-	4/7 (57.1)
Pro21	3	-	2(66.7)	-	1(33.3)	-	-	-	2/7 (28.6)
Pro22	5	1(20.0)	2(40.0)	-	1(20.0)	1(20.0)	-	-	4/7 (57.1)
Pro23	7	1(14.8)	2(28.6)	2(28.6)	1(14.3)	1(14.3)	-	-	5/7 (71.4)
Pro24	6	1(16.7)	2(33.3)	1(16.7)	1(16.7)	1(16.7)	-	-	5/7 (71.4)
Pro25	5	-	2(40.0)	2(40.0)	-	1(20.0)	-	-	3/7 (42.9)
Pro26	5	1(20.0)	2(40.0)	1(20.0)	-	1(20.0)	-	-	4/7 (57.1)
Pro27	3	-	2(66.7)	-	-	1(33.3)	-	-	2/7(28.6)

[a]PA/Pro: total number of private microsatellite alleles (PA) per $C_2S_{1:4}$ progeny (P).
[b]Parents: Par1-'Pinto114', Par2-'A775', Par3-'A429', Par4-'IAPAR57', Par5-'LM21306-0', Par6-'Onix' and Par7-'RGLC'; PA/Par: total number of private microsatellite alleles (PA) per parent (Par).
[c]GR: relative genetic representativeness of the seven parents in the $C_2S_{1:4}$ progenies.

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COMMON BEAN GENOTYPES WITH RESISTANCE TO FUSARIUM WILT

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INTRODUCTION: Fusarium wilt, caused by the fungus *Fusarium oxysporum* f. sp. *phaseoli* (*Fop*), is a major soilborne disease of the common bean in Brazil. The use of resistant cultivars is the most economical method of control of this disease. Our main goal was to phenotypically characterize common bean genotypes from three Brazilian institutions regarding to the reaction to *Fop*.

MATERIAL AND METHODS: We evaluated 197 common bean elite lines or cultivars developed from 2002 through 2012 by the breeding programs conducted by the Universidade Federal de Viçosa, Universidade Federal de Lavras and Embrapa Arroz e Feijão. Some genotypes from international institutions were also included in this evaluation. The experiment was carried out in a greenhouse, where seeds of each line were seeded in trays with substract (Tropstrato HT©, Vida Verde, Mogi Mirim, SP, Brasil). Ten days after sowing, plants were carefully taken from the trays and their roots were washed in tap water. Then, approximately 1/3 of the roots were cut with scissors and discarded. The remaining roots were immersed in a suspension of macro and microconidia of *Fop* (1×10^6 conidia mL^{-1}) for 5 min or in distilled water (control). The isolate of this *Fop* was collected in Coimbra, Minas Gerais, Brazil. After this, the plants were transplanted to plastic pots containing 2.5 L of the substrate. Each plot was represented by a pot with three plants. A randomized block design with two replications (when inoculated) or no replication (control) was used. Twenty two days later, plants were evaluated using this scale of severity: 1 = plants without visible symptoms of Fusarium wilt; 3 = plants with up to 25% reduction in the shoot mass and with low chlorosis levels; 5 = plants with up to 50% reduction in shoot mass or 25% of the leaves with wilt symptoms, and with moderate chlorosis levels; 7 = plants with up to 75% reduction in the shoot mass or with 50% of leaves with wilt symptoms, severe chlorosis, and with dwarfism and limited necrosis; 9 = plants with more than 75% reduction in shoot mass, 75% or more of wilt symptoms and necrosis in leaves, severe dwarfism or dead plants (Pastor-Corrales & Abawi, 1987, adapted by Pereira, 2013). Reduction in the shoot due to Fusarium wilt was obtained by dividing the fresh shoot mass of the diseased plants by the fresh shoot mass of the healthy plants. Genotypes with means of 1.0-3.0 were considered resistant, 3.1-6.0 intermediate, and 6.1-9.0 susceptible.

RESULTS AND DISCUSSION: Out of the 197 genotypes, 24 were considered resistant (Table 1) and could be used in the common bean breeding programs aiming to *Fop* resistance. Among the resistant genotypes were the cultivars 'BRSMG Talismã', 'Ouro Vermelho', 'BRS Embaixador', 'BRS Executivo', 'BRS Radiante', 'Ouro Branco', 'Jalo EEP 558', and 'Manteigão Fosco 11', sources of resistance already available for farmers use.

Table 1 – Common bean cultivars and lines with resistance to *Fusarium oxysporum* f. sp. *phaseoli*

Genotype	Severity value	Commercial group or color[1]
CV55	2.42	carioca
BRSMG Talismã	2.84	carioca
CVIII-32-24	2.84	carioca
CVIII-85-11	1.17	carioca
CVIII-2	2.84	carioca
BJ1	1.92	jalo
BJ2	2.17	jalo
BJ3	2.00	jalo
BJ5	1.33	jalo
BJ6	1.33	jalo
BJ7	1.25	jalo
BJ8	2.17	jalo
BRS Radiante	2.67	cranberry
CNFRJ10571	2.50	jalo
Jalo EEP558	1.42	jalo
Jalo MG65	1.67	jalo
BRS Embaixador	2.17	dark red kidney
BRS Executivo	1.00	cranberry
CAL 96	1.00	purple with pink stripes
Ouro Branco	2.17	white
Manteigão Fosco 11	2.33	cream
VR17	2.00	red
VR18	2.00	red
Ouro Vermelho	2.00	red

[1] carioca (cream with brown stripes), jalo (beige seed). Except for the commercial group carioca and red, the other have large seeds.

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PATHOGENICITY *OF Pseudomonas syringae* pv. *phaseolicola* STRAINS ON DIFFERENT BEAN CULTIVARS.

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INTRODUCTION

Pseudomonas syringae pv. *phaseolicola* [(Burkholder) Young, Dye and Wilkie] causes bean halo blight. The bacteria is seed associated and seed transmitted, therefore the risk of losses is high (1). One of the main bean production areas in Mexico during the Fall-Winter season is the state of Sinaloa at the northwest of the country. Bean cultivars of the Nueva Granada race grown in that state are frequently attacked by halo blight. From 2009 onwards the halo blight disease has become more prevalent and aggressive in Guanajuato in central Mexico showing large damage in experimental plots and commercial fields particularly upon Nueva Granada race cultivars, such as highly priced yellow seeded Azufrado Higuera and Cacahuate Bola (cranberry type landrace). In Guanajuato the disease incidence is particularly higher during the rainy season (second crop in the year). The aim of this work was to determine the pathogenicity of three *Pseudomonas syringae* pv. *phaseolicola* (*Psp*) isolates from different origin on four Nueva Granada race bean cultivars under greenhouse conditions.

MATERIALS AND METHODS

Bean plants of the yellow seeded cultivars Azufrado Higuera, Azufrasin and Janasa, and the white seeded Aluyori, all of the Andean Nueva Granada race, were grown at the greenhouse and inoculated at the R_5 stage with three isolates of *Pseudomonas syringae* pv. *phaseolicola*. The isolates were obtained from the seeds of the same tested cultivars grown at Sinaloa and Guanajuato (Table 1). A bacterial suspension of $3X10^7$ cfu/ml was inoculated by multipuncture and wetting on bean leaves of each cultivar, three replications by cultivar. Different plants were used with each strain and a control inoculated with distilled water was included. Plants were kept at high relative humidity (>80%) for 7 days and the severity was scored at 11^{th}, 16, 20 and 30 days after inoculation scoring with a visual scale from 1:healthy plant and 9:maximum disease (2). The apparent infection rate (AIR) and area under disease progress (AUDP) were calculated using Vanderplank (1963) formula Dx/dt=Xr.

RESULTS

The three isolates caused halo blight on the four cultivars tested (Table 1). The *Psp* isolates from Sinaloa I_1 and I_3 caused from intermediate to susceptible (3.4 – 6.7) reactions, and the isolate from Guanajuato I_2 was more virulent mostly causing susceptible reactions (5.9 – 7.8). Control plants did not show halo blight symptoms (data no shown).

The isolate from Guanajuato I_2 was more severe on cv. Aluyori than the isolates from Sinaloa, although isolate I_1 was obtained from seeds of the same cultivar, Aluyori, and induced an intermediate reaction. Isolated I_3 showed a light reaction on Aluyori.

Janasa (seed from Guanajuato) and Azufrado Higuera were the cultivars with higher susceptibility to the three isolates inoculated. Seed of Janasa from Sinaloa showed intermediate reaction to I_3 and Azufrasin from Sinaloa was intermediate to I_1. The differential reaction of the yellow cultivars could be due to the fact that the isolates belong to different races, none determined yet. At present time is recognized the existence of nine *Pseudomonas syringae* pv. *phaseolicola* races (3). It is known that yellow seeded cultivars of the Nueva Granada race, such as those produced in Sinaloa, are more susceptible to halo blight infection, due to its origin as part of the Andean gene pool (1).

Each *Psp* isolate showed different AIR and AUDP (Table 1); the isolate from Guanajuato I_2 had higher average AIR and AUDP than the Sinaloa strains I_1 and I_3. And the Sinaloa strain I_1 obtained from seeds of Aluyori was the less severe. Azufrado Higuera was susceptible to I_1 and showed the highest AIR and AUDP, nevertheless I_2 had the highest severity score in Azufrasin, the AIR was higher but the AUDP was slightly lower than that of I_1. From the three isolates I_2 had the larger AIR in Aluyori, Azufrado Higuera and Azufrasin, which confirm the aggressiveness of the isolate that developed the disease symptoms faster and more severe.

Table 1. Halo blight severity, apparent infection rate and area under disease progress of three *Pseudomonas syringae* pv. *phaseolicola* isolates inoculated on five bean cultivars under greenhouse conditions.

	Isolate 1			Isolate 2			Isolate 3		
	SEV	AIR	AUDP	SEV	AIR	AUDP	SEV	AIR	AUDP
Janasa (Sin.)	6.5	0.060	24.28	6.3	0.060	23.85	3.4	0.037	21.76
Janasa (Gto.)	5.9	0.060	23.85	5.9	0.060	23.85	6.2	0.060	25.17
Aluyori	4.5	0.050	21.36	7.0	0.076	26.55	4.5	0.037	23.42
Az. Higuera	6.7	0.068	27.72	7.7	0.076	26.55	5.9	0.060	25.17
Azufrasin	4.4	0.037	22.58	7.8	0.076	27.50	6.4	0.060	25.62
Average	5.6	0.050	23.88	6.9	0.069	25.66	5.28	0.051	24.22

Isolate 1: from seed of Aluyori, Sinaloa, 2: from seed of Azufrasin, Guanajuato, 3: from seed of Azufrasin, Sinaloa; SEV: severity, AIR: Apparent Infection Rate, AUDP: area under disease progress

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EVALUATION OF VIRULENCE OF DIFFERENT ISOLATES OF *MACROPHOMINA PHASEOLINA* IN COMMON BEAN (*PHASEOLUS VULGARIS*) USING TWO INOCULATION METHODS

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Ashy stem blight caused by *Macrophomina phaseolina* (Tassi) Godi (Mph) is an endemic disease throughout Puerto Rico and in tropical and subtropical zones worldwide. Hot and dry conditions are ideal for disease development, while ashy stem blight survives in the soil and in plant residues. Common beans (*Phaseolus vulgaris* L.) and other hosts are commonly infected with the pathogen. Three common bean genotypes: BAT 477 (resistant), Verano (intermediate), and G122 (susceptible) and seven different isolates of Mph (Table 1) were evaluated. Two approaches using a wooden "toothpick" and a "band-aid", colonized with Mph, were used for inoculation. Toothpicks were placed on acidified potato dextrose agar media (aPDA) and a disk of a seven day old Mph culture was then use to inoculate the media. A disk was also placed in the mesh area of the "band-aid" previously saturated with aPDA. The toothpicks and the "band-aids" were incubated for two weeks at room temperature using a 12 h photoperiod. For inoculation, a puncture was made in the lower stem for both methods and inoculation took place on 21 day-old plants. The toothpick colonized by Mph was inserted in the puncture wound. The "band-aid" was wrapped around the puncture wound and it was attached with a piece of scotch tape, and wrapped with parafilm to maintain moisture. The three genotypes were grown in Promix. Disease severity ratings (1-9) were taken where 1= no visible symptoms, and 9 = more than 75% of the stem and roots infected, while lesion length was measured 40 days after inoculation. The trial was repeated twice and isolations on acidified potato-dextrose-agar (aPDA) from the site of the inoculation were made. The data were analyzed with the least significant difference (LSD, P=0.05) test.

In the first and second experiments, the "band-aid" method was more efficient in terms of disease development than the toothpick method. Disease severity (DS) and lesion length were higher with the "band-aid" method compared to the toothpick method. Generally, G122 was susceptible to all isolates and isolate Mph 7 was highly virulent (DS 8.9) in experiment 1. The same isolate was also virulent on BAT 477 (DS 5.9). Isolate Mph-55 was highly virulent on Verano (DS 7). In general, BAT 477 was resistant to the isolates but it was infected with isolate Mph-34 (DS 5.6) (data not shown). Isolate Mph-2 were found to be less virulent in the first experiment. In the second experiment (Table 1) significant differences were detected in cultivars and isolates for lesion length and disease severity and the LSDs were lower due to increased replications. Isolate Mph-7 was more virulent and produced a greater lesion size than the other isolates with the "band-aid" method. Isolates Mph-55, Mph-JD, Mph-25, Mph-34, Mph-32, Mph-31 and Mph-2 were similar in virulence. The toothpick method produced similar disease severity and lesion length for all isolates. Using the toothpick method, the average disease severity values of the different isolates ranged from 2.0 to 2.3, showing that the inoculation

method was not efficient in the plant either because the inoculum in the toothpick dried out or the inoculated area was restricted to the point of inoculation. The "band-aid" method is a simple and reliable virulence test for *Macrophomina phaseolina* to screen large numbers of genotypes under greenhouse conditions.

Table 1. Disease severity (1-9) and lesion length (cm) of *Macrophomina phaseolina* isolates with two inoculation methods.

Isolate	Band-Aid Disease severity (1-9) *		Band-Aid Lesion length (cm)		Toothpick Disease severity (1-9)		Toothpick Lesion length (cm)	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Mph-7	8.9	6.0	7.0	6.1	3.6	2.5	3.4	2.0
Mph-25	7.1	4.8	6.3	4.8	3.0	2.6	2.7	2.1
Mph-JD	2.0	4.5	3.0	3.6	1.0	2.5	0.0	1.6
Mph-32	2.8	4.1	3.7	3.2	2.3	2.6	0.4	1.8
Mph-34	2.3	3.6	3.0	3.1	3.0	2.8	1.2	2.7
Mph-55	2.2	3.5	4.3	2.8	5.0	2.6	5.1	2.9
Mph-31	3.6	3.3	4.3	2.3	3.0	2.5	1.5	2.2
Mph-2	1.0	3.0	1.0	2.3	1.0	2.1	0.0	1.7
Control	2.3	1.0	0.0	0.0	1.0	2.3	0.0	0.3
Mean	3.0	3.6	3.4	2.9	2.0	2.3	1.6	1.8
LSD (0.05)	5.6	0.9	2.4	0.7	2.3	0.7	4.1	0.5

**Macrophomina* disease severity score on 1-9 scale with 1= no visible symptoms, and 9 = more than 75% of the stem and roots infected.

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RESPONSE OF COMMON BEAN CULTIVARS TO *MELOIDOGYNE INCOGNITA* RACE 2

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INTRODUCTION: One of the main responsible for the poor yield in bean plant cropping in Brazil is the occurrence of knot-root forming nematodes belonging to the genus *Meloidogyne Göeldi*. The predominance of nematodes of the genus *Meloigogyne* is greater in regions with high temperatures, their being found in the main bean-growing areas in the country (BAIDA et al., 2011). The species *M. incognita* (race 1, 2, 3 and 4) is very common in Brazil. They are present in any kind of soil, with predominance in regions of sandy soil and with high temperatures (above 25°C) (BAIDA et al., 2011). Under the conditions of the state of Tocantins, where high temperatures predominate during most of the year, the occurrence of *M. incognita*, race 2 is favored (CAMPOS et al., 2011). In that sense, there is the need of identifying the possible bean plant cultivars resistant to nematodes. It was aimed with this work to evaluate the resistance of common bean plants to the root-knot nematodes (*Meloidogyne incognita,* raça 2).

MATERIALS AND METHODS: The work was conducted under greenhouse conditions in the Olericulture Department of the Universidade Federal do Tocantins-UFT, Gurupi campus. Isolate of *Meloidogyne incognita,* race 2, obtained from commercial crops of beans of the region was obtained. The following commercial common bean plant cultivars of the Carioca group most grown in the region were evaluated: BRS Requinte, BRS Pontal, CNFC 10470, IPR Tangará, IPR Colibri, Princesa, IPR Siriri, Aporé, Engopa 202 Rubi, IPR Juriti and BRS Majestoso. The experimental design utilized was the completely randomized with four replications, each experimental plot being composed of five pots, with one plant per pot. The number of root-knots on the root system (NG)was evaluated: determined according to the score scale proposed by Huang et al. (1986), in which: 1 – ascribed to root system without egg masses; 2 – ascribed to root system containing between 1 and 5 root-knots; 3 – ascribed to root system containing between 6 and 15 egg masses; 4 – ascribed to root system containing between 16 and 30 egg masses; and 5 – ascribed to root system containing more than 31 egg masses and the reproduction index (IR) of *Meloidogyne incognita*, race 2 was estimated by suing the tomato plant cv. Santa Clara as a standard control (100%) as compared with the bean plant genotypes evaluated, according to the methodology established by Taylor (1967). With the values obtained, the classification based upon resistance levels of each genotype to race 2 of *M. incognita* was conducted by the reproduction criterion established by Taylor (1967), in which: S – genotypes with a susceptible plant, normal reproduction, IR above 51%; LR – genotypes with slightly resistant plants, IR of 26 to 50%; MoR – genotypes with moderately resistant plants, with IR of 11 to 25%; MR – genotypes with very resistant plants, IR de 1 a 10%; AR/I – genotypes with highly resistant/immune plants; IR low 1%.

RESULTS AND DISCUSSION: For the number of root-knots, five statistically different groups with a range of 35.57 a 72.20 root-knots on the root system were formed. The genotypes BRS Requinte, BRS Pontal, CNFC 10470 and IPR Tangará did not differ statistically from each other by the Scott-knott test ($p \leq 0.05$), but they are susceptible to *Meloidogyne incognita,* race 2. The genotypes IPR Colibri, Princesa, IPR Siriri did not differ significantly from each other, being given score equal to 5, their being regarded as susceptible to *Meloidogyne incognita* race 2. Among the genotypes Aporé, Engopa 202 Rubi, IPR Juriti and BRS Majestoso, there was significant difference, but receiving score equal to 5, showing that those genotypes are susceptible to *Meloidogyne incognita,* race 2. For reproduction index of *Meloidogyne incognita,* race 2, among the eleven cultivars evaluated, Engopa 202 Rubi, IPR Juriti and BRS Majestoso are completely susceptible to *Meloidogyne incognita,* race 2, for obtaining reproduction index greater

than 51% (Table 1). The cultivars BRS Requinte, BRS Pontal, CNFC10470, IPR Tangará, IPR Colibri, Princesa, IPR Siriri and Aporé were classified as slightly resistant for having the reproduction index of *Meloidogyne incognita,* race 2 between 26 and 50%, results similar to those found by Simão et al., (2005) and Simão et al., (2010).

Table 1. Average number of root-knots (NG) and Reproduction Index (%) of eleven common bean plant cultivars inoculated with knot-root nematode *Meloidogyne incognita,* race 2, Gurupi-TO, 2011.

Cultivars	Number of root-knots	Reproduction Index (%)
BRS Requinte	35.57 f	30.93
BRS Pontal	36.03 f	31.33
CNFC 10470	36.31 f	31.57
IPR Tangara	37.21 f	32.36
IPR Colibri	44.25 e	38.48
Princesa	45.02 e	39.15
IPR Siriri	45.21 e	39.31
Apore	54.39 d	47.30
Engopa 202 rubi	62.06 c	53.97
IPR Juriti	66.08 b	57.46
BRS Majestoso	72.20 a	62.78
CV (%)	7.36	14.24

CONCLUSIONS: Among the cultivars evaluated, BRS Requinte, BRS Pontal, CNFC 10470, IPR Tangará, IPR Colibri, Princesa, IPR Siriri and Aporé were classified as slightly resistant to the root-knot nematodes *Meloidogyne incognita* race 2 by the reproduction index test.

Cultivars Engopa 202 Rubi, IPR Juriti and BRS Majestoso were classified as susceptible to the root-knot nematode *Meloidogyne incognita* race 2 by the reproduction index test.

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A NEW SCALE FOR WHITE MOLD DISEASE RATING FOR THE COMMON BEAN CUT-STEM METHOD OF INOCULATION IN THE GREENHOUSE

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INTRODUCTION

There are several methods of inoculation in the greenhouse for white mold [caused by *Sclerotinia sclerotiorum* (Lib.) de Bary], and commonly accepted disease ratings for common bean (*Phaseolus vulgaris* L., see Terán and Singh, 2009) including the cut-stem method (Terán et al., 2006) and the straw test (Petzoldt and Dickson, 1996). Terán et al. (2006) modified the rating scale of Petzoldt and Dickson (1996) used for the straw test. While the scale described by Terán et al. (2006) was more descriptive and provided better separation among the nine classes, it differed principally from the Petzoldt and Dickson (1996) scale in that: the score 3 in the latter, i.e., white mold pathogen invasion stopping at the first post inoculation node, was scored as a 4 in the scale described by Terán et al. (2006). The expectation was that we would have genotypes with a consistent score of 2 (i.e., white mold pathogen invasion traveling <1 inch along the inoculated internode) and 3 (i.e., white mold pathogen invasion traveling >1 inch of the inoculated internode but not reaching the first post inoculated node).

For the past several years, we have carried out intensive greenhouse screenings of common bean germplasm, intra- and interspecific F_1 hybrids, segregating populations, families, breeding lines, cultivars, and *Phaseolus* species of the secondary gene pool, using the rating scale proposed by Terán et al. (2006). Unfortunately, we have not found any genotype with consistent ratings of ≤3.0, i.e., white mold infection/disease not reaching the first post inoculation node on the main stem and/or branch. Thus, currently the genotypes with infection stopping at the first post inoculation node are rated as 4, on a 1 to 9 scale, where 1= no sign of inoculated internode infection, immune or completely healthy, and 9= disease infection >1 inch past the second post inoculation node leading to plant death. Often the lowest mean white mold score in any greenhouse screening at 21 days post inoculation or later is a 4, i.e., white mold symptoms stopped at the first post inoculation node, which is considered as a resistant response. In rating scales used for other important common bean diseases such as angular leaf spot, anthracnose, common bacterial blight, and rust, a score of 4 to 6 is considered an intermediate response, and not a resistant response. We therefore feel that to be consistent with the scales commonly used for other common bean diseases there is a need and strong justification to again modify white mold rating scales described by Terán et al. (2006) and Petzoldt and Dickson (1996).

New White Mold Disease Rating Scale:

White mold is rated in the greenhouse at least 7 days after inoculation, using the cut-stem method, and capping it with two or more 48-h-old mycelial plugs stacked in an eppendorf tip, or drinking straw, and periodically verifying the response until the harvest. We propose that the following scale be adopted for future white mold evaluations of common bean and other *Phaseolus* species germplasm:

Resistant Response (scores 1 to 3):

1. No sign of pathogen infection and white mold symptoms adjacent to mycelial plugs or agar inoculants on the inoculated internode when eppendorf tip/drinking straw is removed.
2. Internode infected, but pathogen invasion and white mold symptoms did not reach the first post inoculated node.
3. Internode infected and pathogen invasion and white mold symptoms stopped at the first post inoculated node.

Intermediate Response (scores 4 to 6):

4. Pathogen invasion moves past the first post inoculation node, but white mold symptoms stop at ≤ 50% of the length of the second internode.
5. The second internode infection moves >50% of the length, but white mold symptoms do not reach the second post inoculated node.
6. The second internode infection and white mold symptoms stop at the second post inoculated node.

Susceptible Response (Scores 7 to 9):

7. Pathogen invasion moves past the second post inoculation node, but white mold symptoms stop at ≤ 50% of the length of the third internode.
8. The third internode infection moves >50% of the length, but white mold symptoms do not reach the third post inoculated node.
9. The third internode infection and white mold symptoms either reach or pass the third post inoculated node leading to eventual plant death.

For a reliable white mold disease response in the greenhouse, it is crucial to maintain high humidity (>75%) and moderately low temperatures (<25^0C) for at least the first three weeks after inoculations. Also, multiple inoculations with either the same isolate of *S. sclerotiorum* (isolate-specific) or isolates of different aggressiveness (broad-spectrum) may be used on the same plant for identification of genotypes with a range of resistance levels and to minimize escapes.

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USE OF MUTI SITE SCREENING TO IDENTIFY AND VERIFY PARTIAL RESISTANCE TO WHITE MOLD IN COMMON BEAN IN 2013

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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/pathologists from across the USA with broad and/or specific partial resistance to WM.

Breeders sent seed of 18 bean lines with putative sources of resistance to our laboratory where the seeds were divided in equal amounts for field (400g/line) and/or greenhouse (25 seeds/ line) tests and then sent to nine locations to be evaluated by standardized greenhouse and/or field screening methods. Three bean lines were included in both tests as controls: partially resistant G122, Bunsi with mostly field avoidance and susceptible GN Beryl.

The field tests consisted of two rows of each of the 12 entries and one row of a local semi vine WM susceptible genotype, resulting in a three-row plot 4.6 m (15 ft.) long replicated three times in a randomized complete block design. There were six field tests conducted in six locations. The field nurseries were all evaluated using a CIAT 1 to 9 scale (1 = no visible symptoms to 9 = death) (Van Schoonhoven et al., 1987). Nebraska and North Dakota did not have results due to weather. These problems that resulted in no data were unfortunate, but demonstrate the importance of testing in multiple locations. In the field tests, all 9 lines were significantly more resistant than Beryl (Table 1). The results of the four field tests reported were that 3 bean lines, A195, VRW32 and B10244 were above intermediate resistance while remaining 6 test lines have intermediate resistance.

The greenhouse trials tested 18 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 and fitted over the cut internode. The infected plants were evaluated 8 days later using the modified Petzoldt and Dickson scale (Teran et al, 2006). The greenhouse results (Table 2) indicate that seven bean lines had ratings similar to G122 while eight bean lines had ratings similar to Bunsi; however, greenhouse conditions are more favorable and allow the fungus to grow in optimal conditions which is less likely to be encountered in the field. For example, bean line B10244 had a field rating lower than G122 but was similar to Beryl in the greenhouse test and was likely exhibiting escape or avoidance mechanisms. All field entries including pinto, great northern, black, navy and cranberry seed classes were rated lower than Beryl. Progress in incorporating WM resistance into dry bean lines with commercial potential validates use of multisite screening and National Sclerotinia Initiative support over the last 10 years.

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

ENTRY	SEED CLASS	COLLABORATOR	MI	WA	WI	OR	Mean	t Grouping
BERYL	G. NORTHERN	Susceptible Check	9.0	7.8	9.0	8.0	8.5	A
ASR 1001	DWARF FRENCH BEAN	J. Theuws- BEL	5.0	4.2	7.0	8.3	6.1	B
031-A-11	G. NORTHERN	P. Miklas- WA	5.3	6.0	6.0	4.0	5.3	B C
N 11283	NAVY	J. Kelly- MI	4.0	6.7	4.0	6.3	5.3	B C
G122	CRAN	Resistant Check	5.3	3.8	7.0	4.3	5.1	B C
040-B-2	PINTO	P. Miklas- WA	4.3	4.7	4.0	5.0	4.5	C D
EX RICO (BUNSI)	NAVY	Intermediate Check	4.3	4.3	5.0	4.3	4.5	C D
039-A-5	PINTO	P. Miklas- WA	4.7	4.7	4.0	4.3	4.4	C D
USPT-WM-12	PINTO	P. Miklas- WA	4.0	3.6	5.0	5.0	4.4	C D
B 10244	BLACK	J. Kelly- MI	3.3	.	4.0	3.0	3.4	D
A195	LG CREAM	S. Singh- ID	3.7	2.7	2.0	5.0	3.4	D
VRW 32	SM GREYISH-BROWN	S. Singh- ID	2.3	3.5	2.0	4.7	3.1	D

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

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

ENTRY	SEED CLASS	COLLABORATOR	CO	BEL	MI	NE	NY	OR	WI	WA	Mean	t Grouping
ASR 1001	SNAP	J. Theuws- BEL	8.9	5.0	9.0	5.8	8.8	7.9	9.0	5.9	7.5	A
B 10244	BLACK	J. Kelly- MI	8.6	.	7.2	5.7	6.7	6.8	8.6	6.8	7.2	A B
BERYL	G. NORTHERN	Susceptible Check	8.6	7.7	5.8	7.4	7.2	5.8	9.0	5.9	7.2	A B C
WMG904-13	SNAP	J.Myers-OR	5.9	7.4	5.9	5.2	9.0	5.7	7.5	5.3	6.5	A B C D
N 11283	NAVY	J. Kelly- MI	7.8	.	5.6	5.5	5.4	5.2	9.0	6.3	6.4	A B C D
039-A-5	PINTO	P. Miklas- WA	8.0	6.2	4.0	4.8	8.7	5.8	7.7	5.8	6.4	A B C D
040-B-2	PINTO	P. Miklas- WA	6.4	4.9	4.7	5.3	8.8	7.2	7.4	6.3	6.4	A B C D
OSU 6792	SNAP	J.Myers-OR	7.8	4.8	6.0	5.6	8.4	6.4	6.5	5.3	6.4	A B C D
EX RICO (BUNSI)	NAVY	Intermediate Check	8.8	4.9	4.6	5.2	6.3	5.1	8.8	6.1	6.2	A B C D
WMG377	SNAP	J.Myers-OR	7.7	5.9	4.0	5.1	8.5	5.1	8.0	5.2	6.2	B C D
WMG327	SNAP	J.Myers-OR	6.7	4.5	6.4	4.7	9.0	5.4	5.8	5.9	6.1	B C D
OSU 6772	SNAP	J.Myers-OR	5.5	4.1	5.4	5.1	9.0	6.0	6.0	5.8	5.9	C D
VRW 32	SM GREYISH-BROWN	S. Singh- ID	4.8	4.6	5.1	4.8	8.3	5.3	8.1	5.3	5.8	D E
OSU 6771	SNAP	J.Myers-OR	4.3	5.2	5.2	4.6	8.3	5.1	5.8	5.6	5.5	D E F
OSU 6774	SNAP	J.Myers-OR	8.0	2.6	5.7	4.8	6.5	5.6	5.6	4.9	5.5	D E F
USPT-WM-12	PINTO	P. Miklas- WA	6.0	4.3	3.4	4.2	8.6	4.4	7.0	5.7	5.5	D E F
WMM688	DRY (YELLOW)	J.Myers-OR	6.3	3.6	4.9	4.6	7.4	4.4	6.8	4.4	5.3	D E F
OSU 6743	SNAP	J.Myers-OR	2.9	4.3	4.2	5.5	4.6	4.7	5.3	4.7	4.5	E F G
031-A-11	G. NORTHERN	P. Miklas- WA	5.3	4.6	3.0	4.3	4.9	4.2	4.2	4.3	4.4	F G
G122	CRAN	Resistant Check	4.5	3.2	4.2	4.0	4.3	4.4	4.0	5.0	4.2	F G
A195	LG CREAM	S. Singh- ID	2.7	3.0	3.2	4.0	4.3	4.2	4.2	5.4	3.9	G

*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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GENOME-WIDE ASSOCIATION ANALYSIS FOR REACTION TO WHITE MOLD IN THE BEANCAP MESOAMERICAN PANEL

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INTRODUCTION: The fungal disease white mold, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is significantly detrimental to common bean (*Phaseolus vulgaris* L.) production. To date, no major resistance genes have been reported for this disease, indicating that white mold is a complex, quantitatively inherited trait with several genes distributed throughout in the genome (Miklas et al., 2013). The resistance mechanisms used by the plant can be morphological or physiological and several quantitative trait loci for both have been identified and mapped in common bean. In this study, we used a panel of 96 Mesoamerican common bean genotypes assembled as part of the Bean Coordinated Agricultural Project (BeanCAP) to perform genome-wide association analysis to detect QTL associated with white mold resistance.

MATERIALS AND METHODS: The genotypes were grown at the Montcalm Research and Extension Center located in Entrican, MI under irrigated conditions to promote natural disease development. Disease pressure on susceptible checks was high, incidence scores on all genotypes were recorded on a scale from one to nine at harvest. The plots received a total of 176 mm from precipitation and 102 mm from irrigation from planting (6/18/2013) to harvest (9/20/2013). Other variables recorded were days to flowering, and maturity, lodging and canopy (plant) height. An initial number 6865 single nucleotide polymorphism (SNP) markers obtained from the BARCBean6K_3 Beadchip were filtered for duplicate markers, minor allele frequency (5%), missing data (>70%), and identical markers, and a final group of 2542 SNP markers were used as genotypic data. All missing data in remaining markers were imputed using BEAGLE (Browning and Browning, 2009).

RESULTS: Significant differences ($p<0.0001$) were detected for white mold scores. The five genotypes with the highest (most susceptible) scores were Harold, Gloria, ABCP-8 and Common pinto; in contrast, the five genotypes with the lowest (most resistant) scores were A-55, Rosetta, I9365-31 and Black Rhino (Fig. 1). The markers were fitted to a compressed mixed linear model that included kinship coefficients and principal components to account for population substructure and relatedness (Yu et al., 2006). Genome-wide association (GWA) analysis revealed the presence of a QTL for white mold on chromosome 11 (Pv11) with a peak at SNP ss715649457 (Fig. 2). The four most significant SNP around this peak had effect estimates of 0.5, 0.7, -0.5 and -0.4. QTL WM11.1 for white mold (straw test) was previously detected by Mkwaila et al. (2011) on Pv11 in a black bean Tacana/Landrace inbred backcross population. Additionally, GWA revealed the presence of two QTL peaks for canopy height on chromosomes 1 (Pv01) and 8 (Pv08) in SNPs ss715649728 and ss715647251, respectively (Fig. 3). The four most significant SNPs for plant height had effects of 5.8, -6.7, 6.1 and -5.6.

Acknowledge support from National Sclerotinia Initiative and USDA-NIFA, BeanCAP project.

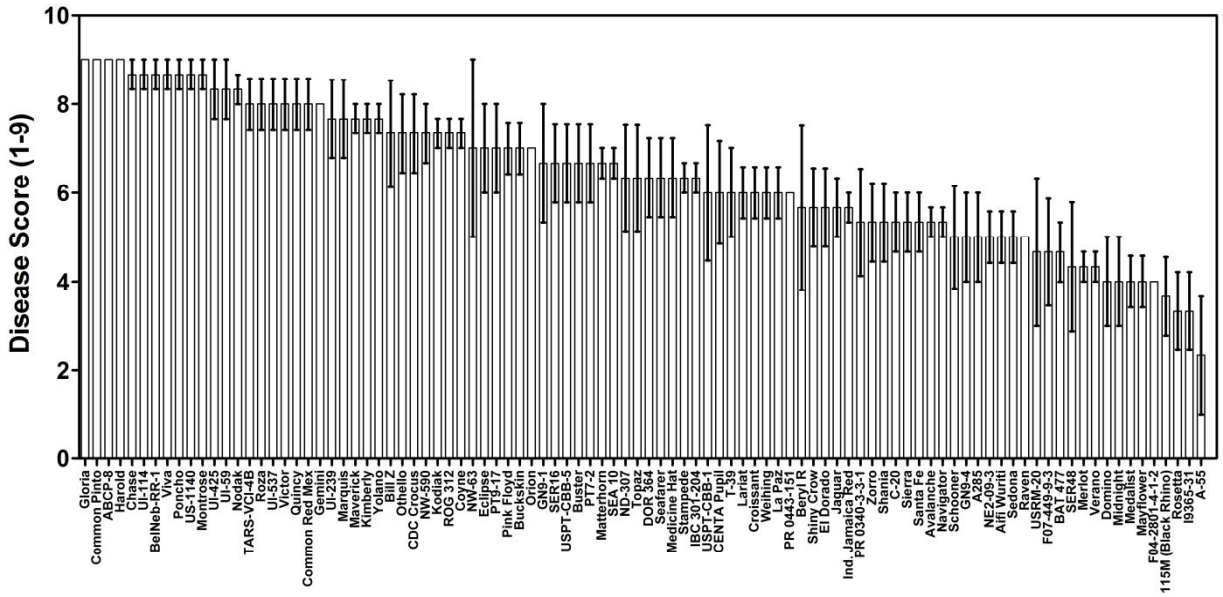

Fig. 1. Means and standard errors for the white mold scorings in the 96-genotype BeanCAP Mesoamerican panel grown in Entrican Michigan in 2013.

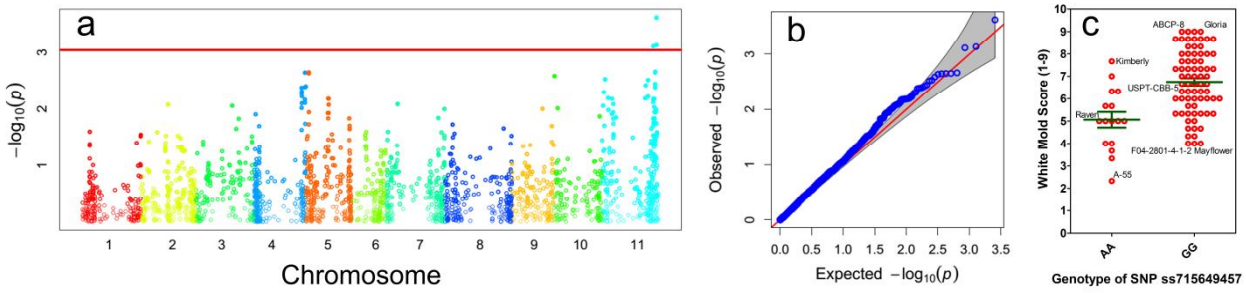

Fig. 2. Manhattan plot (a), quantile-quantile plot (b) and effect of peak SNP on Pv11 including mean and standard error for white mold scores (c).

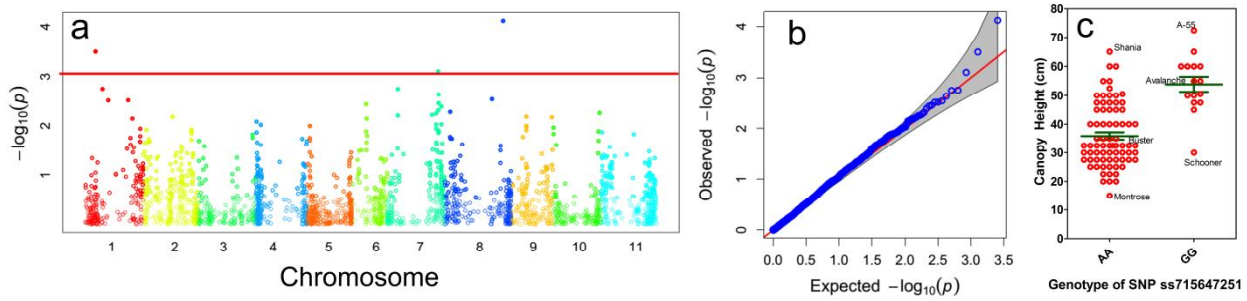

Fig. 3. Manhattan plot (a), quantile-quantile plot (b) and effect of peak SNP on Pv08 including mean and standard error for canopy height (c).

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INVESTIGATION OF SOURCES OF RESISTANCE TO WHITE MOLD IN PHASEOLUS VULGARIS WORKING COLLECTION IN BULGARIA

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INTRODUCTION: White mold, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, could be devastating in the seasons with high rainfalls frequency and low night temperature during flowering in northern temperate regions in Bulgaria. Recent QTL studies showed significant correlations between white mold severity and agronomic traits such flowering, maturity, architecture, lodging, branching patterns and canopy height, indicating that these ones can be useful for indirect selection of lines/cultivars with white mould avoidance (Miklas et al., 2001; Kolkman, Kelly, 2003; Ender and Kelly, 2005). The research objective was to identify snap bean breeding lines with physiological resistance to white mold in Bulgaria.

MATERIALS AND METHODS: The tests were set in a field randomized plots with 48 advanced snap bean lines and susceptible check - Bulgarian common bean cultivar Dobrudzanski ran in 2010. Inoculation was done using the Straw method of Petzoldt and Dickson (1996) at plant stage R6 with a 3 day-old mycelia plug from a colony of *S. sclerotiorum* bean isolate SsPh-2 grown in the dark on PDA at 20°C. Disease severity was rated on the 7th, 14th and 21st day after inoculation using the 1-9 scale of Teran et al., (2006). The following agronomic traits were recorded http://bic.css.msu.edu/_pdf/Bean_Breeding_Scales.pdf following Bean Breeding Scales on: plant type; days to flowering; branching pattern; lodging; pod clearance. Observation on the plant canopy, leaf density and pod distribution were also recorded.

RESULTS AND DISCUSSIONS: 20 resistant and 19 moderately resistant sources were present in the genotypes (Table 1). Only four of them expressed uniform ratings. The majority lines had rates from 3 to 6 which probably are due to genotypes' heterogeneity and less likely due to genotype x environment interaction. All of the screened genotypes possessed determinate growth habit with reproductive terminal bud, but differed in the architectural characteristics. Lodging resistance, branching patterns and pod clearance showed significant positive correlation with disease severity – 0.733, 0.655 and 0.662 respectively. Both leaf density and pod distribution were critical factors in determining disease severity microclimate. Line # 108 and line # 116 were with good branching patterns and longing resistance but had more pods distributed near to the ground and dense canopy. They showed more white mould severity than lines # 80 and # 113 which carried their pods in the upper half of the plant. Susceptible lines (# 90, 91, 98, etc.) had low lodging resistance (rate ≥4), bad pod clearance (≥3.5) and poor branching patterns (≥4). Nevertheless some exceptions were observed such as lines # 105 and #106 which were rated for physiological resistance with 2 to 3 but had poor lodging resistance and bad pod clearance.

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Table 1. Straw test screening to white mold phisiological resistance and plant architecture pattern of 48 advanced snap bean breeding lines in bulgaria

Genotype	Plant type	Lodging (1-5)	Branching pattern (1-5)	Pod clearance (1-5)	Days to flowering	Minimum rate	Maximum rate	Severity	Grouping
116	I	2.0	3.0	2.0	30	2.0	3.0	2.3	A
111	I	1.0	2.0	1.0	29	1.0	3.0	2.5	A
105	I	3.0	3.0	3.0	29	2.0	3.0	2.7	A B
106	I	3.0	2.0	4.0	29	2.0	3.0	2.7	A B
109	I	1.0	2.0	1.0	28	2.0	3.0	2.7	A B
110	I	1.0	2.0	1.0	28	1.0	3.0	2.7	A B
103	I	1.0	2.0	1.0	30	3.0	3.0	3.0	A B C
104	I	2.5	3.0	2.5	30	3.0	3.0	3.0	A B C
108	I	1.0	2.0	1.0	29	3.0	3.0	3.0	A B C
115	I	1.0	2.0	1.0	28	3.0	3.0	3.0	A B C
121	I	3.0	3.0	3.0	29	3.0	3.0	3.0	A B C
136	I	1.0	2.0	1.0	29	3.0	3.0	3.0	A B C
137	I	1.0	2.0	1.0	28	3.0	3.0	3.0	A B C
138	I	1.0	2.0	1.0	29	3.0	3.0	3.0	A B C
140	I	1.0	2.0	1.0	29	3.0	3.0	3.0	A B C
142	I	2.5	3.0	2.5	39	3.0	3.0	3.0	A B C
147	I	2.0	2.0	2.0	29	3.0	3.0	3.0	A B C
148	I	2.5	3.0	2.5	28	3.0	3.0	3.0	A B C
149	I	2.0	2.0	2.0	30	3.0	3.0	3.0	A B C
150	I	2.0	2.0	2.0	29	3.0	3.0	3.0	A B C
614	I	3.0	3.0	3.0	30	3.0	3.0	3.0	A B C
130	I	1.0	2.0	1.0	31	3.0	4.0	3.2	A B C D
89	I	3.5	3.0	3.8	31	2.0	7.0	3.5	A B C D E
131	I	2.5	3.0	2.5	31	3.0	4.0	3.5	A B C D E
113	I	2.0	2.0	3.5	30	3.0	5.0	3.7	A B C D E
144	I	2.0	2.0	2.0	30	3.0	7.0	3.7	A B C D E
99	I	3.5	3.0	3.5	30	3.0	7.0	3.8	A B C D E F
122	I	2.5	3.0	2.5	30	3.0	5.0	4.0	A B C D E F G
128	I	2.0	2.0	2.0	31	3.0	7.0	4.3	B C D E F G H
112	I	1.5	2.5	2.0	30	2.0	7.0	4.5	C D E F G H
134	I	3.5	3.0	3.5	31	3.0	7.0	4.5	C D E F G H
125	I	2.5	3.0	2.5	31	3.0	7.0	4.7	C D E F G H I
80	I	3.0	3.5	2.5	31	4.0	7.0	4.8	D E F G H I
114	I	2.5	3.0	2.5	30	3.0	7.0	5.0	E F G H I
135	I	3.0	3.0	3.0	31	3.0	7.0	5.0	E F G H I
88	I	3.0	3.0	3.0	32	2.0	7.0	5.5	F G H I J
84	I	4.0	4.0	3.5	32	3.0	7.0	5.7	G H I J
91	I	4.5	4.0	3.5	32	3.0	7.0	5.7	G H I J
126	I	3.5	3.0	3.0	32	3.0	7.0	5.7	G H I J
102	I	4.5	4.0	3.5	31	3.0	7.0	5.8	H I J
87	I	4.0	4.0	3.5	32	5.0	7.0	6.3	I J L
93	I	4.5	4.0	3.5	32	4.0	9.0	6.3	I J L
129	I	3.5	3.0	3.0	31	3.0	7.0	6.3	I J L
146	I	4.5	4.0	3.5	32	3.0	7.0	6.3	I J L
97	I	4.0	4.0	3.5	31	3.0	9.0	7.0	J L
145	I	4.5	4.0	3.5	32	7.0	7.0	7.0	J L
90	I	4.5	4.0	3.5	32	7.0	9.0	7.7	L M
98	I	4.5	4.0	3.5	32	7.0	9.0	8.7	M
Susceptible check	I	3.0	4.0	30	35	9.0	9.0	9.0	M
Correlation		0.733	0.655	0.662	0.526				

Degree of significance at P≤0.05 by Duncan's Multiple Range Test (1955)

REACTION OF COMMON BEAN LINES TO *Sclerotinia sclerotiorum* STRAINS IN GREENHOUSE AND FIELD CONDITIONS

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INTRODUCTION

White mold is the disease caused by *Sclerotinia sclerotiorum* and has decreased the common bean production in Brazil. Extensive damage to crops, the absence of high levels of resistance in the host and the great difficulty of controlling diseases caused by *S. sclerotiorum* have driven research on this pathogen (Bolton; Thomma and Nelson, 2006). The sporadic nature of the disease outbreaks makes the search for resistance in hosts under field conditions often problematic without the use of artificial inoculation and irrigation (Kim and Diers, 2000). Therefore, plants inoculation in the greenhouse and in the field should be an integral part of the evaluating methodology for resistance to this pathogen. The straw test is the most widely used method to detect physiological resistance in common bean worldwide (Schwartz and Singh, 2013). The resistance studies of common bean to white mold are scarce and the results have been controversial. So, the goal of this study was to observe if there is an occurrence of divergence in the common bean lines reactions to white mold and level of *S. sclerotiorum* strains aggressiveness, when assessed in the field and under greenhouse conditions.

MATERIAL AND METHODS

Two experiments were conducted, one in the field and the other in a greenhouse to compare the reaction of 14 common bean lines (União, Estilo, Radiante, Pérola, Esplendor, Ouro Negro, Tesouro, Majestoso, RP2, Campeiro, Talismã, Cometa, RP1 and Valente) to four *S. sclerotiorum* strains (UFLA3, UFLA26, UFLA54 and UFLA92). Each pathogenicity test consisted of four experiments which the 14 lines were inoculated with one strain by experiment. The experimental design was a randomized complete block with three replications. The plot was formed by a line of a meter with fifteen seeds per meter. In the greenhouse, the plot consisted of a vase of three liters with three plants. The method of inoculation was the straw test proposed by Petzoldt and Dickson (1996). The inoculation was performed at 42 days after seeding in the field, and 28 days after seeding in the greenhouse. The vases with inoculated plants were maintained in a greenhouse at 16°C during the night, 24°C during the day and 70% relative humidity. Seven days after inoculation, the severity disease was evaluated using a diagrammatic scale from 1 to 9. Average values of notes per plot were analyzed for individual and joint variance using R software and compared by the Scott Knott test at $P \leq 0.05$.

RESULTS AND DISCUSSION

In the joint variance analysis of the average reaction grades of the fourteen lines to the four *S. sclerotiorum* strains evaluated in the field, the source of variation line and the line x strain interaction were not significant, indicating that the reaction of the lines to different strains was

similar. The UFLA26 and UFLA54 strains formed a group of more aggressive strains, while the UFLA92 showed a lower level of aggressiveness. A the source of variation lines and strains were significant in the joint variance analysis of the experiment in a greenhouse,. In general, the Campeiro, Talismã, Ouro Negro and Tesouro lines reacted similarly against four isolates, with lower average grades. The UFLA26 and UFLA92 strains formed a group of more aggressive strains, while the UFLA3 showed less aggressiveness. These results are shown in Table 1. It can be observed that the experiment conducted in the greenhouse best discriminated the lines resistance level.

The inferences about the resistance or susceptibility of a line to *S. sclerotiorum* are complicated, since several factors are involved (Kim; Diers, 2000). For example, field evaluations are difficult because it is necessary a cool and moist environment for the disease development (Kim et al., 2000). Therefore, greenhouse evaluations are important, since controlled conditions provide a suitable environment for the pathogen development and the lines reaction.

Table 1 Result of grouping the means of the each line reaction to the four *S. sclerotiorum* strains by Scott Knott, $P \leq 0.05$.

LINES	UFLA 3		UFLA 26		UFLA 54		UFLA 92	
	Field	Greenhouse	Field	Greenhouse	Field	Greenhouse	Field	Greenhouse
Majestoso	3.613a	4.400a	5.937a	7.767a	4.840a	6.000a	2.110a	6.433a
RP1	4.700a	4.167a	4.703b	7.367a	4.933a	5.800a	2.300a	5.567b
Radiante	4.333a	4.000a	4.710b	6.367a	4.523a	5.667a	1.917a	5.333b
Esplendor	3.023a	4.433a	5.067b	7.333a	4.517a	5.433a	1.677a	6.567a
Valente	3.617a	4.567a	4.873b	6.767a	5.567a	4.767a	1.890a	6.667a
Ouro Negro	3.953a	4.433a	4.843b	5.500b	5.617a	5.767a	1.877a	5.200b
Campeiro	4.387a	4.167a	5.357b	4.367c	5.023a	4.567a	1.973a	5.233b
Tesouro	3.750a	4.467a	4.983b	5.200b	4.767a	4.800a	2.143a	6.467a
Talismã	2.530a	4.100a	5.157b	6.167b	5.203a	4.867a	1.953a	5.967b
União	3.357a	4.167a	4.387b	7.700a	4.727a	4.300a	2.203a	7.233a
Cometa	3.183a	4.433a	6.097a	7.000a	5.223a	6.000a	1.913a	6.867a
Pérola	3.477a	4.567a	6.367a	5.500b	4.813a	5.900a	1.913a	7.767a
Estilo	4.233a	4.500a	4.833b	4.667c	4.883a	6.433a	1.830a	7.667a
RP2	3.410a	4.433a	4.990b	8.000a	5.380a	5.433a	2.263a	7.000a

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RESPONSE OF SIX WILD *PHASEOLUS COSTARICENSIS* ACCESSIONS TO SEVEN BACTERIAL, FUNGAL, AND VIRAL DISEASES OF COMMON BEAN

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Useful genes and QTL for resistance to abiotic and biotic stresses, and other traits are found in the cultivated and wild primary gene pools as well as in the related secondary, tertiary, and other gene pools. In common bean, adequate levels of resistance to some diseases, namely angular leaf spot, anthracnose, bean common mosaic, and rust are found in the cultivated common bean. However, resistance to diseases such as common bacterial blight and white mold is not adequate. Furthermore, high levels of resistance to common bacterial blight from the secondary and tertiary gene pool *Phaseolus* species, and for white mold from the secondary gene pool have successfully been introgressed into common bean (see review by Schwartz and Singh, 2013; Singh and Schwartz, 2010). Moreover, Singh et al. (2009, 2013) introgressed white mold resistance from *P. costaricensis* Freytag & Debouck accession G 40604 into interspecific common bean breeding line VRW 32. Because *P. costaricensis* is a member of the secondary gene pool and is easily hybridized with common bean, our objective was to evaluate G 40604 and other available *P. costaricensis* accessions for their response to bean common mosaic, bacterial brown spot, common bacterial blight, halo blight, Fusarium wilt, rust, and white mold.

MATERIALS AND METHODS

Colorado isolates of rust (2009 field bulk), common bacterial blight (B458), halo blight (B637), bacterial brown spot (B629), bean common mosaic, and Fusarium wilt (B13) pathogens were recovered from cryopreservation for inoculation of plants following standard disease evaluation techniques as described for each disease on the Bean Improvement Cooperative web site at http://bic.css.msu.edu/ResearchTechniques.cfm. Evaluations were made 2 weeks post inoculation for all diseases, except for bean common mosaic which was evaluated at 4 weeks post inoculation. An evaluation scale of 1-6 was used for rust, halo blight, and bacterial brown spot, while a scale of 1-9 was used for common bacterial blight and Fusarium wilt responses. Bean common mosaic was evaluated as susceptible, highly resistant (necrosis, black root) and resistant (no symptoms). The 1-6 scale categories were: 1= immune, 2= highly resistant (near immune), 3 = resistant, 4-6 = susceptible; and the 1-9 scale categories were: 1-3 = resistant, 4-6 = intermediate, 7-9 = susceptible. Response to white mold and common bacterial blight less aggressive (ARX8AC) and aggressive (Xcp25) strains were measured in the greenhouse in Idaho. The cut-stem method of inoculation (Terán et al., 2006) was used for white mold pathogen and multiple needles method for common bacterial blight inoculation of the first trifoliolate leaf. Response to both diseases was recorded 21 days post inoculation, using the 1 to 9 scale as described for common bacterial blight for Colorado.

RESULTS AND DISCUSSION

All wild *P. costaricensis* accessions were either resistant and/or highly resistant (i.e., expressed a hyper sensitive or veinal necrosis) to bean common mosaic (Table 1). Similarly, all accessions had either resistant or near resistant scores for rust and bacterial brown spot. For white mold, G 40804 was resistant, G 40604 had an intermediate score, and all others were susceptible. For the Colorado isolate of common bacterial blight pathogen, G 40604 was susceptible and all others had intermediate scores. But, all accessions were susceptible against FW and CBB isolates ARX8AC and Xcp25. For halo blight, G 40804 was resistant, G 40604 was susceptible, and the remaining four accessions were intermediate. Thus, G 40804 exhibited resistant or intermediate scores for five diseases, namely white mold, rust, halo blight, bacterial brown spot, and bean common mosaic. Use of such germplasm accession should be maximized in interspecific hybridization with common bean for enhanced germplasm and cultivar development.

Table 1. Mean response of six wild *Phaseolus costaricensis* accessions to seven bacterial, fungal, and viral diseases of common bean, evaluated in the greenhouse at Colorado State University, Fort Collins and University of Idaho, Kimberly in 2013.

Name	WM†	Rust	CBB			HB	BBS	BCM	FW
	ARS12D	CO2009B	B458	ARX8AC	Xcp25	B637	B629		B13
G40604	5.6	3.4	6.5	9.0	9.0	5.2	3.0	HR	9.0
G40804	4.0	3.6	3.9	9.0	9.0	1.9	2.9	HR, R	9.0
G40806	6.5	3.4	4.1	9.0	9.0	3.5	3.8	HR,.R	9.0
G40811	8.0	3.0	4.5	9.0	9.0	4.3	3.2	HR	9.0
G40811A	6.5	3.0	4.2	9.0	9.0	3.4	3.6	HR	9.0
G40811B	7.5	2.7	4.0	-	-	3.5	3.6	R	9.0

†WM, white mold; rust; CBB, common bacterial blight; HB, halo blight; BBS, bacterial brown spot; BCM, bean common mosaic; FW, Fusarium wilt.

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EVALUATION OF BEAN CULTIVAR IPR ELDORADO SUBMITTED TO SEED INOCULATION WITH DIFFERENT STRAINS OF *RHIZOBIUM* AND NITROGEN FERTILIZATION

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INTRODUCTION

Nitrogen (N) is essential nutrient for the cultivation of common bean is obtained primarily through fertilizers or by the biological nitrogen fixation process. The use of inoculants favors the biological nitrogen fixation and has been shown to be essential for the sustainability of Brazilian agriculture, with a view to supply nitrogen to crops with low economic cost and reduced environmental impact (Ferreira et al., 2009).

The objective of this study was to evaluate the response of common beans subjected to inoculation with different strains of *Rhizobium* sp. and nitrogen fertilization, analyzing aspects related to nodulation of plants and grain yield in cultivar IPR Eldorado in crop year 2013.

MATERIALS AND METHODS

The experimental design was randomized blocks, factorial 5 x 3 with four replications. The first factor consisted of five inoculation treatments: without inoculation and inoculation with UFLA 04-195 and 02-100 UFLA *Rhizobium etli* strains, UFLA 02-127 *R. leguminosarum* bv. *phaseoli*, SEMIA 4077 (CIAT = 899 BR = 322) of *R. tropici*. The second factor was composed of three N rates: 0, 40 and 80 kg ha^{-1} at sowing divided and coverage that was made in the early developmental stage V4. The N source was urea employed. Variables analyzed were number of nodule, dry weight of nodule, dry weight of shoot, content and accumulation of nitrogen in shoots, number of pods per plant, number of seeds per pod, 100-seed weight and yield. Data were subjected to analysis of variance and where significant treatment effects was performed to compare the means by Bonferroni test.

RESULTS AND DISCUSSION

The treatments without of mineral nitrogen produced a greater number of nodules and dry weight of nodule compared to the doses of 40 and 80 kg N ha^{-1}, confirming the negative effect of added nitrogen on nodulation (Hungria et. al, 1997; Romanini Junior et al, 2007; Ferreira et al, 2009). However, the dry weight of shoots was higher in treatments with N application (Barbosa Filho et al., 2005).

With respect to nitrogen content in shoots, the treatments inoculated with UFLA 04-195, UFLA 02-127 and UFLA 02-100 strains at doses of 40 and 80 kg ha^{-1} were 3.32% to 3.85 % , but did not differ , while in the treatment inoculated with strain CIAT 899 , a dose of 40 kg ha^{-1} reflected a nitrogen content of 4.12%, values above the critical level which Ambrosano et al. (1996) is 3 % during the flowering period . The accumulation of nitrogen in the shoot, the highest value was obtained in the treatment with UFLA 02-127 and strain rate of 80 kg ha^{-1} which consisted of 275.54 mg.

The number of pods per plant was influenced by increasing the dose of nitrogen in the treatments, with the exception of strain UFLA 02-100. As regards the number of grains per pod, there were significant differences in inoculated treatments with UFLA 02-127 and UFLA 02-100 strains, with the best values obtained with the application of 40 kg N ha^{-1} and 0 / 40 kg N ha^{-1} respectively.

In the analysis of 100-seed weight, the N levels differed only in treatments with CIAT 899 and UFLA 02-100 strains.

Yield values achieved in this study were higher than the Brazilian average of crop year 2012/2013 that consisted of 909 kg ha^{-1} (Conab, 2014). The strain CIAT 899 gave the highest grain yield in the absence of nitrogen fertilizer, however, for the other strains there was no significant difference ($P > 0.05$) between treatments.

Table 1. Average values for number of nodule (NN), dry weight of nodule (DWN), dry weight of shoot (DWS), nitrogen content in shoots (NCS), nitrogen accumulation in shoots (NAS), number of pods per plant (NPP), number of seeds per pod (NSP), 100-seed weight (100SW) and yield as a function of the interaction between the nitrogen (kg ha^{-1}) and inoculation with strains in IPR Eldorado cultivar

Dose	NN	DWN	DWS	NCS	NAS	NPP	NSP	100SW	Yield
		---------(g)--------		(%)	(mg)			(g)	(kg ha^{-1})
Without inoculation									
0	60.50 a	0.23 a	4.87 b	2.57 b	124.50 b	9.07 b	3.75 a	22.50 a	1550.80 a
40	12.75 b	0.03 b	5.95 a	3.32 a	197.87 a	10.90 a	3.65 a	24.00 a	1813.26 a
80	11.25b	0.04 b	5.05 b	3.56 a	179.95 a	11.30 a	3.57 a	22.50 a	971.80 b
UFLA 04-195									
0	184.25 a	0.56 a	5.25 b	2.64 b	139.00 b	6.72 b	3.70 a	23.00 a	1434.00 a
40	77.75 b	0.14 b	7.02 a	3.72 a	259.62 a	11.17 a	3.50 a	23.75 a	1365.51 a
80	48.50 c	0.04 c	4.10 a	3.85 a	158.26 b	10.32 a	3.72 a	22.25 a	1539.88 a
CIAT 899									
0	121.25 a	0.20 a	4.70 b	2.50 c	117.50 c	6.97 b	3.67 a	21.75 b	2492.83 a
40	23.75 c	0.02 c	4.00 b	4.12 a	164.77 b	10.07 a	3.70 a	23.00 a	1581.17 b
80	44.75 b	0.04 b	5.60 a	3.65 b	204.27 a	10.00 a	3.67 a	24.00 a	1512.50 b
UFLA 02-127									
0	58.25 a	0.10 a	4.35 b	2.51 b	109.67 c	7.50 c	3.45 b	23.00 a	1505.68 a
40	11.75 b	0.02 b	4.10 b	3.57 a	146.45 b	9.47 b	3.77 a	24.00 a	1465.42 a
80	12.50 b	0.18 b	7.42 a	3.71 a	275.54 a	12.75 a	3.57 b	24.25 a	1575.67 a
UFLA 02-100									
0	24.25 a	0.03 b	2.95 b	2.40 b	70.75 c	7.80 a	3.70 a	22.75 a	1543.28 a
40	22.75 a	0.06 a	6.70 a2	3.44 a	228.86 a	8.02 a	3.55 a	20.00 b	1234.68 a
80	12.50 b	0.01 c	3.67 b	3.45 a	126.86 b	8.17 a	3.35 b	23.25 a	1181.92 a

Means followed by the same letter in columns for each strain. do not differ by Bonferroni test at 5% probability.

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RESPONSE OF ANDEAN AND MESOAMERICAN COMMON BEAN GENOTYPES TO INOCULATION WITH *RHIZOBIUM* STRAINS

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INTRODUCTION: In most common bean (*Phaseolus vulgaris* L.) production regions of Latin America inoculants are very rarely used by farmers in spite of several studies that demonstrate the importance of *rhizobium* inoculation on commercial production of legume crops. Phenotypic variation in nodulation and nitrogen fixation (N2) in response to *Rhizobium* inoculation of beans is common (Graham *et al*. 2003). However, the specific response of diverse Andean and Mesoamerican accessions to different *Rhizobium* species that nodulate and fix N2 with common beans, requires additional studies. A better understanding of specific bean host plant-*Rhizobium* strain interactions will facilitate the selection of superior symbionts with greater response to inoculation, and with the potential to increase bean productivity in N limited soils.

MATERIALS AND METHODS: A study was conducted in a greenhouse at Zamorano, Honduras using 15 cm diameter pots containing 4.5 kg mixture of 1:1 soil: sand substrate, previously sterilized with steam. The substrate contained 1.64% organic matter and 0.08% total N which can be considered a low N soil. Phosphorous was rather low (13 mg/kg), potassium (226 mg/kg) was high, calcium (967 mg/kg) and magnesium (159 mg/kg) were medium, and sodium (35 mg/kg) was normal. Fifteen Andean and 17 Mesoamerican bean genotypes were inoculated with 1 ml per plant of *Rhizobium* liquid inoculum of the strains CIAT 899 (*R. tropici*), CIAT 632 (*R. etli*) and UPR 2010 (*R. leguminosarum*), 4 days after planting (DAP). The study was conducted using a split plot arrangement of a Randomized Complete Block with 4 replications. The *rhizobium* strain treatments were assigned to the whole plots and bean genotypes to the sub-plots. The plants were irrigated alternatively one day with tap water and the other with a nutrient solution without N, to provide conditions where the plant growth was limited only by N deficiency. At flowering stage (40 DAP), plant samples were taken to measure the response to inoculation using a 1 to 9 scale (1= absence or very few small, inactive nodules; 9= > 20 large, active nodules), and shoot, root and total plant dry weights.

RESULTS AND DISCUSSION: The nodulation response of bean genotypes was significantly influenced by *Rhizobium* strains and genotypes treatments, but not by their interaction. On average, Andean genotypes had greater nodulation and plant dry weight than Mesoamerican genotypes (Table 1). Nodulation score was correlated to total plant dry weight (r= 0.375**). Contrasting responses were observed regarding the effects of *Rhizobium* strains on certain genotypes of both gene pools, suggesting specific bean host- *Rhizobium* strain interactions which need to be studied further. A group of six Andean (ICA Quimbaya, G05686, Ervilha, Mantenga, CAL 143 and G06727) and six Mesoamerican (Macuzalito, G21212, Bribri, Tio Canela 75, Carioca and Tacana) genotypes were identified for future studies.

Table 1. Nodulation and total plant dry weight of 32 Andean and Mesoamerican bean genotypes inoculated with *Rhizobium* strains. Zamorano, Honduras, 2013.

Genotype	Nodulation (1 to 9)[Z]			Total dry weight (g/pl)		
	CIAT 899	CIAT 632	UPR2010	CIAT 899	CIAT 632	UPR2010
	Andean			Andean		
ICA Quimbaya	7.75	8.25	8.75	3.44	3.48	3.42
G05686	7.50	7.00	9.00	3.48	3.57	3.63
Ervilha	8.50	7.00	8.25	3.39	3.29	3.72
Mantenga	6.50	7.50	8.25	2.90	3.09	2.73
CAL 143	7.50	6.50	7.50	3.07	2.89	3.33
Montcalm	6.75	7.25	6.50	3,60	3.24	3.77
G06727	6.75	5.00	8.25	2.94	2.55	3.68
PR 0737-1	5.25	7.00	7.25	2.68	3.30	3.25
Badillo	6.75	6.50	5.25	2.80	3.38	3.42
Widusa	5.75	5.00	7.00	2.62	2.84	3.44
Pompadour J	5.75	5.25	6.00	2.75	2.17	2.94
Bolón Bayo	5.75	5.00	5.50	2.95	2.91	2.49
Ind. Jamaica Red	5.50	5.00	5.00	2.46	2.61	2.87
RedHawk	6.75	7.00	4.75	3.41	3.70	3.48
Michigan DRK	4.50	2.75	3.00	3.07	1.72	2.44
Mean (n=15)	6.48	6.13	6.68	3.04	2.98	3.24
	Mesoamerican			Mesoamerican		
Macuzalito	8.00	8.50	4.25	3.00	2.98	2.32
G21212	8.00	6.25	6.75	3.17	2.72	2.75
Bribri	5.00	6.75	6.50	2.46	2.67	2.29
Tio Canela 75	5.75	6.50	5.75	2.62	2.69	2.07
Carioca	5.50	6.75	6.75	2.73	3.04	2.98
Tacaná	5.25	6.00	6.75	2.32	2.75	2.55
Dorado	6.50	5.50	6.25	2.59	2.45	2.61
TARS10IS-2421	5.00	6.25	5.50	2.71	2.79	2.62
A-55	5.25	5.75	7.50	2.93	2.78	3.25
Milenio	6.00	4.25	5.25	2.83	2.18	2.63
Salagnac	6.00	4.75	5.50	2.89	2.73	2.54
Seda	4.00	5.50	5.25	2.27	2.84	3.03
BAT477	4.00	5.00	6.00	2.26	2.38	2.97
Cincuenteño	4.00	5.00	6.25	2.69	3.04	3.27
DPC-40	3.50	5.25	6.00	2.15	2.42	2.96
Flor de Mayo	4.75	5.75	4.00	3.06	3.17	2.87
Aifi Wuriti	4.75	4.00	4.75	2.51	1.95	2.51
Mean (n=17)	5.37	5.75	5.82	2.66	2.67	2.72
LSD .05	1.55	1.40	1.69	0.43	0.38	0.57

[Z] 1-9 scale: 1= absence or very few small, inactive nodules; 9= > 20 large, active nodules.

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GRAIN YIELD OF DRY BEANS WITH APPLICATIONS OF CATTLE MANURE AND RAINFALL CATCHMENT SYSTEMS AT TRIPLE ROW SOWING

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INTRODUCTION

In areas where dry beans are grown in the temperate semiarid Highlands of Mexico, production of this crop is commonly affected by drought, since more than 80% of the cultivated area is under rainfed conditions, with frequent periods of intermittent or terminal drought (Acosta-Diaz *et al*., 2007) and shallow soils with low organic matter content and limited soil moisture holding capacity (Osuna-Ceja *et al*., 2012). Therefore it is required to determine the grain yield potential and adaptation to the environment of the region of the new improved bean varieties. In addition, it is also needed to include efficient agronomic practices to take maximum advantage of the rainfall and the improvement of the physical, chemical and biological soil characteristics (Osuna-Ceja *et al*., 2012).

MATERIALS AND METHODS

During summer 2013 were established two experiments with dry beans under rainfed at Sandovales, Ags., Mexico, at an altitude of 2040 meters above sea level. Rainfall during crop season was 532 mm and average temperature of 16. 3^0C, soil type is a Planosol, with 1% of organic matter content, sandy-clay texture, 2% slope and pH of 6.8. It was evaluated the dry bean sowing in beds of 1.60 m wide with three rows separated 40 cm between them, with "*in situ*" rainfall catchment systems through "furrow ridges" and "Aqueel" (both practices were carried out at sowing time to store water and reduce soil erosion). The dry bean variety Flor de Junio Dalia was sowed in an experiment established on June 21st; this variety has an intermediate to large crop cycle and high leaf area per plant. A second experiment was established on August 5th and the dry bean variety Pinto Coloso was sowed. This variety has an early crop cycle and small leaf per plant area. Grain yield response to the application of three dosages of cattle manure (0, 10, and 20 t ha^{-1}) applied for three consecutive years (2011 to 2013) and compared with the application of chemical fertilizer 80-40-30 (N-P_2O_5-K_2O; kg ha^{-1}) and an absolute check treatment was evaluated in both experiments. The experimental design was a complete randomized blocks with three replicates. Seeds were deposited every 14 cm, this allowed to distribute 7 seeds per linear m, given a density of 140 thousand plants ha^{-1}, considering the three rows. In addition to the grain yield, in each treatment organic matter content, nitrate and electrical conductivity at 10 and 20 cm soil depth after the three years of manure applications were determined. The variables registered were subject to a statistical analysis and mean test analysis was performed using the LSD at 5%.

RESULTS AND DISCUSSION

Average values of organic matter (OM), nitrate (NO_3) and electrical conductivity (EC) for all treatments evaluated at both soil depths (0-10 cm and 10-20 cm) are shown in Table 1. Statistical analysis revealed significant differences ($P \leq 0.05$) among treatments. Mean test showed that the treatments of 10 and 20 t ha^{-1} manure showed higher OM content with 1.85 and 2.05% respectively. Nitrate concentration also showed statistical differences ($P \leq 0.05$), where treatment

of 20 t ha^{-1} had the highest NO_3 content with 14.85 mg kg^{-1}. Application of manure did not increase statistically ($P \leq 0.05$) soil EC.

Table 1. Organic matter (OM), nitrate (NO_3) and electrical conductivity (EC) at 10 and 20 cm soil depth after three years of manure application.

Manure Treatments	OM %	NO_3 mg kg^{-1}	EC dS m^{-1}	OM %	NO_3 mg kg^{-1}	EC dS m^{-1}
t ha^{-1}	---------- 0-10 cm soil depth ----------			---------- 10-20 soil depth ----------		
0	1.19 b	5.2 c	0.29	0.82 b	3.60 c	0.39
10	1.85 a	7.93 b	0.49	1.30 a	7.43 b	0.47
20	2.05 a	14.85 a	0.53	1.39 a	11.85 a	0.50
[1]ChF	1.02 b	7.22 b	0.3	0.88 b	7.04 b	0.38
[2]LSD_{05}	0.457	1.75	NS	0.524	2.88	NS
[3]CV (%)	12.65	18.54	13.64	14.22	20.56	12.08

[1]ChF = chemical fertilization treatment (80-40-30 NPK kg ha^{-1}), [2]LSD = least significant difference, [3]CV = Coefficient of variation,

Manure treatments significantly ($P \leq 0.05$) increased grain yield in the two experiments established (Table 2). The mean test showed a grain yield range from 1.33 to 2.31 t ha^{-1} in early planting date with Flor de Junio Dalia, while in late sowing experiment the absolute check treatment obtained lower grain yield with Pinto Coloso (0.71 t ha^{-1}). Average grain yields obtained with both dry bean varieties was higher than that observed in region of study (0.35 t ha^{-1}), without using improved dry bean varieties neither more efficient agricultural practices.

Table 2. Grain yield of two dry bean varieties sowed in triple row beds with rainfall catchment systems and the effect of continues application for three years of cattle manure.

Manure Treatments	Grain yield of dry beans	
	FJD[€]	PC[¥]
t ha^{-1}	**---------- t ha^{-1} ----------**	
0	**1.33 c**	**0.71 b**
10	**2.31 a**	**1.35 a**
20	**2.12 a**	**1.10 a**
ChF	**1.90 b**	**1.06 a**
LSD_{05}	**0.2106**	**0.338**
CV (%)	**5.51**	**16.31**

[€]FJD = Flor de Junio Dalia, [¥] PC = Pinto Coloso

CONCLUSIONS

After three years of continuous application of 10 and 20 t ha^{-1} of cattle manure, some chemical soil characteristics, such as organic matter and nitrates content were modified in a positive way. Thus, grain yield of dry beans was increased up to 2.31 and 2.12 t ha^{-1} in the early sowing with Flor de Junio Dalia, while in the late sowing with Pinto Coloso grain yield was 1.35 and 1.10 t ha^{-1}, when 10 and 20 t ha^{-1} of cattle manure were applied respectively, suggesting that those soil chemical characteristics may have a great influence on grain yield of rainfed crops in the area.

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VARIABILITY IN NITROGEN USE EFFICIENCY OF DRY BEAN GENOTYPES

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INTRODUCTION

Nitrogen (N) is usually the most limiting nutrient for growth and yield of crops, including dry beans (*Phaseolous vulgaris* L.). Global use of N has increased dramatically which increases input cost and results in contamination of ground and surface water with nitrate. Therefore, N-efficient genotypes, characterized by stable yields even at low soil N, are required to improve crop yields, reduce production cost and maintain environmental quality.

MATERIALS AND METHODS

A field experiment was conducted in 2012 at Lethbridge Research Centre, Lethbridge, AB. Sixteen dry bean genotypes (N-efficient and N-inefficient) which had previously been selected from a greenhouse experiment were grown in low-N (30 kg N ha^{-1}) and high-N (90 kg N ha^{-1}) soil. The experimental site was N-deficient (13 kg N ha^{-1}). The treatments were arranged in a split-plot design with N levels as main plots and dry bean genotypes as sub-plots with four replications. At flowering (R1) stage, a 30-cm length of row was hand-harvested in each plot for estimating dry matter (DM) content and N uptake efficiency. At physiological maturity, grain yield was determined by mechanically harvesting of two rows in each plot. Nitrogen use efficiency (NUE = grain yield per unit of available N in the soil and fertilizer) was measured in terms of N uptake efficiency and N utilization efficiency. Nitrogen uptake efficiency was calculated as plant N uptake per unit soil and fertilizer N, and nitrogen utilization efficiency was calculated as seed yield per unit plant N uptake (Moll et al. 1982). The experiment was repeated in 2013.

RESULTS AND DISCUSSION

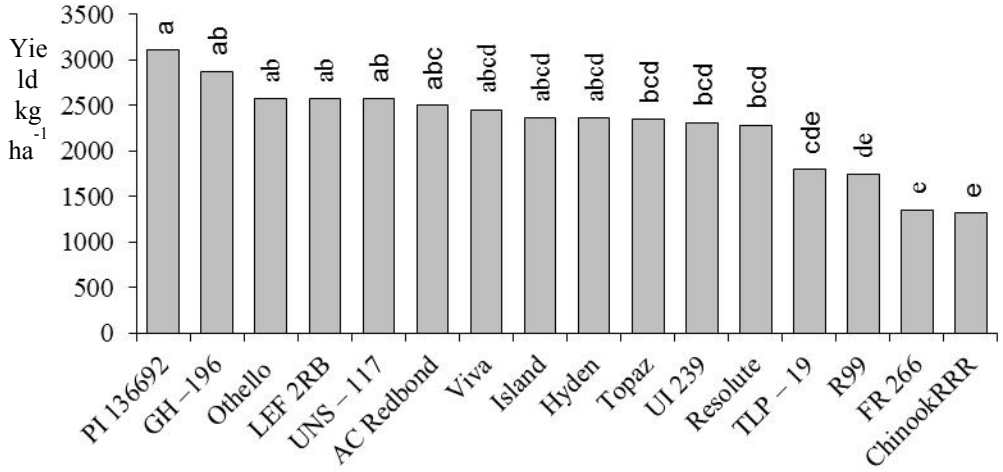

Figure 1. Mean grain yield of dry bean genotypes (averaged over the two N levels) in 2012. Yields of genotypes with the same letters above the bars were not significantly different at 5% significance level.

Interaction between genotypes and N levels for grain yield was not significant; therefore, the graph presented in Fig. 1 is the average of the two N levels. Genotype PI 136692 had the highest grain yield (3105 kg ha^{-1}) followed by GH-196 (2864 kg ha^{-1}), Othello (2575 kg ha^{-1}) and LEF 2RB (2575 kg ha^{-1}) compared to other genotypes (Fig. 1). The differences between genotypes were significant ($P < 0.05$) for Nitrogen use efficiency. Genotypes PI 136692, Othello, LEF 2RB and GH-196 were the most N-efficient genotypes at both N levels, but PI 136692, GH-196, LEF 2RB and Hyden had the highest NUE at low N (Table 1). NUE is positively associated with high grain-yield, and the genotypes that are superior in NUE capture a relatively high amount of the soil available N in the grain, thereby reducing the risk of losing N to the environment (Anbessa et al. 2009).

Table 1. NUE of dry bean genotypes in different soil-N levels

Genotypes	NUE (Kg grain kg^{-1} N supplied)	
	Low N	High N
Othello	73.31^{de}	29.52^{ab}
Island	75.24^{cde}	24.8^{bcd}
Resolute	73.16^{de}	23.7^{bcd}
Topaz	77.29^{cd}	23.77^{bcd}
GH-196	95.52^{ab}	28.64^{ab}
Viva	80.62^{bcd}	24.91^{abcd}
UNS – 117	74.91^{cde}	28.93^{ab}
AC Redbond	80.17^{bcd}	25.94^{abc}
UI 239	68.6^{de}	25.64^{abc}
PI 136692	100.38^{a}	31.99^{a}
TLP – 19	59.57^{ef}	18.19^{de}
ChinookRRR	41.55^{g}	13.97^{e}
FR 266	45.33^{fg}	13.34^{e}
Hyden	85.88^{abcd}	21.47^{cd}
LEF 2RB	91.89^{abc}	26.08^{abc}
R99	49.21^{fg}	20.09^{cde}
LSD ($P = 0.05$)	17.34	7.10

Means followed by the same letter are not significantly different at 5% significance level.

CONCLUSION: This study demonstrated high genotypic variability among dry bean genotypes in NUE. PI 136692, LEF 2RB and GH-196 were N-efficient. These genotypes will be used in the breeding program to sustainably increase dry bean productivity. Grain yield and N-uptake were predominant traits responsible for improved NUE of the above genotypes. These traits contributed to the growth of dry beans on low-N soils with no reduction in yield per unit of available N.

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EFFECTS OF DOSES AND TIME OF NITROGEN APPLICATION ON GROWTH AND YIELD OF COMMON BEAN (*Phaseolus vulgaris* L.)

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INTRODUCTION

Nitrogen fertilization has raised relevant questions that involve the reactions and mechanisms which control the availability of this nutrient and the reactions of different sources of nitrogen in the soil, as well as the use of doses and time of application during the crop cicle. The present work aimed to evaluate the responses of common bean to different nitrogen doses and methods of application.

MATERIAL AND METHODS

Two field experiments were conducted from March to July 2012, in the area of Experimental Farm of Vale do Rio Verde University, Três Corações, Minas Gerais State, Brazil. Sowings were made under conventional tillage, using the cultivar BRS Cometa. The experimental design was a randomized complete block with five treatments and three replicates. Each plot consisted of four 5-m rows spaced 0.50 m apart, with seeding of 15 seeds per meter. Treatments were carried out as follows: T1 = 0 $kg.ha^{-1}$; T2 = 60 $kg.ha^{-1}$; T3 = 90 $kg.ha^{-1}$; T4 = 120 $kg.ha^{-1}$; T5 = 150 $kg.ha^{-1}$ of urea. In the first experiment, the application of urea was restricted to the time of sowing, while in the second experiment the fertilization was split so that 1/3 was applied at sowing and 2/3 in topdress manuring 21 days after planting. The fertilizer was diluted in 1000 mL of water and applied near the ranks of bean. The features evaluated were: plant height (PH), height of the first pod insertion (HFPI), number of pods per plant (NPP), number of grains per pod (NGP), weight of 100 grains (WG) and yield (Y). The data were submitted to the analysis of variance and in the cases of significance they were subjected to regression analysis.

RESULTS AND DISCUSSION

In the first experiment no significant differences between treatments were observed, indicating that the application of varying doses of urea at sowing does not result in gains in the development and yield of bean. Carvalho et al. (2001) also found no interference of nitrogen fertilizer, applied at sowing, on grain yield. But for the second experiment there was a significant effect only for NPP and Y variables (Table 2 and Figure 1). The NPP increased in a quadratic manner reaching a maximum value of 20.26 pods per plant at a dose of 70 $kg.ha^{-1}$ of urea approximately. Nitrogen doses also influenced the grain yield, as also reported by Sant'ana et al. (2010) for cultivar BRS Horizonte. In this work, yield reached a maximum value of 5528.75 kg. ha^{-1} at a dose of 66 kg ha^{-1}, evidencing the participation of number of pods per plant, since there was a significant correlation between these two variables.

Table 1. Average values of PH (cm), NPP, HFPI (cm), NGP, Y (kg.ha^{-1}) e WG (g) concerning the doses of urea, applied at sowing.

Doses of urea (kg.ha^{-1})	PH ^{ns}	NPP ^{ns}	HFPI ^{ns}	NGP ^{ns}	Y ^{ns}	WG ^{ns}
0.0	39.57	11.00	7.94	4.57	2548.00	25.49
60.0	40.57	10.00	7.02	4.93	2632.00	26.27
90.0	46.53	11.67	6.31	5.04	2954.00	26.01
120.0	46.40	10.67	9.03	4.70	2534.00	25.87
150.0	40.83	11.33	7.86	4.66	2646.00	26.17
CV (%)	7.74	15.26	12.14	9.33	14.06	4.78

PH – plant height; NPP – number of pods per plant; HFPI – height of first pod insertion; NGP – number of grains per pod; Y – yield; MG – weight of 100 grains.
^{ns}: non significant

Table 2. Average values of PH (cm), NPP, HFPI (cm), NGP, Y (kg.ha^{-1}) e WG (g) concerning the doses of urea, applied at sowing and at topdressing manuring 21 days after planting.

Doses de urea (kg.ha^{-1})	PH ^{ns}	NPP *	HFPI ^{ns}	NGP ^{ns}	Y *	WG ^{ns}
0.0	54.07	18.67	8.93	5.22	5418.00	27.85
60.0	60.33	17.33	8.63	4.78	4634.00	27.50
90.0	58.15	21.00	10.15	4.75	6146.00	30.35
120.0	62.45	21.00	10.35	4.84	5670.00	27.59
150.0	58.93	16.00	8.93	5.17	4704.00	27.40
CV (%)	6.58	5.49	8.59	6.19	4.38	4.75

PH – plant height; NPP – number of pods per plant; HFPI – height of first pod insertion; NGP – number of grains per pod; Y – yield; MG – weight of 100 grains.
^{ns}: non significant; *: significant

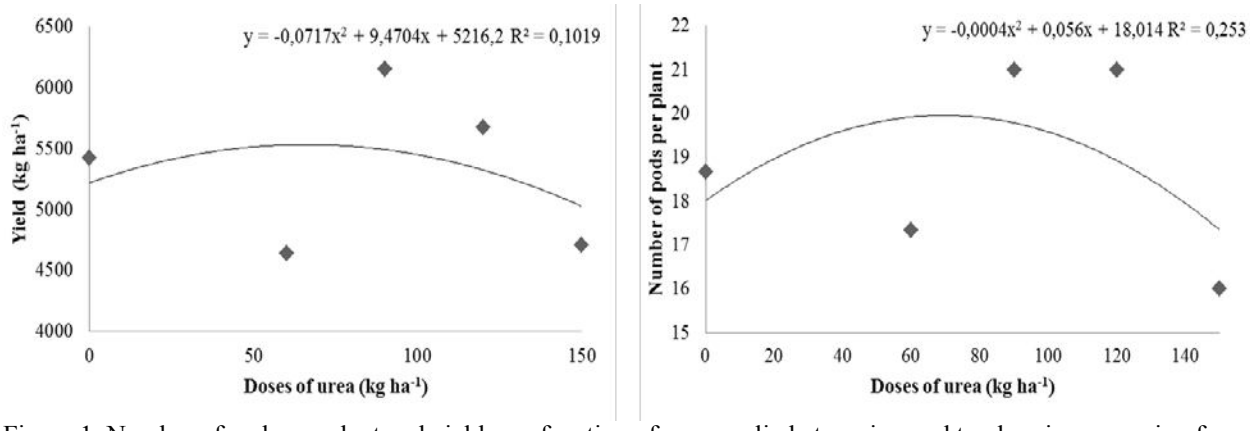

Figure 1. Number of pods per plant and yield as a function of urea applied at sowing and topdressing manuring for common bean under conventional tillage.

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EARLY GENERATION INHERITANCE OF INCREASED LATERAL AND BASAL ROOTS PRODUCTION AND HIGHER SHOOT MASS PRODUCTION AS TRAITS FOR TOLERANCE TO LOW PHOSPHOROUS AVAILABILITY IN COMMON BEAN

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INTRODUCTION: In common bean and other crop plants, morphological and physiological characters that are important to low phosphorous (P) tolerance have been reported such as the number and length of lateral roots, adventitious rooting and top soil foraging. Lateral roots in particular have been reported to play an important role by increasing soil exploration, the absorptive surface of the root system and P solubilization, which results into increased P uptake. Liao and Yan, (2001) reported that morphological characteristics of the basal and lateral roots contribute more to P efficiency than those of the tap roots. Higher shoot growth of bean plants under limited soil phosphorous supply is another adaptation criterion for P deficiency (Araujo *et al*, 2005). The present study was conducted using three parental genotypes contrasting in their P efficiency in order to investigate if root traits, in particular increased lateral and basal root production and higher shoot mass production under low phosphorous availability were heritable traits in common bean so as to provide a basis for genetic improvement for tolerance to low phosphorous availability.

MATERIALS AND METHODS: Low P tolerant common bean genotypes RWR 1946 and RWR 2075 were crossed with a sensitive genotype K 132 to generate the following F_1 crosses: K 132 x RWR 1946 and K 132 x RWR 2075. 30 seeds of each of the parental genotypes RWR 1946, RWR 2075 and K 132, and their F_1 crosses were planted in 16 kg containers in low phosphorous treatment consisting of 10 mg P/kg of soil and high phosphorous treatment consisting of 160 mg P/kg of soil as tripple super phosphate. For each treatment, base fertilizers, i.e 100 mg K/kg of soil (as potassium choloride), 10 mg Zn /kg of soil (as Zinc sulphate) and 1 mg B/kg of soil (as boric acid) were added. A completely randomized block design was used. 6 days after emergence, thinning was done leaving only 24 plants for each of the parents and the F_1s, under each treatment. Nitrogen fertilisers i.e 200 mg N /kg of soil (as ammonium sulphate) and 200 mg N/kg of soil (as Urea) were also applied via dilute solution 12 and 25 days after emergence respectively. Watering was done as adequately as possible. At 45 days after planting, all shoots were harvested per 16 kg container. Roots including nodules were separated from soil by washing with water. Basal and lateral root production in response to phosphorus availability was visually scored based on a scale of 1-9, where 1 is excellent and 9 is very poor according to the common bean Shovelomics. Broad and narrow sense heritability was also calculated.

RESULTS AND DISCUSSION: Under low P treatment, all the plants for each of the parental genotypes RWR 1946 and RWR 2075 showed obvious greater lateral and basal roots production (Plate 1) and shoot mass production than the plants of the in-efficient genotype K 132. The response of lateral and basal roots production and shoot mass production of the F_1 progenies K 132 x RWR 1946 and K 132 x RWR 2075 was similar to parental genotypes RWR 1946 and RWR 2075 indicating that these traits are heritable in the early generation.

Plate 1: Lateral and basal root production of parental genotypes RWR 1946, RWR 2075 and K 132 and their F_1 progenies at 45 days after planting (DAP): F_1 3246 = F_1 K 132 x RWR 1946; F_1 3275 = F_1 K 132 x RWR 2075

Under high P treatment, there was no obvious genotypic variation for the measured traits. All the parents and F_1s responded to the high P treatment and showed vigorous morphological traits. Narrow sense heritability was generally higher than broad sense heritability (Table 1), strongly suggesting that early generation inheritance of increased lateral rooting and higher shoot mass production under low phosphorous availability in genotypes RWR 1946 and RWR 2075 was largely due to additive genetic effects.

Table 1: Heritability of increased lateral and basal root production and shoot mass production in the F_1 progenies

P level	F_1 generation	Heritability (%)	Increased lateral and basal root production	Shoot mass Production
Low p	K 132 x RWR 1946	Broad sense heritability	0.38062	0.5681
		Narrow sense heritability	0.75443	0.5851
	K 132 x RWR 2075	Broad sense heritability	0.43214	0.5673
		Narrow sense heritability	0.60178	0.4652
High P	K 132 x RWR 1946	Broad sense heritability	0.30280	0.54039
		Narrow sense heritability	0.44816	0.67209
	K 132 x RWR 2075	Broad sense heritability	0.54179	0.5386
		Narrow sense heritability	0.50734	0.63104

CONCLUSION

From this study early generation inheritance of increased lateral and basal roots production and higher shoot mass production as traits for tolerance to low phosphorous availability in genotypes RWR 1946 and RWR 2075 is largely controlled by additive genes (main effects). Hence there is potential for genetic improvement using these traits to select for desirable genotypes. Further studies are recommended to better understand the traits in advanced generations and the number of genes involved.

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RESPONSE OF SOWING METHODS AND FERTILIZATION ON FLOR DE MAYO AND FLOR DE JUNIO TYPE DRY BEAN CULTIVARS

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INTRODUCTION

Under irrigations conditions, in the state of Aguascalientes and in many other producing areas of the semiarid region of the North-Central part of Mexico, Flor de Mayo and Flor de Junio dry bean cultivars are classified as preferable on the national market. Then, these cultivars are sowed most commonly (Acosta-Díaz *et al*., 2007). However, traditional cultivation of these varieties often limits the yield and the response to fertilization. Therefore, it is necessary to increase the productivity and competitiveness of bean cultivation. Thus, it is important to explore new ways of cultivating to determine components of the cropping system allowing to the new Flor de Mayo and Flor de Junio type bean varieties to optimize its yield. One of these components is the increase of the plant population density by means of sowing in narrow rows (Osuna *et al*., 2012). The objective of the present study was to determine the effect of two methods of sowing and chemical and biological fertilization treatments on pods per plant, seed weight and grain yield of two Flor de Mayo and two Flor de Junio type bean varieties under irrigation conditions.

MATERIALS AND METHODS

The experimental design was randomized blocks with four replications and a 2x4x6 factorial treatment arrangement including the following factors: 1) Two sowing methods, a) conventional sowing at 0.76 m between rows and a plant density of 90 thousand plants ha^{-1} and b) planting beds with triple row at 0.40 m between them and a plant density of 145 thousand plants ha^{-1}), 2) Four bean cultivars and 3) Six fertilization treatments. The cultivars were Flor de Mayo Dolores, Flor de Mayo Eugenia, Flor de Junio Dalia and Flor de Junio León. All of them are of intermediate cycle, 95 days to maturity. Fertilization treatments evaluated were: 1) 40-60-30 (current soil fertilization dosage recommended for bean in that region), 2) 40-60-30 + Foliar Fertilization "FF" applied in grain filling stage, 3) Bio-fertilization (inoculation of seeds with 0.350 kg mycorrhize), 4) Mycorrhize + FF, 5) FF and 6) 00-00-00. Sowing methods corresponded to the large plots and cultivars and fertilization treatment to the subplots. Planting was on April 19th, and the harvest, on August/2013. Soil fertilization was totally applied at planting time and Urea (46 % N) and simple superphosphate (20% P_2O_5) were used as N and P sources. The nutrient sources used in the FF treatment were Urea 46%N and phosphoric acid 54% P_2O_5 at a concentration in the sprayed solution of 2% and 1%, respectively by volume. The total amounts of nutrients applied by FF were 5.5 and 4.5 kg of N and P_2O_5 per hectare. Each plot consisted of four and six row with 50 m length and spacing of 0.76 m and 40 m between rows. Three irrigations were applied during the crop season. At harvest, samples of 10 plants for were collected to determine pod number per plant and weight of hundred grains.

RESULTS AND DISCUSSION

Sowing method in beds with three rows showed the highest grain yield, while the conventional single row 0.76 m sowing method had the lowest grain yield. Grain yield was increased by 165% as compared to the traditional sowing method used by most farmers in the region of study and it is attributed to a higher plant population and greater soil surface cover. A similar trend was

observed in both yield components, seed weight and pod number, although a significant difference ($p \leq 0.05$) was only found on seed weight, in the combined analysis.

Table 1. Average values of grain yield (kg ha^{-1}), pods per plant and seed weigh of four bean cultivars cultivated under two sowing methods and fertilization treatments under irrigation. Aguascalientes, México. 2013.

Evaluation factors	Grain yield (kg ha^{-1})	Pods plant^{-1}	Weight of 100 seed (g)
----------Sowing methods ----------			
Single plant row	598.9 b	10.42	33.62 b
Three plant row beds	1584.9 a	12.23	35.72 a
Mean	**1091.9**	**11.33**	**34.69**
DMS$_{05}$	**238.10**	**Ns**	**1.326**
---------- Cultivars ----------			
Flor de Mayo Dolores	1112.0	10.56	32.65 b
Flor de Mayo Eugenia	1041.5	10.83	34.96 a
Flor de Junio León	1113.6	12.47	36.10 a
Flor de Junio Dalia	1100.6	11.43	35.05 a
Mean	**1091.9**	**11.33**	**34.69**
DMS$_{05}$	**ns**	**ns**	**1.644**
---------- Fertilization ----------			
40-60-30	1092.0 bc	13.37 a	35.86 a
40-60-30 + FF	1486.9 a	14.56 a	36.55 a
Mycorrhize	1012.8 bc	10.83 ab	34.72 ab
Mycorrhize + FF	1130.2 b	9.56 ab	33.45 ab
Foliar Fertilization (FF)	983.8 bc	8.96 b	33.60 b
00-00-00	845.9 c	8.77 b	33.50 b
Mean	**1091.9**	**11.33**	**34.69**
DMS$_{05}$	**279.36**	**3.047**	**2.057**

Grain yield of all bean cultivars evaluated revealed no significance differences among them, as well as pod number, whereas seed weight of Flor de Mayo Dolores was significantly lower as compared to the other cultivars (Table 1). The fertilization treatment 40-60-30 + FF, significantly ($P \leq 0.05$) increased grain yield (1486.9 kg ha^{-1}), while the 00-00-00 treatment had the lowest yield (845.9 kg ha^{-1}). However, foliar application of NP increased grain yield average by 16% (from 845.9 to 983.8 kg ha^{-1}) as compared to the treatment without any fertilization, although this difference between both fertilization treatments was statistically no significant. Thus, it is concluded that any of the four bean cultivars evaluated are suitable for the area of study and soil fertilization of NPK + FF could increase grain yield as much as 36% compared with no fertilization application. For those farmers having low economical sources, it is recommended to apply at least the foliar application of NP, since it is cheap and can be cost-effective.

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VALIDATION OF DRY BEAN SOWING PROTOTYPES IN AGUASCALIENTES, MEXICO

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INTRODCUTION: Mexican agriculture is a strategic sector for national development. Dry bean has an important role on diet since it is a source of protein for the rural population. The production of this crop is affected by drought, as approximately 85% of the area planted with dry beans in Mexico is located in regions where seasonal rainfall present frequent periods of intermittent or terminal drought (Acosta-Díaz *et al*., 2007), as well as shallow soils having low organic matter content and limited water holding capacity. Another factor affecting yield is the limited use of appropriate technology, which make rainfed agriculture be of subsistence, but it is not less important than that under irrigated conditions. Sowing of dry beans in this area is usually performed by rustic mechanical seeders which do not allow a work of quality, since they cannot regulate the depth of sowing, distance between seeds and number of seeds per hole. This is due to lack of access to modern equipment, mainly because to its high cost. This study was conducted with the aim to validate a versatile experimental machine for sowing dry beans in beds with two, three or four rows, equipped to capture rainwater and applying organic fertilizer in one operation and compare their performance with other commercial sowers.

MATERIALS AND METHODS: A field experiment was established on a soil type Calcisol, sandy-loam texture, alkaline pH of 7.9 and 2% of organic matter content at the Experimental Station of Pabellon, Aguascalientes, Mexico. Three seeders machines with various characteristics were evaluated: 1) versatile experimental precision mechanical sower, for sowing in beds at two, three and four rows, with integrated water catchment system (Aqueel) and dispenser of organic fertilizer, 2) commercial precision mechanical sower for single row and 3) commercial mechanical seeder for single row sowing. Cultivar dry bean Pinto Centenario was used in the study. Different plant densities were established with each one of the evaluated equipment. Plant density with the versatile experimental mechanical precision sower was 100, 170 and 190 thousand plants ha^{-1} in beds of two, three or four rows, respectively, while plant density with the other two mechanical seeders was of 100 thousand plants ha^{-1} in single rows. The combinations of treatments were laid out in a complete randomized block experimental design with four replications. The experimental plot consisted of two beds 1.60 m wide and 30 m long for the sowing at two, three and four rows (0.80, 0.40, and 0.30 m wide between lines) for the experimental precision sower and four rows of 30 m long and 0.80 m wide between rows for the other two commercial planters with sowing to single rows. Distance between plants, number of plants per linear meter, density of plants per hectare and amount of seed used, as well as the grain yield production per hectare were determined.

RESULTS AND DISCUSSION: Table 1 shows the mean values of distance between plants, number of plants per linear meter, plant density per hectare, amount of used seed and grain yield obtained with the different sowing mechanical devices evaluated. Statistical analysis revealed no significance ($P \leq 0.05$) for distance between plants and number of plants per linear meter. On the

other hand, equipment and methods of sowing showed significant differences for plant density, amount of used seed and grain yield. The highest plant density and amount of seed used were obtained in the sowing method in beds at four rows, followed by sowing at three rows; both sowings treatments were performed with the experimental precision mechanical sower.

Table 1. Average distance between plants, number of plants per meter, plant density, amount of seed used and grain yield of dry beans obtained with three different mechanical sowing prototypes. Pabellón, Aguascalientes, México. 2013.

Sowers	Treatment	D/p (cm)	Pl m^{-1}	PD (10^3 plants $ha^{-1)}$	Seed (kg ha^{-1})	Grain yield (t ha^{-1})
EPMS	4 rows	14.07	7.11	187.63 a	72.00 a	1.68 a
	3 rows	12.22	8.18	162.03 b	62.00 b	1.44 b
	Single plant row	13.94	7.17	94.64 c	36.20 c	1.05 c
CPMS	Single plant row	13.75	7.27	96.00 c	36.50 c	0.90 c
CMS	Single plant row	14.22	7.03	92.83 c	35.30 c	0.88 c
DMS_{05}		Ns	ns	2.356	2.866	0.203

EPMS = experimental precision mechanical sower; CPMS = commercial precision mechanical sower; CMS = commercial mechanical sower; D/p = distance between plants; Pl = plants per meter; PD = plant density per hectare and ns = no significant.

Significant differences ($P \leq 0.05$) were observed in grain yield among sowing methods established with the different mechanical seeders. Mean test analysis showed that grain yield ranges from 0.88-1.68 t ha^{-1}, and sowing methods in beds at three and four planting rows obtained higher yields with 1.44 and 1.68 t ha^{-1}, respectively, which represent a 64 and 91% respect to the conventional single row sowing method at 0.76 m. The lower yield of 0.88 t ha^{-1} was observed with the single row sowing method when the commercial mechanical seeder was used. These results demonstrate that experimental precision mechanical sower is a viable option for sowing dry beans under the rainfed conditions of the semi-arid region of the North-Center of Mexico. Therefore, it is concluded that with this experimental precision mechanical sower several operations can be performed simultaneously, such as: sowing at three or four rows, use of the Aqueel system for in-site water catchment and application of organic fertilizer, which allows obtaining grain yields above those with traditional mechanical equipment.

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BIODIVERSITY SCORE AND ITS EFFECTS ON COMMON BEAN (*Phaseolus vulgaris* L.) CROP GENETIC DIVERSITY

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INTRODUCTION

The concept that agriculture research systems currently under way are not suitable to attend future global food needs, under the picture of decreased land availability, population increase and climatic change, has growing lately (JORGENSEN, 2012). However technological innovation alone, it is believed, is not enough to alter in the favorable way such systems. We must also change our values, beliefs and aspirations towards sustainability (STATE OF THE PLANET DECLARATION- New Knowledge Towards Solutions, 2012). Foreseeing such of these perceptions the common bean research team of Embrapa Temperate Climate - CPACT, designed the methodology identified as Biodiversity Score– BS(Partitura de Biodiversidade; ANTUNES and BEVILAQUA, 2009). The BS comprises a group of Landraces – LR, that form the common bean germplasm bank of CPACT. The LR are variable in morphological and physiological characteristics, as well as in origin. The theoretical aim of the BS comprises the LR germplasm preservation, increase of genetic diversity, dietary improvement and greater income to the farmers. Another important point related to the BS, is the role of the farmer in the process of evaluation. Besides using its usual technological framework in the implementation of the BS, that varies from farmer to farmer, the criteria for evaluation of the LR that form the BS, are under his determination. Part of the BS system is also the presence of the extension agent, which also evaluates the LR compounding the interaction among research, extension and the farmer.

This article shows results obtained from testing of BS by farmers from 2007 to the present and their effects on the common bean genetic diversity.

MATERIALS AND METHODS

The Biodiversity Score – BS comprehends a group of Landraces – LR obtained through direct field collection or through the reception from farmers or extension agents. Usually comprises 10 LR and three cultivars released by Embrapa as checks. Experimental plots are formed by four 4m rows with 12 seeds/m with no replication. Cultural practices are the usual for each farmer in order to permit the evaluation exactly according to his traditional way. It is suggested that both the farmer and the extension agent judge the BS appointing to the three best and the three worst LR, informing the reasons for such judgment. Data is registered on disease incidence; plant architecture; adaptation; sowing, flowering and harvest dates; and seed quality. At harvest, the two central rows are collected upon which seed yield is determined.

The BS system was set in 2007/08 crop year and, up to now, about 200 have been sent for evaluation to all common bean production regions of Rio Grande do Sul State (RS). One hundred twenty LR have been distributed to farmers through the BS system, adjoining about 140 farmers

directly to the process. Mostly of the BS had the official extension service participation in the evaluation.

RESULTS AND DISCUSSION

Some of the results obtained since 2007, reveal that LR displayed differential adaptation to the environments where they have been tested. Besides, different farmers took different characteristics as of most importance in electing the best cultivars.

In such way, both the farmer and the extension agent, located in Estrela (RS) elected as the best cultivars the LRs Preto Graúdo and Milico, based on the high probability of good market performance. In Venancio Aires (RS) the reason for selection of the cultivar Chumbinho as the best, was its excellent cooking quality, whereas superior yield and disease resistance presented by the LRs BalimGrosso, Guabiju and Felipe where the characteristics for selection as the best made by the farmer located in Sertão Santana(RS).

Another important feedback from the farmers came from TenentePortela (RS), where a farmer which can be considered as a seed keeper, declared that the BS is an important instrument as source of new cultivars for use in family farming systems, in which the farmers are able to produce their own seeds.

Based in the results, the BS system mechanism resulted in improved genetic diversity since a group of new cultivars was added to the common bean cropping system

Results obtained up to now also suggest that the BS system is attending the main objectives present at the moment it was designed and point forward to its use as a model for other crops in order to accomplish with the ideas for new research systems.

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A COMMON BEAN GERMPLASM BANK AS SOURCE FOR RECOVERY OF CULTURAL RICHNESS

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INTRODUCTION

It is known that the scientific knowledge has ways of construction and application, which differ from those of the traditional knowledge. The first imposes itself as an absolute truth until the appearance of a new universal paradigm; the traditional knowledge frequently houses, equally with skepticism or confidence, divergent explanations, where validity is just local (CARNEIRO DA CUNHA, 2009). Manipulation of varieties of food plant species by indigenous groups is an example of a concrete expression of a simultaneously ancient and local knowledge.

Embrapa Temperate Climate (CPACT), located in Pelotas, Rio Grande do Sul State (RS), Brazil, an agricultural research institution, has been conducting works that deal with research-action with indigenous seed keepers, starting with those located at Terra Indigena Guarita Indian Reserve, located Northwest Rio Grande do Sul. Results from this first approach revealed that many landrace varieties (LR) used for food had been lost, mainly due to adverse climatic conditions (FEIJÓ et al., 2012).

More recently, CPACT amplifying its participation in relationship to the theme started a joint study with the Mbya Guarani group presently located in the Rio Grande do Sul coastal region. Preliminary talks have shown that the group had lost many LR, as had happened at Terra Indigena Guarita Indian Reserve. Among those, many common bean (*Phaseolus vulgaris* L.) LR.

The existence of a common bean germplasm bank at CPACT suggested the possibility of recovering of the lost landraces.

This article shows results obtained from such attempt.

MATERIAL AND METHODS

In order to give continuity to the activities related to the search for the lost Mbya Guarani varieties, eight Mbya Guarani farmers and three researchers from the Foundation for the Support of the University Extension and Research Services (FAPEU), Santa Catarina State, Brazil, on September 13, 2013, went to Low Lands Experimental Station (ETB), part of CPACT structure. On this day, the visitors were able to get acquainted with some of the work done at the institution involving research with landrace varieties and seed keepers, as well as with sustainability of family farms and social and economic development.

Specifically, before the start of the practice of selection of landrace varieties by Guarani farmers, it was established a dialogue with the participation of researchers and the Head of CPACT, in which it was achieved the institutional partnership. At the same meeting the agenda to be conducted on the day and the sequence of the work for the agricultural year 2013/2014, were discussed.

Prior to the beginning of the selection activity, it was explained to the farmers the way the seeds have been stored and the way the collections have been built. Likewise, the Guarani farmers explained the types of seeds they sought, including particularly those which form part of their diet.

At ETB Research Station, where the Guarani were welcomed, is maintained the common bean landrace collection. In this occasion, seeds of 35 common bean landraces varieties were exposed to the farmers for their observation and selection.

Based on their own criteria, the Mbya Guarani performed their selection (Figure 1).

RESULTS AND DISCUSSION

From the dialogue established among them, always in the Guarani language, of 35 LR varieties, have been identified and selected seven, called Rim de Porco, Unha de Princesa, Preto Comprido, Vermelho Anchieta, Amendoim Unaic , Fogo na Serra and Mourinho.

Among these, Mourinho was the only one that the Mbya Guarani stated being identical to the ones have been lost. The six other varieties, despite the fact they were nor identical to the original ones, were selected due to favorable phenotypic characteristics under their criteria.

The experience shared with the Mbya Guarani shows that the common bean germplasm bank of CPACT, that has many LR entries, can contribute not only to the maintenance of the available genetic diversity, but also in the recovery and even in the increase of such diversity.

Figure 1. Selection of common bean landraces by Mbya Guarani farmers

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ANALYSIS AND CHARACTERIZATION OF BLACK BEANS (*Phaseolus vulgaris* L.) LANDRACES FROM PARANÁ STATE (BRAZIL) BASED ON MORPHOAGRONOMIC TRAITS FOR GERMPLASM INCREMENT

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INTRODUCTION

Common beans (*Phaseolus vulgaris* L) is one of the most important crops in Brazil. This grain legume is considered one of the main sources of amino acids and carbohydrates for the Brazilian people (BROUGHTON et al., 2003).

The diversity among accessions is essential for breeding gain. The landraces are valuable sources of alleles that turn them into adapted plants, and therefore more productive in specific environments. In order to maintain the biodiversity and keep the variability available for research and breeding programs, it is essential to analyze and preserve these germplasms (RODRIGUES et al., 2002). This study aimed to analyze and characterize black bean landraces collected in Paraná state to assess their value in a breeding program as source of variability and integrate the UNICENTRO's germplasm bank.

MATERIALS AND METHODS

The 28 black beans (*P. vulgaris*) landraces were collected in different small farms in Paraná state (Southern Brazil) to integrate the UNICENTRO's germplasm bank.

The assay was carried out at Universidade Estadual do Centro Oeste – UNICENTRO, Campus CEDETEG, in Guarapuava, Paraná, Brazil (latitude 25º 22' S, longitude 51º 28'). The landraces were analyzed in a randomized blocks essay with three replications. One hundred and twenty seeds of each landrace were sowed (40 each replication), and 90 of them (30 each replication) were randomly chosen and evaluated, resulting in a total of 2,520 analyzed plants. The crop management was done according to Vieira et al. (2006).

A set of features were studied for the accessions' characterization including: plant height (cm), height of the insertion of the first pod (cm), days to flowering and ripening, days from flowering to ripening, amount of pods per plant, amount of seeds per pod, weight of 1,000 seeds (grams) and yield (kg ha^{-1}). The data acquired was then statistically analyzed with the software SISVAR (Sistema de Análise de Variância) by Scott-Knott (1974) test at 5% probability.

RESULTS AND DISCUSSION

The results of the statistical analysis is shown on Table 1. The amount of pods per plant and seeds per pod could not differ the landraces at 5% probability by the Scott-Knott test. On the other hand, seven traits were able to do so, including: plant height, height of the insertion of the first pod, days to flowering and ripening, days from flowering to ripening, weight of 1,000 seeds and yield. Plant height and yield were the most effective traits for landraces segregation since five divergent groups were found.

It is important to mention that variability was found between individuals of the same landrace. This may occur based on the fact that farmers usually exchange seeds in order to get more productive cultivars.

Table 1. Mean values and grouping for seven traits of 28 black bean landraces. Guarapuava, Paraná.

Landraces	Height (cm)		1st. pod (cm)		Flowering (Days)		Flowering to ripening (Days)		Ripening (Days)		1,000 seeds weight (g)		Yield (kg ha^{-1})	
L1	65.92	a5	15.08	a1	43.00	a2	39.00	a1	93.33	a1	221.53	a2	1050.00	a3
L2	57.30	a3	16.53	a2	43.67	a2	49.67	a2	93.33	a1	194.87	a1	1075.00	a3
L3	55.46	a3	15.32	a1	41.00	a2	53.67	a2	94.67	a2	192.57	a1	1483.33	a4
L4	52.65	a2	15.98	a2	41.00	a2	65.67	a3	108.67	a4	223.90	a2	725.00	a1
L5	58.91	a3	18.61	a3	38.33	a1	59.00	a3	97.33	a2	201.03	a1	975.00	a2
L6	55.78	a3	16.90	a2	40.33	a1	54.67	a2	99.33	a3	254.70	a3	1325.00	a3
L7	60.61	a3	18.11	a3	39.67	a1	53.33	a2	92.67	a1	201.23	a1	925.00	a2
L8	50.31	a2	15.71	a1	36.00	a1	61.00	a3	97.00	a2	182.10	a1	1100.00	a3
L9	58.18	a3	17.11	a2	43.67	a2	57.67	a3	101.67	a3	200.53	a1	600.00	a1
L10	66.48	a5	18.10	a3	39.67	a1	64.33	a3	104.00	a3	212.97	a2	1816.67	a5
L11	57.25	a3	14.17	a1	43.00	a2	58.33	a3	101.33	a3	209.27	a2	1100.00	a3
L12	54.34	a3	13.98	a1	40.00	a1	57.00	a3	97.00	a2	198.80	a1	1158.33	a3
L13	57.40	a3	14.30	a1	37.33	a1	63.33	a3	99.67	a3	189.83	a1	1058.33	a3
L14	63.00	a4	19.26	a3	38.67	a1	63.67	a3	110.00	a4	172.60	a1	1583.33	a4
L15	45.45	a1	15.65	a1	42.00	a2	54.67	a2	96.67	a2	190.30	a1	1175.00	a3
L16	63.26	a4	16.51	a2	41.67	a2	60.00	a3	103.33	a3	203.87	a1	1225.00	a3
L17	59.15	a3	15.33	a1	39.00	a1	54.33	a2	91.67	a1	189.93	a1	1241.67	a3
L18	54.77	a3	16.81	a2	38.67	a1	54.00	a2	92.67	a1	215.80	a2	941.67	a2
L19	52.30	a2	14.55	a1	43.67	a2	51.67	a2	95.33	a2	201.47	a1	866.67	a2
L20	58.44	a3	16.71	a2	37.00	a1	61.00	a3	98.00	a2	197.67	a1	867.33	a2
L21	62.16	a4	15.33	a1	43.67	a2	55.33	a2	99.00	a3	187.43	a1	566.67	a1
L22	51.05	a2	14.52	a1	38.33	a1	61.33	a3	99.67	a3	139.13	a3	625.00	a1
L23	57.98	a3	19.12	a3	40.00	a1	51.00	a2	91.00	a1	222.63	a2	475.00	a1
L24	58.97	a3	17.92	a3	41.00	a2	49.67	a2	90.67	a1	212.77	a2	1341.67	a3
L25	55.60	a3	15.14	a1	41.67	a2	50.67	a2	92.33	a1	217.43	a2	525.00	a1
L26	63.27	a4	19.96	a3	40.67	a2	54.33	a2	95.00	a2	194.63	a1	1158.33	a3
L27	68.25	a5	16.78	a2	42.67	a2	48.00	a2	99.00	a3	216.60	a2	1591.67	a4
L28	66.60	a5	20.44	a3	41.00	a2	59.67	a3	104.33	a3	202.83	a1	650.00	a1
Mean	58.24		16.56		40.58		55.93		97.81		205.30		1043.77	

Note: Means followed by same number in column do not differ by Scott-Knott test at 5% probability.

The characterization of the 28 landraces is still in progress and, in the near future, traits related to insect and disease resistance will be evaluated in order to provide researchers with more information about these accessions.

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EFFECT OF AZADIRACHTIN ON REPELLENCY AND MORTALITY OF *Helicoverpa armigera* (HÜBNER) LARVAE ON *Phaseolus vulgaris* L.

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INTRODUCTION: *Helicoverpa armigera* can occur in bean crops, causing damages in plants and considerable reductions in the production (Pawar, 2003). Azadirachtin is as bioactive compound extracted from neem plant *Azadirachta indica* A. Juss (Meliaceae), which has insecticidal action and also exerts antifeeding effect on larvae (Schmutterer, 1990). Thus, this study aimed to evaluate the antifeeding effect and the mortality of different concentrations of neem oil, commercial product with 1.2% azadirachtin, on *H. armigera* larvae.

MATERIALS AND METHODS: The experiment was carried out in the Laboratório de Resistência de Plantas a Insetos, of the Faculdade de Ciências Agrárias e Veterinárias – UNESP, Jaboticabal, São Paulo, Brazil. The experiments were conducted at 25 ± 2 ºC temperature, 70 ±10% relative humidity, and 12 hours photophase. Six different concentrations of the commercial product Azamax® (azadirachtin 1.2 EC) were evaluated: 0.1%, 0.2%, 0.3%, 0.4% and 0.5% and two treatments as control. Control treatments consisted of distilled water, and a physiological insecticide of the benzoylureas chemical group (lufenuron - Match®), at 1.5% concentration. Leaf discs were used, which were prepared from trifoliates of 30 day-old plants of common bean (cultivar Pérola) using a metallic puncher (2.5 cm diameter). These discs were immersed into the emulsions for one minute and next they were exposed in the environment for 30 minutes to dry, protected of light to avoid degradation of the product. After drying, the leaf discs were placed in Petri dishes (9.0 cm diameter and 1.5 cm height) lined with moistened filter paper. One third-instar larva was released in each Petri dish.

At 5, 10, 15 and 30 minutes and 1, 2, 6, 12 and 24 hours after the insects release attractiveness by the larvae was assessed towards the treatments, and at the end of the tests, the leaf area consumed (L.A.C.) was quantified using an electronic leaf area meter, model LI-COR 3100A®. Evaluations of larval mortality were performed at three, four and five days after the beginning of the experiment, and the insect weight (mg) was assessed after the end of the experiment. A two-day period of feeding was chosen in order to assure the product was consumed by all larvae. After this period, the larvae were transferred to leaves without any treatment.

The experiments consisted of seven treatments set in a completely randomized design, with 10 replications. Data obtained were transformed in $(x + 0.5)^{1/2}$ for normalization and submitted to the analysis of variance (ANOVA) by F test, and means were compared by Tukey's test ($P = 0.05$). Efficiency percentage (%E) was calculated by Abbott's formula (Abbott, 1925).

RESULTS AND DISCUSSION: Significant differences were observed at 15 and 30 minutes, and 1 hour after the insects release. The *H. armigera* larvae were more attractive to leaf discs treated with Match® at 15 and 30 minutes, not differing from the control at 30 minutes. After 1 hour, the control treatment was the most attractive. This may indicate repellency effect by

azadirachtin to the larvae. With respect to leaf area consumed, significant differences were not found among the bean leaf discs treated with the different doses of azadirachtin (Table 1).

Table 1. Number of *Helicoverpa armigera* larvae attracted at different times and leaf area consumed (L.A.C.) of *Phaseolus vulgaris* leaf discs immersed into doses of neem oil. Jaboticabal, SP, Brazil, 2014.

Treatments	Conc. p.c. (%)	5'	10'	15'	30'	1h	3h	6h	12h	24h	L.A.C. (cm^2)
Control	-	0.10	0.00	0.10b	0.40a	0.20a	0.10	0.40	0.40	0.50	1.59
Azamax	0.1	0.10	0.20	0.10b	0.00b	0.00b	0.00	0.00	0.50	0.60	0.75
Azamax	0.2	0.20	0.10	0.00b	0.00b	0.00b	0.10	0.10	0.40	0.10	0.49
Azamax	0.3	0.10	0.20	0.00b	0.00b	0.00b	0.10	0.20	0.50	0.40	0.66
Azamax	0.4	0.20	0.00	0.00b	0.00b	0.00b	0.00	0.30	0.60	0.40	0.67
Azamax	0.5	0.00	0.00	0.00b	0.10b	0.00b	0.10	0.30	0.60	0.50	1.05
Match	0.15	0.30	0.40	0.50a	0.40a	0.00b	0.20	0.40	0.60	0.50	1.38
F (Treatments)		0.75^{ns}	2.17^{ns}	4.88**	3.95**	2.25*	0.84^{ns}	1.25^{ns}	0.30^{ns}	1.04^{ns}	1.66^{ns}
C.V.(%)		23.62	21.49	17.82	20.13	11.43	18.17	26.56	27.63	27.73	29.78

Table 2. Average number of dead larvae (N), and efficiency percentage (%E) on the control of *Helicoverpa armigera* at three, four and five days after treatment on bean leaf discs and weight of larvae (W). Jaboticabal, SP, Brazil, 2014.

Treatments	Conc.	3		4		5		$W^{1(n)}$
	c.p. %	N	E%	N	E%	N	E%	(mg)
Control	-	0.00	-	0.10b	-	0.10b	-	173.0a $^{(09)}$
Azamax	0.1	0.10	10.00	0.30ab	22.22	0.50ab	44.44	80.5ab $^{(05)}$
Azamax	0.2	0.20	20.00	0.30ab	28.57	0.40ab	33.33	78.4ab $^{(06)}$
Azamax	0.3	0.30	30.00	0.70ab	66.67	0.70ab	66.67	23.7b $^{(03)}$
Azamax	0.4	0.10	10.00	0.20ab	11.11	0.20b	11.11	22.9b $^{(08)}$
Azamax	0.5	0.20	20.00	0.70ab	66.67	0.80a	77.78	$^{-2}$
Match	0.15	0.30	30.00	0.80a	77.78	0.80a	77.78	$^{-2}$
F (Treatments)		0.85^{ns}		4.01**		3.97**		5.37**
C.V.(%)		24.86		24.62		24.06		6.05

[1]Means followed by the same letter in the column do not differ significantly by Tukey's test at 5% probability. [n]Number of larvae analyzed. [2]Insuficient number of larvae for statistical analysis (For analysis, data were transformed to $(x+0.5)^{1/2}$.

At four days after treatment with azadirachtin, all treatments differed significantly from the control (water). At five days after the exposure of the larvae to the treatments, Azamax® at 0.5% concentration and the chemical lufenuron provided the highest mortality. Regardless of the azadirachtin concentrations, there were deleterious effects on the insects, interfering on the insect weight gain. Under the conditions in which this work was performed, Azamax® showed satisfactory insecticidal effect on *H. armigera* larvae, and this efficiency was similar to the chemical (Table 2).

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FEEDING NON-PREFERENCE OF *Helicoverpa armigera* (HÜBNER) (LEPIDOPTERA: NOCTUIDAE) BY BEAN GENOTYPES

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INTRODUCTION

Helicoverpa armigera (Lepidoptera: Noctuidae) is a highly polyphagous pest, and due to its feeding behavior it seriously compromises yield of several crops with agronomical importance worldwide. *Helicoverpa armigera* was recently introduced in Brazil (Czepak et al., 2013) and has been causing losses up to 80% of the production in bean crops where it was found.

Cultivation of bean genotypes that posses any resistance category provides lower production costs, especially the reduction of pesticide use to control agricultural pests. The aim of this work was to evaluate the feeding non-preference of *H. armigera* larvae on bean genotypes in laboratory.

MATERIALS AND METHODS

The study was performed in the Laboratório de Resistência de Plantas a Insetos of FCAV/UNESP, Jaboticabal, SP, Brazil, under environmental controlled conditions. We tested 10 bean genotypes and evaluated the attractiveness and leaf consumption by *H. armigera* immatures in free-choice and no-choice tests, with 10 replicates. We used the following genotypes of common beans: Pérola, RAZ 49, BRS Supremo, IAC Tibatã, IAC Galante, IAC Diplomata, IAC Harmonia, IAPAR 81, IAC Una and IAC Carioca-Eté.

For the free-choice test, glass arenas (26 cm diameter and 5 cm height) were used, and the genotypes were distributed on filter paper moistened with distilled water and 10 larvae were released in each replicate. In the no-choice test, Petri dishes (9 cm diameter and 1.2 cm height) were used with one leaf disc (2.5 cm diameter) per replicate on filter paper moistened with distilled water. One larva of *H. armigera* was introduced into each Petri dish. Next, attractiveness was recorded at several times, and the leaf area consumed (cm^2) on each genotype after 24h of the release of the larvae in both tests.

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

RESULTS AND DISCUSSION

Means of attractiveness of the *H. armigera* immatures in the feeding preference assays were significant only at the first three times of evaluation in the free-choice test (Table 1). The genotypes RAZ 49 and IAC Galante were the most attractive to the larvae, while IAC Diplomata, IAC Tybatã, and BRS Supremo were the least attractive. Concerning the leaf area consumed, there were significant differences in both free-choice (Table 1) and no-choice

(Table 2) tests. Genotypes BRS Supremo, IAC Carioca-Eté and IAC Tybatã were the most consumed in the former test, while Pérola was the most consumed in the latter test. IAC Harmonia was the least consumed genotype in the free-choice (0.20 cm^2) and no-choice (0.24 cm^2) tests.

In summary, we concluded that genotype IAC Harmonia was the least consumed by *H. armigera*, and the other genotypes were susceptible to the insect.

Table 1. Number of *Helicoverpa armigera* larvae attracted by bean genotypes at different minutes and leaf area consumed (LAC), in free-choice test. Jaboticabal, SP, Brazil, 2013.

Genotypes	1'	5'	10'	15'	30'	60'	180'	360'	720'	1440'	LAC (cm^2)
Pérola	0.4ab	0.2ab	0.3ab	0.3	0.4	0.2	0.2	0.7	0.8	0.5	1.39ab
RAZ 49	0.8b	0.2ab	0.1ab	0.1	0.1	0.2	0.2	0.5	0.3	0.4	0.84ab
BRS Supremo	0.3ab	0.2ab	0.0a	0.1	0.0	0.0	0.2	0.6	0.6	0.5	1.80b
IAC Tibatã	0.3ab	0.0a	0.1ab	0.1	0.0	0.2	0.1	0.7	1.0	0.4	1.66b
IAC Galante	0.5ab	0.8b	0.6b	0.6	0.4	0.0	0.5	0.1	0.4	0.4	0.49ab
IAC Diplomata	0.0a	0.1ab	0.3ab	0.1	0.2	0.0	0.0	0.3	0.5	0.4	0.73ab
IAC Harmonia	0.5ab	0.3ab	0.3ab	0.3	0.3	0.2	0.3	0.2	0.2	0.1	0.20a
IAPAR 81	0.3ab	0.2ab	0.1ab	0.1	0.2	0.2	0.1	0.3	0.2	0.4	0.37ab
IAC Una	0.4ab	0.1ab	0.1ab	0.2	0.1	0.1	0.2	0.5	0.5	0.6	1.41ab
IAC Carioca-Eté	0.2ab	0.3ab	0.5ab	0.2	0.3	0.3	0.3	0.7	0.7	0.4	1.80b
F (Genotypes)	1.63^{NS}	2.00*	2.41*	1.21^{NS}	1.13^{NS}	0.61^{NS}	0.79^{NS}	1.38^{NS}	1.64^{NS}	0.48^{NS}	3.74**
C.V.(%)	29.16	27.35	25.47	27.31	27.13	25.49	27.84	31.34	30.17	31.88	31.86

Table 2. Number of *Helicoverpa armigera* larvae attracted by bean genotypes at different minutes and leaf area consumed (LAC), in no-choice test. Jaboticabal, SP, Brazil, 2013.

Genotypes	1'	5'	10'	15'	30'	60'	180'	360'	720'	LAC (cm^2)
Pérola	0.0	0.1	0.1	0.1	0.6	0.6	0.2	0.4	0.6	1.65b
RAZ 49	0.1	0.1	0.2	0.1	0.1	0.1	0.4	0.4	0.6	0.86ab
BRS Supremo	0.2	0.3	0.2	0.2	0.4	0.3	0.7	0.7	0.5	1.22ab
IAC Tibatã	0.0	0.0	0.0	0.0	0.3	0.3	0.6	0.5	0.6	1.13ab
IAC Galante	0.0	0.1	0.0	0.0	0.5	0.5	0.7	0.8	0.8	1.44ab
IAC Diplomata	0.2	0.2	0.3	0.2	0.3	0.2	0.5	0.6	0.8	1.10ab
IAC Harmonia	0.2	0.2	0.1	0.1	0.5	0.5	0.4	0.2	0.3	0.24a
IAPAR 81	0.1	0.1	0.1	0.1	0.4	0.3	0.5	0.6	0.7	0.52ab
IAC Una	0.0	0.1	0.2	0.2	0.3	0.3	0.5	0.5	0.7	0.96ab
IAC Carioca-Eté	0.2	0.2	0.3	0.3	0.3	0.3	0.5	0.5	0.6	0.98ab
F (Genotypes)	0.97^{NS}	0.56^{NS}	0.90^{NS}	0.77^{NS}	0.84^{NS}	1.00^{NS}	0.87^{NS}	1.14^{NS}	0.91^{NS}	2.09*
C.V.(%)	20.59	23.63	23.78	22.83	28.15	27.91	27.09	26.45	24.67	30.80

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FEEDING NON-PREFERENCE OF *Heliothis virescens* (FABRICIUS, 1781) (LEPIDOPTERA: NOCTUIDAE) TO BEAN PODS

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INTRODUCTION

Common bean, *Phaseolus vulgaris* L., can be attacked throughout its cycle by several insect pests. Among them, *Heliothis virescens* (Fabricius, 1781) (Lepidoptera: Noctuidae) stands out by attacking many crops (FITT, 1989) and by causing significant reductions in productivity, especially at the reproductive stages of the crop, when the insect can attack leaves, flowers and pods. In addition, larvae of *H. virescens* can also entail indirect damage by the entry of pathogens into the feeding holes of the pods (SILVA, 2013).

An alternative to control the pest is the use of resistant varieties, which has as advantages the reduction of populations of insect pests, decrease of the cost of production and risks of imbalances in the agro-ecosystem, persistence under low pest populations, and the possibility of use with other control tactics harmoniously (BOIÇA JÚNIOR, et al., 2013). Thus, the aim of this work was to evaluate the feeding non-preference of *H. virescens* by pods of bean genotypes.

MATERIAL AND METHODS

The experiment was conducted in the Laboratório de Resistencia de Plantas a Insetos, Departamento de Fitossanidade, UNESP/FCAV, Jaboticabal, SP, Brazil, in an acclimatized room at 25 ± 1 °C temperature, 70 ± 10% relative humidity and 12 hours photophase. Free-choice and no-choice test were performed, using a completely randomized design with 10 treatments for the first test and eight treatments in the second test, both with 10 repetitions.

In the free-choice test, the bean pods were placed equidistantly from the center in circular glass arenas (23 cm diameter and 5 cm height) lined with moistened filter paper, where one third-instar larva of *H. virescens* was released per each genotype tested. For the no-choice test, the pods were placed individually in Petri dishes (9 cm diameter) lined with moistened filter paper, and one larva was released per plate. In both tests, attractiveness of the larvae towards the genotypes was recorded at 3, 10 and 30 minutes, and 2, 6, 12 and 24 hours after the release of the larvae. At the end of the experiment, the larvae were removed from the plates and the pods were cut lengthwise for the observation of injuries caused by the larvae. According to the injuries, scores were assigned by three assessors, ranging from 1 to 5: score 1 = pod without injury; 2 = pod scraped; 3 = pod with a hole without penetration of the larvae; 4 = pod with two holes without penetration of the larvae; and 5 = pod with penetration of the larvae. Also, the injury percentage was estimated ranging from 0 to 100%, for uninjured pods and pods fully injured, respectively.

Data recorded from larvae attractiveness and injury scores on the pods were transformed to $(x + 0.5)^{1/2}$ and data of the injury percentage was transformed to arcsine $(x/100)^{1/2}$. All data were subjected to variance by F test, and when significant means were compared by Tukey test at 5% probability.

RESULTS AND DISCUSSION

In the free-choice test, the number of larvae attracted differed significantly among the genotypes at 3 minutes and 12 hours (Table 1). In the first evaluation, it was not observed the presence of larvae on the genotypes BRS Supremo and IAPAR 81, which differed from the genotype Raz 49. At 12 hours, it was not observed the presence of larvae on the genotype IAPAR 81, which differed from the genotype IAC Carioca Eté, which had the highest number of larvae attracted. There were no significant differences in the injury scores and injury percentage.

In the no-choice test (Table 1), a significant difference occurred only at 30 minutes. Lowest number of larvae was found on the genotypes Pérola, BRS Supremo and IAPAR 81, in relation the genotype IAC Carioca Eté. There was no significant difference in the injury scores and injury percentage. We concluded that the genotypes tested did not exhibit resistance of the category feeding non-preference against *H. virescens*.

Table 1. Number of third-instar larvae of *Heliothis virescens* attracted, injury and injury percentage caused in free-choice and no-choice test, Jaboticabal, SP, Brazil, 2013.

Genotypes	Number of larvae attracted[1]							Injury scores[1]	Injury (%)[2]
	Minutes			Hours					
	3	10	30	2	6	12	24		
Free-choice test									
Pérola	0.1 ab	0.1	0.1	0.4	0.2	0.2 ab	0.2	2.1	13.5
Raz 49	0.6 b	0.1	0.1	0.0	0.1	0.5 ab	0.5	1.8	13.8
BRS Supremo	0.0 a	0.1	0.2	0.3	0.4	0.3 ab	0.3	1.7	8.5
IAC Carioca Tybatã	0.1 ab	0.4	0.1	0.4	0.4	0.4 ab	0.4	2.0	16.5
IAC Galante	0.2 ab	0.0	0.2	0.3	0.5	1.0 ab	1.3	3.6	21.0
IAC Diplomata	0.3 ab	0.2	0.2	0.3	0.4	0.7 ab	0.8	2.4	11.5
IAC Harmonia	0.4 ab	0.4	0.3	0.1	0.5	0.5 ab	0.5	2.5	15.5
IAPAR 81	0.0 a	0.0	0.1	0.0	0.0	0.0 a	0.3	1.8	12.0
IAC Una	0.2 ab	0.1	0.0	0.3	0.3	0.6 ab	0.4	2.5	20.1
IAC Carioca Eté	0.1 ab	0.2	0.2	0.3	0.9	1.4 b	1.0	3.9	33.0
F (Genotypes)	2.26*	1.48^{ns}	0.50^{ns}	1.25^{ns}	1.68^{ns}	2.20*	1.70^{ns}	2.26^{ns}	0.99^{ns}
CV (%)	25.39	24.32	24.62	23.75	33.48	38.35	38.28	27.72	130.03
No-choice test									
Pérola	0.2	0.3	0.1 a	0.7	0.8	0.9	0.8	2.9	11.6
BRS Supremo	0.2	0.3	0.3 a	0.6	1.0	1.0	1.0	4.0	18.0
IAC Carioca Tybatã	0.4	0.4	0.4 ab	0.5	0.7	0.7	0.9	2.9	19.5
IAC Galante	0.5	0.6	0.7 ab	1.0	1.0	1.0	1.0	4.0	18.0
IAC Diplomata	0.3	0.2	0.4 ab	0.5	0.5	0.7	0.8	2.6	10.0
IAPAR 81	0.2	0.4	0.3 a	0.6	0.6	0.8	0.9	3.5	16.7
IAC Una	0.3	0.4	0.6 ab	0.7	0.7	0.7	0.9	3.5	32.70
IAC Carioca Eté	0.8	0.6	1.0 b	1.0	1.0	0.9	0.9	3.5	18.5
F (Genotypes)	2.09^{ns}	0.78^{ns}	3.78**	1.98^{ns}	2.56^{ns}	1.27^{ns}	0.59^{ns}	1.61^{ns}	1.58^{ns}
CV (%)	26.17	28.63	24.86	21.73	17.97	16.61	13.73	17.39	58.89

Means followed by the same letter in the column did not differ significantly by Tukey test at 5% probability. [1]For analysis, data were transformed in $(x + 0.5)^{1/2}$. [2]Data transformed in arcsine $(x/100)^{1/2}$. ^{ns}Non-significant, *Significant at 5% and **Significant at 1%.

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COMPATIBILITY BETWEEN THE ENTOMOPATHOGENIC FUNGI *METARHIZIUM ANISOPLIAE* AND PESTICIDES USED IN BEAN CROP (*PHASEOLUS VULGARIS* L.) TO CONTROL *BEMISIA TABACI* BIOTYPE B (HEMIPTERA: ALEYRODIDAE)

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INTRODUCTION

Bemisia tabaci Gennadius (Hemiptera: Aleyrodidae) (biotype B) is an important pest of tropical and subtropical regions (Ahmad et al., 2002; Nauen; Deholm, 2005). Highly toxic pesticides are used to control this pest in bean crop (*Phaseolus vulgaris* L.), which results selection pressure, in many cases of resistance, and environmental contamination (Ahmad et al., 2002; Silva et al., 2009). Thus, the combined use of the chemical method with entomopathogenic fungi can minimize the pesticide impact on the environment (Alves et al., 1997). Among the biological control agents, *Metarhizium anisopliae* (Metsch.) Sorok. is considered a promising species to reduce whitefly populations (Schlick-Souza, 2011). The objective of this research was analyze the compatibility between the entomopathogenic fungi *M. anisopliae* and pesticides recommended to control *B. tabaci* biotype B in bean crop.

MATERIAL AND METHODS

The recommended insecticides for *B. tabaci* biotype B in bean crop used in this research were Actara® 250WG (i.a. thiamethoxam) (100g $*$ $100L^{-1}$), Karate Zeon® 50 CS (i.a. lambda-cyalothrin) (240mL $*$ $100L^{-1}$), and Oberon® (i.a. spiromesifen) (250mL $*$ $100L^{-1}$). The toxic effect of these insecticides on *M. anisopliae* (Metarril® PM) was assayed in vitro adding these chemicals in PDA (Potato-Dextrose-Agar) culture medium in Petri dishes of 5-cm-diameter. The entomopathogen was inoculated in the respective substrates (culture medium + active ingredient), at $1.0 * 10^8$ viable conidia $*$ mL^{-1}, with an amount of 5.0 µL of the entomopathogen solution distributed in the center of each dish, totaling 8 replicates/treatment.

The colony growth (cm^2) was measured after fifteen days, using a leaf scanner (CID Bio-Science, Camas, Washington, USA, CI-202 model). The conidia counting were performed by Neubauer chamber in optical microscope. The mean size of the colonies and the number of conidia for each treatment were compared by Tukey test ($P < 0.05$). The data were standardized by the compatibility rate developed by Alves et al. (1998).

RESULTS AND DISCUSSION

Vegetative growth of *M. anisopliae* was negatively affected when in contact with lambda-cyalothrin ($F = 19.61$; df = 3, 28; $P < 0.001$). However, vegetative growth of *M. anisopliae* was not affected by thiamethoxam (Table 1). The number of conidia of the entomopathogenic fungus *M. anisopliae* was significantly affected when in contact with the active ingredients ($F = 38.97$; df = 3, 28; $P < 0.001$). The mean number of conidia was lower in thiamethoxam treatment when compared with the control (Table 1).

Table 1. Vegetative growth and number of viable conidia/mL (mean ± SE) of *Metarhizium anisopliae* on solid culture medium with active ingredients of pesticides, and toxicological classification of these chemical products on the entomopathogenic fungi.

Treatment	Vegetative Growth (cm^2)	Number of Conidia/mL (10^8)	*T	** Toxicological Classification
Control	39.93 ± 1.56 a	1.00 ±0.051 a	-	-
Thiamethoxam	35.73 ± 1.15 a	0.08 ± 0.003 c	24.30	*HT*
lambda-cyalothrin	22.80 ± 3.10 b	0.53 ± 0.090 b	53.82	*MoT*
Spiromesifen	24.15 ± 1.12 b	0.46 ± 0.064 b	48.90	*MoT*

*T = correlation between the values of vegetative growth and conidia production, according Alves et al., 1998.
**C = compatible; *MoT* = moderately toxic; T = toxic; *HT* = high toxic (Alves et al., 1998).

The active ingredient thiamethoxam was classified as high toxicity when in contact with *M. anisopliae*. However, lambda-cyalothrin and spiromesifen were moderately toxic to this entomopathogen (Table 1). The positive correlation between lambda-cyalothrin and spiromesifen with *M. anisopliae* suggests that the combined use of these two strategies constitutes an important tool in the integrated management of *B. tabaci* biotype B in bean crop, due to maximum exposure of the microorganism to the active ingredient that confirms the pesticide selectivity to the fungi (Alves et al., 2001).

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OVIPOSITION NON-PREFERENCE OF *Bemisia tabaci* BIOTYPE B ON BEAN GENOTYPES

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INTRODUCTION

The whitefly *Bemisia tabaci* (Genn., 1889) biotype B (Hemiptera: Aleyrodidae) is one of the major pests of common bean. Amongst the main control methods adopted to its management, plants with resistant-related traits can constitute an important tool against the pest, with the advantages to reduce the pest population below economic injury levels, not overtax production costs, in addition to be compatible with other pest management tactics (BOIÇA JÚNIOR et al., 2012).

Thus, the aim of this research was to evaluate the oviposition non-preference of *B. tabaci* on common bean genotypes in no-choice tests.

MATERIALS AND METHODS

The study was conducted in an experimental area of the Departamento de Fitossanidade of Faculdade de Ciências Agrárias e Veterinárias – FCAV/UNESP, located in Jaboticabal, SP, Brazil. Ten bean genotypes were used: Pérola, BRS Horizonte, BRS Supremo, Raz 49, IAC Galante, IAC Diplomata, IAC Harmonia, IAPAR 81, IAC Una and IPR Eldorado.

In order to evaluate the oviposition non-preference, a no-choice test was set up in which each individual cultivar was individualized in a cylindrical metal cage (60 cm height x 40 cm diameter), coated with *voile* fabric, and 100 non-sexed adults of the whitefly were released per cage, with four replications.

The adults were removed 48 hours after the release and the number of eggs on the abaxial face of all the leaves were recorded using a stereoscope. Leaf area was measured using an electronic area meter LI-COR® model 3100, for calculate the number of eggs per cm^2. Data of the mean number of eggs, nymphs and adults of *B. tabaci* biotype B were transformed in $(x + 0.5)^{1/2}$ for normalization, submitted to the analysis of variance by F test and means were compared by Tukey's test, at 5% probability.

RESULTS AND DISCUSSION

Significant differences were observed in the number of eggs of *B. tabaci* biotype B on bean cultivars studied. The genotypes IPR Eldorado, BRS Horizonte, IAC Diplomata, Pérola, IAC Harmonia and IAC Una had fewer eggs (0.79, 0.9, 1.08, 1.12, 1.17 and 1.35), differing from Raz 49, which had higher oviposition (3.54 eggs per cm^2) (Table 1).

Similar results were found by SILVA (2012), who found the lowest oviposition on the cultivars IPR Eldorado, IAC Una, IAC Harmonia and Pérola in infestation assays with *B. tabaci* in bean cultivars Jesus et al. (2009) observed lower oviposition on IAC Harmonia and Pérola in infestation tests with the whitefly.

Table 1. Mean number (± SE) of eggs per cm^2 of *Bemisia tabaci* biotype B obtained on the abaxial face of the leaves of *Phaseolus vulgaris* genotypes in no-choice test. Jaboticabal, SP, Brazil, 2014.

Genotypes	Number of eggs per cm^2
IPR Eldorado	0.79 ± 0.05 a
BRS Horizonte	0.90 ± 0.04 a
IAC Diplomata	1.08 ± 0.22 a
Pérola	1.12 ± 0.08 a
IAC Harmonia	1.17 ± 0.22 a
IAC Una	1.35 ± 0.20 a
IAPAR 81	1.47 ± 0.06 ab
BRS Supremo	2.23 ± 0.13 bc
IAC Galante	2.92 ± 0.34 cd
Raz 49	3.54 ± 0.13 d
F(Genotypes)	24.76^{***}
P-value	0.0001

Means followed by same letter in column do not differ significantly by Tukey's test at 5% probability. For statistical analysis, data were transformed in $(x + 0.5)^{1/2}$. (± SE) standard error of the mean.

It is possible to conclude that the bean genotypes less oviposited by *B. tabaci* biotype B in the no-choice test were IPR Eldorado, BRS Horizonte, IAC Diplomata, Pérola, IAC Harmonia and IAC Una.

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PHENOTYING FOR ROOT WHORLS AND BASAL LATERAL ROOTS IN OPAQUE BLACK COMMON BEAN GENOTYPES IN TROPICAL HUMID MEXICO

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INTRODUCTION: Opaque black common beans are grown under two agricultural conditions, rainfed and/or residual moisture in tropical humid areas in southeastern Mexico. Periods of lack of soil humidity, particularly towards the end of the growing cycle, could coincide with the grain filling stage of development and a resulting significant seed yield reduction. In common bean, drought tolerance has been associated with depth of rooting, and root architecture is an important factor for the acquisition of underground resources (Lynch, 1995). Basal roots developed from 2 to 4 definable whorls at the root-shoot interface, in conjunction with the lateral roots that emerge from them, basal roots usually comprise the majority of total root length (Liao *et al*., 2001). The objective of this study was to assess the variability of basal lateral roots of a group of tropical drought tolerant black-seeded improved recombinant breeding lines.

MATERIALS AND METHODS: Thirteen opaque black common bean genotypes, with different origins, were used in this study. Six of them are from the CIAT Bean Program (SEQ-344-21, NCB-229, CIAT 103-25, SEN-59, SEN-70 and SCN-2), one from the University of Puerto Rico (X02-33-153), four breeding lines (ELS-9-27, NGO-17-99, NGO-07022, Jamapa plus) from the INIFAP National Bean Breeding Program and two commercial cultivars (Negro Tacana and Negro Jamapa), also from INIFAP, which were used as checks. Twenty seeds (one replication consisted of five seeds) of each genotype were surface sterilized with 5% bleach solution for 1 minute, then seeds were rinsed for 1 minute with water. Seeds were placed (micropyle side downward) on paper towels, which was rolled up and placed in a beaker. The beaker was filled with 6 centimeters of 0.5 mM calcium sulfate ($CaSO_4$) solution. Aluminum foil was placed over the beaker and paper rolls to prevent desiccation and contamination. Beakers were placed in a germination chamber (at 28^{o}C) for three to four days. Number of root whorls (NRW) and lateral basal roots (NBLR) were counted in seedlings 3 to 4-days after germination had begun. Data was statistically analyzed, the ANOVA was calculated as a CRD design with four replicates.

RESULTS AND DISCUSSION: There were highly significant differences among genotypes for both root variables, NRW and NBLR. The highest number of root whorls was 2.10 observed with genotypes SEQ-344-21, NCB-229, and X02-33-153, which were similar to another group of six common bean cultivars (LSD = 0.15 at 0.05% probability), but different from the rest of the genotypes (averaged 1.75 - 1.95 NRW). Genotypic variability was higher among common bean genotypes for NBLR, which fluctuated from 6.15 to 8.10. Genotypes SEQ-344-21, NCB-229 and the controls, Negro Jamapa and Negro Tacana, formed a group of cultivars that developed statistically similar number basal roots (from 7.85 to 8.10), but were different to the rest of genotypes (6.15 to 7.15 NBLR). Both root variables measured were correlated (r=0.81**) indicating that, as expected, the higher the number of number of whorls the higher the number of basal lateral roots. Moreover, seed size of the common bean cultivars evaluated varied from 20

to 27 g/100 seed weight. The correlation between seed size and root variables was low and not significant. It could indicate that seed size was not a factor in determining the assessed root architectural traits. There was no particular tendency for the root variables measured and the origin of the common bean genotypes. The genetic material from CIAT-Colombia, UPR-Puerto Rico and INIFAP-Mexico comprised a group of four genotypes with the highest number of whorls and the highest number of basal lateral roots (Table 1).

Table 1. Name, origin and plant characteristics including number of root whorls and basal lateral roots of 13 tropical black common bean genotypes grown in Cotaxtla, Veracruz, Mexico. 2013.

Genotype name	Origin	Flowering (days)	Maturity (days)	100 seed wt. (g)	# of whorls	# of basal lateral roots
SEQ-344-21	CIAT-Colombia	43	77	20	2.10*	8.10*
NCB-229	CIAT-Colombia	37	70	27	2.10*	7.45*
Negro Jamapa[&]	INIFAP-México	42	75	20	2.00*	7.40*
X02-33-153	UPR-Puerto Rico	41	73	20	2.10*	7.85*
Negro Tacaná[&]	INIFAP-México	42	75	22	2.00*	7.15
Jamapa Plus	INIFAP-México	43	75	20	2.05*	7.30
CIAT-103-25	CIAT-Colombia	43	75	22	2.05*	7.00
SEN-56	CIAT-Colombia	36	67	26	2.05*	7.00
NGO-07022	INIFAP-México	41	73	22	2.00*	6.90
NGO-17-99	INIFAP-México	42	75	23	1.90	6.80
SEN-70	CIAT-Colombia	36	67	24	1.95	6.50
SCN-2	CIAT-Colombia	36	69	27	1.95	6.20
ELS-9-27	INIFAP-México	43	75	20	1.75	6.15

[&], Control checks, improved common bean cultivars released for commercial use in tropical humid Mexico.
*, LSD (0.05) for # of whorls = 0.15 and for # of basal lateral roots = 0.71.

CONCLUSIONS: The assessed opaque black common bean genotypes varied for both architectural root traits measured, number of whorls and number of basal lateral roots. Seed size was not a factor in determining root architectural traits. There was no particular tendency for the root variables to be associated with the origin of the common bean genotypes.

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GENETIC DIVERGENCE IN SNAP BEAN BASED ON AGRONOMIC TRAITS

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INTRODUCTION: Snap and dry beans are crops that are taxonomically classified in the same botanical species, *Phaseolus vulgaris* L.. However, some important differences between these plants are related to plant management and their consumption. In dry bean, the final product is the grain, which is an important protein source for populations in less developed areas in the world. Snap beans are cultivated as a vegetable crop, and their pods, with a juicy mesocarp and reduced fibre content are the final products (Blair et al., 2010). In Brazil, snap beans is mainly cultivated by small farmers, who use a reduced number of cultivars, with restricted genetic variability and limited use of technology and inputs, resulting in low yield. The objective of this study was to estimate the genetic divergence in snap bean accessions based on agronomic descriptors.

MATERIAL AND METHODS: Evaluations were performed for 25 snap bean accessions of determinate habit from the vegetable germoplasm collection from Universidade Estadual de Londrina (UEL). The experiment was conducted in the experimental area of the UEL, in Londrina, PR, Brazil, in randomized block design with three replications. The experimental plots consisted of two 2.0 m rows, spaced 0.8 m between rows and 0.2 m between plants. Fertilization consisted of application of organic compost at a dose of 2 kg/m^2 of fresh matter. There was no need to control pests and disease. The following traits were studied: plant height (PH), mean pod length (PL) (cm), mean pod diameter (PD) (cm), number of pods per plant (NPP), pod weight (PW) (g) and yield (g planta^{-1}). Mahalanobis generalized distance was used to quantify the genetic divergence among accessions. The following methods were used: hierarchical clustering UPGMA (Unweighted Pair-Group Method using an Arithmetic Mean) and the canonical variables. The cophenetic correlation coefficient (CCC) was calculated to test the efficiency of UPGMA clustering. The statistical analyses were performed using the program R (www.r-project.org).

RESULTS AND DISCUSSION: The cluster analysis of accessions by the UPGMA method, based on Mahalonobis generalized distance as dissimilarity measure, had a CCC value of 0.82, demonstrating good reliability of the dendrogram. The analysis of the statistics of pseudo t^2 showed that the peak was reached with the formation of five groups (Figure 1). The group I was formed by HAB 8 accession, where has the largest NPP compared with other accessions. Group II also consisted of an accession, which it has the highest values for PH and PL. In relation, III, IV and V groups were formed for five, two and 16 accessions, respectively. The accessions that have the greatest yields were allocated to group V, except HAB 08 and HAB 09 accessions that were allocated in group I and II, respectively. The first two canonical variables explained 73.97% of the total variation. The results of grouping based on the canonical variables were similar to those of the UPGMA clustering technique, with a subdivision of the group V in three groups.

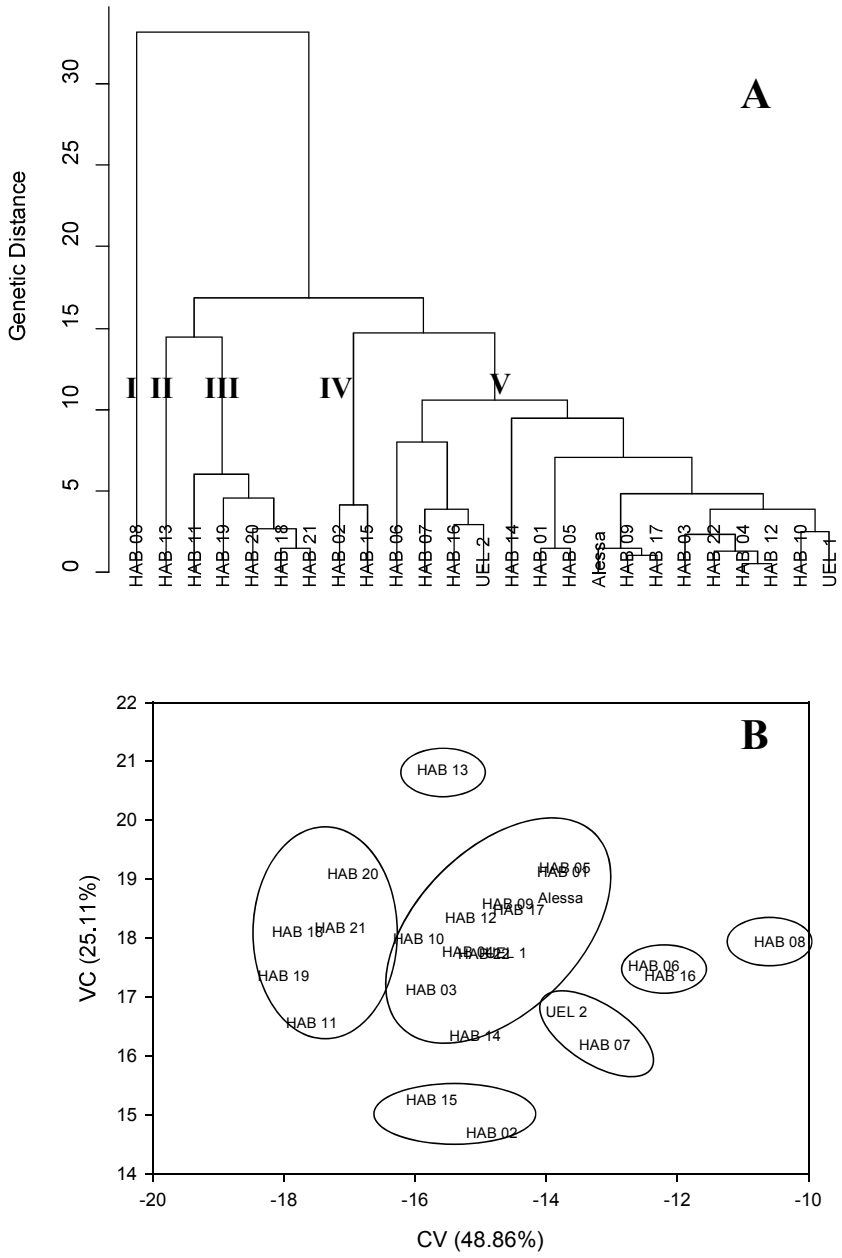

Figure 1. (**A**) Dendrogram by the UPGMA method based on genetic dissimilarity; and (**B**) graphic dispersion in relation to the two first canonical variables in 25 snap bean accessions.

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OXIDANT COMPOUNDS IN COMMON BEAN SEEDLINGS UNDER DROUGHT STRESS

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INTRODUCTION

The water deficit submitted to common bean frequently promotes stomatal closing, with consequent decrease in stomatal conductance and increase in diffusive resistance to water vapor and reduction in transpiration rate. This combination represents a problem in relation to gas exchange with repercussion on photosynthesis rate. Therefore, the stomata act as modulators of water loss (Nascimento et al., 2011). The aim of this research was to evaluate the electrolyte leakage and oxidant compounds in common bean seedlings submitted to water restriction.

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 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 two cultivars (IPR-Siriri 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 electrolyte leakage, glutathione and hydrogen peroxide, being measured in root tissue. An analysis of variance was performed, and when significant differences were present, a Scott-Knott test with a 5% level of error probability was used.

RESULTS AND DISCUSSION

In relation to electrolyte leakage, both cultivars presented increase when submitted to concentrations of -0.6 MPa PEG (Fig. 1 A), however the IAPAR 81 cultivar had higher sensibility to water deficiency. The electrolyte leakage presented a behavior to increase under limited water availability, suggesting the occurrence of cell damages (Martins-Júnior et al., 2008).

The water deficit occasioned higher values to glutathione under concentrations of -0.6 MPa PEG to both cultivars evaluated. The IAPAR 81 cultivar also presented higher sensibility, when compared with IPR-Siriri, to water limitation (Fig. 1 B). The increase in glutathione probably occurred due to lower activity linked to d*ehydroascorbate reductase* (DHAR), because this enzyme catalyzes the reaction of glutathione formation (GSH), consumption and consequently formation of *glutathione disulfide* (GSSG) (Gill and Tuteja, 2010). Similar results were showed by Chang et al., 2012 working with *Pluchea indica* submitted to water deficit.

The values of hydrogen peroxide were progressive with increase in concentrations of PEG, being the two cultivars sensitive to water deprivation, but the IAPAR 81 cultivar was more

affected (Fig. 1 C). The water deficit promoted an increase in values linked to hydrogen peroxyde, being this fact related to increase in respiration rate, that will produce reactive oxygen species, such as H_2O_2 (Carneiro et al., 2011).

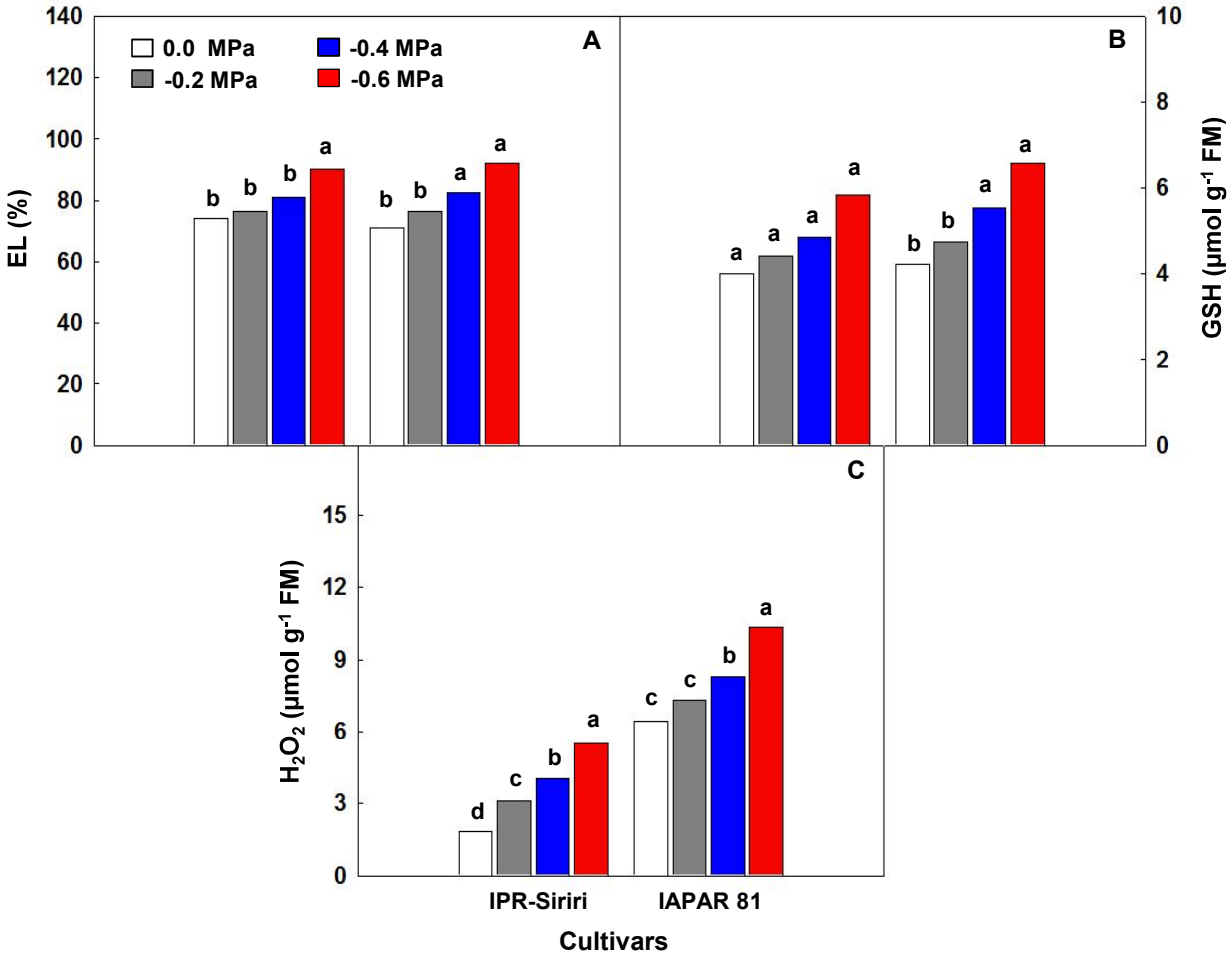

Fig. 1: Electrolyte leakage (A) glutathione (B) and hydrogen peroxide (C) of two common bean cultivars exposed to concentrations -0.6, -0.4, -0.2 and 0.0 (control) MPa of PEG 6000. Means followed by the same letter into each cultivar are not significantly different by the Scott-Knott test at 5% of probability.

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1-OXYPHENAZINE FROM *PSEUDOMONAS AUREOFACIENS* KMBU PHZ 127/11 BACTERIA INDUCES DROUGHT STRESS TOLERANCE OF BEAN (*PHASEOLUS VULGARIS*)

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INTRODUCTION

More than third part of earth territory are the arid regions (annual rainfall is lower than 250 mm). Half of this area (approximately 12% earth) is the extremely arid. Annual crop losses from drought are enormous because plants evaporate more water than precipitation falls during the growing season even in the temperate zone. Thus, it is very important to know the mechanisms of plants resistance to water deficit and ways to improve it.

It is well known that rhizospheric bacteria of PGPR-groups are capable to induce the systemic resistance in plants to different groups of different phytopathogenic microorganisms as well as environmental factors (Van Loon L.C., 1998; Whipps J.M., 2001). Inducers of systemic resistance may be siderophores, lipopolysaccharides, bacterial natural antibiotics, that is, compounds belonging to biogenic elicitors

In this paper, we describe the induction of resistance in common bean (*Phaseolus vulgaris L.*) to soil drought with antibiotic of phenazine series: 1-oxyphenazine, synthesized by rhizosphere bacteria *P. aureofaciens* KMBU phz 127/11.

MATERIALS AND METHODS

We have used the strain *P. aureofaciens* KMBU phz 127/11 resulted from chemical mutagenesis of strain *P. aureofaciens* KMBU phz 127 extracted from bed silt of Halong Bay (South China Sea, Vietnam) with further sampling for resistance to toxic analogue of metabolites of "aromatic pathway" – 6-diazo-5-oxo-L-nor-leucine. Mass-spectrophotometric analysis of the substance extracted from culture liquid of bacteria *P. aureofaciens* KMBU phz 127/11 revealed the presence of 1-oxyphenazine – $C_{12}H_7N_2OH$ (molecular weight 196 Da, maximum absorption corresponds to 260 and 387 nm). The level of 1-oxyphenazin synthesis using the strain under study was 180 mg/l.

Domestic breeding line 354-18-36/56 (candidate variety of French bean) have used in the experiment. This line is improved by green pods yield increasing, also by complex of resistance to Halo blight and BCMV. Water scarcity was simulated by creating soil drought by stopping of watering plants during the appearance of the first pair of trifoliate leaves. Experimental variants have been treated by a solution of 1-oxyphenazine (30 μg/ml) at 3 days prior to the termination of irrigation, the pure water was used at the control variants. Plants were grown in climatic camera at 25 ° C, 14 h of light and 10 h of darkness. The plants were evaluated by weight of seedlings, as well as on the content of photosynthetic pigments in bean leaves on the 30th day of cultivation.

RESULTS AND DISCUSSION

It was found that bean plants treatment by the solution of 1-oxyphenazine in drought conditions results leads to improved physiological status of the plant. The plant height is increasing at 30% and plant mass is increasing at 15% in comparison with the variant without treatment.

It is clearly demonstrated that the growth of plants under water deficit reduces the photosynthetic pigments of 55% compared with the control group (Table 1). At the same time the use of 1-oxyphenazine as resistance elicitor can increase the concentration of chlorophyll A, chlorophyll B and carotenoids in plant tissues beans at 20%.

Table 1. The content of pigments in the leaves of bean grown in drought conditions/

Pigment	Содержание пигментов, μg/g		
	Without watering	Without watering + 1-oxyphenazine	With watering (control)
Chlorophyll A	1,12±0,03	1,52±0,05	2,03±0,06
Chlorophyll B	0,97±0,02	1,40±0,04	1,79±0,05
Carotenoids	0,93±0,02	1,29±0,03	1,71±0,04
Sum of pigments	3,02	4,21	5,53

It is known that some bacteria, in particular, *Paenibacillus polymyxa*, *Bacillus subtilis* and *Pseudomonas chlororaphis* strain O6 cause induction of systemic plant as resistance to phytopathogens, so as tolerance to drought (Aroca R., 2012). However, the mechanisms of such a microbe-mediated resistance to drought are still not completely understood.

The photochemical reactions and reduction reactions of CO_2 are inhibited in plant cells during the water deficit. Limitation of the photosynthetic apparatus is activated by closing the stomata and reduction of carbon dioxide proceeds, as well as disruption of the structure of chloroplasts and chlorophyll synthesis, uncoupling of electron transport and phosphorylation. This leads to a shortage of ATP within the cell. This contributes to inhibition of synthetic processes in the plant as a whole in conjunction with the inhibition of respiration ETC.

We suggest the following mechanism of tolerance to drought using antibiotics phenazine series. Phenazine antibiotics induce the formation of reactive oxygen, which have extremely high reactivity. Phenazine mainly stimulates the formation of hydrogen peroxide in the extracellular space. Hydrogen peroxide stimulates deposition of the cell wall lignin plants, which are known to provide greater drought resistance.

Demonstrated induced drought resistance in beans has a practical value for use in the growing of beans in the areas of risk farming in drought.

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ABAXIAL AND ADAXIAL STOMATAL DENSITY, STOMATAL CHARACTERISTICS BETWEEN GENOTYPES OF BEANS (*Phaseolus vulgaris* L.)

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INTRODUCTION

The stomata are small openings on the leaf surface that regulate water loss through transpiration and CO_2 uptake during photosynthesis. Stomatal density may vary among leaves of the same species, in the different phenological growth stages and due to environmental factors (Al Afas et al., 2006).

Leaf anatomy plays an important role regarding crop production, being a tool that can assist in genetic improvement of beans, allowing the choice of cultivars with potential capacity for tolerance to several environmental conditions, since the environmental factors directly influence leaf anatomy (Bussis et al., 2011). Therefore, the present study was aimed to assess stomatal density in the abaxial and adaxial surfaces the leaves and document the dimensional characteristics of stomata in different genotypes of bean.

MATERIAL AND METHODS

The observation of the density and the distribution stomatal was performed using the replica method, where an impression of the leaf surface was obtained with cellulose acetate film adhesive tape over an area of approximately 1.0cm2. An optical microscope eyepiece lens 15x and objective lens 40x was used, which provided 600x magnification and a field of view of 0.39mm2. The images used for analysis were captured by a capture system composed of a microscope equipped with camera AxioCam ICc3 and Bel View software. Image processing and analysis was performed with public domain software Image J 1.43a, version 64, with the measurement of five fields replicated for each analyzed leaf. Was determined; number of epidermal cells; polar diameter of stomata; equatorial diameter of stomata. Calculations were made of: stomatal density (number of stomata per mm^{-2}); stomatal index (percentage of stomata in relation to total epidermal cells per area) and stomatal functionality (considered the polar/equatorial diameter ratio of the stomata). Analysis of Variance (ANOVA) was performed for statistical analysis of data, and Scott-Knott test, at 5% probability, was used for comparison of means.

RESULTS AND DISCUSSION

According to the analysis of variance, the stomatal density of the different genotypes of bean on the two surfaces of the leaf (abaxial and adaxial) showed significant effects. There were more stomata on the abaxial surface of the leaf than on the adaxial surface, and the difference was statistically significant, for all the genotypes studied (Table 1).

The highest stomatal index was observed for genotype “G9”, which is an important parameter because it is related to the plant response to the environment, and their higher values indicate greater adaptability to increased radiation intensity (Oliveira & Miglioranza, 2013). There were no differences between the means of the treatments for the other variables in both abaxial as the adaxial surface of the leaves of bean genotypes (Table 1).

Table 1. Stomatal density (mm^{-2}), adaxial/abaxial and stomatal indices in different genotypes beans.

Genotypes	Stomatal density (mm^{-2})		Stomatal index	
	Adaxial	Abaxial	Adaxial	Abaxial
G1	38.2 bB	172.6 gA	18.0 aA	18.1 bA
G2	37.4 bB	170.2 gA	18.2 aA	18.4 bA
G3	46.2 aB	205.3 eA	18.0 aA	18.1 bA
G4	40.5 bB	183.8 eA	18.2 aA	18.1 bA
G5	43.5 aB	187.5 eA	18.4 aA	18.8 bA
G6	40.7 bB	185.1 fA	18.6 aA	18.7 bA
G7	40.5 bB	180.5 dA	18.6 aA	18.6 bA
G8	45.4 aB	198.2 bA	18.3 aA	18.8 bA
G9	44.8 aB	213.1 bA	18.6 aB	22.4 aA
G11	38.6 bB	187.0 eA	18.1 aA	18.0 bA
G12	43.5 aB	185.5 eA	18.3 aA	18.2 bA
G13	46.1 aB	218.0 aA	18.4 aA	18.7 bA
G16	44.6 aB	209.5 bA	18.4 aA	18.3 bA
G19	45.7 aB	209.5 bA	18.3 aA	18.7 bA
G21	45.0 aB	180.5 fA	18.1 aA	18.4 bA
G23	40.3 bB	179.5 fA	18.0 aA	18.3 bA
G25	40.0 bB	181.5 fA	18.6 aA	18.7 bA
G30	45.0 aB	205.3 cA	18.3 aA	18.5 bA
G34	45.9 aB	204.1 cA	18.2 aA	18.4 bA
G38	43.7 aB	185.0 eA	18.4 aA	18.5 bA
G39	45.0 aB	184.6 eA	18.0 aA	17.9 bA
G40	44.6 aB	185.6 eA	18.0 aA	17.9 bA
UEL1	42.0 bB	209.5 bA	18.1 aA	18.0 bA
UEL2	44.5 aB	184.0 eA	18.0 aA	18.1 bA
ALESSA	42.5 aB	182.7 fA	18.4 aA	18.5 bA

Means followed by the same lower case in the column and upper case in the line do not differ statistically from one another according to Scott-Knott test ($p<0.05$).

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EFFECTS OF DIFFERENT IN-FIELD RAINWATER HARVESTING TECHNIQUES FOR COMMON BEAN SEED YIELD ON DURANGO, MÉXICO

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the most important cash-crops in the state of Durango and other states of the Highlands of North-Central, México. In this region, the rainwater is the most limiting input for crop production under rainfed conditions. Traditional crop agricultural techniques have been related to a significant water and soil losses. Efficient soil management and agriculture machinery adjustment are needed in order to increase in-field rainwater harvesting and ensuring common bean production under rainfed conditions. Contour furrow and tie ridging are considered useful techniques to harvest rainwater and to prevent soil erosion in dry areas (IIRR and ACT, 2005). In Durango and Chihuahua, significant increments for common bean seed yield have been obtained using tie ridging (Cuéllar, 2005). Contour furrow is a less used technique due to an increase in machinery and labor costs. The objective of this research was to evaluate the effects of different water and soil conservation techniques on common bean seed yield in three locations in Durango, México.

MATERIALS AND METHODS

In 2013, four rainwater harvesting techniques were evaluated: traditional method with straight furrow (SF), compared to contour furrow (CF), SF + tie ridging (TR) and CF + TR. Semi-commercial plots were sown in three locations (Durango, Francisco I. Madero and Canatlán), consisting in 2,500 m^2 for each technique. Early maturity 'Pinto Centauro' common bean cultivar (Rosales *et al*., 2012) was sown at delayed planting dates (late July and early August) due to late precipitation registered under rainfed conditions. Foliar fertilizer was sprayed at the rate of 2.5 liter/hectare during reproductive period. At maturity, five plant samples consisting in two furrows of 5 m in length by 0.81 m in width (8.1 m^2) were taken in each plot for yield determinations. Random seed samples were also obtained in each replication (field sample) in order to evaluate 100 seeds weight in a digital balance with an accuracy of 0.1 g. Analysis of variance was obtained under randomized complete block design combined over locations with five replications and mean comparison tests were performed by Tukey's honestly significant difference (HSD, $P \leq 0.05$).

RESULTS AND DISCUSSION

Highly significant ($P \leq 0.01$) differences for seed yield were observed among rainwater harvesting techniques across locations. On average, highest yield value was obtained for combinations of contour furrow plus tie ridging (1762.1 kg ha^{-1}) and straight furrow plus tie ridging (1493.0 kg ha^{-1}). Similar results were obtained in previous experiments testing different techniques for rainwater harvesting techniques (Cuéllar, 2005). Actual sowing method (straight furrow = SF) registered the lowest average yield across locations (1260.0 kg ha^{-1}). Yield

increments observed in SF + TR (18 %) and CF + TR (40 %), compared to traditional method (SF), justify government programs to encourage the adoption of combined in-field rainwater harvesting techniques in the state of Durango. Highest average seed yield was obtained in Canatlán (1881.0 kg ha^{-1}) and this response was related to the high amount of rainfall. On average, lowest seed yield was registered in Francisco I. Madero in despite of the higher seasonal amount of rainfall (318 mm), compared to Durango (283 mm). Results were related to soil type observed in Francisco I. Madero (low depth-sandy soils), suggesting that soil traits are relevant for an efficient selection of an appropriate in-field rainwater harvesting technique. Higher values for 100 seed weight was observed in Canatlán (32.4 g), showing that this treatment is responsive to water availability. In contrast, seed size reduction caused by intermittent water stress registered in Francisco I. Madero (31.5 g) is an undesirable trait in common bean production due a significant decrease for grain commercial value. Significant yield increments were obtained by contour furrow and tie ridging combination. Tie ridging combined with straight furrows was also identified as an important agricultural practice that increases seed yield under rainfed conditions. Incentive programs need to be implemented in order to encourage farmer's adoption of the in-field rainwater harvesting techniques. The adoption of techniques that increase the rainwater harvesting leads to a higher seed yield and natural resources conservation in Durango.

Table 1. Yield and seed size registered on common bean cultivar 'Pinto Centauro' grown in three locations in Durango, México (2013).

Land Management Technique	Seed Yield (kg ha^{-1})			Mean
	Durango	Francisco I. Madero	Canatlán	
Straight Furrow (SF)	1348.0^{c}	967.9^{d}	1464.2^{c}	1260.0^{d}
Contour Furrow (CF)	1363.0^{b}	1138.3^{b}	1328.3^{d}	1276.5^{c}
SF + Tie Ridging (TR)	1229.6^{d}	1281.5^{a}	1967.9^{b}	1493.0^{b}
CF + TR	1404.9^{a}	1116.0^{c}	2765.4^{a}	1762.1^{a}
Average[1]	1336.4^{b}	1125.9^{c}	1881.5^{a}	
Accumulated rain (mm)	283	318	331	
100 seed weight (g)	32.2	31.5	32.4	

$^{a-d}$ = Letters in each column indicate significant differences according to Tukey´s Honestly Significant Difference ($P \leq 0.05$). Average[1]: obtained for comparison between locations.

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CANOPY GROWTH, YIELD, WATER USE EFFICIENCY AND HEAT UNITS OF BEAN UNDER RAIN CONDITIONS IN TEMPERATE CLIMATE

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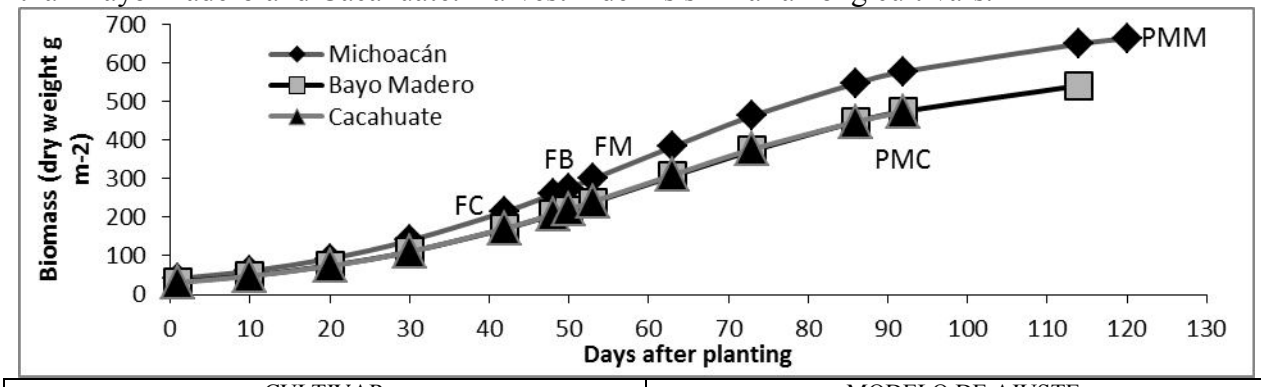
INTRODUCTION: In Mexico, 87% of area sown to bean (*Phaseolus vulgaris* l.) is during the season of rains (SIAP, 2010). Thus, the growth and yield of beans depends on the variability in the amount and distribution of rainfall and is reflected in a low yield. A strategy to achieve increases in yield under these conditions is that a greater proportion of rain water is used in transpiration in relation to the loss by evaporation of the soil (Escalante, 1995). For which required cultivars of rapid growth and large canopy appropriate for each region. So, the knowledge of the dynamics of dry matter (DM) or biomass (BT)**,** that can be explained through mathematical models (Escalante *et al*., 2012) it is important to achieve that increase. The objective of the study was to determine in cultivars of different habit of growth, planted in rainy season in temperate climate: a) phenology and heat units; (b) production of biomass, harvest index and grain yield; and (c) the water use efficiency.

MATERIALS AND METHODS: The study was conducted during the rainy season in Montecillo Méx., temperate climate. Treatments consisted of planting on June 16 of cultivars (CV) Cacahuate 72 (Cacahuate) of determinate type I growth habit, Michoacán 12-A-3 (Michoacán) and Bayo Madero (Bayo) of indeterminate growth habit type II shrub, with 100 kg of N and P ha^{-1}, density of 24 plants m^{-2}. The experimental design was randomized blocks with four replications. Four plants were sampled every 10 days to register total biomass (BT (dry matter, MS) g m^{-2}). In addition, the occurrence to emergency days, to flowering (F) and physiological maturity (PM), heat units (HU,^{o}Cd) using the residual method (Snyder, 1995) with temperature base 10^{o}C. To physiological maturity (PM), biomass (BT, dry matter, DM), the harvest index (HI), grain yield (at 10% of moisture, GY), the seasonal average of maximum and minimum temperature (^{o}C), the seasonal amount of rainfall (mm,PP) and in the reproductive stage. Water use efficiency (WUE, g m^{-2} mm^{-1}) was calculated based on the BT, GY and PP, (WUEBT and WUEGY, respectively) for the total cycle and reproductive stage were recorded. An analysis of variance and the Tukey multiple comparison test was applied. For the adjustment of the curves, Curve Expert 1.3 was used.

RESULTS AND DISCUSSION: The emergency for the CV was 8 days after planting (dap) for which the heat requirement was 68 ^{o}C d, the onset of flowering (F) for Cacahuate, Bayo Madero and Michoacán was 42, 50 and 53 dap, with 266, 317 and 335 ^{o}C d, respectively; and the PM 92, 116 and 122 dap with 581,710 and 746 ^{o}C d, respectively. The reproductive stage duration was higher in Michoacán, followed by Bayo Madero and Cacahuate. The sum of the total rainfall (374-380mm) and during the reproductive stage (140-219 mm) was different between cultivars. During the crop development, the average minimum and maximum temperature was 8.2 and 28.6 ^{o}C. The dynamics of BT followed a sigmoid curve [$Y = a / (1 + e^{-cx})$] and indicate differences in the size of the canopy between CV (Figure 1), that was reflected to PM in the BT and WUEBT (Table 1) . Thus, the highest BT and WUEBT corresponded to Michoacán, followed by Bayo

Madero and Cacahuate. HI (efficiency in distributing DM to the grain) was similar between CV. The highest GY and WUEGY were met with Michoacán and were followed by Bayo Madero and Cacahuate that presented the lower yield. These results indicate the importance of the canopy size of bean to achieve a greater water use efficiency, biomass and grain yield.

CONCLUSIONS: The dry matter dynamics in the bean cultivars fits a sigmoid model. Bean Michoacán exceeds in biomass and water use efficiency to Bayo Madero and Cacahuate. The cultivars show differences in the days to flowering and to physiological maturity, which was related to heat units. The CV Michoacán was the latest, followed by Bayo Madero and Cacahuate. The biomass, grain yield, grain yield water use efficiency of Michoacán is higher than Bayo Madero and Cacahuate. Harvest index is similar among cultivars.



CULTIVAR	MODELO DE AJUSTE
Michoacán	$Y= 696 / (1 + e^{-0.048 x})$
Bayo Madero	$Y= 588 / (1 + e^{-0.047x})$
Cacahuate	$Y= 573 / (1 + e^{-0.05 x})$

Figure 1. Dynamics of dry matter in bean cultivars. Montecillo Méx. Summer. FC, FB, FM, refers to the flowering of Cacahuate, Bayo Madero and Michoacán, respectively; PMC, PMB and PMM refers to physiological maturity of Cacahuate , Bayo Madero and Michoacán, respectively.

Table 1. Biomass (BT), harvest index (HI), grain yield (GY), water use efficiency (WUEGY) to physiological maturity (PM) in bean cultivars. Montecillo Méx. Summer.

CV	BT gm^{-2}	HI (%)	GY gm^{-2}	WUE (1) gm^{-2} mm^{-1}	WUE (2) gm^{-2} mm^{-1}
Michoacán	664 a	38 a	254 a	0.67 a	1.83 a
Cacahuate	474 c	39 a	186 c	0.50 b	0.91 b
Bayo Madero	542 b	39 a	210 b	0.57 b	1.11b
F prob.	*(40)	*(3)	**(23)	**(0.10)	**(0.20)

WUE(1) and WUE (2) refers to the water use efficiency calculated with the seasonal PP and during the reproductive period, respectively.

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PHENOTYPIC CHARACTERIZATION OF THE RUDÁ x AND 277 COMMON BEAN RIL POPULATION

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Populations composed by recombinant inbred lines (RIL) are very useful to develop genetic linkage maps. Keep the genetic structure of these populations over the generation advancement is important to develop accurate and reliable linkage maps. The existence of genetic variability within those populations is essential for detection of the significant association between molecular markers and loci controlling quantitative or qualitative traits. For this reason, the main objective of this work was to quantify the phenotypic diversity of the RIL population derived from crosses between Rudá and AND 277, a potential new core mapping population for common bean.

As described by Sanglard *et al.* (2013), F_2 plants derived from the cross Rudá x AND 277 were conducted under greenhouse condition up to the F_{10} generation using the single seed descent (SSD) method to obtain the RIL population. In this work, a group of 393 RIL's, the parents, and five commercial control cultivars were screened in the field, in a 20 x 20 triple lattice design, for seven quantitative traits. Because of the low efficiency of the lattice, the data were analyzed in randomized blocks with additional treatments (parents) with three replications. The genetic dissimilarity of the RIL's and parents was estimated by the Mahalanobis generalized distance (D^2). The Tocher agglomerative method was used to group the genotypes into clusters of dissimilarity.

The RIL effect was significant for all evaluated traits ($P < 0.01$), showing the existence of genetic variability in this population. The RIL's *vs* parents contrast was significant for the traits number of days to flowering (DF), days to harvest (DH), grain yield (YLD), and weight of 100 seeds (W100), but no significant for architecture of plants (ARC), seed flattening (H), and seed shape (J). The significance of the mentioned contrasts indicates that the phenotypic mean of RIL's differs from the mean of the parent cultivars. Coincidence between these means is expected only in the absence of epistasis. Thus, these results indicate the occurrence of additive x additive epistatic interactions for DF, DH, YLD and W100. Heritability values for the evaluated traits ranged from 82.81 to 97.09%. The 393 RIL's were grouped into 10 different groups based on the Tocher agglomerative method, using the Mahalanobis generalized distance indexes (Table 1). The traits that most contributed to the genetic dissimilarity were W100 and DF, while ARC was the less one. In geral, it was observed that the population formed by the 393 RIL's (Rudá x AND 277) presented genetic variability for all evaluated traits, what is essential for detecting associations between these traits and molecular markers in coming up efforts of genetic mapping and QTL analysis.

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Table 1. Clustering of 393 RIL's (Rudá x AND 277) and parent cultivars of common bean obtained by the Tocher agglomerative method based on the Mahalanobis generalized distance indexes.

Cluster	N[1]	Rudá x AND 277 RIL's (ID Code: UFV-RA)
I	329	34 106 223 354 94 78 147 57 97 344 301 5 83 329 115 289 7 173 2 287 376 359 4 357 196 185 215 163 193 213 22 145 246 8 88 89 132 39 253 197 25 272 384 232 347 342 91 60 211 381 84 76 201 337 310 130 353 139 131 269 41 318 181 280 160 43 221 36 126 268 12 50 70 283 53 28 149 27 205 202 326 317 325 52 349 373 291 166 46 65 87 251 62 167 298 188 273 81 15 379 74 44 1 189 276 146 24 371 48 80 104 29 255 161 294 154 285 346 172 47 334 295 218 42 388 13 119 231 300 77 314 240 361 293 250 236 331 93 10 111 284 351 116 308 31 303 368 73 151 239 237 123 142 169 216 281 33 96 207 262 171 254 174 103 316 156 319 49 124 138 90 19 82 137 26 122 358 121 217 134 21 92 292 32 263 370 17 369 153 341 264 219 233 11 377 150 30 203 6 279 85 71 14 305 313 112 177 9 100 374 117 247 378 392 206 58 278 304 228 311 56 327 40 186 257 212 155 222 296 282 178 190 141 101 140 175 309 307 258 364 133 393 290 363 210 176 37 338 199 345 183 227 336 238 105 383 328 302 180 179 113 120 61 324 195 200 184 356 45 389 267 157 67 18 3 64 252 72 107 209 164 274 129 391 271 330 367 386 16 321 339 192 109 375 312 230 229 198 158 352 118 382 385 286 98 55 244 372 260 59 136 54 220 306 235 226 99 38 102 51 148 182 159 362 320 214 256 315 355
II	16	95 162 143 108 245 288 114 20 266 333 350 204 69 135 225 35
III	7	66 265 348 323 380 168 187 Rudá
IV	2	297 387
V	31	152 194 366 275 241 208 125 332 75 248 335 249 299 234 242 340 261 224 170 343 322 365 390 128 68 191 79 243 86 165 259
VI	3	23 110 63
VII	2	144 270
VIII	1	127
IX	1	360
X	1	277
XI	-	AND 277

[1]Number of RIL's in each cluster.

BIOSTIMULANTS ON THE GROWTH AND YIELD OF WINTER COMMON BEAN

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INTRODUCTION

Biostimulants are natural or synthetic substances considered plant growth regulators and they can be applied directly on the plants (leaves, fruits, seeds), causing changes in vital and structural processes, in order to increase production, improve quality and facilitate harvest. The objective of this study was to evaluate the effect of commercial chemical upon the productive characteristics of winter common bean.

MATERIAL AND METHODS

The experiment was conducted at the Experimental Farm of Vale do Rio Verde University, Três Corações, Minas Gerais State, Brazil. The planting carried out in April 2013 manually, with 0,5 m between rows and 15 seeds per meter. The randomized complete block design was used, with six treatments and six replicates, in plots consisting of four rows ten feet long. Doses and forms of application of biostimulant Booster® ZnMo were tested on cultivar BRS Majestoso. The treatments were: T1 = Control (without application); T2 = 0.2 $L.ha^{-1}$ in the seed and two leaf sprays during the reproductive and grain filling stages, with 0.3 $L.ha^{-1}$ in each; T3 = 0.2 $L.ha^{-1}$ in the seed and three leaf sprays on vegetative, reproductive and grain filling stages, with 0.3 $L.ha^{-1}$ in each; T4 = three leaf sprays on vegetative, reproductive and grain filling stages with 0.3 $L.ha^{-1}$ in each; T5 = two leaf sprays in reproductive and grain filling stages with 0.3 $L.ha^{-1}$ in each; T6 = 0, 2 $L.ha^{-1}$ in the seed. Number of pods per plant (NPP), number of grains per pod (NGP), weight of 100 grains (WG), plant height (PH), height of the first pod insertion (HFPI) and yield (Y) were evaluated. The data were submitted to the analysis of variance adopting a significance level of 5% for the F test.

RESULTS AND DISCUSSION

The application of biostimulant Booster® ZnMo did not cause significant differences in any of the variables analyzed in this study (Table 1). These results agree with those obtained by Spring et. al. (2011) in which the application of Booster® ZnMo did not result either yield gains or yield components gains of common bean. These results also corroborate the data obtained by Cato (2006) and Ferreira et al. (2007) regarding the average yield of wheat and soybean, respectively, when the authors used the biostimulat Booster® ZnMo. According to a study of Abrantes et al. (2011), the application of growth regulators on vegetative stage of the bean crop did not affect characteristics such as height, height of the first pod insertion and weight of 100 grains. However, the application in the reproductive stage resulted in gains in the number of grains per plant and yield. In the present work, these gains were not observed. In this context, we conclude, for the conditions of this study, that the use of the growth promoter Booster® ZnMo does not

affect development and grain yield of common bean, regardless of the dose and form of application.

Table 1. Average values of PH (cm), NPP, HFPI (cm), NGP, Y ($kg.ha^{-1}$) e WG (g) concerning the doses and forms of application of biostimulantg.

Treatments	PH^{ns}	NPP ^{ns}	HFPI ^{ns}	NGP ^{ns}	Y ^{ns}	WG ^{ns}
T1	20.7	4.0	15.7	3.6	574.6	17.4
T2	20.6	4.0	16.1	4.0	630.3	17.9
T3	21.4	4.0	16.7	3.8	581.4	18.2
T4	20.1	3.0	15.7	4.0	550.8	17.9
T5	19.1	3.0	14.7	3.3	426.2	17.1
T6	20.3	3.0	15.9	3.5	446.7	17.8
CV (%)	9.8	22.8	8.2	19.94	30.2	4.2

PH – plant height; NPP – number of pods per plant; HFPI – height of first pod insertion; NGP – number of grains per pod; Y – yield; MG – weight of 100 grains.
^{ns}: non significant

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ASSOCIATION OF INFLORESCENCE WITH GRAIN YIELD IN COMMON BEAN

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INTRODUCTION: The number of pods per common bean plant is one of the primary grain yield components. This depends on the number of flowers produced and that will develop into pods. Thus, to increase yield, one of the alternatives is to have a larger number of flowers per plant. Lines were identified in which plants have few clusters, but in each inflorescence, the number of flowers is quite large, in contrast with common bean plants now under cultivation that have many clusters with two to six flowers (Figure 1). The scarcity of studies seeking to analyze the type of inflorescence on the common bean plant and the implications of the number of flowers for an increase in yield potential of grains led to the present study.

Figure 4. Exotic lines with compound inflorescence used as parents in the two crosses and the cultivar 'BRSMG Talismã' used as the female parent.

MATERIALS AND METHODS: Two different crosses were made: 'BRSMG Talismã' (P1) x Line 59583 (P2) and 'BRSMG Talismã' x Line 59692 (P3). The 'Talismã' cultivar has inflorescences with two to six flowers per cluster. It is the cultivar recommended for growing in the region due to yield and tolerance to some stresses, especially of biotic nature (ABREU et al. 2004). Lines 59583 and 59692 have inflorescences of the compound type with growth of multiple flowers with a pink color. Crosses were made in a greenhouse of the Department of Biology at the Federal University of Lavras (UFLA), Lavras, MG, Brazil, and the F_1 and F_2 generations, together with the parents, were assessed in the field, sown in May 2012. All the data were collected from individual plants at the time of harvest. The traits assessed were length of the inflorescence (LI); number of pods per inflorescence (NPI); number of pods per plant (NP) and plant grain yield (GY). The phenotype and genotypic correlations of all the pairs of traits were estimated using the procedure proposed by Falconer e Mackay (1996).

RESULTS AND DISCUSSION: Estimates of the phenotypic and genetic correlations had a wide variation among the traits and showed very similar magnitude and sign. Correlations between the traits two by two were mostly of small magnitude for the two crosses carried out, except between NP and GY (Tables 2 and 3). For LI, associations were not observed with any of the other traits assessed in the 'Talismã' x L.59583 cross. As for the other cross, the estimates involving LI were also of small magnitude, even though they were significant ($P \leq 0.01$) with NPI

and NP. Grain yield per plant was positively correlated with NP in both crosses. Observe that the estimate of correlation involving length of the inflorescence and grain yield was even negative, although not significant. Nevertheless, the wide variability for the traits assessed allows one to infer that it is possible to increase the yield potential of a common bean plant through increasing its number of flowers.

Table 1. Estimates of genetic correlations (above the diagonal) and phenotypic correlations (below the diagonal) between the traits: length of inflorescence (LI), number of pods per inflorescence (NPI), number of pods (NP) and grain yield (GY). Data obtained for the cross 'Talismã' x L.59583.

	LI	NPI	NP	GY
LI	1	-0.19	0.02	-0.06
NPI	-0.13	1	0.13	0.24
NP	-0.03	0.11	1	0.60
GY	-0.11	0.23**	0.71**	1

** Estimates significant at 1% of probability by the t test.

Table 2. Estimates of the genetic correlations (above the diagonal) and phenotypic correlations (below the diagonal) between the traits: length of inflorescence (LI), number of pods per inflorescence (NPI), number of pods (NP) and grain yield (GY). Data obtained for the cross 'Talismã' x L.59692.

	LI	NPI	NP	GY
LI	1	-0.85	-0.44	-0.02
NPI	-0.53**	1	-0.30	-0.07
NP	-0.28**	-0.21**	1	0.79
GY	-0.02^{ns}	-0.05^{ns}	0.70**	1

** Estimates significant at 1% of probability by the t test.

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PRE-HARVEST DESICCATION OF COMMON BEAN CULTIVARS

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INTRODUCTION: The use of desiccant herbicides on pre-harvests, in addition to allow harvests in late high weed reinfestation cultivations, has been highlighted as an alternative in accelerating and, especially, homogenizing plant drying, allowing for a more precocious harvest. However, a few fundamental aspects, such as the choice of herbicide and of cultivar, must be considered in order to prevent productivity loss and seed quality. This study was performed with the intent of evaluating the efficiency of different desiccants over new bean genotypes.

MATERIALS AND METHODS: A field experiment was conducted with the 2013 summer-autumn harvest, in Madre de Deus de Minas, Minas Gerais, Brazil. We used a random block experimental design, with three replicates in a 3 x 3 factorial scheme, involving three bean genotypes (BRS Estilo, IAC Formoso and IAC Imperador) and the desiccants paraquat dichloride, diquat dibromide and a witness without desiccant. The cultivars, of a type II undetermined growing and upright habit, present normal (Estilo cv.), demi-precocious (Formoso cv.) and precocious (Imperador cv.) culture cycles. The sowing was mechanized, in the spacing of 0.5 m between lines and a density of 17 seed m^{-1}. The fertilization was equivalent of 180 kg ha^{-1} of MAP and the seeds were treated with commercial inoculant. The desiccants, in the dose of 2 L ha^{-1} p.c., were applied 93 days after the emergence of the seedlings with a constant pressure pulverizer, at a solution volume of 400 L ha^{-1}. Seven days after the application we evaluated plant phytointoxication (**FITO**), employing the nine degree scale proposed by EWRC (1964), in which 1represents the absence of FITO and 9 represents the plants' total destruction. At the harvest we evaluated the physical (water content – WC and tegument color L index) and physiological quality (% of normal seedlings – NS, speed index – SI, emergence percentage – EP and electric conductivity – EC) of the seeds. We also determined the weight of one hundred seeds (WHS) and productivity (PROD). The data were submitted to analysis of variance and, in case of significant effect of the treatment, the means were compared by the Scott-Knott test at a 5% level of probability.

RESULTS AND DISCUSSION: The analysis of variance revealed that the experimental precision was good, with the exception of EC (Table 1). The FITO was influenced by the cultivars (C), desiccants (D) and by the interaction C x D. There was also the significant effect of the C factor over the L color index, NS and WHS, and of the D factor over WHS. The effect of the desiccants over FITO depended on the cultivar (Table 2). Generally, both desiccants provided elevated FITO and overcame the witness treatment. When applying the paraquet dichloride, the Formoso cv. behaved in a similar manner as the Imperador cv, responding more to the herbicide than the Estilo cv. In regard to the diquat dibromide, the Formoso and Estilo cv. means ranged little and remained below that of the Imperador cv. The application of paraquat dichloride on the Estilo cv. provided an inferior FITO to that obtained with diquat dibromide; still, there was effect on the product when compared to the witness (Table 2). In regard to WC, we expected higher content on the Formoso cv. due to its demi-precocious cycle. However, the higher permanence of this cultivar on the field did not favor the desiccation, which leads us to believing that other factors, such as relative air humidity, influenced the evaluation. The reading values of the color L index, which reflect seed clarity, respected the decreasing order: Imerador cv. > Formoso cv. > Estilo cv. (Table 1). The cultivar with the most accentuated coloration

(Estilo cv.) also presented the lowest seed weight and incidence of normal seedlings; however, the productivity was not affected and has maintained equivalent to the other genotypes.

Table 1 – Coefficient of variation (CV%) and the average values of bean water content (WC), color L index and emergence speed (ES), emergence percentage (EP) and normal seedlings (NS), electric conductivity (EC), weight of on hundred seeds (WHS) and productivity (PROD).

Cultivars	WC (g kg^{-1})	L Index	ES	EP	NS	EC	WHS	PROD
				----- (%) ----		(µS cm^{-1} g^{-1})	(g)	(kg ha^{-1})
Estilo	171 A	48.3 C	5.4	89.6	58.7 B	103.6	21.5 C	1894
Formoso	199 B	53.6 B	4.6	75.8	74.2 A	63.1	24.1 A	2174
Imperador	154 A	57.9 A	5.4	87.8	87.6 A	78.4	23.3 B	2352
Desiccants								
Paraquat dichloride	169 A	52.6	5.9	94.9	69.1	94.3	22.4 B	2246
Diquat dibromide	159 A	54.3	5.2	81.8	82.0	64.7	22.7 B	2115
Witness	197 B	52.8	4.4	76.4	69.3	86.0	23.9 A	2058
Means	175	53.3	5.1	84.4	73.5	81.7	23.0	2140
CV %	14.4	6.3	24.3	20.6	24.4	43.3	3.1	17.1

Inside each factor, means followed by different letters on the columns belong to distinct groups, by the Scott-Knott test at the level of 5% of probability.

Table 2 – Bean phytointoxicationin the maturation stage (EWRC grade scale).

Cultivars	Phytointoxication			Means
	Paraquat dichloride	Diquate dibromide	Witness	
Estilo	7.3 Bb	8.0 Ba	1.0 Ac	5.4 C
Formoso	8.7 Aa	8.3 Ba	1.0 Ab	6.0 B
Imperador	9.0 Aa	9.0 Aa	1.0 Ab	6.3 A
Means	8.3 a	8.4 a	1.0 b	5.9

Means followed by the same letter, upper case in the columns and lower case in the lines, belong to the same group, according to the Scott-Knott test at the level of 5% of probability.

The color results from transformations induced and intensified by the cultivating and storage conditions, which may negatively influence seed quality. In the present work, there was no difference between cultivars in regard to solute leaching (EC) and seedling emergence (ES and EP) parameters. However, Estilo cv. seeds showed compromise of the cellular membrane integrity, draining 46% more exudates to the test solution then the other cultivars (Table 1). Even if this increase in EC was not significant, the smaller occurrence of normal seedlings of the genotype in question confirms the inferior quality of these seeds. Seed weight, as well as FITO and WC, was influenced by desiccation. However, despite the significance, the average values were very close, which restricts the practical importance of the differences, probably detected due to the high experimental precision with which the characteristics were estimated (Table 1). It must also be mentioned that, despite these significant differences, the productivity of all three treatments was equivalent, reaching a mean superior to 2,000 kg ha^{-1}. Considering that the desiccants reduced the humidity without interfering with tegument color, physiological quality (EC, ES, EP, NS) of the seeds and productivity, the employment of the tested products may be recommended in the pre-harvest of the evaluated cultivars.

CONCLUSIONS: The employment of paraquat dichloride and diquat dibromide based desiccants accelerates the harvest without compromising bean seed quality and productivity. The BRS Estilo cv. presents dark tegument and low % of normal seedlings, however, its productivity is equivalent to that of the IAC Formoso and IAC Imperador cvs.

CORRELATIONS BETWEEN MORPHO-AGRONOMIC TRAITS IN COMMON BEAN

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INTRODUCTION

The knowledge of the association between characters is of great interest in plant breeding as it allows evaluating how the modification of a character can affect the others (SILVA et al., 2009). In the case of common bean, it is generally known that large grain cultivars, between 40g to 60g per 100 grains, like Jalo, Diacol Calima and Pintado, also have large leaves and pods. Furthermore, small grain cultivars such as Small White and Ex Rico 23 whose 100 grains weight are less than 20g also have small pods and leaves. However, there are few studies to check if this correlation is really significant because in plant breeding in some regions there is the interest of upright plants with smaller leaves, however, with intermediate grain sizes. Therefore, this study was done with the aim of evaluating segregating progenies, from the crossing of cultivars Jalo and Small White, through the weight of 100 grains, leaf and pod length, and of estimating correlations between these traits.

MATERIAL AND METHODS

The crossing between Jalo and Small White lines was performed in greenhouse and the F_1 seeds obtained were sown in the experimental field of the Federal University of Lavras (UFLA), MG State, Brazil, to obtain the F_2 generation. From individual F_2 plants, $F_{2:3}$ and $F_{2:4}$ progenies were obtained, totaling 190 progenies that were used in the study.

Phenotypic evaluations were set up in the field using the $F_{2:3}$ e $F_{2:4}$ progenies. Two evaluations were performed, one in the winter crop (evaluation of the $F_{2:3}$) and the other in summer crop (evaluation of the $F_{2:4}$), both in Lavras-MG. The experimental design used in the evaluations was the triple lattice 14 x 14, the treatments being the 190 progenies and the checks, both parents (Jalo and Small White) and also the lines, Cornell 605, G-122, Corujinha and CNFC 9506.

The experimental plot consisted of one-meter row spaced 0.60m, and sowing density of fifteen seeds per meter. The 100 grains weight (W) was obtained by weighing on a precision balance a random sample of 100 grains. Measures of the central leaflet length of a full developed leaf (LL) of the middle third of the plant were taken. To measure the length of the pod (PL), the longitudinal extent between the base and the apex of the pod (mm) was taken. It was measured a mature pod per plant, collected in the middle third of the plant. Ten plants of the plot were used to obtain the data for each character. The Genes software (CRUZ, 2006) was used for statistical analysis.

RESULTS AND DISCUSSION

Wide genetic variability was observed among the progenies, with significant effects ($P < 0.01$) of treatments in the analysis of variance for all traits, as well as the medium to high values of heritability (Table 1). Considering that the existence of genetic variability in a population is crucial to any breeding program (RAMALHO et al., 2000), the population used proves to be at first, promising for selection, and even for checking the correlations between the characters. The high value of selective accuracy (r_{gg}%) and the small values of CV indicate that the

experiment was conducted with a high degree of experimental precision. Therefore, the correlation estimates can be more accurate and the selection can be performed efficiently.

Table 1. Analysis of variance of the length of the central leaflet (LL) and pod (PL), and 100 grains weight (W) of common bean progenies.

Source of variation	DF	MS		
		LL	PL	W
Replications	2	4.64*	0.15 ^{ns}	10.68**
Block/rep	39	5.96**	4.70**	28.53**
Progenies	195	1.40**	2.44**	61.94**
Error	351	0.60	0.47	6.68
CV(%)		11.07	7.32	11.01
r_{gg}(%)		75.60	89.85	94.95
h^2(%)		57	81	89

**,* e ^{ns} Significant at 1% , 5% level of probability and not significant, respectively, by F test.

The correlations between the characters are described in Table 2. All estimated correlations were positive and significant. This shows that the same physiological mechanisms have influenced the causes of genetic, phenotypic and environmental variation of all traits. For all pairs of characters, the genetic correlations were greater than the phenotypic and environmental values, showing an increased contribution of genetic factors in the association of these traits. The genetic correlation is responsible for the heritable fraction of the parents to their progeny (COIMBRA et al., 2000), and can be caused mainly by pleiotropy or linked genes. If two characters have significant genetic correlation, it is possible to use the indirect selection to obtain higher gains or else the correlation may became difficult the selection. Specifically, the leaf size and the grain weight correlation indicates that longer leaves are associated with higher grain weight, however its magnitude indicates the possibility of selection of lines with upright plant type with smaller leaves and grains of intermediate sizes, which are the ideal phenotypes for cultivars in the region.

Table 2. Genetic (r_g) phenotypic (r_p) and environmental (r_e) correlations between the central leaflet length (LL), pod length (PL) and 100 grains weight (W) in common bean progenies.

Characters	Correlations		
	r_g	r_p	r_e
LL x PL	0,668^{+}	0,5927^{**++}	0,4883^{**++}
LL x W	0,5135^{+}	0,3985^{**+}	0,1598*
PL x W	0,6572^{++}	0,5914^{**++}	0,2505^{**+}

** e * Significant at 1% and 5% of probability by t test respectively.
++ e + Significant at 1% and 5% of probability in 5000 *bootstrap's* simulations.

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MORPHOLOGICAL ALTERATIONS IN COMMON BEAN SEEDLINGS EXPOSED TO SALT STRESS

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INTRODUCTION

The excessive salinity of the soil promotes several problems in growth and development of plants, and it can cause necrosis and premature senescence, affecting the water relations, photosynthesis and nutrient assimilation. During salinity occurs a decrease in soil osmotic potential, reduction in water availability, toxicity, and negative interferences in metabolism that will have as consequence morphological modifications (Mortele et al., 2006). Aim of this study was to describe the interferences of salt stress in three seedlings of common bean largely cultivated in Brazil.

MATERIALS AND METHODS

Study was implemented in Núcleo de Pesquisa Vegetal Básica e Aplicada of the Universidade Federal Rural da Amazônia, Brazil with seeds of *Phaseolus vulgaris* cvs. IPR-Siriri, IPR-Uirapuru and 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 hypocotyl length and root length, being expressed in cm. The images were obtained with *Olympus* digital camera with eight megapixels. An analysis of variance was performed, and when significant differences were present, a Scott-Knott test with a 5% level of error probability was used.

RESULTS AND DISCUSSION

To hypocotyl length, under concentration of 0 mM NaCl the IPR-139 cultivar presented better result (Fig. 1 A), as well as in concentration of 50 mM NaCl the IPR-Siriri cultivar demonstrated higher value. In concentration of 100 mM NaCl was showed that occurred not significant difference between cultivars and when these cultivars were exposed to 150 mM NaCl, the better result was obtained with IPR-Siriri. The decrease in hypocotyl length (Red boxes) as consequence of the salt stress is due to salinity to interfere negatively in energy balance, difficult the cell division and consequently reducing the growth rate of the seedlings (Kaymakanova, 2009).

The root length was affected by the increase in salinity (Red boxes). The IPR-Siriri cultivar presented better result in concentration of 0 mM NaCl (Fig. 1 B). To concentration of 50 mM NaCl the IPR-Uirapuru cultivar revealed high value. Additionally, the IPR-139 cultivar in concentration of 100 mM NaCl has high performance. In treatment under application of 150 mM NaCl occurred not significant difference between cultivars. The decrease linked to root length

showed in all cultivars of *Phaseolus vulgaris* can be explained by the lower water absorption, ionic toxicity and physiologic and metabolic damages induced by the salt stress (Maia et al., 2012).

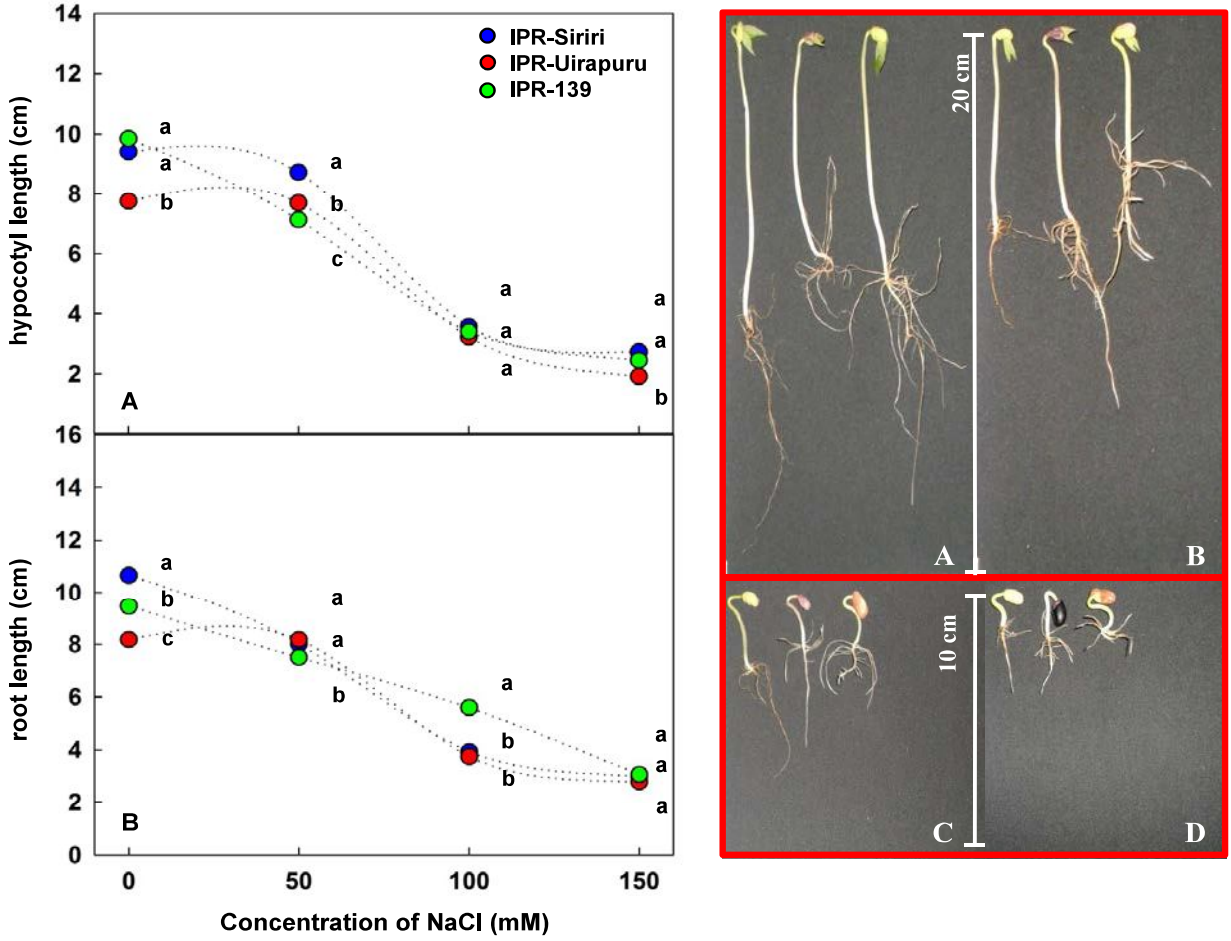

Fig. 1: Hypocotyl length (A) and root length (B) of three common bean cultivars exposed to concentrations of 0, 50, 100 and 150 mM NaCl. Means followed by the same letter in equal concentrations are not significantly different by the Scott-Knott test at 5% of probability. Red boxes are presented the symptoms linked to salt toxicity in seedlings of IPR-Siriri (left), IPR-Uirapuru (middle) and IPR-139 (right) submitted to concentrations of 0 (A), 50 (B), 100 (C) and 150 (D) mM NaCl.

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CARIOCA BEAN CULTIVARS UNDER DIFFERENT SOWING DENSITY

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INTRODUCTION: In order that the farmers can explore the richness and the diversity of the new bean cultivars improved by genetic breeding, the agronomic research must answer with studies that improve the management system, making possible to obtain high yield allied to economic and environmental sustainability, supporting the practice of the modern agriculture. One of the most important aspects of management that have a substantial impact in the performance of the production systems, is the sowing density, primordial to a good plant arrangement in the field and good bean yield. The aim of this paper was evaluate the behavior of eight different bean cultivars type ‘‘Carioca’’ under six different sowing densities.

MATERIALS AND METHODS: Eight field trials were installed simutaniously in no-tillage system, each one with a different bean cultivar type Carioca: BRS 9435 Cometa, BRS Estilo and BRS Notável, from EMBRAPA Rice and Beans Centre, IAC Alvorada, IAC Formoso and IAC Imperador from Campinas Agronomic Institute, IPR Tangará from Paraná Agronomic Institute and BRSMG Madrepérola from the bean genetic breeding program of Minas Gerais State, which envolves Epamig, Embrapa, UFLA and UFV. The experiment was conducted in a dystrophic red-yellow latosol located at ‘’Liberdade Farm’’, at 21°26’ S, 44°18’ W and 1.000 m above sea level, on Madre de Deus de Minas, Minas Gerais State, Brazil. According to the Köppen classification, the local climatic conditions can be classified as Cwa. In all trials the statistical design adopted was randomized blocks with eight replications and six treatments (density of 6, 8, 9, 12, 14 e 16 plants.m^{-1}, which correspond to 133, 178, 200, 222, 267 e 311 thousand plants.ha^{-1}). The sowing in the treatment of 0,45 m between rows, was done by a seed drill equipped with a vacuum system for the seed distribution, monitored by an electronic panel. Twenty seeds were sown per meter, and after the seedling emergence, the thinning was done to obtain the correct population. The experimental plot consisted of nine rows 5 m long , with effective area of 20,25 m^2.The base fertilization was composed by 180 kg ha^{-1} of MAP, and the seed were inoculated with commercial rhizobium. Seed treatment and other phytosanitary treatments were performed following the pattern used in the farm. The experiments were irrigated by sprinkler. Harvesting was performed with Ceiflex® cutting the nine rows of each experimental plot, where we obtain the sample corresponding to 1,5 m long, with 6,075 m^2. The plants were threshed at Sector of Commercial Crops in the Agriculture Department of UFLA. The obtained grains were weighted to estimate the yield. Data were subjected to variance analysis of in each experiment and the joint analysis was also done. In cases of significant effect of densities, regression analysis was performed and selected representative equations of the relationships between variables, based on the significance of the model and the R^2 value. In case of significance of cultivar, means were grouped by the Scott-Knott test at 5% probability.

RESULTS AND DISCUSSION: The cultivars influenced all response variables and populations influenced the moisture and weight of 100 grains, while the interaction was significant only to the weight of 100 grains. In the Scott-Knott test the cultivars formed three groups to the yield. The cultivars BRS Notável and BRSMG Madrepérola showed the highest yields. The intermediate group was formed by IAC Alvorada, IPR Tangará, BRS 9435 Cometa e BRS Estilo. The cultivars with the lowest yield were IAC Formoso and IAC Imperador. This results corroborate with previous results, as ALVES et al. (2009) and JUNIOR; BACKES, (2008). The bean yield was not influenced by plant population size (Table 1).

Table 1. Average values of productivity, 100-grain weight and grain moisture content due to cultivars and populations of common bean

Treatment	Productivity (kg ha^{-1})	100 grain weight (g)	Content moisture average (%)
Cultivars			
BRS Notável	2644.35 a	23.83	8.97 d
BRSMG Madrepérola	2481.63 a	23.80	7.01 f
IAC Alvorada	2302.50 b	26.66	7.94 e
IPR Tangará	2259.80 b	27.44	14.51 a
BRS 9435 Cometa	2214.19 b	23.98	10.30 c
BRS Estilo	2175.37 b	24.78	10.17 c
IAC Formoso	1957.40 c	25.00	11.65 b
IAC Imperador	1947.46 c	23.27	9.90 c
Plant Population (m^{-1})			
6	2289.69	25.47	10.72
8	2233.79	24.81	10.12
9	2162.18	24.73	10.04
10	2342.39	24.79	10.07
12	2302.36	24.35	9.67
14	2156.62	24.90	9.74

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

CONCLUSIONS: The studied density interval, do not interfere in the grain yield of the tested cultivars. The cultivars BRS Notável and BRSMG Madrepérola showed the higher yields. Those cultivars that showed higher weight of 100 seeds was not show the higher yield.

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SEED YIELD AND ITS COMPONENTS OF WILD AND CULTIVATED *Phaseolus vulgaris* L.

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INTRODUCTION:. Wild bean is a basic source of germplasm which can be used to incorporate genes for tolerance to unfavorable abiotic and biotic factors. The preservation of plant genetic resources is important due to the role of genetic diversity in the development of new varieties (Acosta-Gallegos *et al.* 1999; López *et al*. 2005). The comparison of the wild and cultivated forms of *P. vulgaris*, as well as the investigations about the evolutive forces which relate both forms will provide a better understanding of how the gene pool of common bean has developed (Gepts and Debouck, 1991). México is one of the two centers of origin of *Phaseolus* with great diversity of wild, landraces and improved populations (Peña Valdivia *et al.* 2012). The objective of the present work was to determine for a cultivated variety, a wild bean and five progenies derived from the crossing of both forms: 1) the seed yield, 2) the 100-seed weight and 3) the number of seeds per plant.

MATERIALS AND METHODS: The study was carried out during 2013 under greenhouse conditions. The experiment consisted of seven treatments with five replications. The treatments consisted of seven *Phaseolus vulgaris* genotypes: Negro Tacaná (NT) a cultivated variety of determinate growth (type I, CIAT, 1982), W13, a wild genotype of indeterminate growth (type III), and five progenies (53b, 11.1, 3.3, 51b and 118b) derived from crossing NT and W13. This material was provided by Dr. Jorge A. Acosta Gallegos of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Seeds were sown April 17. The lapse from seeding to harvest were different for the various genotypes. The longest one corresponded to the wild genotype. Complete Steiner nutritive solution (Steiner, 1984) and 4.5 kg of red "tezontle" (inert volcanic cinder substrate) per pot were employed. The variance analysis were performed with the Staistical Analysis System (SAS, 2012) and for the comparison of means Tukey's test was used.

RESULTS: *Seed yield* per plant did not show statistical difference among the cultivated variety Negro Tacaná (NT), the progenies 3.3, 51b and the wild genotype (W13). The progenies 11.1, 118b and 53b exhibited the lower yield (Table 1). *100-seed weight* was the highest for NT and the lowest for the W13. The progenies 51b, 11.1 and 3.3 showed intermediate values (Table 1). *Number of seeds per plant,* the wild genotype produced the highest number of seeds per plant followed by the progenies 3.3, 51b, 118b and 11.1 while the lowest values corresponded to NT and 53b (Table 1). It was noted a strong contrast for the number of seeds per plant between the wild genotype (1120 and the cultivated 244 seeds). However, *the seed yield* was not statistically different between them. This indicates a compensation of a higher number of seeds, by a smaller seed (in this case 4.6 times less than the cultivated variety). This situation, observed en the wild plants enables them a higher probability to survive as species in critical environments. The response in the variables studied showed that two of the progenies (3.3 and 51b) the seed yield per plant were statistically equal to the cultivated genotype which exhibited a bigger seed than the wild one.

Table 1. Seed yield, 100-seed weight and seeds per plant of seven genotypes of *Phaseolus vulgaris* L. Montecillo, México, 2013.							
Genotype	[1]Seed yield (g plant^{-1})		Genotype	100-seed weight (g)		Genotype	Seeds plant^{-1}
NT	63.4 a		NT	26.1 a		W13	1120 a
3.3	60.0 ab		51b	13.7 b		3.3	448 b
51b	58.0 abc		11.1	13.7 b		51b	424 bc
W13	56.7 abc		3.3	13.5 bc		118b	368 bcd
11.1	46.6 bcd		118b	11.6 cd		11.1	341 bcd
118b	42.8 cd		53b	10.9 d		53b	277 cd
53b	30.5 d		W13	5.1 e		NT	244 d

[1]Seeds with 10.1% of moisture content.

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CHARACTERIZATION OF FINAL TRIALS FOR CULTIVAR RELEASE OF THE BRAZILIAN COMMON BEAN ASSAY NETWORK COORDINATED BY EMBRAPA

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The number of final trials for cultivar release conducted by the Embrapa common bean breeding program and partners is very large, once these trials are accomplished in all four growing areas in Brazil (Pereira *et al.*, 2010), representing different environmental conditions. These environmental variations directly influence the agronomic performance of the tested advanced lines, including yielding and disease reaction. The characterization of the final trial network can support decision making and optimize breeding program efforts to ensure more security on recommendation process of new cultivars. Therefore, the present work aimed to characterize the Brazilian common bean assay network coordinated by Embrapa. Information of experiments conducted from 2003 to 2012 for the market classes "carioca" and black was used. These market classes represent 70% and 17%, respectively, of the bean consumption in the country. The data used in this study were generated from final trials conducted during all growing seasons in the common bean growing areas 1, 2 and 3, as showed in Figure 1, where 98% of the Brazilian common bean is produced. All trials were conducted in randomized block design, with three replicates, using plots with four rows of four meter long.

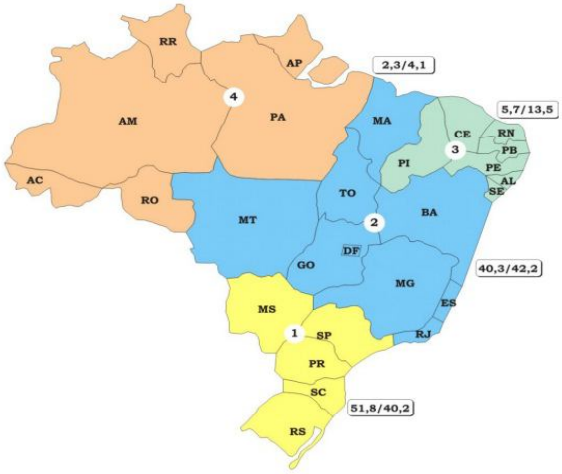

Figure 1. Brazilian common bean growing areas 1-to-4. The numbers into the boxes are, respectively, common bean grain production and planted area in percentage for each growing area. Source: Feijão (2010), adapted by Pereira *et al.* (2010).

During the period of 2003-2012, 68 "carioca" seeded genotypes (59 advanced lines and nine control cultivars) were evaluated in 364 trials. A total of 55 black seeded genotypes (44 advanced lines and 11 control cultivars) were evaluated in 334 trials. The tested advanced lines are replaced each new cycle, that begin after two years. This process permitted the evaluation of eight to twelve bean lines in each cycle. The area 1 represents 51.8% of the grain production and 40.2% of the common bean grown area in Brazil, showing the larger percentage of final trials for both market classes (45.3% of "carioca" and 45.5% of black) (Tables 1 and 2). The area 2 represents 40.3% of the grain production and 42.2% of the grown area in the country. It presented 40.4% of the "carioca" final trials and 40.1% of the black trials. The area 3 only represents 5.7% of the grain production and 13.5% of the grown area, presenting around 14.0% of the final trials for both market classes. Area 3 has only one growing season per year while the

other areas have at least two. The Brazilian common bean assay network coordinated by Embrapa has been successful to conduct the final trials for cultivars release, what can be verified by the adequate experimental precision of the trials. It has been observed CV% scores, in all regions, lower than 15%, with mean yield potential over 2,100 kg.ha^{-1}, being all trials conducted without the use of fungicides. For the area 1, there was significative increment in grain yield to genotypes of both classes. This will reflect at genetic progress of the breeding program in a growing area where the common bean crop is very important and competitive.

Table 1. Trials (T), mean yield (MY) (kg.ha^{-1}) and experimental coefficient of variation (CV%) by growing areas and by biennial final trial cycles of the Brazilian common bean assay network for the market class "carioca", period from 2003 to 2012.

Trial cycle	Growing areas									Overall		
	1			2			3					
	T[1]	MY	CV	T[2]	MY	CV	T[3]	MY	CV	T	MY	CV
2003/04	31 (12^{D}-19^{R})	2,335	13.6	40 (24^{W}-16^{R})	2,052	15.7	5	2,541	12.2	76	2,309	13.8
2005/06	23 (8^{D}-15^{R})	2,358	15.5	23 (12^{W}-11^{R})	2,257	13.5	7	1,685	11.0	53	2,100	13.3
2007/08	43 (9^{D}-34^{R})	2,215	14.3	28 (16^{W}-12^{R})	2,173	13.8	10	2,330	12.5	81	2,239	13.5
2009/10	34 (12^{D}-22^{R})	2,282	15.3	27 (10^{W}-17^{R})	2,194	16.1	15	2,128	13.9	76	2,201	15.1
2011/12	34 (14^{D}-20^{R})	2,629	13.8	29 (14^{W}-15^{R})	2,137	15.0	15	2,016	11.9	78	2,261	13.6
O[4]/M[5]	165	2,364	14.5	147	2,163	14.8	52	2,140	12.3	364	2,222	13.9

[1]Number of trials realized in the dry and rainy growing seasons in the States of Rio Grande do Sul, Santa Catarina, Paraná, São Paulo and Mato Grosso do Sul. [2]Number of trials realized in the rainy and winter growing seasons in the States of Mato Grosso, Goiás/Distrito Federal, Minas Gerais, Rio de Janeiro, Espírito Santo, Bahia and Maranhão. [3]Number of trials realized in the rainy growing season the States of Sergipe, Alagoas, Pernambuco, Paraíba, Rio Grande do Norte, Ceará and Piauí. [4]Overall. [5]Mean. [D]Dry. [R]Rainy. [W]Winter.

Table 2. Trials (T), mean yield (MY) (kg.ha^{-1}) and experimental coefficient of variation (CV%) by growing areas and by biennial final trial cycles of the Brazilian common bean assay network for the market class black, period from 2003 to 2012.

Trial cycle	Growing areas									Overall		
	1			2			3					
	T[1]	MY	CV	T[2]	MY	CV	T[3]	MY	CV	T	MY	CV
2003/04	30 (11^{D}-19^{R})	2,260	12.9	40 (23^{W}-17^{R})	2,148	14.8	5	2510	11.4	75	2,306	13.0
2005/06	23 (7^{D}-16^{R})	2,477	13.0	20 (9^{W}-11^{R})	2,476	13.9	7	1907	11.4	50	2,287	12.8
2007/08	41 (10^{D}-31^{R})	2,148	14.1	28 (15^{W}-13^{R})	2,188	14.4	9	2606	10.4	78	2,314	13.0
2009/10	37 (10^{D}-27^{R})	2,304	15.7	26 (10^{W}-16^{R})	2,294	14.8	12	1937	13.9	75	2,178	14.8
2011/12	21 (4^{D}-17^{R})	2,481	13.7	20 (9^{W}-11^{R})	1,926	14.1	15	2012	13.1	56	2,140	13.6
O[4]/M[5]	152	2,334	13.9	134	2,206	14.4	48	2194	12.0	334	2,245	13.4

[1]Number of trials realized in the dry and rainy growing seasons in the States of Rio Grande do Sul, Santa Catarina, Paraná, São Paulo and Mato Grosso do Sul. [2]Number of trials realized in the dry and rainy growing seasons in the States of Mato Grosso, Goiás/Distrito Federal, Minas Gerais, Rio de Janeiro, Espírito Santo, Bahia and Maranhão. [3]Number of trials realized in the rainy growing season in the States of Sergipe, Alagoas, Pernambuco, Paraíba, Rio Grande do Norte, Ceará and Piauí. [4]Overall. [5]Mean. [D]Dry. [R]Rainy. [W]Winter.

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MOLECULAR CHARACTERIZATION AND GENETIC DIVERSITY OF BRAZILIAN COMMON BEAN CULTIVARS AND ELITE LINES

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INTRODUCTION

The characterization and quantification of genetic diversity of common bean (*Phaseolus vulgaris* L.) cultivars and elite lines are useful for the breeding programs in the selection process of parents as well as in the determination of the genetic identity of superior genotypes (Cabral *et al.,* 2011). Currently, several classes of molecular markers are available for this purpose of which microsatellite markers (SSR – Simple Sequence Repeats) should be highlighted for being abundant throughout the genome, multi-allelic and suitable for semi-automated genotyping. Thus, the main goal of this work was to characterize and estimate the genetic diversity of "carioca" and black-seeded common bean cultivars and elite lines developed by the Brazilian Agricultural Research Corporation (Embrapa) and partners in Brazil, using 33 microsatellite loci developed and validated for common bean (Valdisser *et al.,* 2013).

MATERIAL AND METHODS

Seventeen cultivars and elite lines were analyzed, all selected for their high yield potential and consumer acceptance. Of these genotypes, 14 belong to carioca the commercial group ('Pérola', 'BRS Pontal', 'BRS Ametista', 'BRS Notável', 'BRS Cometa', 'BRS Horizonte', 'BRS Estilo', 'BRSMG Majestoso', 'BRSMG Madrepérola', 'CNFC 10429', 'CNFC 10431', 'CNFC 10432', 'CNFC 10467', and 'VC-6'), and three to the black group ('BRS Esplendor', 'BRS Supremo' and 'BRS Valente'). The DNA was extracted using samples composed of leaf tissue from 10 plants of each genotype collected in bulk, using the CTAB protocol. The 33 microsatellite loci were amplified as described by Valdisser *et al.* (2013). The amplified fragments were separated by capillary electrophoresis using an ABI 3500 Genetic Analyzer (Applied Biosystems ®) and markers were genotyped using software GeneMapper 3.5 (Applied Biosystems ®). The number of alleles per locus, allele frequency, genetic diversity, and the polymorphic information content (PIC) were calculated using the program PowerMarker (Liu & Muse, 2005). The genetic distances between genotypes were estimated by the method proposed by Smouse and Peakall, and grouped by Tocher's method of sequential clustering (Vasconcelos *et al.,* 2007), using software Genes (Cruz, 2013).

RESULTS AND DISCUSSION

Of the 33 SSR markers used in the analysis of 17 common bean genotypes, 26 (78.8 %) were polymorphic. A total of 109 alleles were identified, with an average of 4.0 alleles per locus, ranging from two alleles for the loci BM189, BM202 and PV169 to eight alleles for PV163. The mean genetic diversity of the SSR loci was 0.54, ranging from 0.06 for locus PV169 to 0.80 for BM187. In the genetic characterization of common bean cultivars from different commercial groups with AFLP and SSR markers, Cabral *et al.* (2011) and Perseguini *et al.* (2011) found mean scores of genetic diversity of 0.45 and 0.47, respectively, which are consistent with those estimated in this study. The PIC ranged from 0.05 for marker PV169 to 0.77 for BM187, with a

mean of 0.50. These results confirmed estimates of Díaz *et al.* (2010) who analyzed a set of 92 common bean landraces with 52 SSR markers (PIC = 0.54). The shortest genetic distance was found between 'BRS Ametista' and 'Pérola', while the longest was observed between 'BRS Majestoso' and 'CNFC 10432'. By Tocher's sequential clustering analysis, four groups were formed based on genetic distances estimated between genotypes according to Smouse and Peakall (Table 1). The results showed that the microsatellite markers were efficient to discriminate the analyzed genotypes. The black seeded genotypes were all clustered into a single group, along with the "carioca" seeded cultivars 'BRS Notável' and 'BRS Estilo'. The other "carioca"-seeded cultivars and elite lines were clustered into three distinct groups, which is consistent with the contrasts of these genotypes for several agronomic traits. The results confirm the existence of genetic variability among the tested genotypes, which can be exploited by the breeding programs in Brazil.

Table 1. Grouping of common bean cultivars and elite lines developed in Brazil by Embrapa and partners obtained by Tocher's sequential clustering, based on genetic distances of Smouse and Peakall estimated by 26 polymorphic SSR loci.

Groups	No. of Genotypes	Genotypes
I	4	Pérola[a], BRS Ametista[a], BRSMG Majestoso[a], and CNFC 10467[a]
II	7	BRS Cometa[a], BRS Pontal[a], BRS Horizonte[a], VC-6[a], CNFC 10429[a], CNFC10431[a], and CNFC 10432[a]
III	5	BRS Esplendor[b], BRS Valente[b], BRS Supremo[b], BRS Estilo[a], and BRS Notável[a]
IV	1	BRSMG Madrepérola[a]
Total	17	

[a]carioca and [b]black-seeded genotypes.

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GENETIC VARIABILITY IN LIMA BEAN GENOTYPES (*PHASEOLUS LUNATUS*) FROM RIO GRANDE DO SUL, BRAZIL

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INTRODUCTION: The introduction of alternative crops dual-purpose in order to produce biomass and grain in family-based agriculture is a fundamental strategy in sustainable agriculture. The plant is well suited to complex system, characteristic of family farming, defined as sequential arrangements or consortia of herbaceous, shrub and tree species in different strata (HENZ, 2009). This practice aims to disseminate experiences to enrich the diet of the population, currently restricted to little diversity of grains and cereals, with quality products.

The lima beans or fava beans, leguminous plant of the Fabaceae family, is characterized by its genetic diversity, high adaptability and productivity for being a kind of dual-purpose and can be used in human food, animal and green manure. In Brazil, the production of this species is concentrated in the northeast, where it is grown intercropped with maize, cassava, castor or tropical grasses, using them as a support (AZEVEDO et al, 2003). However in temperate region is great variability of this kind which can become an economically viable alternative.

The aim of the study was to describe the genetic and phenotypic variability of lima bean genotypes from temperate region.

MATERIAL AND METHODS: Embrapa Temperate Climate has a germplasm bank of lima beans composed of 70 cultivars collected throughout the southern region of Brazil and some coming from the northeast. These genotypes have been evaluated over the past few years and show a large variation between the grains, cycle and size. The soil where the genotypes were evaluated Haplaquult is typical of floodplains, presenting poorly drained and low fertility. After preliminary analysis of the soil with limestone correction was performed, and adding organic compost, rock phosphate powder and granodiorite rock, manually entered. For installation of observation units were sown in november, four lines of each variety, 6m long, spaced 0.50 m apart at a density 2 to 3 plants m^{-1}.

RESULTS AND DISCUSSION

The grain types showed high variability expressed for size and coat color, as showed in Figure 1. The grain production ranged of 2 to 7 ton ha^{-1}, and the red Canguçu variety (G1), showed highly significant values in relation to other cultures, which can be used in summer, as cowpea (*Vigna unguiculata*).

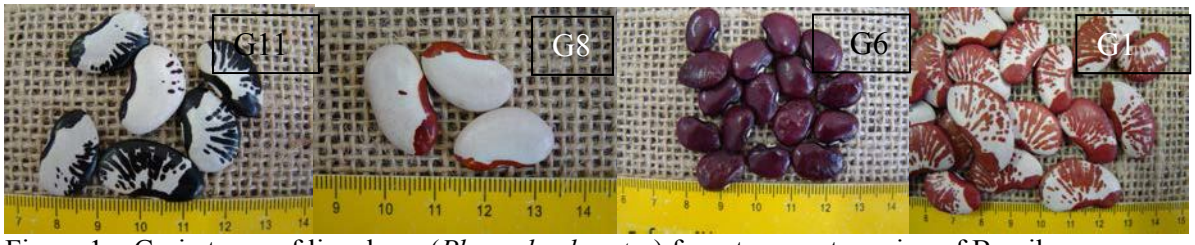

Figure 1 – Grain types of lima bean (*Phaseolus lunatus*) from temperate region of Brazil.

The vast majority of the varieties analyzed showed indeterminate growth habit. The date of flowering occurred between 90 and 98 days after emergence, extending to the beginning of

winter, in some varieties. Another aspect observed was the forage potential of the later varieties, due to its high strength and vegetative growth that lasted until the month of june. The flowering in black Canguçu (G11) and red Canguçu (G1), was considered late. The G6 variety were classified as early. The maturation of pods occurred between 126 and 140 days after emergence.

Table 1. Seed caracterstics in 27 lima bean genotypes (*Phaseolus lunatus*).

Genotypes	Background collor	Pattern collor	2ª pattern collor	Coat	Shape
G1	White	red	absent	9	12
G2	white	Purple red	absent	9	12
G3	white	Purple red	absent	9	12
G4	white	Dark brown	absent	9	12
G5	white	Purple red	absent	6	12
G6	Purple red	absent	absent	0	8
G7	white	red	absent	9	12
G8	white	red	absent	4	7
G9	white	Purple red	absent	9	10
G10	white	Purple red	absent	9	10
G11	withe	Black	absent	13	7
G12	white	Dark brown	absent	10	6
G13	white	absent	absent	0	7
G14	white	Dark brown	absent	8	11
G15	white	red	absent	13	10
G16	white	Purple red	absent	9	12
G17	gray	brown	black	5	6
G18	gray	brown	black	5	6
G19	gray	brown	black	5	6
G20	gray	brown	black	5	6
G21	gray	brown	black	5	6
G22	gray	brown	black	5	6
G23	gray	brown	black	5	6
G24	gray	brown	black	5	6
G25	gray	black	Absent	11	6
G26	white	brown	absent	10	6
G27	gray	black	absent	5	6

*Coat color and seed shape pattern according IPGRI (2001).

In some cases, the botanical descriptors used for seeds weren't efficient to differentiate the genotypes, as observed in the group G17 to G24. In this case is necessary to use complementary the plant descriptors.

CONCLUSION: the lima beans is a species with high genetic and phenotypic variability, very promising for use in intercropping diversified systems due to its versatility; the seeds botanical descriptors don't are effective to differentiate the genotypes.

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2013 FINANCIAL STATEMENT
BEAN IMPROVEMENT COOPERATIVE

BALANCE AS OF January 1, 2013			**$ 8,474.37**
INCOME			
		2013	
2013 Dues	$	4,002.00	
Extra Articles for 2013 Report	$	25.00	
2014 Dues	$	80.00	
2013 BIC meeting excess	$	4,500.00	
Back Issues	$	150.00	
Bank Interest	$	104.29	
TOTAL INCOME	$	8,861.29	
EXPENSE			
Labor Charges	$	780.37	
Postage, Copy Charges and Office Supplies	$	1,459.85	
Printing and shipment – Volume 56	$	1,467.19	
Google Checkout and PayPal Fees	$	107.26	
Awards for 2013 Portland meeting	$	450.00	
TOTAL EXPENSE	$	4,264.67	
BALANCE AS OF December 31, 2013			**$ 13,070.99**