ANNUAL REPORT OF THE

BEAN IMPROVEMENT COOPERATIVE

BIC

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

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BEAN IMPROVEMENT COOPERATIVE

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Coordinating Committee

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Cover: Seed mixture from the Andean region (photo courtesy of H. Schwartz)

THE 55th ANNUAL REPORT OF THE BEAN IMPROVEMENT COOPERATIVE

The Bean Improvement Cooperative (BIC)celebrated the twenty-sixthBiennial Meeting in San Juan, Puerto Rico. This was the first BIC meeting to be held outside of the continental U.S., and based on the positive responses of the ~120 participants it was an enjoyable success. The meeting began with an Abiotic Stress Symposium and ended with a session on Nutrition and Utilization. Overall there were 37 oral and 38 poster presentations. The Frazier-Zaumeyer Distinguished Lectureship entitled '*Applications of the Common Bean Genome Sequence: Genomics in the Age of Plant Breeding'* was presented by Dr. Phillip McClean, Professor at North Dakota State University. This lecture, usually given at the beginning of the meeting, led off an afternoon session on Bean Genomics instead.

The meeting received generous support from: Harris Moran Seed Company; Monsanto (Seminis) in honor of David Webster;Syngenta Seeds, Inc.; U.S. Dry Bean Council; Puerto Rico Convention Bureau; Provita, Inc.; Office of International Programs, University of Puerto Rico, Mayaguez Campus; Central Bean; Michigan Bean Commission; and Pure Line Seeds, Inc.On behalf of the BIC, I wish to acknowledge the substantial role of the organizing committee, James Beaver and Timothy Porch, 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. Ken Grafton, and three colleagues: Dr. Juan Jose Ferreira, Dr. Tim Porch, and Dr. Carlos Urrea, were recognized for significant contributions to bean research with the BIC Achievement Award. Two student awards were presented for the best oral and poster presentations at the BIC meeting.

The outstanding student oral presentation entitled: *'Determining the Genomic Structure of a QTL Linked to Angular Leaf Spot Resistance'*was presented byPaula Oblessuc from Campinas University, Brazil –Drs. L.B. Rubiano and M. Melotto (University of Texas – Arlington), co-advisors [p.39-40].

The outstanding poster presentation entitled: '*Influence of Leaf Color in a Dry Bean Mapping Population on Emposca sp. Populations and Host Plant Resistance'* was presented by Elizabeth Brisco, from Michigan State University, - Dr. James Kelly, advisor[p.83-84].

The BIC Coordinating Committee added four new members: Dr. Kirstin Bett, University of Saskatchewan; Dr Juan Osorno, North Dakota State University; Dr. Talo Pastor-Corrales, USDA-ARS-Beltsville; and Dr. Dan Wahlquist, Syngenta. The four members stepping down have contributed a combined 59 years of service to the committee: Dr. Howard Schwartz (26 years – 10 as President), Dr. Jim Beaver (14 years), Dr. Bert Vandenberg (14 years) and Dr. Ron Shellenberger (5 years). The BIC community applauds their service which contributedto the continued improvement and success of the organization.

The next BIC Biennial Meeting is planned forPortland or Corvallis, Oregon in October, 2013. The local organizing committee consists of Jim Myers. Details for the 2013 BIC meeting will be posted on the BIC Web page www.css.msu.edu/bic.

Dr. Phillip Miklas, BIC President

BIC COMMITTEE MEMBERSHIP - 1957 to 2012

Coordinating Committee (approximate year of appointment):

1957 Dean, Enzie, **Frazier* (BIC Coordinator/President)**, McCabe, Zaumeyer
1960 Anderson, Atkin, Dean, Enzie, **Frazier**, McCabe, Zaumeyer
1962 Anderson, Atkin, Dean, **Frazier**, Pierce, Polzak, Zaumeyer
1968 Anderson, **Coyne**, Dean, Jorgensen, Polzak, Zaumeyer
1971 Briggs, **Coyne**, Dean, Jorgensen, Polzak, Zaumeyer
1972 Burke, **Coyne**, Dean, Jorgensen, Kiely, Polzak, Zaumeyer
1974 Ballantyne, Bravo, Burke, **Coyne**, Dickson, Emery, Evans, Kiely, Saettler, Zaumeyer
1977 Ballantyne, Bliss, Coyne, **Dickson**, Emery, Evans, Graham, Meiners, Morris, Saettler, Zaumeyer
1978 Atkin, Ballantyne, Bliss, Coyne, **Dickson**, Graham, Meiners, Morris, Saettler, Sprague
1979 Atkin, Bliss, **Dickson**, Graham, Hagedorn, Meiners, Morris, Sprague, Wallace
1980 Atkin, Bliss, **Dickson**, Hagedorn, Morris, Sprague, Steadman, Temple, Wallace
1982 Atkin, Coyne, **Dickson**, Hagedorn, Sprague, Steadman, Temple, Wallace, Wyatt
1983 Coyne, **Dickson**, Hagedorn, Saettler, Silbernagel, Steadman, Temple, Wallace, Wyatt
1985 Coyne, **Dickson**, Mok, Saettler, Silbernagel, Steadman, Temple, Wallace, Wyatt
1986 Coyne, **Dickson**, Mok, Saettler, Schoonhoven, Schwartz, Silbernagel, Steadman, Wallace
1988 Brick, Dickson, Emery, Magnuson, Roos, **Schwartz**, Singh, Steadman, Uebersax
1992 Dickson, Emery, Grafton, Magnuson, **Schwartz**, Singh, Stavely, Steadman, Uebersax
1994 Antonius, Dickson, Grafton, Magnuson, Park, **Schwartz**, Singh, Stavely, Uebersax
1996 Antonius, Grafton, Park, **Schwartz**, Singh, Stavely, Myers, Kotch, Miklas, Riley
1998 Antonius, Beaver, **Kelly**, Kotch, Miklas, Myers, Park, Riley, Schwartz (ex officio), Singh, Vandenberg
2001 Antonius, Beaver, **Kelly**, Kotch, Miklas, Myers, Park, Riley, de Ron, Schwartz (ex officio), Vandenberg
2003 Beaver, **Kelly**, Kmiecik, Kurowski, Miklas, Myers, Park, Riley, de Ron, Schwartz (ex officio), Vandenberg
2006 Beaver, **Kelly**, Kmiecik, Miklas, Myers, Park, Riley, de Ron, Schwartz (ex officio), Shellenberger, Vandenberg
2008 Beaver, **Kelly**, Kmiecik, Miklas, Myers, Pauls, Riley, de Ron, Schwartz (ex officio), Shellenberger, Vandenberg
2010 Beaver, Kelly(ex officio), Kmiecik, **Miklas**, Myers, Pauls, Riley, de Ron, Schwartz, Shellenberger, Vandenberg
2012 Bett, Kelly(ex officio), 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
2012 Noffsinger, Schwartz, Singh

Genetics Committee

2008: Bett, Blair, Gepts, McClean, Miklas, **Porch**, Urrea, Welsh (ex officio).
2011: Bett, Blair, Gepts, Kelly, McClean, **Porch**, Urrea, Welsh (ex officio).
2012: Bett, Blair, Gepts, Kelly, McClean, **Porch**, Urrea, Welsh (ex officio).

GENETICS COMMITTEE MINUTES
2011 Meeting, Puerto Rico

Meeting location: Verdanza Hotel, San Juan, Puerto Rico
Date: Nov. 2, 2011
Time: 2:50 PM to 4:10 PM

Attendance (committee members present)

Kirstin Bett	University of Saskatchewan, Saskatoon	Committee member
Paul Gepts	University of California, Davis	Committee member
James Kelly	Michigan State University	Committee member
Phil McClean	North Dakota State University	Committee member
Tim Porch	USDA/ARS, PR	Chairperson
Carlos Urrea	University of Nebraska	Committee member
Molly Welsh	USDA/ARS, WA (ex officio)	Committee member

Old Business

1. **Approval of the Genetics Committee meeting minutes from the W2150 Meeting in Tucson, Arizona on Feb 17th, 2011. Decision**: Minutes approved by those attending.

2. **Update of the Fusarium wilt gene symbols** (Mark Brick)
   There are two gene symbols for Fusarium wilt, *Fop-1* and *Fop-2*. Ribeiro and Hagedorn (1979) first described the two genes, *Fop-1* conditioning resistance to a race from Brazil that has since been named race 2 (Woo et al., 1996), while *Fop-2*confers resistance to a race from South Carolina that has since been named race 1. It was decided at the previous meeting in Arizona to leave these designations as they are. Current research has shown an additional gene in LEF2RB, for which there are mapped flanking markers, for which *Fop-3* gene designation is being proposed. **Decision**: The Genetics Committee has requested additional evidence for this gene before the gene symbol is approved.

3. **Update on Angular leaf spot gene symbols** (Talo Pastor Corrales)
   There are a number genes that have been named, but not mapped, thus additional evidence is needed before the gene symbols are approved. There are new gene symbols published for ALS (Mahuku et al., 2009) on Pv04 and Pv09 and include Phg_{G5686A}, Phg_{G5686B}, and Phg_{G5686C}. Another gene symbol, $Phg_{G10909B}$, was published by Mahuku et al. (2010) on Pv08, but allelism tests were not completed. **Decision:** These gene symbols, in addition to other gene symbols published by a Brazilian group, need to be reviewed by the genetics committee before approval. These gene symbols can be reviewed by email after the meeting.

New Business

1. **Rust resistance gene nomenclature in tepary, *Ur-Pa1*.** (Carlos Urrea)
   Carlos Urrea presented evidence for a gene for rust resistance in tepary. The *Ur-Pa1* symbol was proposed for this gene. **Decision**: It was decided to continue discussion and review of the evidence for this gene symbol by mail.

2. **Review of anthracnose gene symbol, *Co-14*.** (Celeste Gonclaves-Vidigal)
   The evidence for the *Co-14* gene symbol in cv. Pitanga was reviewed including allelism tests. **Decision**: The *Co-14* gene symbol was approved and can be referred to in the following publication:
   Gonçalves-Vidigal, M.C., Meirelles, A.C., Poletine, J.P., Sousa, L.L., Cruz, A.S., Nunes, M.P., Lacanallo, G.F., and Vidigal, P.S., 2012. Genetic analysis of anthracnose resistance in 'Pitanga' dry bean cultivar. Plant Breed. doi:10.1111/j.1439-0523.2011.01939.x

3. **Membership and Next meeting**
   No changes in Committee membership.

   The next meeting will be held on Monday November 5, 2012 in Niagara Falls, Canada at the Marriott Gateway on the Falls.

   Meeting adjourned at 4:10 pm

2011 BIC AWARD RECIPIENTS

THE BEAN IMPROVEMENT COOPERATIVE

Proudly Presents the

Frazier – Zaumeyer Distinquished Lectureship

to

PHILLIP E.MCCLEAN

Department of Plant Sciences,
North Dakota State University

the

Distinuished Achievement Award

to

JUAN JOSE FERREIRA FERNÁNDEZ
Servicio Regional de Investigación y Desarrollo Agroalimentario
del Gobierno del Principado de Asturias, Spain

TIMOTHY G. PORCH
USDA-ARS Tropical Agriculture Research Station
Mayagüez, Puerto Rico

CARLOS A. URREA FLOREZ
Panhandle Research and Extension Center
University of Nebraska

and the

Meritorious Service Award

to

KENNETH F. GRAFTON
Department of Plant Sciences
North Dakota State University

PHILLIP E. MCCLEAN

Dr. Phillip E. McClean received a BS degree in Biology from the Metropolitan State College of Denver in 1977, and M.S. (1980) and Ph.D. (1982) in Genetics at Colorado State University, with Dr. Donald Wood as his advisor. His M.S. research studied methionine content in beans while his Ph.D research focused on the effect of water stress in protein and carbohydrates. After graduation, Phil had two postdoctoral fellowships at the Universities of Virginia and Missouri working on tomato and maize molecular biology, respectively. In 1985, he joined the department of Plant Sciences at North Dakota State University as an Assistant Professor working on bean genetics and molecular biology. Currently, Phil is a Professor in the department and has been recognized for an outstanding career both as a teacher and scientist. He has served as major advisor for seven M.S. and five Ph.D students, and has received numerous Teaching awards. Phil is a leader in the bean genomics community, and major contributor to the recent momentum in bean genomics research and development. He received the BIC Distinguished Achievement Award in 1999. One of his first scientific contributions to beans was in providing a better understanding of the organization of the genetic diversity in cultivated beans in North America. The article is considered a landmark in bean research as it proved the hypothesis of a narrow genetic base in U.S. cultivars at that time and raised awareness about the need to increase efforts towards broadening the genetic diversity. Given Phil's interest in computers and technology, another important early contribution was the establishment of BeanGenes, a computer database that organized existing and new genetic information, so it could be accessed by bean scientists worldwide. He has published more than 50 peer reviewed articles in areas such as bean genetic diversity and evolution, basic genetics, molecular biology, and disease resistance. Phil conducts research in other crops as well, such as soybean, where he has contributed significantly to understating and leveraging the genome synteny between soybean and common bean, which is providing useful information for both scientific communities. Another area in which Phil has excelled is education and the use of technology to improve teaching and learning in the classroom. Phil teaches both Intermediate Genetics and Plant Molecular Genetics at NDSU using class websites he developed. These websites are open to the public and average 12,000 visitors per week. He was one of the leaders of the Virtual Cell Animation project (Vcell), in which more than 18 computer animations of complex cellular processes have been created for use as learning tools in science classes. The Vcell website has around 7,000 members and its YouTube channel to date has more than 3.5 million views. Phil has contributed more than 20 publications in this area of education and technology and has received awards for his excellence in teaching in 1994 and 2000, and innovative teaching in 1998 and 1999. Phil's most recent accomplishment is the assembling and successful funding of two major projects: the Bean Coordinated Agricultural project (BeanCAP) and the Whole Genome Sequencing of Bean Project. The BeanCAP is a multi-state, multi-disciplinary project focused on the study of the nutritional traits that make beans a healthy food, developing high throughput SNP markers for, association mapping, and training and extension. It is apparent to breeders that Phil is very conscious about the importance and impact of plant breeding and is always looking for ways to adopt new technological tools, such as INDEL markers, to improve the efficiency of the breeding process. The bean sequence project will provide the world a complete genome sequence of beans in the near future. These two projects will have tremendous impact in the future direction of bean research. In summary, Phil has contributed significantly to the bean community during his career as a scientist and educator.

JUAN JOSE FERREIRA FERNÁNDEZ

Dr. Juan José Ferreira Fernández was born on November 2, 1965 in Pravia, a small village in Asturias, northern Spain. He attended primary and secondary schools in Pravia. He studied Biology at the University of Oviedo and earned a Licence title in June 1988. Subsequently, he earned "Tesina de Licenciatura" (an equivalent of M.S. degree) from the Misión Biológica de Galicia, Pontevedra, Spain under the guidance of Dr. Antonio de Ron. There he studied genetic diversity among local *Phaseolus vulgaris* L. collections. Juan José returned to the University of Oviedo where he earned his Ph.D. degree in plant genetics under the supervision of Prof. Ramon Giraldez in 1996. While conducting his doctoral research, he spent three months at CIAT, Cali, Colombia with the dry bean breeding and genetics program of Shree Singh.

At the SERIDA (Servicio Regional de Investigación y Desarrollo Agroalimentario del Gobierno del Principado de Asturias, Spain), in 1997, Juan José initiated breeding of highly priced extremely large (100 to 120 g 100^{-1} seeds) white 'fabada' climbing bean native to the region for resistance to viral and fungal diseases, and determinate growth habit. In 1999, he spent three months learning molecular techniques in the laboratory of Prof. Paul Gepts at University of California-Davis.

Juan José dedicated his early career in characterizing and conserving genetic resources of *Phaseolus* spp., *Triticum* spp, *Corylus avellana* L., and horticultural crops of the region. In common bean, he identified germplasm resistant against the local pathogen races causing anthracnose, white mold, root rots, and *Bean common mosaic virus;* studied the genetics of anthracnose and white mold resistance; and identified and mapped resistance genes and QTL on the integrated linkage map. His breeding program has emphasized development of multiple disease resistant climbing fabada germplasm lines and cultivars. He also successfully developed disease resistant determinate Type I fabada bean germplasm lines (e.g., X2776) and cultivars ('Xana') through a backcross breeding program. His breeding program has made a long lasting impact on production of fabada beans in the region. Juan José has freely shared his improved germplasm lines, cultivars, and accessions of common bean.

He has authored and co-authored 20 refereed articles, maintains a collection of 1500 accessions, and developed 21 breeding lines and cultivars of common bean. He has mentored 6 graduate students in a relatively short period of his professional life despite the fact that he does not have a formal teaching responsibility and is located at a provincial agricultural research station. He currently leads a team of two researchers and two students working in plant genetics and breeding.

TIMOTHY G. PORCH

Dr. Timothy Porch received formal training in plant breeding and genetics at Cornell University. His dissertation research dealt with the genetics and applications of heat tolerance in common bean. A portion of this research was conducted in Honduras which reflects his interest in conducting research of potential benefit to developing countries. As a Postdoctoral Associate at the University of Florida, Dr. Porch cloned and characterized the role of the *Vp10* allele in maize development. He showed that *Vp10* encodes the ortholog of *Cnx1*, which catalyzes the final common step of Moco synthesis, an ABA cofactor. Since 2003, Dr. Porch has worked as a Research Geneticist at the USDA/ARS, Tropical Agriculture Research Station in Mayaguez, Puerto Rico. In a short period of time, he initiated research dealing with a wide range of topics related to common bean breeding and genetics. A major objective of his research program is the utilization of tropical bean germplasm to introduce traits of economic value and to broaden the genetic base of bean germplasm in the U.S. He has developed and released improved bean germplasm with greater tolerance to high temperature and resistance diseases such as common bacterial blight and root rot. In Puerto Rico, he participated in the release of the white bean cultivar 'Verano' and the light red kidney bean cultivar 'Badillo' which have enhanced levels of resistance to common bacterial blight. Dr. Porch is a willing and generous collaborator. He established a shuttle breeding program with the University of Nebraska to increase drought tolerance in common bean. He also conducted collaborative research that led to the identification of bean lines with greater water use efficiency. Dr. Porch has played a key role in the generation, use, and maintenance of the first mutagenesis population for TILLING (targeting induced local lesions in genomes) in common bean. This population, which can be useful for the identification and mapping of important traits in common bean, has been distributed to colleagues in the U.S. and Canada. He participated in collaborative research that led to the identification of the first gene for resistance to common bacterial blight. He has developed innovative field and laboratory techniques for screening beans for disease resistance and tolerance to abiotic stress. Dr. Porch is an active member of the Bean Improvement Cooperative. He currently serves as the Chair of the BIC Genetics Committee which is responsible for the evaluation of requests to assign symbols for genes and QTLs. Dr. Porch has adjunct professor appointments in the Department of Crop and Agro-Environmental Sciences at the University of Puerto Rico and the Department of Plant Science at North Dakota State University. He has provided numerous formal and informal training opportunities for students from the U.S. and developing countries. He currently serves as a Co-Principal Investigator for a Dry Grain Pulse CRSP project working in Central America, Haiti and Angola. He utilized marker-assisted selection to develop bean populations for Haiti and Angola that have enhanced levels of disease resistance. Given the current trajectory of his research program, it can be expected that Dr. Porch will continue to make significant contributions to bean research.

CARLOS A. URREA FLOREZ

Dr. Carlos Urrea, University of Nebraska in Scottsbluff, received his B.S. in Agronomy fromUniversidad Nacional de Colombia, Palmira in 1984. He earned his M.S. degree in Agronomy from the University of Puerto Rico, Mayagüez in 1996. Dr. Urrea earned his Ph.D. degree in Plant Breeding/Minor in Statistics from North Dakota State University, Fargo in 2000. Carlos has worked in barley and corn as breeder. As barley breeder, he released the first non-Chevron, six-rowed barley cultivar with putative resistance to Fusarium head blight, and collaborated in identification of a QTL associated with Fusarium head blight resistance in barley accession CIho4196. As corn breeder, he worked in CIMMYT, Mexico and Nepal. He released 6 subtropical corn testers, and Open Pollinated Varieties and hybrids currently grown in Nepal. Carlos has been working in common beans for almost 19 years, but only 6 years post-graduation. Before joining CIAT, Carlos worked for his B.S. thesis in dry beans (drought/phosphorus). Carlos worked at CIAT, Cali, Colombia, as a Research Assistant in the Bean Breeding and Genetics Program from 1985 to 1994 with Shree Singh. He was in charge of all common bean breeding and genetics studies, field and greenhouse operations, and supervised four field technicians and laborers at CIAT-Quilichao Research Station. His work resulted in the release of six small and medium-seeded common bean cultivars possessing multiple abiotic and biotic stress resistances in Argentina, Bolivia, Brazil, and Mexico, and publication of several papers in refereed journals. While working with Dr. Phil Miklas as a M.S. student, Carlos identified a codominant PCR-based marker linked to the recessive resistance allele *bgm-1* conferring a high level of resistance to *Bean golden yellow mosaic virus* (BGYMV). This marker has been used extensively to introgress BGYMV resistance in dry and snap bean. He also worked on PCR markers for tagging resistance to Macrophomina root rot and common bacterial blight, and collaborated on a research project to develop a RAPD linkage map of disease resistance alleles and QTL in dry bean. Carlos joined the University Nebraska as Dry Bean Breeder in 2005. Carlos' efforts focus on the genetics, germplasm evaluation, and development of dry bean and chickpea cultivars adapted to western Nebraska. Screening bean germplasm for desirable agronomic traits and resistance to major abiotic and biotic stresses is an integral part of his breeding program. This includes evaluating exotic germplasm from CIAT and cultivars, breeding lines, and germplasm from other USA breeders. Recently, Carlos has released great northern cultivars 'Coyne' and 'ABC-Weihing' and germplasm lines MST-1, and SB-DT-1, and one garbanzo germplasm line PHREC-*Ca*-Comp. #1. 'Coyne' is expected to generate a gross income of 2.3 million dollars in 2011 with the royalty fees coming back to his program. Carlos has identified and is mapping a new source of resistance to the African and American isolates of bean common rust pathogen from an interspecific cross. Carlos has developed strong relationships with local (Nebraska Dry Bean Growers Association, Nebraska Dry Bean Commission, Stateline Cooperative Bean Producers) and regional (Bean Coordinated Agricultural Project, BIC, and W-1150/2150) dry bean organizations and individual growers. This facilitates the exchange of information and has resulted in strong financial support from the Nebraska Dry Bean Commission and several speaking invitations. Carlos interacts with about 300 growers each year, providing timely information to assist growers in their dry bean production decisions. Carlos has co-authored more than 33 refereed publications, 27 on beans. Carlos is an active member of the BIC, currently serving on the Bean Genetics and Phaseolus Crop Germplasm Committees. He has also been a member of the W-1150/2150 Multistate Regional Project since 2006 and has served as Secretary, Vice-President, and Chairman (in 2010). Carlos has also served as the coordinator for the Western Regional Bean Trial since 2006. Carlos has graduated 2 M.S. students.

KENNETH F. GRAFTON

Dr. Kenneth F. Grafton is a native of Cleveland, Ohio. He received both his B.S degree in Agriculture and M.S. in Plant Breeding and Genetics from The Ohio State University and his Ph.D. in Plant Breeding and Genetics from the University of Missouri in 1980. Ken joined the Department of Plant Sciences at North Dakota State University first as a research associate in 1980 and as an assistant professor in 1981 when he initiated the first bean breeding program in North Dakota. He advanced through the ranks to Professor in 1994, Associate Dean, Graduate School in 2001; Director, ND Agricultural Experiment Station in 2002; to Dean of the College of Agriculture, Food Systems, and Natural Resources (CAFSNR) in 2005. In those many roles he embodies the Experiment Station's motto '*For the Land and its People'* in not only expanding the facilities for staff and faculty at NDSU that includes a modern state-of-the-art research greenhouse complex valued at ~$36 million to advancing current research capabilities and enhancing student training and education at NDSU; downtown agri-business office and convention center in Fargo; to the hiring of vibrant young faculty in a wide array of disciplines related to agricultural research including bean improvement and new breeding programs for other important pulse crops in the region. Ken has received many recognitions over his career among those was the Distinguished Achievement Award from the BIC in 1995. He has served on a wide array of national committees; he travels extensively in support of research needs of North Dakota and the nation; and he has trained many successful graduate students the least of who is our very own BIC President Phil Miklas. Ken is recognized as establishing the most recent and highly successful public bean breeding program in the country. Many young faculty have the advantage of assuming an active breeding program but Ken had to start the program at NDSU from scratch, with minimum resources and infrastructure, and in a new species as he previously worked in cereal crops. He quickly met that challenge and has developed some of the most widely grown bean varieties in the country. These include Eclipse black, Norstar and Avalanche navy, Maverick, Stampede, and Lariat pinto bean varieties. He collaborated widely in the development and release of over 50 improved germplasm and variety releases. He readily shared germplasm among fellow breeders as attested to by their use as parents in other public and private breeding programs. In addition, Ken has made significant contributions to the control of major bean diseases (especially white mold, rust, and root rots), research on the genetics of seed micronutrients, and improvement of agronomy/production practices in the region. The MinDak area is known worldwide for its bean production and expansion. That industry would not have advanced at the same pace without the intervention of Dr. Grafton in nurturing it in its early stages, meeting the challenges to produce short-season high-yielding varieties suited to its varied environmental conditions; to confronting the many pathological and nutritional challenges the new crop faced in these soils. Even with a busy administrative agenda, plant breeding is still one of his passions. Ken remains involved with the dry bean breeding program, providing technical advice to the new breeder, but still allowing for independence and freedom of thinking. He makes room in his schedule every summer to go to the field, see the beans, and share his vast knowledge and expertise with scientists, stakeholders and growers who hold him in high recognition. Ken has been very successful promoting NDSU agriculture at the international level, with academic/research agreements with several countries such as New Zealand, Australia, Chile, and Puerto Rico, among others. Many of these programs allow for student and faculty exchange, as well as collaborative research. Most of this is recognized by past NDSU President, Joseph Chapman who proclaimed, "NDSU cannot become the great university we want to be without agriculture. NDSU is the Land-Grant University uniquely situated in the most agricultural state in the nation and serving agriculture, the largest industry in the nation. NDSU has become a thriving campus growing rapidly in enrollment, programs, buildings, research and stature. NDSU is truly on the move!" ---so we add in large part due to the efforts and dedicated professional service and vision of Dr. Ken Grafton.

IN MEMORY OF GEORGE FREYTAG

GeorgeF.FreytagofFt.Collins, Colorado passed peacefullyat his homeon September 9, 2011inthepresenceof his wifeanddaughterafter battlingmany years with Alzheimer's disease. Georgewas born February4,1928,inLaramie,Wyoming toFrederick and EvelynFreytag, one offivesiblings.HegraduatedfromtheUniversityofWyomingwitha degreeinBotany and went on to earn the MAandPhD degreesfromWashingtonUniversity,St. Louis,Missouri.

Dr.Freytagspentmore than20years inCentral andSouthAmerica,andtheCaribbean where he was employed in teaching, research and administrative positions. Employers included the Rockefeller Foundation in Mexico, the Government of Nicaragua and the University of Wisconsin in a USAID supported project in Brazil. Dr. Freytag and his wifeAlcinia raisedtheirfivechildrenin El Zamorano,Honduras where he was a professorofagronomyatEscuelaAgrícolaPanamericana. In 1974, they moved to Mayaguez,PuertoRico, where he was employed bytheUSDA-ARS as aresearch geneticist. He participated in the development and release ofbean germplasm with enhanced levels of disease resistance and higher levels of protein. He also served as a Principal Investigator for Bean/Cowpea CRSP projects in the Dominican Republic and Honduras. Dr. Freytag was a member of the American Society of Agronomy, the Crop Science Society of America, the American Genetics Society and the Bean Improvement Cooperative. He maintained a lifelong interest in bean taxonomy. Dr. Freytag's lifelong work with genetic diversity and taxonomy of bean culminated with the collaboration of Dr. Daniel Debouck to publish the book "Taxonomy, distribution, and ecology of the genus *Phaseolus* (Leguminosae-Papilionodeae) in North America, Mexico and Central America".

Georgewas a devotedhusbandwho enjoyed gardening,camping, nature walks, and visitingwithfriends and neighbors. Georgeis survived byhis wifeof 59years of marriage Alcinia;daughter Evelyn;sons Melvin, Paul, Edward andwifeLucyfrom New Orleans,Louisiana;theirchildrenSofiaand David; andJay, (precededin death2009) and his wifeMary;theirchildrenMichael, Hazel, and AlciniaJillfromAnchorage,Alaska. Heis alsosurvived byhis brothers: PhillipandwifeJanefromBelize;Johnandwife JoanniefromYuma,Arizona; Paul andwifeAnn,from Lexington,Kentucky,andRobert, (precededindeath2008) and his wife PatriciafromLaporte,Colorado.

YELLOW BEANS IN LATIN AMERICA

Oswaldo Voysest

Bean Program, Centro Internacional de Agricultura Tropical (CIAT)
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A set of stereotypes are common in the language used to refer to common beans (*Phaseolus vulgaris* L.). One of the most prevalent is that beans are "food for the poor", a statement which is partly correct given that beans are consumed mainly by the lower economic levels of society. However, it is not correct to say that bean consumption is limited only to the poor; actually, beans are consumed by all levels of society with one important difference: the poor eat beans more frequently that the rich.

Despite their many differences in social standing, nationality or race, one thing distinguishes all bean consumers: their marked preference for specific grain types, based on exacting requirements of the right color, size and shape. The opposite of these preferences by bean consumers is a distinct discrimination of grain types other than the ones they are accustomed to. One can draw a parallel between languages and bean preferences to better understand this discriminatory behaviour. Take for example the English language and the small red beans. Both, in the USA and the UK people speak English but users and uses of English can be characterized in terms of variation in region, society, style and medium that actually we could talk about English languages. The same, people in Honduras, Nicaragua, Costa Rica and El Salvador all eat small red beans, but preferences for particular variations in color intensity, brilliance and hilum characteristics of the grain mark the differences among countries and even regions within countries. In summary, like the subtle differences between accents within a single language, a single seed class can have very subtle variations, that in turn affect whether a variety is accepted in a region or not. The subtle variations described for the small red grain type occur in the eight basic colors of bean seeds and in all countries it seems the bean consumer is as particular as the most demanding gourmet. In this article, I will discuss the origins and importance of yellow beans in Latin America.

Countries that consume yellow beans in Latin America

The principal countries that consume yellow beans in Latin America are Peru, Ecuador and Mexico (Table 1). Yellow beans are also planted to a lesser extent across a wide geographic area from Brazil, Bolivia, Chile, Colombia and Panama (Table 2). Bean consumers in these countries have invented a number of descriptive names for the yellow beans they prefer, based on the many tones found in this seed class. If they are widely grown, some of these names are the epithet for a distinct, recognizable commercial seed class.

TABLE 1.- The relationship between different commercial yellow seed classes and the regions within countries that consume yellow beans.

PERU			ECUADOR			
COAST	HIGH-LANDS	JUNGLE	COAST	NORTHERN HIGHLANDS	SOUTHERN HIGHLANDS	MEXICO
Canario	-	-	-	-	-	Peruano[1]
	-	-	Mantequilla	Matahambre	Mantequilla	Canario
-	-	-	-	-	-	Azufrado
-	-	-	-	-	-	Garbancillo
-	Amarillo	-	-	-	-	-
-	-	Ucayalino Huallaguino	-	-	-	-
-	-	-	-	Canario Ojo Negro (BolónAmarillo)	-	-
-	Canario (serrano)	-	-	-	Canario (Bola Amarillo)	-
-	Q'osqo Poroto	-	-	-	-	-

[1] Since 1978 when Azufrado Pimono 78 (Mayocoba) was released

The following conclusions can be made from the above table:

- The term "canario" does not refer to the same type of bean in the three principal countries that consume yellow beans. There are at least 11 different yellow bean types grown and consumed in Latin America
- Each region has its own widely distributed, specific type of yellow bean
- The most demanding region, inasmuch as preference for a particular yellow bean, is the Peruano coast where only one type of yellow bean is accepted, "canario" the very same color that in Mexico is called Peruano or Azufrado Peruano

1. Peru. Yellow color is very important in Peru. This country produces yellow cassava, yellow potato, yellow pepper and yellow beans. Peru is probably the most important center of diversity for yellow beans with two commercial classes that are found nowhere else in the world ("canario" and "Q'osqo Poroto"); two other seed classes ("Amarillo Gigante" and "Ucayalino") that probably originated there but that have now been distributed more widely within but not outside of Latin America. A short description of these beans follows:

a) "Canario" beans, named after the cheerfull little Canary bird with the bright yellow plumage, have been grown along the Peruano coast since ancient times. The Mexicans received from Peru a sample of the variety (Peruano) "canario", a type III bean which they called "Peruano" to distinguish it from their own "canario" type which is a different type of yellow bean with a dark hilum. "Peruano" was later crossed with "Canario 107" a commercial type I bean to produce in 1979 the variety "Azufrado Pimono 78", which was later renamed "Mayocoba", the first Mexican variety to resemble the bright yellow color of the Peruano original "canario". This new (for the Mexicans) yellow color was from then on called "Peruano" or "Azufrado Peruano"

Recently, a private company in the USA, claims to have developed a new variety called "Enola" and has taken out patent rights on the yellow color of the original "Peruano" or "Azufrado Peruano". The company patent wrongly alleges to have introduced yellow beans for the first time to the USA market, when the USDA has several "Azufrado Peruano" plant introductions (P.I. under their original name "canario"), such as the variety "Canario LM", in the germplasm collection at Prosser, Washington. Furthermore, Voysest in 1960 made available the variety to breeders in the USA (BIC Annual Report, Bean Improvement Cooperative BIC Vol 3. p. 23, 1960). The germplasm bank at CIAT, Colombia also has a diverse set of yellow bean varieties in its FAO designated germplasm collection of 26,000 bean accessions that are held in trust for the world community.

b) "Q'osqo Poroto" is another class of yellow beans which is unique to Peru. It is a small, round, yellow popping bean (known locally as "frijol reventón", "ñuña" or "poroto") that is grown exclusively in the southern Andes of Peru. We hope not to see popping beans patented elsewhere out of Peru under an odd assumption.

c) In the highlands there are also large-seeded golden yellow, non-popping beans that are found infrequently in other areas of Andean South America. Similar beans of smaller size are found in the highlands and valleys of Bolivia. Another type of yellow bean, called "Canario Serrano" is grown in the Peruano Andes and also in the southern highlands of Ecuador. In the lowland rainforest of Peru there is a small-seeded golden yellow bean that is found nowhere else in Latin America.

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2. Ecuador. The large round "bolón" beans (above 60 g/100 seed) are characteristic only of Ecuador and Colombia. The Ecuadorian Canario has the same color as the Mexican Canario (light tan with yellow patches; dark hilum) but the size and shape are different

3. Mexico. Up to the late seventies, the Mexican consumers have traditionally had three commercial types of yellow beans: "azufrado", "canario" and "garbancillo". The differences between these seed classes especially between "azufrados" and "canarios" are subtle, albeit important to yellow bean consumers. Beginning in 1979, with the release of the new variety "Mayocoba", the commercial class "Peruano" began to be grown in Mexico. Mayocoba represented a new type of yellow bean for Mexico, with a different tone than the previous three classes: an intense yellow color without the dark hilum ring that is found in all the previous yellow seed classes. It is interesting to note that the original Mexican "canarios" belong to the Andean gene pool and must have been introduced from South America a long time ago. In northern Mexico as in many other countries (Tables 1 and 2) many land race varieties of "canario" beans are sometimes known as "mantequilla" beans, which translated from Spanish are butter beans. In germplasm collections there are many accesions of Mexican type "canario" beans that are called "beurre" beans, which translated from French would also mean butter beans. This French denomination for yellow beans may have been translated inaccurately into "burro" beans which are found in Chile. Alternatively, "burro" the Italian word for butter is also used to describe the "mantequilla" (butter) beans. The other category of yellow beans, the "garbancillos" are truly Mexican and has been classified as belonging to the Jalisco race within the Mesoamerican gene pool.

Yellow beans of Latin America can be considered to be a unique genetic resource found mainly in Peru, Ecuador and México (Tables 3, 4, 5). As such this resource should be protected from misappropriation by individuals who would not give the due recognition of the valuable role that Latin American farmers have had in developing and preserving this interesting set of bean varieties.

TABLE 2. Yellow bean seed classesin Latin America and the countries that produce them on a minor scale

Yellow bean classes	**BRASIL**	**BOLIVIA**	**CHILE**	**PANAMA**	**COLOMBIA**
Jalinho	Jalinho	Mantequilla	-	-	-
Liborino	-	-	-	-	Liborino
Canario	Jalo	Manteca	Mantequilla	Mantequilla	-
Azufrado	Enxofre Enxofrão	-	Burros Azufrado	-	-
Garbancillo	-	-	-	-	-
Peruano	-	-	-	-	-
Amarillo Gigante	-	-	-	-	-
Ucayalino	-	-	-	-	
Q'osqo Poroto	-	-	-	-	-
Canario Bolón	-	-	-	-	-
Canario Bola	-	-	-	-	-

TABLE 3. Varieties of yellow beans developed in Mexico, and Ecuador. 1930 - 1999

COMMERCIAL CLASS	COUNTRY	VARIETY	ORIGIN	YEAR
CANARIO	Mexico	Canario 101	selection	1950's
		Canario 107	selection	-
		Canario Guanajuato 43	selection	-
		Canocel	hybridization	1959
		CIAS 72	hybridization	1972
		Ahome	hybridization	1978
AZUFRADO	Mexico	Amarillo 153	selection	-
		Amarillo 154	selection	1954
		Azufrado 33	selection	1970's
		Azufrado Bolita	selection	-
		Azufrado Regional	selection	-
		Culiacán 200	hybridization	1976
		Cahita 100	hybridization	1978
		Azufrado Tapatio	hybridization	1990
GARBANCILLO	Mexico	Garbancillo Zarco	selection	-
		Garbancillo Supremo	selection	-
BOLA CANARIO	Ecuador	INIAP-419 Canario	selection	1994
BOLON AMARILLO	Ecuador	Canario Ojo Negro	selection	-

TABLE 4. Varieties of unique yellow color from Peru. 1930 – 1999.

COMMERCIAL CLASS	COUNTRY	VARIETY	ORIGIN	YEAR
PERUANO	Mexico	Mayocoba	hybridization	1978
		Azufrado Peruano 87	hybridization	1987
		Azufrado Regional 87	hybridization	1987
		Azufrado Noroeste	hybridization	1995
		Azufrado Higuera	hybridization	1995
	Peru	Canario	land race	-
		Canario LM 1	selection	1944
		Canario LM-2- 57	selection	1957
		Canario Camanejo	land race	-
		Canario Divex 8120	hybridization	1965
		Canario Divex 8130	hybridization	1966
		Canario PF 210	hybridization	1967
		Canario Barranquino	hybridization	1970
		Canario Chinchano	hybridization	1970
		Canario Huaralino	hybridization	1970
		Canario Molinero	hybridization	1970
		Canario Centinela	hybridization	1991
		Canario 2000 INIA	hybridization	1991
		Pata Amarilla	hybridization	1998
AMARILLO GIGANTE	Peru	Q'ello Poroto	land race	-
		Kori Inti	hybridization	1989
		Jacinto INIA	hybridization	1994
HUASCA POROTO	Peru	Ucayalino	land race	-
		Huallaguino	land race	-
Q'OSQO POROTO	Peru	Q'osqo Poroto INIA	selection	1996

TABLE 5. Varieties of yellow beans grown in Brazil, Bolivia, Chile, Colombia y Panama. 1930 - 1999

COMMERCIAL	COUNTRY	VARIETY	ORIGIN
CANARIO	Brasil	Jalo EEP 558	selection
		Jalo Precoce	selection
		Novo Jalo	hybridization
	Bolivia	Manteca Mairana	hybridization
	Chile	Burros Argentinos	selection
	Panama	Mantequilla	selection
		Primavera	-
AZUFRADO	Brasil	Enxofre	selection
		Enxofrão	selection
	Chile	Azufrado	selection
JALINHO	Brasil	EMGOPA 201- Ouro	hybridization
	Bolivia	Mantequilla Mairana	hybridization
LIBORINO	Colombia	Liborino	selection
		Guarzo amarillo	selection

FINAL COMMENTS ON TRUTHS AND LIES IN THE CASE OF ENOLA BEAN VARIETY

"The problem is, yellow beans have been grown in Mexico for Millennia". This argument has been mentioned repeatedly by various opponents of the Enola patent, however, this simply is not true.

a. While it is true that in Mexico there are many yellow beans until 1960-1970 there were no beans in Mexico with Mayocoba characteristic yellow color.

b. The "yellow" bean color Mayocoba "(or Peruano) was only introduced in Mexico presumably in the 1960's. Inexplicably INIFAP, although you can, so far has not provided the exact date when it was introduced from Peru beans Canario (Canario variety presumably LM-2-57) even though this data is recorded in the input files of materials from the Germplasm Bank. It is the ONLY yellow material "Mayocoba" (or Peruano) that owns Mexico. These have subsequently entered the bank of the yellow beans such as Canario Divex 8120 and 8130 also Divex Peru. After Mayocoba Mexican breeders have developed other varieties of Peruano type but always the source of yellow was the Canario Peruano.

Table 6. Mexican Yellow Bean Varieties, Genealogy, Year of Release

VARIETY	YEAR	GENEALOGY
Azufrado Pimono 78 (Mayocoba)	1979	Canario 107 x **PERUANO**
Azufrado Peruano 87	1988	Azufrado 100 x (Canario 107 x **PERUANO)**
Azufrado Noroeste	1995	Azufrado 100 x (Canario 107 x **PERUANO)**
Azufrado Higuera	1995	(Red Kidney x **PERUANO**) x Royal Red

Peruano Canario Bean was introduced from Peru and may be the only source in developingMayocoba type varieties. Those yellow beans from the Peruano type "occur in Mexico for thousands of years ago", simply is incorrect because they have never been grown for that time.

c. Upon receipt of the variety Canario Peru, the Mexicans recorded it as Peruano. They did not use its original name (Canario) in the country (Peru) because Mexico has a commercial class of pale yellow bean very popular also called Canario. The author spent a year working on the Bean Program in Mexico (1958-1959) and never saw a yellow bean color Mayocoba Peruano type or the field or in the germplasm collection. Mexico has a great diversity of Creole yellow beans that have existed in Mexico "for millennia" and which varieties derived by selection: the canario types (Canario 101, Canario 107, etc.). Sulfur types (Yellow 53, Yellow 154, 33 Azufrado etc.) the type garbancillo (Garbancillo Zarco, Supreme Garbancillo, etc). Only from the 70s in Mexico began creating yellow grain varieties by hybridization. In the period 1970-78 were created canario type varieties and sulfur (CIAS 72, Ahome, Azufrado Cahita 200 and 100) using Mexican canario parents. In the development of Mayocoba or Peruano color they never used local germplasm for one simple reason: they did not have in their germplasm collection any bean of that color. Germplasm used only introduced in 1978 (Peru) to develop a new type of bean yellow, or Mayocoba Peruano type (Table 6). This is due to the introduction of Canario Peruano bean type (presumably the variety Canario LM-2-57). With this parent created the variety Azufrado Pimono bean 78 the first Peruano type developed in Mexico. The author was in Los Mochis watching the demonstration plots of 78 Pimono Azufrado planted next to an old

acquaintance of mine the Canario, beans, habit III, most popular and preferred in Peru and which works for 17 years. It is therefore not true that such planting Mexico yellow bean "for millennia. Yellow beans were introduced in 1960s, used in crosses in 1970s, sowed commercially in the 1980s and the Azufrado Pimono became Mayocoba 78 in 1979.

d. The Peruano beans or Mayocoba in Peru: Canario Beans (yellow Peruano or Mayocoba) is the most prized beans kindof in Peru, mainly in central and south coast and parts of the mountains. Its cultivation dates back to the "dawn of time" without being able to determine exactly from where. The variety (a land race) was practically the only Canario sown. It's a habit III of a growing season varying from 120-150 days. In 1944 Boza Barducci made a selection in the variety and launched Canario LM 1 (LM for the station La Molina). With the creation in 1957 the National Bean Program was launched Canario range 2-57 LM (possibly that was sent to Mexico later) derived by selection in the Canario LM 1. This bean was sown in Canario Central Coast to the mid-60's fall between the rows of "ratoon" cotton (cotton pruning) after summer for regrowth in the spring). The beans planted at relatively low temperatures and little sunshine yielded a beautiful yellow bean, unique among all the yellow, just like the plumage of birds canary. A similar custom observed in California in the Salinas Valley where the furrows harvested lettuce was planted in autumn, the California Small White. People called the canario seed in these coastal valleys of Chincha Canario (the valley of Chincha cotton).

On the south coast seeding system in the valleys of Majes rice and beans Camaná also sown in autumn in the same fields in which rice had been harvested (Table 7). The beans planted in the residual moisture of the rice reached the highest yields of Peru and the bean known as Canary Camanejo (through the valley of Camana was a favorite among housewives. In the decade of 60 for health reasons is banned "ratoon" (pruning) of cotton but the cotton farmers in the new variety found Canario Divex 8120, a bush beans, early, which gave greater flexibility in planting and adaptation to farming systems. Canario was the first bean Divex Peruano product of hybridization. Then came another canary beans hybridized products such as Canario Divex 8130 (1966), Canario PF 210 (1975), Canary 2000 (1991), Canary Sentinel (1991), Canary CIFAC 90,105 (1998), Canario CIFAC 90106 (1998), all having the original in Canario genealogy. The valleys of Majes Camana and in the south of Peru did not take any of these varieties as the Canario Camanejo, late and habit III continued to be the best option in their traditional rice and beans.In short, Peru never bought a gram of Enola or Mayocoba seed. It has from time immemorial the yellow color that has generated this absurd controversy. Doubters can travel to Peru and see in different bean valleys, thousands of hectares planted with Canario Peruano, the true color of yellow.

Table 7. Bean Production in Peru. Information: Angel Valladolid (2004)

PERUVIAN VALLEYS	HAS.
Majes y Camaná	5,000
Costa Central (Lima, Huaura, Barranca, etc)	4,000
Costa Sur Medio (Nazca e Ica)	300
Costa Norte (Lambayeque)	200
San Miguel (Ayacucho)	500
Condebamba (Cajamarca)	300
Ancash	300
Huánuco	300

THE 2011 FRAZIER-ZAUMEYER LECTURE

APPLICATIONS OF THE PHASEOLUS VULGARIS GENEOME SEQUENCE: GENOMICS IN THE AGE OF PLANT BREEDING

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A milestone in common bean research (*Phaseolus vulgaris* L.) will soon be reached with the release of a draft of the sequence of the genome. The genome is being sequenced and analyzed by a consortium involving researchers at US universities (Scott Jackson, University of Georgia; Phillip McClean, North Dakota State University), private research institutes (Jeremy Schmutz, Hudson Alpha Genome Sequencing Center), and federal laboratories (Perry Cregan, USDA, Beltsville, MD; Dan Rokshar, Department of Energy, Joint Genome Institute). Early results from the preview release (www.phytozome.net/commonbean.php) based on a Newbler2.6 assembly of Roche 454 sequences found 26,374 protein-coding loci, 4,347 alternatively spliced transcripts, and that the genome contains 41% transposable elements. The important next question is how can the common bean improvement community use the genome sequence information to improve the crop. This is especially urgent in light of the concern for climate change (McClean et al. 2011a) and the continuing trend to move common bean production to more marginal lands regardless of the climate change conditions. The following are examples of how the sequence can be used by itself and in conjunction with other plant species to better understand the crop and address important production and nutrition issues.

Origins, domestication, and genomic organization of the species. It has long been established that common bean is organized into two gene pools, Mesoamerican and Andean, and that domestication occurred independently in these two gene pools. What has not been known is the origin of the ancestral population or the features of the domestication process. Bitocchi et al. (2012) utilized sequence data from multiple genes and placed the ancestral population in Mexico, a result consistent with the geographical location of the closest *Phaseolus* relatives to common bean. The domestication process was modeled by Mamidi et al. (2011) using data from multiple genes across the genome and discovered that each wild gene pool underwent a domestication bottleneck that begin about ~8500-8200 years before present (Ybp) and lasted until ~7000-6300 Ybp, respectively, for the Andean and Mesoamerican gene pools. Associated with the domestication was a post domestication expansion in population size and symmetrical migration between the wild and domesticated populations.

The genome sequence will allow us further understand the history of the species at a much greater degree of granularity. For example, resequencing efforts will provide many more loci for modeling these evolutionary processes. From a plant breeding perspective, resequencing of modern breeding programs will define the extent of similarities/differences that define our major market classes. An in-depth, comparative evaluation of the data may reveal critical regions of the genome that lead to the agronomic success of the crop. This has recently been done with the domesticated forms of rice (Huang et al. 2012) and soybean (Lam et al. 2010).

Molecular breeding and *in silico* marker selection. Bean breeders and geneticists have been pioneers in the application of marker-assisted selection (MAS; Miklas et al. 2006). The next generation of markers, based on extensive sequencing efforts, are being developed by the USDA Common Bean Coordinated Agricultural Project (BeanCAP; www.beancap.org). A SNP

platform that is useful across major market classes is being developed and will be implemented shortly. This will allow breeders to assess the variation within their breeding program for parental selection purposes and mine core collections for genotypes with unique sequence variation (McClean et al. 2011b). The SNP data will also be the important genotypic component for a national association mapping project that is funded by the BeanCAP. The goal is to discover genetic factors associated with important nutritional and agronomic traits, the results of which will further inform breeding decisions. The marker system can also be used to discover markers more tightly linked to major genes of agronomic importance. The sequencing data that supported the SNP discovery program also provided important structural information that has been used to develop an indel-based marker system that will be inexpensive to implement (Mafi Moghaddam et al. 2012).

The Gepts lab, as part of the BeanCAP project, has developed the PhaseolusGenes database (phaseolusgenes.bioinformatics.ucdavis.edu/) that compiles molecular marker data based on genomic sequence data. Using the genome as a reference, breeders and geneticists are able to leverage the growing suite of markers and make educated decisions regarding the best markers to trace a specific gene of interest. The approach was first demonstrated by Gonsalves-Vidigal et al (2011) with their efforts to map disease resistance loci. With the release of the common bean genome sequence this approach will become more robust.

Phenotypic synteny: leveraging legume phenotypic and genotypic data. The most recent estimate is that common bean and its important economic relative, soybean, diverged about 19 million years ago (Lavin et al. 2005). Recent evidence using a gene-based map of common bean (McConnell et al. 2010) has uncovered extensive synteny between common bean and soybean (McClean et al. 2010; Galeano et al. 2011). It may prove useful to leverage knowledge from this two species to discover genes of importance given the extensive investment in research for these crops and their close syntenic relationship. This principle was shown recently by the work of Repinski et al (2012) and Tian (2010) who cloned the *Fin* and *Dt1* gene from common bean and soybean, respectively. These genes control determinate/indeterminate growth habit, and these groups showed that these legume genes were functionally orthologous to *TFL1* the Arabidopsis growth habitgene. Importantly, *Fin* and *Dt1* map to syntenic regions based on genetic and physical map data. Other such physical relationships can be envisioned and once the common bean draft genome sequence is available, collaborations with soybean and other *Phaseoleae* legume researchers may accelerate the discovery of genes important to multiple legume species.

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BREEDING FOR ABIOTIC STRESS TOLERANCE IN COMMON BEAN: ALUMINUM TOLERANCE

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INTRODUCTION

An estimated 40% of the common bean (*Phaseolus vulgaris* L.) growing area is affected by aluminum (Al) toxicity, resulting in yield reductions from 30% to 60% (Wortmann et al., 1998; Thung and Rao, 1999). Aluminum is one of the most abundant minerals in the soil comprising approximately 7% of soil mass. At neutral or weakly acidic pH, it exists in insoluble forms of alumino-silicate or oxide; however, in an acidic soil, it is solubilised into a phytotoxic form. Toxic Al levels damage roots, restrict plant size, and lower yield in most crops (Villagarcia et al., 2001). Root stunting is a consequence of Al-induced inhibition of root elongation.Developing bean genotypes tolerant to acid soil conditions is an ecologically friendly, energy-conserving, and economical solution for resource-poor farmers in the tropics. Wild *Phaseolus vulgaris* evolved in soils with little stress from aluminium, and thus the species tends to be less tolerant that other crops such as cowpea. Sister species of the *Phaseolus* genus that evolved in harsher conditions of acid soil may offer more promise for superior adaptation.

MATERIALS AND METHODS

Two broad strategies were employed to improve the tolerance of common bean to aluminum toxicity. Genotypes of the Andean gene pool were found to present excellent tolerance in greenhouse tests in nutrient solution (Rangel et al., 2010). ICA Quimbaya proved to be especially tolerant. Andean lines were crossed to lines of the Mesoamerican gene pool that were susceptible in nutrient solution but that had a vigorous root system in the field, such as VAX 1. This strategy sought to combine traits from each gene pool to obtain superior tolerance as reflected in yield.

A second strategy sought to employ *Phaseolus coccineus* as a source of aluminum tolerance (Butare et al., 2011a, b). A core collection of *P. coccineus* and *P. dumosus* was evaluated in an aluminum toxic soil in Santander de Quilichao, Colombia. One especially vigorous accession, G35346, was identified, and proved to be tolerant in greenhouse tests both in acid soil in soil cylinders and in nutrient solution as well. This was backcrossed to SER 16, a drought tolerant line that has expressed excellent combining ability, readily transmitting its characteristics of drought resistance, short bush habit, and productive branching. Therefore, SER 16 was employed to produce a backcross-1 population of (SER 16 x (SER 16 x *P. coccineus*)) for improving resistance to aluminum toxicity.

RESULTS AND DISCUSSION

The strategy of using intergene pool crosses (Mesoamerican x Andean) of common bean gave irregular results. While one or more lines presented yields superior to the VAX 1 check in every planting season, no line was consistently superior across seasons. This strategy was eventually abandoned, given the difficulty of obtaining consistent results.

The cross between SER 16 and G35346 generated a population of more than 150 lines that were coded as ALB lines. These displayed wide variability in plant morphology, confirming significant introgression of coccineus genes into the genome of SER 16. Phenotypic evaluation of 94 lines served to confirm that these presented ample variability in their reaction to aluminum toxic acid soil in the field, and also in greenhouse soil and nutrient tests. Sampling of biomass demonstrated that many lines had acquired the capacity for greater biomass accumulation from coccineus (Figure 1). Whereas SER 16 yielded about 1000 kg/ha, several lines yielded 25-30% more, while harvest index also varied widely.

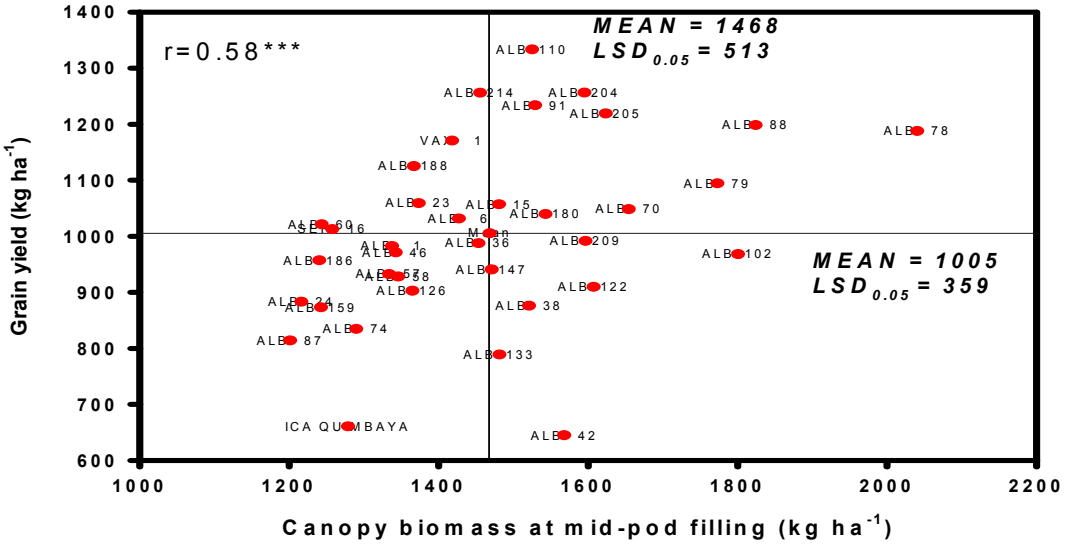

Figure 1: Yield of interspecific lines in an acid soil site, as a function of canopy biomass.

The population was found to be variable for many traits in both nutrient solution and/or soil cylinders: total root length, rooting depth in acid soil, specific root length, number of root tips, leaf area, and shoot biomass. Traits in the acid soil cylinders showed low correlations with yield under acid soil in the field (for example, shoot biomass with r = 0.29**), while traits measured in the nutrient solution showed little tendency to correlate with yield.

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ROOT HAIRS: THE LOW HANGING FRUIT FOR IMPROVING PHOSPHORUS ACQUISITION IN COMMON BEAN

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Low soil phosphorus availability is a primary constraint to bean production: Low soil phosphorus (P) availability is a primary, pervasive constraint to bean production, affecting well over half of the bean production area in Latin America, the Carribean, and subSaharan Africa (Wortmann et al 1998, Lynch 2007). Phosphorus availability is suboptimal for crop growth in many weathered tropical soils and volcanic soils important for bean production, which is exacerbated by the soil degradation and inadequate fertilization common in smallholder agriculture. As a legume, bean has a relatively greater P requirement than cereal crops. An array of root traits have been identified that are associated with variation among bean genotypes for phosphorus acquisition (Figure 1). Several are now being used as selection criteria in bean breeding programs for Africa and Latin America. Of these traits, one of the most promising and straightforward is root hair length and density.

Root hairs are important for phosphorus acquisition: It is well established that root hairs are important for phosphorus acquisition by expanding the effective phosphorus depletion zones around the root. In bean, genotypic variation in root hair length and density is important for phosphorus acquisition regardless of the mycorrhizal status of the plant (Miguel, 2004). Physiological analysis of wildtype and hairless *Arabidopsis* genotypes, and contrasting RILs of maize, indicates that the direct metabolic cost of root hairs is negligible (Lynch and Ho, 2005). Root hair length and density are attractive targets for bean breeding programs because they vary substantially among genotypes (Figure 2), are directly associated with phosphorus acquisition regardless of mycorrhizal status (Figure 3), they are under relatively simple genetic control, and are amenable to direct phenotypic selection (Lynch, 2007). Root hair length and density in bean are controlled by several QTL, which show cosegregation with yield in low phosphorus soils (Yan et al., 2004).

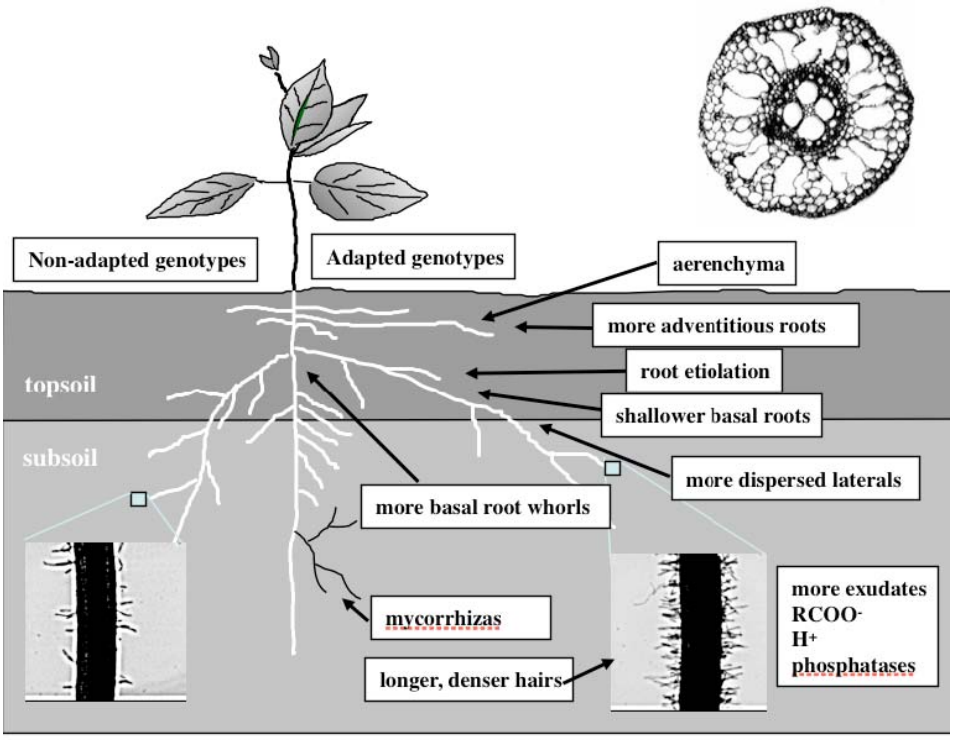

Figure 1. Root phenes associated with genotypic differences in adaptation to low phosphorus From Lynch 2007.

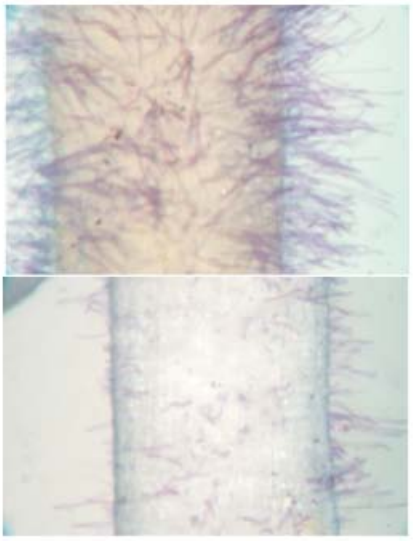

Figure 2. Genotypic variation for root hair length and density in common bean.

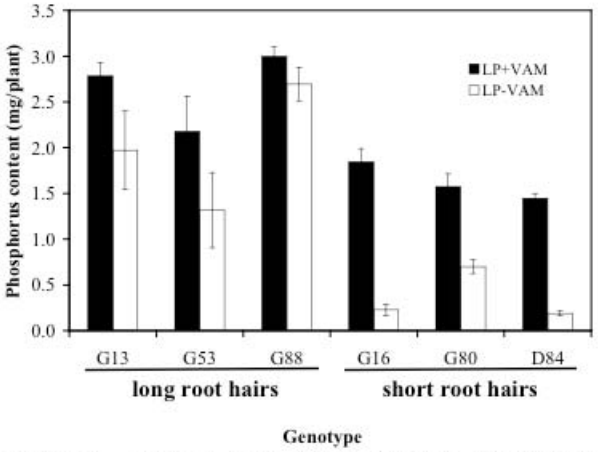

Figure 3. Longer root hairs improve phosphorus acquisition in the presence and absence of mycorrhizal inoculation in common bean. Plants were grown for 28 days in low-P soil in pots with (+VAM) or without (-VAM) mycorrhizal inoculum. Genotypes are recombinant inbred lines having long or short root hairs. Each bar is the mean of 4 replicates, bars = SEM. From Miguel, 2004.

Root hairs are easily phenotyped in bean seedlings: The length and density of root hairs is readily phenotyped in bean seedlings (Vieira et al 2007, http:// roots.psu.edu/en/node/46). Seeds are surface sterilized with 0.5 % NaOCl for one minute, rinsed thoroughly and scarified with a razor blade, then placed 2 cm from the top of brown germination paper (Zhu et al., 2005) soaked in 0.5 mM CaSO4 with radicles pointing downwards. The paper is then rolledinto a moderately tight cigar roll configuration and placed in a 1 L beaker with 100 mL of 0.5 mM CaSO4. Beakers are wrapped with cellophane plastic punctured with holes before being placed in a germination chamber at 28 °C. Five days later, roots of each genotype are conserved in 25 % v/v ethanol immediately after harvest. Root hairs are visually evaluated after staining with 0.05 % trypan blue using a rating scale of 1-9 with a rating of 1 corresponding with no root hairs and 9 corresponding with abundant root hairs (Figure 4, from Vieira et al 2007).

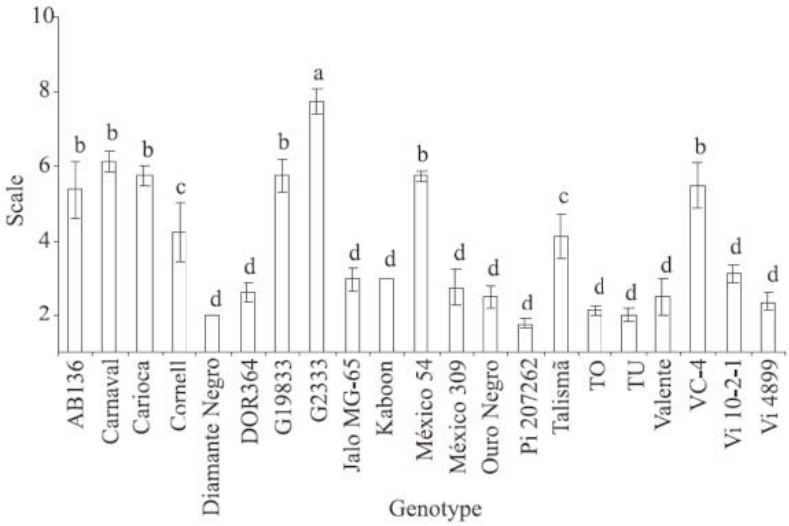

Figure 4. Root hairs in basal roots 5 d after germination of 21 genotypes of common bean. Data shown are means ± SE (n = 4). Columns with equal letters belong to homogenous groups by Scott-Knott test at 5 % of probability.

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BIOLOGICAL NITROGEN FIXATION IN COMMON BEAN: RESEARCH CHALLENGES

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Response to *Rhizobium* inoculation in common beans (*Phaseolus vulgari*s L.) is variable due toedaphic and biotic conditions that affect nodulation and nitrogen fixation. Moreover, genotypic variation in common bean indicates the importance of strain x genotype selection for an efficient interaction.Therefore, improvement of nitrogen fixation in common beans will depend on the selection of genotypes for their ability to nodulate, fix nitrogen and tolerate environmental stresses. *Rhizobium tropici* CIAT 899 and *R. etli* UMR 1597 inoculated on*Phaseolus vulgaris*genotypes have showed significant bean line x rhizobia strain interaction in growth pouches and field studies (Estevez de Jensen, et al. 2011).Significant variation in nodulation, shoot, root and total plant dry weight (DW), and shoot: root ratio were also found in greenhouse studies conducted at Zamorano, Honduras with 180 different genotypes and inoculation of strains CIAT 899 and CR 477 when compared with controls with and without mineral nitrogen (Rosas, Pers. Communication).Two greenhouse experiments were conducted at Zamorano, Honduras, with inoculation of*Rhizobium tropici* strains CIAT 899 and CR 477 in different *Phaseolus vulgaris* genotypes. Relatively greater nodule and total plant dry weight (DW) with strain CIAT 899 was observed in pots containing a soil: sand mixture (1:3) low in N (0.007%), and using nutrient solution with (70 ppm) and withouth nitrogen (Broughton and Dilworth, 1970).In the first experiment, significant differences were found in nodule, plant and root biomass between strains CIAT 899, CR 477, control without inoculation and the nitrogen treatment. No differences for the interaction treatment x genotype were detected. *Rhizobium tropici* strain CIAT 899 increased nodule and plant biomass in Macuzalito and Don Silvio RR when compared with strain CR 477 that resulted in lower nodule and plant biomass (Table 1). In contrast G21212 performed better with strain CR 477 producing greater nodule and plant biomass than with CIAT 899. Contrasting differences were observed with Negro Jamapa and Carioca where nodule and plant biomass were not increased with neither strain compared with the other genotypes (Table 1). In the second experiment Negro Jamapa consistently resulted in the lowest nodule dry weight (240 mg/plant) with CIAT 477 compared to G21212 and Cincuenteño (data not shown). Don Silvio RR nodule biomass was 170 mg/plant with strain CR 477 compared to 347 mg/plant with CIAT 899. These results indicate that genotype by strain interaction are determinant for an efficient nodulation and nitrogen fixation. Also that strain evaluation with different genotypes needs to be assessed before recommending strains for use in commercial inoculants. Superior and inferior common bean genotypes in their ability to nodulate and fix nitrogen will aid breeding programs to define genetic variation in nitrogen fixation.

Table 1. **Effects on nodulation and plant dry weight of *Rhizobium tropici* strains CIAT 899 and CR 477 in *Phaseolus vulgaris* genotypes, Zamorano, Honduras, Trial 1, 2011.**

Genotype	Nodule DW (mg/pl)		Plant DW (g/pl)	
	CIAT899	CR477	CIAT899	CR477
Macuzalito	542	250	4.7	3.6
G21212	384	501	4.7	5.2
Don Silvio RR	359	254	5.2	4.0
IJR	322	367	4.8	4.3
Cincuenteño	226	332	4.4	4.1
Carioca	145	260	2.4	4.0
Negro Jamapa	199	134	3.7	2.5
Range (n= 40)	145-542	71-501	2.4-5.5	1.4-5.3
Mean	274	236	4.1	3.6

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STRATEGIES TO IMPROVE ADAPTATION OF COMMON BEAN TO DROUGHT

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Drought, due to insufficient or unpredictable rainfall, has been identified worldwide as a bean production problem exceeded in magnitude only by bean diseases. Drought affects over 60% of dry beanproduction worldwide and is endemic in the major production areas of northeastern Brazil and north-central highlands ofMexico where it cause yield losses up to 80%. Dry beans cannot be produced in the Western U.S. without supplementalirrigation (Singh, 2007). Problems of water shortage have been accentuated with demographic expansion and climatic changes. The importance and urgencyof developing high yielding drought resistant bean cultivars thatuse water efficiently, and reduce dependence on irrigation watershould help reduce associated production costs and stabilize yieldin drought-prone environments, while increasing profit margins for commercialproducers.

Drought, whether intermittent or terminal in expression, can be confounded with high temperatures, or aggravated by shallow infertile soils, and root rotting pathogens. Recognizing that drought stress varies throughout the Latin American and East African regions, the identification of the nature of drought stress and the type of genotype best suited to the particular stress is critical. Intermittent or sporadic drought occurs when rainfall is not adequate or unpredictable during the growing season whereas terminal drought occurs during the reproductive period and the plant matures during a period when precipitation ceases and moisture becomes limiting. Terminal drought may be even more critical for bean crops grown during a post-rainy season and are entirely reliant on stored soil moisture. Intermittent drought is the most difficult to simulate experimentally and usually requires extensive field testing over years and locations. The type of genotype functional in each system will be different, although adaptation mechanisms such as earliness, remobilization, partitioning might be useful under both types of drought. Bean breeders are most interested in selecting for drought resistance not drought tolerance. Drought resistance is more important since it is related to yield and is defined as the relative yield of a genotype compared to other genotypes subjected to the same drought stress. Those genotypes with high yield potential under non-stress and limited yield loss under stress are most desirable and can be identified based on their geometric mean when grown under both stress and non-stress treatments.

The authors have worked on improving drought tolerance in dry beans in different production countries of Mexico, Honduras, and Ecuador and collaborated with colleagues in the semiarid western production states of Idaho. Successful dry tolerant varieties such as Pinto Villa were developed for production in Mexico and Matterhorn in the US. Under drought stress conditions in the western US the great northern cultivar Matterhorn developed in Michigan in absence of irrigation had the highest average seed yield under stress in that commercial class (Singh, 2007). To further enhance the level of drought tolerance in future bean varieties, different mechanisms such as high pod harvest index (Beebe et al., 2008) functional in drought resistance need to be combined in individual genotypes. Root depth has also been demonstrated as a key architectural

trait in drought tolerance, as the most productive genotype (Frahm et al., 2004) under severe drought stress were shown to possess the deepest root system (Henry et al., 2010).
Foliar traits such as osmotic adjustment, sensitive stomates, and efficient root/shoot transport are recognized as traits functional in drought resistance. Combining foliar and root traits should serve to further enhance the level of drought tolerance in beans. Given the complexity of these traits and absence in most current varieties, there exists a need to screen germplasm to identify potential sources and develop populations where QTL analysis can be conducted to identify the genomic regions controlling these complex traits. Phenotypic screening for specific physiological traits would need to be conducted under controlled greenhouse or growth chamber conditions and final performance under drought could be conducted internationally in locations were persistent drought occurs. Field sites on research stations with access to irrigation would be critical to ensure comparative results under stress and non stress conditions. Finally various recombinant inbred line (RIL) populations are available at present that could be studied for drought tolerance. These include the L88 black bean population studied by Frahm et al. (2004) in Honduras and Mexico, RIL population between Pinto Villa/Pinto Saltillo and bean populations introgressed with wild *P. vulgaris* (Acosta et al., 2007).

Previous research in Mexico has demonstrated the vital importance of phenology and local specific adaptation of genotypes adapted to intermittent drought. These findings underscore the need for local testing and the limitation of identifying single genotypes with broad application across a wide range of environments. Knowledge of gene action of phenological traits such as photoperiod, phenotypic plasticity is limited but genotypes can be developed with these specific traits once recognized as being key in controlling adaptation to local conditions. Earliness is recognized as a key trait for both photoperiod sensitive and neutral genotypes planted under either drought stress. Earliness is confounded with traits like growth habit, and factors such as planting dates (with photoperiod sensitive materials), altitude, temperature and these factors need to be considered in the selection and screening for drought resistance. Finally, the majority of lines recognized as being resistant to intermittent drought were race Durango and possess a high degree of photoperiod sensitivity, as well as phenotypic plasticity. Lines recognized with resistance to terminal drought were race Mesoamerica with type II growth habit and are generally photoperiod neutral. Few Andean sources are known but these generally are race Chile with indeterminate growth habit or are early determinate types from race Nueva Granada which exhibit drought escape. There is a real need to expand the Andean group since these are the preferred types in many regions of the world. One suggestion would be to test the core collection or a sub-core of Andean lines for drought resistance across a selected number of sites with different drought stress conditions.

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STRATEGIES TO IMPROVE ADAPTATION OF COMMON BEAN TO HIGH AMBIENT TEMPERATURE

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High ambient temperatures are a significant constraint to low-land tropical areas and an intermittent constraint to temperatecommon bean production. Temperature increases associated with climate change are predicted to continue this warming trend. To offset this effect, efforts are being made to understand the genetic and physiological response to high-temperature stress and to develop stress tolerant germplasm adapted to heat stress and to targeted diseases.

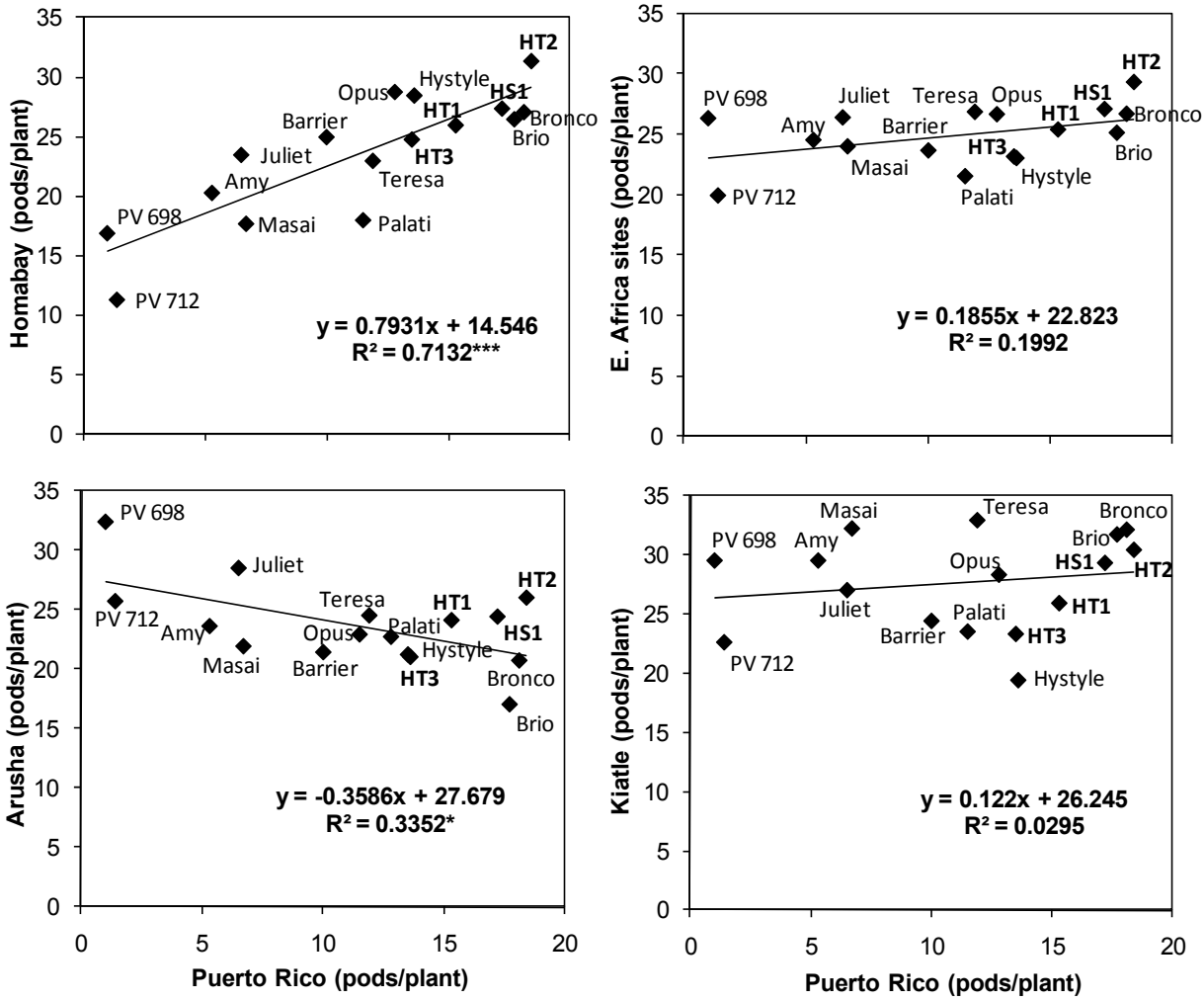
Global warming in pulse growing areas worldwide has recently been shown, with the large majority of countries producing common beans experiencing warming since 1960 (Santana, personal communication). The asymmetric warming trend, with minimum temperatures increasing at a more rapid rate than maximum temperatures, poses an increased threat to common bean due to its higher sensitivity to increases in night-time temperatures. Common bean production regions, such as the lowland tropics, have temperatures close to, or often exceeding, critical average temperatures for bean production, of 30/20°C. Thus, additional global warming, with an estimated increase of 3-5°C by 2100, will result in more frequent high temperature periods in the U.S. and other temperate areas, and a constant high temperature stress environment for beans produced in the lowlands of Central America, the Caribbean, and other regions of the lowland tropics.

Reproductive development is more sensitive than vegetative development to high ambient temperature stress, consequently most genetics and plant breeding efforts have been directed towards understanding the response during this phase. Tolerance to reproductive heat stress, measured as yield, has been shown to be heritable and additive (Roman-Aviles and Beaver, 2003), while reproductive organ abscission has been shown to be controlled by a single gene (Rainey and Griffiths, 2005).Studies have found different numbers of genes involved and their genomic location has yet to be determined. Stress tolerance indices, based on yield, have been employed for selection for heat tolerance (Porch, 2006). High temperature during reproductive development results in increased abscission of buds, flowers, and to a lesser degree, pods. Sensitive plants show a larger number of small pods with aborted seeds, and low yields of smaller seeds. Biotic constraints that coincide with higher temperatures, e.g. Ashy stem blight, bean golden yellow mosaic virus, and common bacterial blight, also need to be considered in breeding programs.

Breeding efforts directed towards specific production regions, including Central America, the Caribbean and East Africa, have resulted in significant advances in tolerance to high temperature stress. In the Americas and the Caribbean, a number of germplasm and cultivars in both dry and snap bean market classes have been released with heat tolerance including, DOR 364, DOR 557, Tio Canela, Amadeus, CENTA Pipil, Cornell 502 and 503, TARS-SR05, Verano, SB-DT1, and TARS-HT1, -HT2, and -MST1. These efforts have resulted in significant yield increases in specific high temperature growing environments, such as El Salvador with the release of CENTA Pipil. Ongoing efforts in East Africa have resulted in the combination of rust resistance and heat tolerance in snap bean germplasm. The pyramiding of *Ur-4* and *Ur-11*, combining both Mesoamerican and Andean sources of rust resistance, and heat tolerance in small

sieve types, has resulted in significant yield gains in trials in Kenya and Tanzania (Wasonga et al., 2010). Recent evidence from these efforts indicate that the heat tolerance trait has broad adaptation. Pod yield in trials in a high temperature site in Kenya (Homabay) are highly correlated with those under high temperature stress in Puerto Rico (Figure 1; top left), while correlations with lower temperature sites have smaller R^2 values.

Figure 1. Regression analysis of pod yield of 16 selected snap bean genotypes from three East African sites: Arusha, Homabay and Kitale, combined with yield from Puerto Rico.



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ADVANCING THE PRODUCTIVITY FRONTIER FOR COMMON BEANS: OPPORTUNITIES AND CHALLENGES

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Pulses (grain legumes) are gaining increased attention from the international agriculture research and development community for their critical role in enhancing the nutritional quality of diets, the lack of gains in grain legume productivity by smallholder farmers, and regarding concerns about increasing grain prices in global markets. As pulses are traditional staple foods for many in developing countries around the world, the fear is that grain prices are approaching levels that will make pulses unaffordable to the poor who are food and nutritional insecure.

Overcoming abiotic and edaphic constrains remain formidable constraints to increasing pulse productivity as insufficient research investments have been made in pulse crops such as common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata*). Bean production in Sub-Saharan Africa and Latin America is commonly on degraded and problem soils which receive limited fertilizer amendments or are eroded due to cultivation of hillsides. With increasing evidence of climate change, beans are also exposed with greater frequency to drought (either intermittent or terminal), excess soil moisture due to episodic major rain events, and to high temperatures which adversely affect pollination and seed set.

In May 2011, the United States Agency for International Development (USAID) released a *Global Food Security Research Strategy* as part of the Presidential Initiative "Feed the Future". The three research themes in the strategy include: (1) advancing the productivity frontier, (2) transforming key production systems, (3) and improving nutritional quality of diets and food safety. This nutrition objective was proposed to be most effectively addressed by increasing the availability of, access to, and consumption of nutrient-dense foods, such as legumes and animal source foods, particularly by women and children. The consensus is that the best approach to improve "access" by food and nutritional insecure poor people to pulse grain is to reduce prices through increases in "productivity" and total production.

To this end, the Pennsylvania State University under the leadership of Dr. Jonathan Lynch convened a workshop (August 14-17, 2011) of nearly 50 grain legume scientists from around the world to formulate recommendations on future research foci "to achieve major increases in pulse productivity under edaphic (soil related) and abiotic stress conditions in small-holder farm settings". The following recommendations, abstracted from the Executive Summary of this workshop, are available from the Dry Grain Pulses CRSP website (www.pulsecrsp.msu.edu/).

A multi-disciplinary research approach to enhancing pulse productivity was advocated with focus on (1) genetic improvement, (2) phenomics, (3) BNF plant/microbe interactions, and (4) systems analysis and management.

The rapidly emerging capacity to affordably sequence the genome of individual species and plants affords plant breeders with incredible tools and a genetic foundation to address the edaphic and climatic constraints to limit legume yields. To realize the potential afforded by genomics, legume communities must develop breeding platforms that: create networks to evaluate many lines for multiple traits across multiple stressful environments; outsource

sequencing of germplasm sets; share, access and interpret information, and convert genomics and phenotypic information into selection strategies (e.g., GWAS, MAS, etc.). The goal is to reveal and deploy genes for tolerance to drought, heat, low soil fertility (low P, N and K plus toxicities to Al and Mn), and grain filling under stress so as to develop improved varieties of bean and cowpea.

Workshop participants also advocated for a comprehensive effort to discover key traits, characterize their utility for specific stress environments, and to phenotypically profile the diversity of grain legume germplasm. For improving adaptation to low soil fertility, phenomics research should include: (1) root traits that enhance acquisition of P and mineral N; (2) physiological processes that enhance utilization of P and N in the plant and adaptation to low soil K, Ca and Mg. For improving adaptation to drought and heat, research priorities should include: (1) traits for efficient water acquisition and utilization; and (2) physiological processes for improved carbon partitioning and grain filling under stress. Phenotypic information would be extremely valuable for guiding breeding strategies, development of high-throughput phenotype screens to identify adapted germplasm and molecular markers, and for the development of integrated crop management strategies that achieve synergies between genetic and agro-ecological technologies.

A third research goal was to identify and capitalize on favorable microbial-plant interactions so as to improve Biological Nitrogen Fixation (BNF) by grain legumes and improved farming system productivity and sustainability on degraded soils. Recommended research actions included: (1) increasing understanding of the effects of environment and soil management practices on microbial populations and symbiotic relations with legumes; (2) systematic breeding to identify superior legumes and *Rhizobium* strains for enhanced BNF; and (3) the establishment of regional centers for *Rhizobium* germplasm curation, inoculant standardization and training.

Systems Analysis and Management was also recommended as a priority research area. The goal would be to identify and promote promising crop management options for grain legume-based agricultural systems that emphasize more efficient use of existing or readily available resources and that ultimately improve the productivity, profitability, and sustainability of small-holder agriculture. Quantitative Systems Analysis (QSA), which combines simulations with geo-spatial analyses, would be highly useful in evaluating the benefit of alternative crop and soil management scenarios on the productivity of diverse legume-based farming systems. Research is also needed to utilize eco-physiological models to better understand the value of specific plant traits in target environments on crop development and gain yield. Participatory multi-disciplinary team research should evaluate strategies identified by QSA under field conditions in target smallholder farm contexts. Increased use of diagnostic and decision management tool kits were recommended to identify options by which farmers might achieve multifunctional goals (productivity, sustainability, nutritional security) for pulse-based farming systems.

The way forward to achieve major increases in pulse productivity requires that research be multi-disciplinary, collaborative, coordinated and accountable for outputs and outcomes. The recommended research priorities will require a sustained long-term commitment by an international community of scientists plus necessitate the leveraging of research investments from diverse donors and sources.

THE APPLIED BEAN GENOMICS AND BIOPRODUCTS PROJECT: SEQUENCING THE BEAN GENOME

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INTRODUCTON

The Applied Bean Genomics and Bioproducts project initiated in Ontario, Canada, in 2011 aims to accelerate the rate of genetic improvement in dry beans by developing genomics resources and tools for genomics- assisted improvement of Phaseolus for bio-product development. The "Phaseolus Genomics for Improved Bio-Product Development" project is organized around five themes, namely:

- development of a draft genomic sequence for *P. vulgaris*
- identification of genes involved in resistance to a bacterial pathogen that causes bacterial blight, a major disease of bean throughout the world,
- characterization of genes that code for the phenylpropanoid pathway that results in an enormous variety of compounds with significant disease resistance, seed quality and nutraceutical importance,
- characterization of the genes that code for protein composition in bean, which is the major reason it is grown throughout the world, and
- determination of the economic consequences of bio-product demand for dry beans.

The results will be incorporated into the combined bean breeding programs of the University of Guelph and Agriculture and Agri-Food Canada, co-located at the University of Guelph. They will be used to develop molecular markers to accelerate the development of improved bean varieties with enhanced disease resistance, healthful qualities, enhanced consumer appeal and utility for protein-based bioproduct manufacture.

Financial support for the project comes from the Ontario White Bean Marketing Board, the Ontario Coloured Bean Growers, Hensall District Co-operative, Agriculture AgriFood Canada and the Ontario Ministry for Research and Innovation. It is a collaborative effort among researchers at the University of Guelph, the University of Windsor and Agriculture Agri-Food Canada scientists with adjunct appointments at these two universities plus the University of Western Ontario.

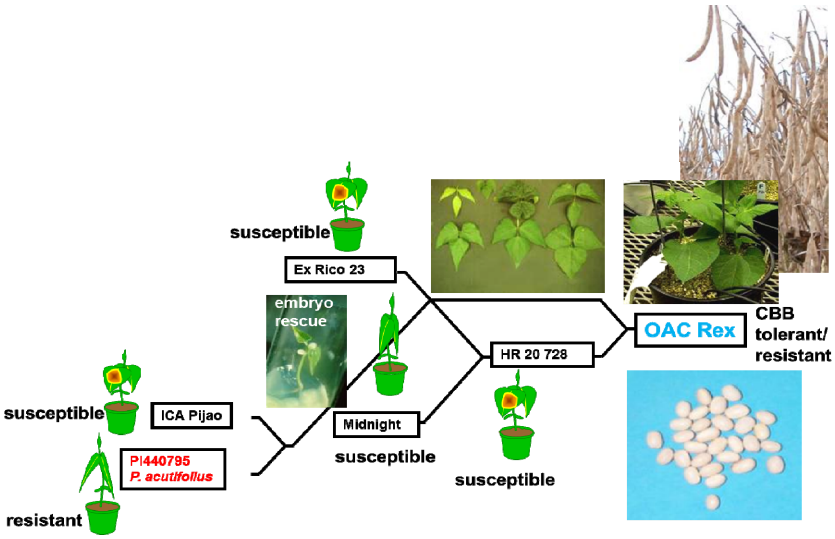
This project will contribute to genomics efforts in bean by sequencing the genome of OAC Rex, a bacterial blight resistant variety grown in Ontario (Micheals et al, 2006). This variety was developed from an interspecific cross between *Phaseolus vulgaris* and *Phaseolus acutifolius* to transfer resistance to bacterial blight from the related wild species to dry bean varieties.

METHODS

Nuclear DNA was extracted from nuclei isolated from three week-old leaves of OAC Rex.

After removal of the plastids, the DNA was extracted using a Qiagen Plant Maxi Kit.

2.5 and 8 kb mate-pair (2 x 100bp) libraries and a paired-end shotgun library (2 x 100bp) were sequencing at the Centre for Applied Genomics (TGAC) SickKids Hospital, Toronto using the Illumina HiSeq 2000 platform.



Pedigree for OAC Rex, a bacterial blight resistant white bean variety derived from an interspecific cross between *Phaseolus vulgaris* and *Phaseolus acutifolius* (PI440795).

RESULTS

Good sequence reads were obtained for all three DNA libraries. Read lengths ranged from 64 to 120bp and the depth was estimated to be greater than 60 fold for the Mate-Pair libraries, and 122x for the shotgun sequencing.

Table 1: Sequencing results for OAC-Rex genomic DNA Libraries

Library	**Number of Reads**	**Read Length**	**Depth**	**GC Content**
2.5Kb Mate-Pair	407,086,824	101.0	62x	41.3
8Kb Mate-Pair	428,996,344	97.8	65x	41.2
Shotgun	778,369,180	102.0	122x	41.3

Assembly is being performed with SOAPdenovo and Abyss.

A description of the project and preliminary results will be available at http://www.beangenomics.ca/

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TRANSCRIPTOMIC ANALYSIS OF *PHASEOLUS VULGARIS* USING 454 PYROSEQUENCING

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INTRODUCTION: This BIC report represents a summary of the work that we have carried out recently in the area of common bean transcriptome analysis. The goal of our work has been to increase molecular genetic and genomic resources in common bean (*Phaseolus vulgaris*), the world's most important food legume. In order to better understand the expression and regulation of genes in common bean, we undertook a global transcriptome sequence analysis of four tissues, leaves, root, flowers and pod using 454 pyrosequencing. From a total of 1,692,972 reads with an average read length of 207 nucleotides (nt), 59,295 unigenes including 39,572 contigs and 19,723 singletons were identified. At least 53.4% of these transcripts had no matches to the common bean ESTs in Genbank, and could be considered as new transcripts. Gene ontology assignments to *Arabidopsis* and soybean indicated that this collection covered a broad range of GO categories. At least 21% of the unigenes match to the common bacterial artificial chromosome (BAC) end sequences and this dataset is further useful for data mining for important genes as well as discovery of molecular markers in common bean.

MATERIALS AND METHODS: Leaves, flowers, and root tissue from the common bean cultivar Sierra, and pods from the common bean genotype BAT93 were collected into envelopes and frozen in liquid nitrogen. RNA isolation, library construction for 454 sequencing and bioinformatic analysis are as discussed in Kalavacharla et al. (2011).

RESULTS: cDNA was derived from the RNA of the four tissues described above. After tagging to identify sequences derived from specific tissues, the libraries were normalized, and the 454 libraries were sequenced and assembled. This resulted in the generation of ~1.6 million reads, with an average length of 207 nucleotides (nt). The total length of the sequences was 350 Mbp derived from three bulk 454 runs. These reads were assembled using gsAssembler (Newbler, from Roche, www.roche-applied-science.com), into 39,572 contigs and 55,051 singletons. Of these singletons, 35,328 were less than 100 nucleotides (nt) in length. A BLASTn search (e-value < $1e^{-10}$) of the 454 sequences against the common bean ESTs in GenBank showed that 27,631 (46.60%) of the 454 unigenes matched known ESTs. Therefore, approximately 31,664 unigenes (53.40%) can be considered as new *P. vulgaris* unigenes.

We were able to map 12,725 unigenes (9,199 contigs and 3,256 singletons) to the available 8,823 BAC-end sequences from genotype G19833. Due to tagging with a molecular barcode, we are able to distinguish between the different transcripts that are present in the various tissues. Based on this analysis, we were able to determine that approximately 69% (41,161 unigenes) of the unigenes were present in leaves, 52% (30,914 unigenes) were present in flowers, 42% (24,725 unigenes) were present in roots, and 36% (21,063 unigenes) were present in pods. Among all the unigenes, 27% (16,155 unigenes) were observed only in leaves, 8% (4,805 unigenes)only in roots, 11% (6,810 unigenes)onlyin flowers, and 6% (3,321 unigenes) only in pods. In order to verify expression of the common bean transcripts identified in this work, we randomly selected 48 contigs for validation. We designed PCR primers from these 48

contigs and analyzed the cDNA under standard PCR conditions. We observed that almost all the amplifications yielded products ranging from 100 bp-150 bp, and that these transcripts are derived from actual expression of genes in common bean.

APPLICATIONS: A wide variety of pathogens, such as common bacterial blight (CBB), anthracnose, bean golden yellow mosaic virus (BGYMV), and rust, can severely constraint common bean yield and reduce seed or pod quality. The transcriptomic sequences derived from the 454 sequencing and the public ESTs in Genbank offer a good opportunity to mine functional genes of interest and develop molecular markers. We used data-mining to reveal putative expressed resistance gene analogs (RGAs). As a result, a total of 365 RGAs were identified which correspond to five different classes of known *R* genes. 105 RGAs were integrated into the existing common bean physical map by in silico mapping. 237 RGAs were mapped onto the common bean genetic map based on the conserved blocks shared by common bean and soybean. 11 STS and 19 CAPS markers were developed for 25 unique RGAs. The expressed RGAs provide a large sequence resource for RGA-tagged marker development and will be useful for genetic mapping of disease resistance. Molecular markers are very important in exploring genetic variation, linkage and quantitative trait loci (QTL) mapping, and marker-assistant selection. In the past two decades, the bean community has exploited a variety of molecular markers such as RFLP, RAPD, AFLP, SSR and SNP, to analyze common bean genetic variation. Gene-based simple sequence repeats (EST-SSRs) are more conserved and informative than genomic SSRs due to their genic origin. Previous efforts of gene-based SSR marker development have provided important information on common bean genetic diversity. However, more markers are still need to saturate the bean linkage map. We have discovered 1,516 gene-based SSRs from our 454 transcriptomic unigenes. Among them, 712 SSRs were newly developed. In order to transfer the new gene-based SSRs into genetic markers, we have designed 508 primer pairs and screened for polymorphism between Bat93 and Jalo EEP558, the two parental lines of the BJ population widely used by the bean community. A total of 76 SSRs were polymorphic. The new gene-based SSR markers will facilitate to enhance molecular density of common bean linkage map and benefit for bean genetic improvement.

CONCLUSIONS: In summary, we identified 59,295 common bean unigenes of which 31,664 unigenes are newly discovered sequences. Combined with existing transcriptomic and genomic sequences available for common bean, this dataset will be very useful for functional genomics research in common bean.The new sequences identified by this study increase by ~150% the number of common bean transcripts that can be used for the discovery of new genes, identification of molecular markers for future genetic linkage and QTL analysis, and comparative studies with other legumes. Comparison of the number of common bean unigene matches to other legumes shows that there may be many more legume unigenes that are yet to be discovered. Therefore, high throughput transcriptome sequencing will continue to help in identifying genes associated with biotic and abiotic stress, development of high resolution genetic maps, and automated phenotyping which will lead to crop improvement.

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OVERVIEW OF GENOMICS-ASSISTED BREEDING OF COMMON BEAN FOR RESISTANCE TO COMMON BACTERIAL BLIGHT (CBB) AT GPCRC, AAFC

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INTRODUCTION

Progresses on structural and functional genomics of bean (*Phaseolus, vulgaris* L.) have provided bean breeders with novel and efficient tools in marker-assisted selection (MAS) for variety development. The availability of bean genome sequence and single nucleotide polymorphism (SNP) markers has changed the traditional MAS practice. The molecular biology and genomics laboratory at the AAFC-GPCRC and the bean breeding program at AAFC/Guelph in collaboration with researchers from USDA and South Africa have adapted the novel genomics approaches in developing co-dominant candidate gene markers associated with CBB resistance through sequencing, and in adapting association mapping method to integrate the two processes, namely CBB resistance QTL (quantitative trait loci) mapping and the CBB resistance variety development, into one process.

MATERIALS AND METHODS

Development of co-dominant candidate gene markers (CO-CGM)

A bacterial artificial Chromosome (BAC) library of HR45, a CBB resistance line, was developed (Yu et al. (2006). Eighteen BAC clones were physically mapped to the genomic region containing the CBB resistance quantitative trait loci (QTL), BC420 (Liu et al. 2010). One of the BAC clone, 4k7, selected with BC420 sequence characterized amplified region (SCAR) marker, was sequenced and candidate genes were predicted using the FGENESH software using Medicago gene model (www.softberry.com). Sequences were annotated according to their homology with known EST sequences. PCR primers were designed from the candidate genes with EST support for marker development. A population of 392 bean lines or cultivars tested in a CBB resistance nursery were used to identify candidate gene markers associated with CBB resistance. A recombinant inbred line (RIL) population was also used to confirm the association of the markers from SU91 with CBB resistance.

Association mapping of breeding lines for CBB resistance

Four hundred and sixty-nine bean cultivars or lines were grown in a CBB resistance testing nursery and 395 of them were evaluated for CBB resistance. One hundred thirty-two single nucleotide polymorphism (SNP) markers were used for genotyping. Associations between SNPs and CBB resistance were analyzed with MLM (Mixed Linear Model) analysis, taking both population structure and kinship into consideration (Shi et al. 2011).

RESULTS AND DISCUSSION

CO-CGM development

Nine annotated candidate genes (CG) derived from the 4k7 BAC sequence containing the BC420 SCAR marker were supported by EST sequences of either *Phaseolus vulgaris*, L. or *P. acutifolius,* L. Six annotated candidate genes derived from the 32H6 BAC sequence containing the SU91 SCAR marker were supported by EST sequences of either *P. vulgaris,* L. or *P. acutifolius*, L or *Glycine max* L. Among the nine BC420 related CG markers, four of them are co-dominant and two of the 6 SU91 related CG markers are co-dominant. Of the 4 BC420 related co-dominant CG markers, only BC420-CG14 is significantly associated with CBB resistance with the CBB resistance data from the 392 lines or cultivars. The BC420 SCAR marker, however, was not significantly associated with CBB resistance for the population. In contrast, both of the SU91 related co-dominant CG markers, SU91-CG10 and SU91-CG11 are highly significantly associated with CBB resistance with the CBB resistance data from the 392 lines or cultivars. The SU91 SCAR marker, however, was also highly significantly associated with CBB resistance for the population. Analysis of the SU91 related CG markers in a recombinant inbred line population indicated that the SU91-CG11 marker was better than the SU91 SCAR marker in that population.

Association mapping

A total of 132 SNPs (single nucleotide polymorphism) were used to carry out association mapping with 395 bean lines or cultivars. Of the 132 SNPs, 94 of them were useful for data analysis. The 94 SNPs were well distributed across the 11 bean chromosomes. Six of the 11 bean chromosomes (2, 3, 6, 7, and 8) were found highly significantly associations with CBB resistance. Significant associations with CBB were also detected on Chromosomes 5, 9, and 10. SNPs g321 on chromosome 2, g471 on chromosome 6 and g796 on chromosome 8 as well as BC420 and SU91 SCARs were all highly significantly associated with CBB resistance for CBB data collected both at 14DAI (day after inoculation) and 21DAI.

CONCLUSIONS

1) Because co-dominant markers are superior to dominant markers for use in marker assisted selection (MAS), we recommend to replace the BC420 SCAR with the new BC420-CG14 and the SU91 SCAR with the new SU91-CG10 and SU91-CG11 markers in MAS of bean for CBB resistance when XAN 159, HR45, HR67, or OAC-Rex were used as CBB resistance source.
2) The SNPs identified in the association mapping study should also be useful in MAS of dry bean for CBB resistance.

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LINKAGE GENETIC MAP DEVELOPED IN THE XANA/CORNELL 42492 RIL POPULATION: A REVIEW

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An inter-gene pool population of 104 $F_{2:7}$ recombinant inbred lines derived from the cross Xana x Cornell 49242 (XC) was used to develop a linkage genetic map. MAPMAKER V 2.0 software was used for the map construction considering a minimum LOD (logarithm of the odds) of 3.0. This work summarizes the current situation of the XC genetic map.

The map consisted of 350 loci which were distributed across 11 linkage groups aligned to the bean core map using common molecular markers as anchor points (Figure 1). XC genetic map includes the following qualitative loci:
- 175 AFLPs named according to their corresponding primer pair combinations (M__E__).
- 76 microsatellites named according to the respective authors (prefixes AT-, BM-, BMd-, Bng-, GA-, and PV-). Nine of them, were developed from the BAC clones available at GenBank: BAC 78L17 (78L17c), BAC 2470 (2470a), BAC FZ-E9 (FZ-E9b, FZ-E9m, FZ-E9n, and FZ-E9o), and BAC-B4-410 (contig-ah, contig-IIIh, and contig-IIIi).
- 40 SCAR markers: 19 SCARs, previously described in common bean: SAA19, SBC6, SW13, SBA8, SF6Em3, SQ4, SR13, SW12, SW6-800R, ROC11, SAS8, SI19, SBB14, SCAreoli (for references see http://www.css.msu.edu/bic/), Bng21 (Murray et al. 2002), AI10 (Vallejos et al. 2006), 254-G15F (David et al. 2008), and CU542014, and TGA1.1 (Gonçalves-Vidigal et al. 2011). Eighteen SCARs developed by Pañeda et al (2008): SAS15, SO15, SZ13, SD3, SD8, SE15, SF8, SG14, SH8, SH13, SH15, SCG5, SH18b, SZ4, SR20, SY4, SU8, and SI19b.
- 25 ISSR markers named according to their respective primers.
- 14 RAPD markers. The name of each RAPD marker is derived from an 'O' prefix for Operon primers, and the letters identifying the Operon primer number.
- 14 loci coding for seed proteins: locus, *Pha*, and 13 other loci, named *SpA* to *SpM*.
- The genes involved in the genetic control of seed coat colour (genes *Asp,asp* and *P,p*), growth habit (gene *Fin,fin*), resistance to BCMV (gene *I,i*), resistance to races 38 and 73 of *Colletotrichum lindemuthianum (*genes *Co-1,co-1* and *Co-2,co-2)* were directly mapped.

Quantitative trait loci (QTL) were located on the genetic map using the QTL Cartographer V2.5 software (Figure 1). Significant QTLs were mapped by composite interval mapping (CIM) analysis using a window size of 10 cM (walkspeed). A LOD score of 2.5 was used as the threshold to determine the presence of significant QTLs. Quantitative trait loci mapped in XC RIL population were: days to flowering (**DF**), days to end of flowering (**DE**), days to harvest as green pod (**DG**), and days to maturity (**DM**); seed length (**SL**), seed height (**SH**), seed width (**WI**), seed weight (**SW**): water absorption (**WA**), coat proportion (**CP**); response to the five isolates (A, B, C, D, and E) of white mold (**WM**); plant height (**PH**), first internode length (**IL**), first internode width (**IW**); response to a local isolate of *Pythium ultimum* based on the seedling vigor (**SV**); cotyledon content in amylose (**A**), apparent amylose (**AA**), protein (**Pt**), starch (**S**);

seed coat content in calcium (**Ca**), dietary fiber (**DFi**), magnesium (**Mg**), and uronic acids (**UA**); and seed content in ashes (**As**).

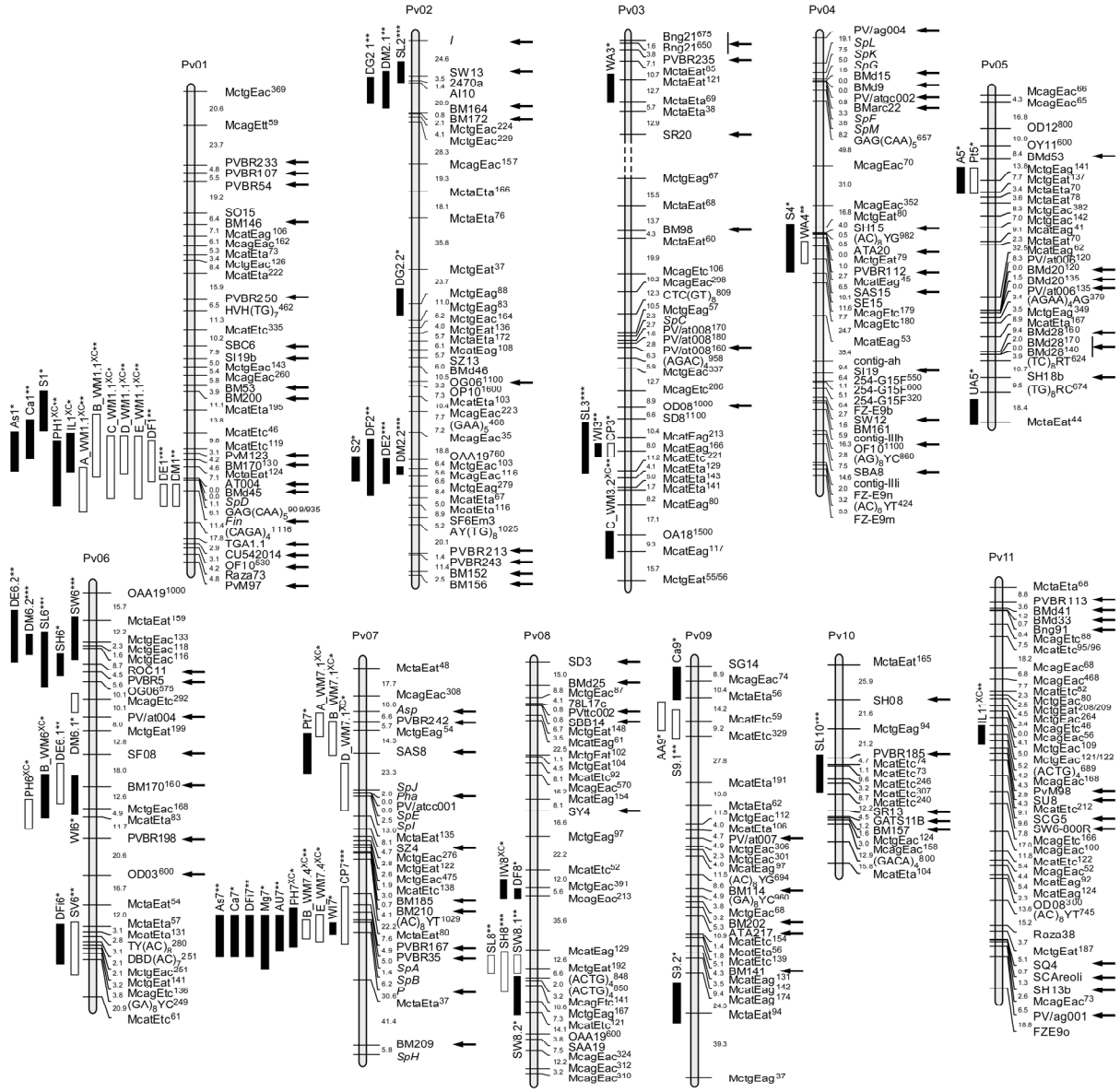

Figure 1. Genetic linkage map for the XC RIL population showing the location of significant quantitative trait loci. Black and white vertical bars represent QTL in which the increase is supported by the alleles of Xana and Cornell 49242, respectively. The number of asterisks near the name of the QTL indicate the number of environments in which the QTL was detected. Arrows indicate common loci to previously published maps.

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SSR MARKERS LINKED WITH SLOW DARKENING TRAIT IN PINTO BEAN WERE DISCOVERED BY SNP ASSAY AND WHOLE GENOME SEQUENCE

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INTRODUCTION

Seed quality of pinto bean (*Phaseolus vulgaris* L.) is assessed by visual factors such as size, color and shape. Postharvest changes in seed color, characterized by a gradual darkening of the light cream background color, can have a detrimental effect on pinto bean quality. The seed darkening is exacerbated by certain storage conditions, such as high relative humidity and high temperatures as well as exposure to light.

Several slow darkening (SD) pintos, conditioned by the presence of the recessive *sd* gene, exist but are poorly adapted(Junk-Knievel et al., 2008; Elsadr et al., 2011). Introgression of *sd* into pinto bean is complicated by the recessive inheritance and expression of the trait in maternal tissue. Our objective was to identify genetic markers tightly linked with *sd* to facilitate breeding for slow darkening pinto beans.

MATERIALS AND METHODS

Three F_2 populations (159 total individuals) segregating for the slow darkening trait were genotyped, following exposure to ultraviolet C (UV-C) light for 72 h (Junk-Knievel et al., 2007), as homozygous slow darkening *sd//sd*, homozygous darkening *Sd//Sd*, or heterozygous darkening *Sd//sd* pintos. The UV-C exposure represents an accelerated darkening test. Source of the *sd* gene was SDIP (Singh et al., 2006) and 1533-15 (commercialized as CDC WM-1 in Canada in 2009).

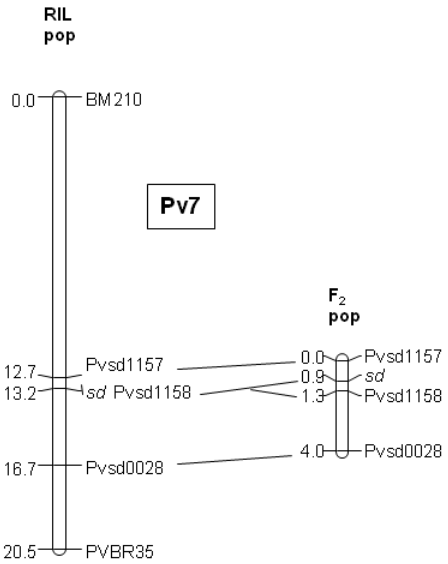
For each population, two bulk samples of DNA were generated. One bulk consisted of DNA pooled from six to eight F_2 plants that were homozygous *Sd//Sd* for the darkening genotype. The other bulk consisted of six to eight F_2 plants that were homozygous *sd//sd* for the slow darkening genotype. These DNA pools were used for bulked-segregant analysis (Michelmore et al., 1991) for identification of SNP markers potentially linked with the *sd* gene. A 1536 SNP array as described by Hyten et al. (2010) revealed two SNPs with alternative alleles between the homozygous bulks. The SNP-containing DNA sequence was aligned to the 20X assembly of the DNA sequence of common bean, using standalone Megablast at a stringency of W=50, p=95. The sequence scaffold possessing the SNP-containing sequence was interrogated for presence of SSRs using the Perl script "MISA" (Thielet al. 2003) as described by Song et al. (2010).

RESULTS AND DISCUSSION

Three of 13 SSR markers generated, Pvsd-1157, Pvsd-1158, and Pvsd-0028 were observed to be tightly linked with the *sd* locus at 0.9, 0.4, and 3.1 cM, respectively across the F_2 populations (Table 1). The SSRs assayed across a recombinant inbred line mapping population (CDC Pintium x 1533-15) placed the *sd* gene on bean linkage group Pv7, between framework SSR markers BM210 and PvBR35 (Fig. 1). A survey of SD and RD advanced lines and cultivars (data not shown) revealed the SSRs will have utility for marker-assisted selection of the slow darkening trait in pinto bean and perhaps in other dry bean market classes as well.

Table 1: Three *sd*-linked SSRs discovered by SNP assay and whole genome sequence.

SSR Name	Primer sequence (5`- 3`)	Expected Size upper/lower band (bp)	Tm (˚C)	Linkage (cM) from *sd* locus		
				Pop I	Pop II	Pop III
Pvsd1157	F AATGGGGAAGATGGTTGGTT R GTGAGGGTTGAAAATTGCGT	170-*sd* / 160-*Sd*	57	0.9	0.0	1.9
Pvsd1158	F GCAATTGACAAAAAGCTTCG R TTGTCATGCGGTTTT	140,130-*Sd* / 120-*sd*	57	0.0	0.0	2.1
Pvsd0028	F TGAAACGCCTAGATAAAATTTAAAAC R TGGTACAATTTTATGAATGATGCC	160-*Sd* / 140-*sd*	57	1.9	1.1	5.5



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IDENTIFICATION OF QTLS AND GENES ASSOCIATED WITH POD LENGTH AS A SNAP BEAN (*PHASEOLUS VULGARIS L.*) DOMESTICATION EVENT

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INTRODUCTION

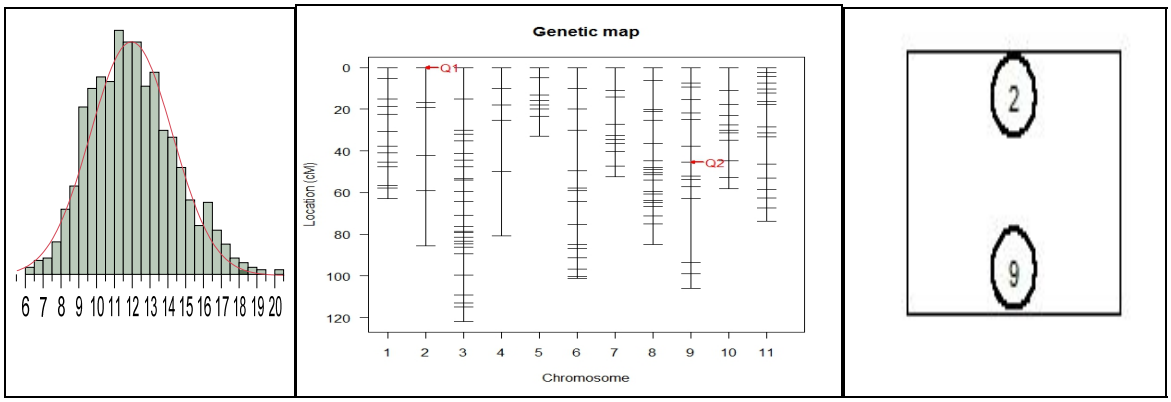
Large genotypic and phenotypic variation exists within common bean (*Phaseolus vulgaris L.*). Based on phaseolin type, snap beans are thought to be derived from the Andean center of origin (Gepts et al., 1986). However, based on molecular marker analysis and genetic diversity, some contemporary snap bean cultivars are derived from both Andean and Mesoamerican origin (Skroch and Nienhuis, 1995). The dry bean evolved from the wild-growing vine type into a major grain crop over a period at least 7000 to 8000 years (Gepts and Debouck, 1999). Selection during domestication has in changes in the size, shape, and color. For example, in wild type beans, the seeds are small, ranging from 6 to 14g/100 seeds, where in domesticated beans, the range is from 20-100g/100 seeds, a 7 fold increase (Gepts and Debouck, 1999). Contemporary snap beans cultivars derived from the dry bean, have long pods with greatly reduced fiber (<20%), and thickened pod wall (Myers, 2000). Variation associated with snap beans is often due to the effects of multiple genetic loci as well as environmental effects. Based on previous research, QTLs for the pod length trait ware mapped on linkage group 2 and 7 on the cultivated common beans x wild bean (Midas x accession G12873) (Koinange et al., 1996). However, pod length has not been mapped in domesticated snap bean population. The objectives of this study are to identify the quantitative trait loci associated with the domesticated traits of snap beans, and to use developing genomic tools to identify the genes associated with the snap bean domestication.

MATERIALS AND METHODS

A recombinant inbred line (RIL) population was developed for Andean x Mesoamerican, EP (Eagle x Puebla). Eagle is a white seeded, green podded snap beans cultivar, with a determinate plant growth, and of Andean origin. Puebla 152 is a black seeded dry bean landrace with an indeterminate of growth habits (IV) of Mesoamerican origin. One hundred fifty RAPD markers have been mapped to the EP population and are being used to identify the QTLs for the domestication traits on this population. R software is being used to detect QTLs. Single interval mapping is being used, by performing a genome scan with single QTL model, where the purpose is to detect loci with important marginal effect.

RESULTS AND DISCUSSION

From the analysis goodness of fit, the means of pod length is not normally distributed (Figure 1a). Pod length in the EP population ranged from 6cm to 20cm with a mean of Eagle and Puebla 152 are 14.7cm and 11.36cm respectively. In the research done by Koinange et al. (1996), the phenotypic variance was 37% and the pod length of the parents were 7.5cm (wild bean, accession G12873) and 6.5cm (Midas). There was no association between the marker and QTLs alleles in the recombinant inbred line population, where the alleles are inherited from one of the parents, G12873 (Koinange et.al, 1996).



(a) (b) (c)

Figure 1: (a) Distribution on EP pod length in cm, (b) location of the QTLs on the genetic map, (c) Plot model in the EP population for pod length traits

Quantitative trait loci (QTLs) have been detected on Chromosomes 2 and 9 on the total pod length trait with EP population (Figure 1b). A plot model has been done to plot a graphical representation of a multiple-QTL model (Figure 1c). The plot shows that the QTL are additive, where no interaction between chromosomes 2 and 9. This result can be used to guide further experiments, perhaps with the aim of fine-mapping the QTL and ultimately identify the gene or genes associated with the total pod length traits. It should be determined using cloning, sequencing and synteny to the soybean genome.

In conclusion, divergence between two gene pools may have led to different, independent suites of genes controlling overall performance. Quantitative trait loci are being detected for domesticated traits such as total pod length. This result is similar to the previous research by Koinange et al (1996). In the future, analysis to identify more QTLs will be continued with other domestication traits such as growth habit, seed color, pod fiber, and presence for string suture.

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RECENT ADVANCES IN BREEDING AND VARIETAL RELEASE OF GRAIN LEGUMES AT UC DAVIS

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Grain legume breeding at UC Davis focuses on lima bean (*Phaseolus lunatus* L.), common bean (*P. vulgaris* L.), and garbanzos (kabuli types of *Cicer arietinum*). A few years ago, UC Riverside (P. Roberts and J. Ehlers) have become the sole program responsible for blackeyed pea [or cowpea, *Vigna unguiculata* (L.) Walp.] breeding. While some older varieties were produced at UC Davis (e.g., CB5 and CB 46), newer varieties are in the UCR pipeline. Lima beans are the most important grain legume, followed by blackeyes, and lastly, a mixed category including several grain legumes, including light and dark red kidneys, lupin, pigeon pea, lablab, velvet bean, adzuki, faba bean, vetch, and pea (Fig. 1.a).

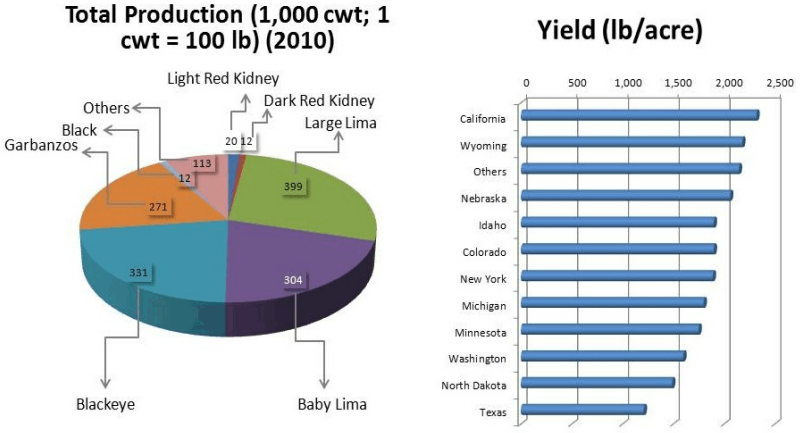

Figure 1. California grain legume production (USDA 2010).a. Various types of grain legumes produced in the state. b. Yield differences among states.

The Grain Legume breeding program at UC Davis has a long history of varietal releases (http://sbc.ucdavis.edu/old_files/57360.pdf ; e.g., Robert Allard, FrancesSmith: http://www.plantsciences.ucdavis.edu/plantbreeding/history/figures/figures.html) and has continued to do so most recently under the guidance of S. Temple. The climatic and edaphic conditions, the agronomic systems of the state, and the specialized production of specific bean classes warrant a California-centric breeding program, when compared to other bean production areas.

California's conditions for bean cultivation include a number of distinctive features. In the Central Valley, it is mostly irrigated desert with deep, well-drained soils. However, some production of lima beans and garbanzos also takes place along the coast (e.g., Santa Maria, Oxnard). Consistent with the Mediterranean climate of the state, rains are distributed only in winter and not in summer. Hence, summer cultivation (of lima and common beans) is done under irrigation, plants are generally devoid of foliar diseases, and yields are quite high (Fig. 1.b). In contrast, garbanzos are grown as a winter crop with a risk of diseases such as *Ascochyta* and white mold. For summer production, the biggest production challenges are insects (*Lygus hesperus*, mites) and some aphid-borne viruses (e.g., BCMV, CPMV).

Grain legume production in California represents a mixture of seed and grain production. Often grain legumes are part of a rotation centered on other crops, such as tomato or wheat. Most commercial varieties in the state originated from UC breeding programs.

Lima beans are produced for their dry seeds (all with white color). They consist of four unique classes: large (Andean) vs. baby (Mesoamerican), both of which can be further subdivided in bush (determinate) and vine (indeterminate). The export market is an important factor, driving a constant push towards improved seed quality. Breeding for *Lygus* resistance has targeted primarily baby limas and has led to two recent releases: UC Beija-Flor (Bush Baby Lima) and UC Haskell (Viny Baby Lima). A future challenge will be to combine *Lygus* resistance (currently mostly in baby limas) with resistance to nematodes (currently in large limes). This will involve an inter-gene pool cross and, therefore, require careful handling of progenies through inbred backcross or other procedures. An additional challenge is the near-absence of molecular linkage map and marker information in lima bean. There is a potential for applying next-generation sequence combined with synteny with common bean and soybean to relatively quickly generate a molecular linkage map, QTL information, and markers for marker-assisted selection in this species. One of the seed production concerns associated with lima beans is their higher level of outcrossing, which warrant larger isolation distances than currently adopted by the industry.

The focus in garbanzo breeding is on yield, grain quality (seed size, color, absence of seedcoat checks), and canning quality. Varieties should also be adapted to winter growing conditions, including resistance to *Ascochyta* blight and white mold (*Sclerotinia*). Along the coast, they should have resistance to *Fusarium* yellows. Plants should also have strong root systems, be upright for direct harvesting, and show resistance to pod shattering. There is a potential for developing varieties for consumption in the green (shell) state, similar to *edamame* soybeans. A company (Califresh: http://califresh.net/) currently markets such edamame chickpeas. Currently, the program relies on the early generation materials that were produced by the USDA-ARS at Washington State University (F. Muehlbauer and K. McPhee). With program changes at the USDA-ARS, other sources of genetic diversity, including a crossing program at UC Davis, will be pursued.

One of the challenges of common bean are the multitude of market types, each with their specific breeding objectives. S. Temple has emphasized the production of varieties for "traditional" types such as dark red kidney (UC Nichols), cranberry (UC 0801), and pink (UCD9634), but has also sought to introduce newer market types to address small-scale demand from organic production, farmer's markets, and "locavores." The latter include yellow beans (UC Canario 707), Flor de Mayo, Jacob's Cattle, and Andino types. Important traits are overall yield, upright growth habit and the incorporation of the *I* gene. Pod shattering remains a problem in some market classes such as the black turtle soup and cranberry. Varieties such as UC 0801 are resistant to shattering (Fig. 2).

Figure 2. Comparison of UC0801, a recent cranberry release with upright growth habit and pod shattering resistance, with a commercial checkEtna

LIMA BEAN BREEDING AND GENETICS RESEARCH AT THE UNIVERSITY OF DELAWARE

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Lima bean (*Phaseoluslunatus*) is an important processing vegetable crop in Delaware. There are approximately 5,670 ha of lima beans planted in the state each year, making limas Delaware's most widely planted vegetable crop. A lima bean breeding program was initiated at University of Delaware in 2004 with the goal of developing varieties with improved yield and disease resistance for the Mid-Atlantic region of the United States. The breeding program now includes three different commercial classes: baby limas, Fordhooks and large-seeded pole limas.

Green Baby Lima Breeding: Green baby lima lines from the program have been tested in yield trials since 2008. In separate trials the breeding lines have also been field screened for downy mildew (caused by *Phytophthora phaseoli*) race E and F resistance each year.

Table 1. Days to Harvest, Yield and *Phytophthora phaseoli* Resistance of University of Delaware Baby Lima Bean Breeding Lines and Standard Varieties in Trials 2009-2011

Line Name	DTH	Yield of Shelled Succulent Beans (kg/ha)	*Phytophthora phaseoli* Reaction*	
			Race E	Race F
DE0505002A	92.0 bc	4532 a	R	S
DE0407907	93.1 ab	3878 b	S	R
DE0407905	91.5 c	3773 bc	S	S
C-elite Select	93.9 a	3747 bc	S	R
DE0402701	92.5 bc	3648 bc	S	S
DE0407903	89.4 de	3611 bc	S	R
DE0505002B	89.5 d	3369 c	S	S
184-85	94.1 a	3349 c	R	S
DE0407906	92.6 bc	3311 cd	R	S
Cypress	87.6 e	2821 d	R	S

*R=resistant, S=susceptible

Based on a combined analysis of yield trial results from 2009 through 2011, one of the Delaware breeding lines, DE0505002A, produced a significantly higher yield than the three standard varieties (C-elite Select, 184-85, and Cypress) and all of the other experimental lines (Table 1). DE0505002A does not have the desirable green seed coat, green cotyledon trait. Green-seeded backcross lines derived from DE0505002A are being developed and the first such line will be trialed in 2012. Another of the Delaware breeding lines, DE0407907, produced significantly higher yields than 184-85 and Cypress. As with all of the currently available commercial lima bean varieties, none of the Delaware breeding lines have resistance to both races of downy mildew present on Delmarva at this time.

Downy mildew resistance to race E is conferred by a single dominant resistance gene, as is resistance to race F. Breeding for combined resistance to downy mildew races E and F in baby limas is being pursued separately from breeding for yield. In 2009 the F_2 progeny of eight different E x F resistant or F x E resistant crosses were field screened for Race F resistance.

Seed was collected from the resistant plants and 705 F_3 seeds were planted in the field in 2010 for screening with race E. Seed was collected from 50 individual E resistant F_3 plants, and the F_4 generation was grown in the greenhouse in winter 2010/11. Half of the F_5 seed of each individual line was field screened for Race E resistance and the other half for Race F resistance. Based on resistant : susceptible segregation ratios in the F_5 populations, the number of F_4 plants lines of each genotype is as follows:

Genotype	EEff	EEFf	EEFF	EeFF	EeFf	Eeff	eeFF	eeFf	eeff
# F_4 Lines	24	0	0	0	8	2	6	9	1

It did not appear that any of the F_4 individual plants were homozygous for both dominant resistance genes. Resistant individuals from the eight heterozygous F_4 lines were selected and seed of more than 80 of these individual selections is being increased in the greenhouse in winter 2011/12 for field screening in 2012. The resistance genes for downy mildew races E and F appear to be at least weakly linked in repulsion.

Fordhook Lima Breeding: Delaware Fordhook breeding lines were evaluated in 2010 and 2011 in small-plot yield trials. In the 2011 trial, 18 of the 22 Delaware lines had significantly higher yields than the standard variety, Concentrated Fordhook (CFH). The three top yielding lines in the 2011 trial DE0600102B, DE0600605C, and DE0600602B, had significantly higher yields than the other the standard variety, Fordhook 242 and were also high yielding in the 2010 trial (Table 2). This is notable because yield stability is the major limiting factor for Fordhook production on the Delmarva Peninsula. CFH produced less than one quarter of the yield in 2011 that it did in 2010.

Table 2. Days to Harvest and Yield of University of Delaware Fordhook Lima Bean Breeding Lines and Standard Varieties in Trials 2010-2011

Line Name	**2010**		**2011**		**2011 Yield as % of 2010**
	DTH	**Yield (kg/ha)**	**DTH**	**Yield (kg/ha)**	
DE0600102B	95	5536 abc	91	4060 a	73.4
DE0600605C	99	6680 a	91	3893 a	58.3
DE0600602B	97	4820 bcde	92	3653 a	75.8
DE0600101D	94	4700 bcde	91	2777 b	59.1
DE0600601D	100	5080 bcde	92	2597 bc	51.1
DE0600605A	94	4284 cde	96	2511 bc	58.6
DE0600602C	95	5033 bcde	97	2399 bc	47.7
DE0601001D	103	4643 bcde	97	2196 bcd	47.3
DE0601001B	95	4567 bcde	101	2024 bcd	44.3
DE0600101A	95	5414 abcd	96	2013 bcd	37.2
Fordhook 242	not tested in 2010		92	2010 bcd	NA
DE0501201A	94	3691 e	101	1884 cde	51.0
DE0504103C	99	5938 ab	95	1840 cde	31.0
DE0501103A	99	4653 bcde	101	1425 def	30.6
DE0600104A	100	5327 abcd	99	1188 ef	22.3
DE0504101D	95	4607 bcde	103	1026 f	22.3
Concentrated FH	98	4066 de	95	940 f	23.1
p-value	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	

BEAN TECHNOLOGY DISSEMINATION MODELS IN HAITI, GUATEMALA AND NICARAGUA: EARLY LESSONS LEARNED FROM SEVERAL PARTNERSHIP MODELS AND KEY ELEMENTS OF SUSTAINABILITY

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The Bean Technology Dissemination (BTD) project ("*Strategic Investment in Rapid Technology Dissemination: Commercialization of Disease Resistant Bean Varieties in Guatemala, Nicaragua, Honduras and Haiti* ") addresses the shortage of high-quality bean seed of improved varieties available to resource-poor farmers in Haiti, Guatemala, Honduras and Nicaragua. The objectives of the project are aligned with the goals of the U.S. Government's "Feed the Future" (FTF) Initiative which involves a multi-agency response to increasing staple food prices and the persistent food insecurity in many developing countries.

Technology dissemination sustainability strategies

The BTD project has placed priority on implementing activities that contribute to the establishment of "sustainable" bean multiplication and dissemination systems in each country. This sustainability goal has also been given nearly equal priority as the dissemination targets (number of beneficiary farmers) by the NARSs partnering in the project. The consensus of the bean sector based on years of experience in both developed and developing countries is that community preoccupation for and commitment to ensuring "seed security" plus farmer ownership of the production of high quality seed (of large-seeded self-pollinated staple grains such as beans) to meet local planting needs are critical "sustainability factors" that should be considered in the design of any bean technology dissemination project. In this regard, the BTD project has paid considerable attention to promoting the establishment of Community Seed Banks (CSBs) in partnership with INTA (Instituto Nicaragüense de Tecnología Agropecuaria) in Nicaragua. INTA's vision has been to implement a sustainable community-based approach for the multiplication of quality seed of improved varieties of staple grains (e.g., open pollinated maize, common bean, sorghum, etc.) to meet the needs of small-holder resource-poor farmers in Nicaragua. Through the BTD project, INTA has been able to effectively implement a national level program that establishes and provides technical assistance to 200 Community Seed Banks (CSB) and promotes the adoption of improved small-red bean varieties with high yield potential and adaptation to the agro-ecologies of Nicaragua.

Early lessons learned from the CSB model

Bean/Cowpea and Dry Grain Pulses CRSP supported research on the socio-economic aspects of bean value-chains revealed that resource-poor smallholder farmers frequently have limited access to quality seed of bean varieties with high yield potential due in large part to a lack of access to financial resources and government assistance programs and to geographic dispersion. Economic shocks and natural disasters (e.g., hurricanes, earthquakes) exacerbate the disconnect that resource-poor bean farmers experience. Community Seed Banks represent a model by which small groups of farmers with social linkages collectively take measures to assure their own "seed security". Through the multiplication and effective storage of seed, farmers in a community are assured of having access to affordable quality seed to plant their next crop, even if they have experienced a crop failure due to whatever reason. As resource-poor small-holder

farmers clearly understand, seed security directly translates to both household food security and the opportunities to generate needed income.

The experience in Nicaragua during the first year of implementation of the BTD project has been highly positive and motivated the CRSP Management Office to promote the Community Seed Bank model in other Central American countries where common bean is extensively grown and consumed. Key aspects of the CSB model that are conducive to sustainability include:

- Building upon existing social networks and capital within a community
- Establishment of long-term seed and grain reserves of critical staple crops to reduce vulnerability and increase resilience of small-holder farmers to episodic environmental and economic shocks
- Farmer access to and understanding of the potential of improved technologies (including quality seed for planting, "registered" seed of improved varieties, fertilizers, post-harvest grain storage silos, etc.) and management practices to enhance bean yields
- Provision of training and technical assistance by the NARS through a network of progressive farmers in a region ("Promotores") which provide leadership to Community Seed Banks and serve as catalysts for transformative change of farming systems.

CSB model transferability to Haiti and other countries

Assessment of the Community Seed Bank model has revealed that transference to other countries and socio-economic-political contexts requires flexibility and adaptation. Each country is distinct due to differences in national seed policies, roles of government entities in the production of foundation and "registered" seed stocks and the certification of "seed" production, government agriculture development priorities, and the existence of functional extension programs required to provide technical assistance to rural farm communities. Clearly one rigid model cannot be forced upon communities of farmers in other contexts (i.e., Sub Saharan African countries). One must have insights into community social structures and decision making, plus understand the keys to success of alternative approaches that might facilitate small-holder farmer access to and adoption of yield-enhancing technologies such as seed of improved varieties within that context.

Project experience in Guatemala in 2011 revealed that the National Agriculture Research System (ICTA) considered the CSB model to be compatible with their mission of ensuring national seed and food security. Constraints encountered however included the capacity of the national extension service (SNEA) and the cost for implementation. With certain modifications, the average cost to establish a CSB in a rural community in Guatemala was determined to be only $600 including the provision of inputs of registered seed, training, grain silos, etc. This modest cost however required a commitment of cost share by the SNEA in the form of support for salaries and transportation for field extension agents.

In Haiti, government receptivity to the CSB model has been less than enthusiastic. Key informants expressed concerns about its viability in the current socio-economic and political environment. Due to the post-earthquake socio-economic hardships, community cohesion and collaboration to improve rural livelihoods does not seem to be embedded in the Haitian value and social systems as in other locations in Latin America.

SCREENING DRY BEAN GENOTYPES FOR NITROGEN-USE EFFICIENCY

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INTRODUCTION

Nitrogen (N) is one of the major essential nutrients in increasing yield of dry bean (*Phaseolus vulgaris* L.) and other crops. Excess application of N fertilizer increases input cost and results in contamination of ground and surface water with nitrate. Therefore, selection of dry bean genotypes with improved N-use efficiency (NUE: dry matter yield per unit N available from soil and fertilizer) is required to reduce input cost and negative environmental effects of N fertilizer application.

MATERIALS AND METHODS

A greenhouse experiment was conducted to screen 22 dry bean genotypes for differences in NUE at low (15 mg N kg^{-1} soil, equivalent to 30 kg N ha^{-1}) and high N supply (50 mg N kg^{-1} soil, equivalent to 100 kg N ha^{-1}). Three bean seeds of each genotype were planted in 12 L pots with soil from Purple Spring, AB (pH: 7.5; NO_3-N: 5; P: 26 and K: 175 mg kg^{-1}), gravel and vermiculite in a 1:1:1 mixture (by weight). Seven days after emergence, seedlings were thinned to one per pot. The experiment was performed as a randomized complete block design with four replications over time. Plants were fertilized weekly with a modified Hoagland's solution lacking nitrogen. At the R1 (flowering) stage of each genotype, plants were harvested by cutting the stem at the surface of the growing mix. The root system was separated into two parts by soil depth: 0-20 cm and 20-40 cm depth. After washing the roots with water, root surface area of each of the two parts was estimated by analysis of scanned images. Cleaned roots were placed in clear Plexiglas tray and submerged in water, and scanned with an Epson Perfection 4990 Photo scanner. Scanned samples were analyzed and root surface area was determined by using Image Pro Plus v4.1. Shoot and root dry weight were determined after drying at 55ºC for one week. All data were subjected to analysis of variance, using the PROC GLM procedure of SAS (Ver. 9.1, SAS Institute Inc. Cary NC). Data were combined where no genotype by treatment interaction occurred.

RESULTS AND DISCUSSION

All investigated traits of dry bean genotypes were significantly ($P<0.0001$) influenced by soil N levels. Genotypes TLP-19, Othello, Topaz and ChinookRRR had highest shoot dry weight (DW) at both N levels. The higher shoot DW of TLP-19, Othello and Topaz at low N level indicates their potential to grow in N-deficient soil (Ahmad et al., 2008). Dry bean genotypes Othello, Topaz, UNS-117 and LEF 2RB had larger root DW (Fig. 1A) and root surface area (Fig. 1B) at both N levels. Increasing root surface enables plant to take up N from a larger soil volume for adequate N acquisition (Fageria et al., 2010). All of the genotypes grown in low N level (Fig. 2A) had a greater N-uptake efficiency than the genotypes grown in high nitrogen level except R99 (Fig. 2B). On average, the plants grown in low N had a better nitrogen-uptake efficiency of 0.665 g g^{-1} DM N compared to 0.317 g g^{-1} DM N for plants grown with high N level.

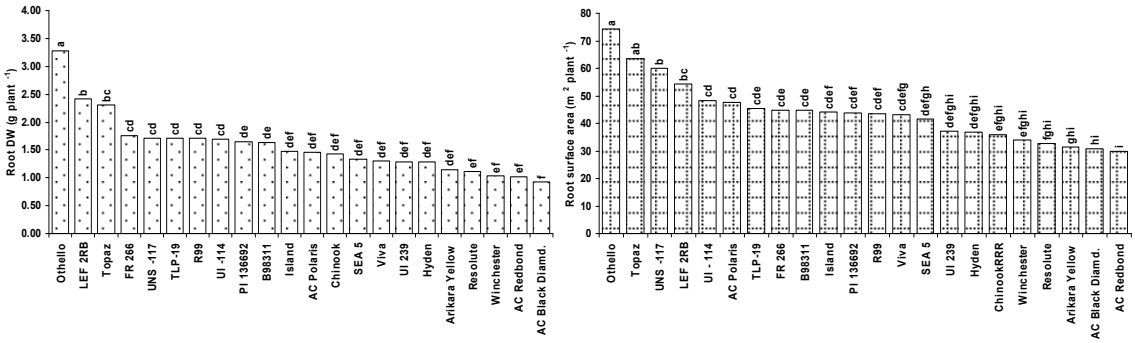

Figure 1: Root Dry weight (A) and root surface area (B) of dry bean genotypes averaged over two N levels. Different letters above the bars indicate the differences between genotypes are significant (P<0.0001).

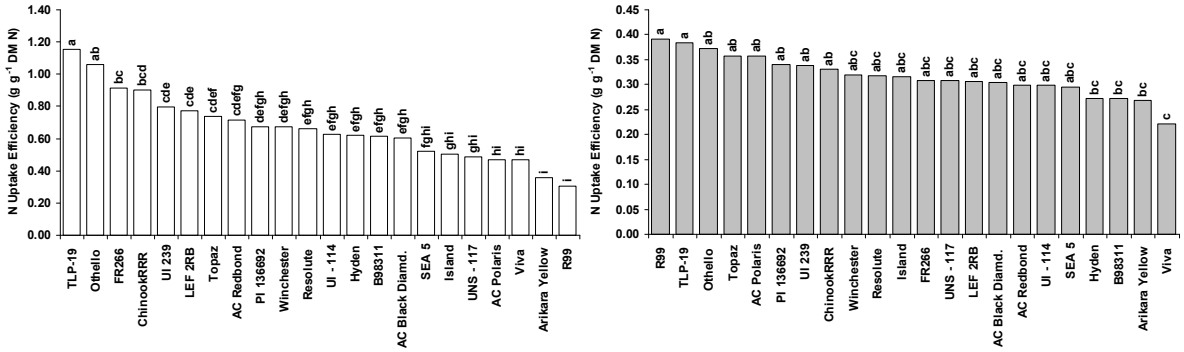

Figure 2: N uptake efficiency of dry bean genotypes at low (A) and high N (B) supply. Different letters above the bars indicate the differences between genotypes are significant (P<0.0001).

CONCLUSIONS

Othello, TLP-19, FR 266 and Topaz were the most N-efficient genotypes with the highest dry matter yield per unit of available N under low N conditions. Shoot and root dry weights and N-uptake efficiency were the main determinants of N use efficiency of dry beans and contribute to the growth of dry bean plants in low N soils with no reduction in shoot and root dry matter yield per unit of available N.

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REINTRODUCING A NOVEL BEAN SPECIES: *APIOS AMERICANA* (MEDIKUS)

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Apios americana, a species in the Phaseoleae tribe, is native to central and eastern North America. Apios produces edible tubers that were a staple of American Indian groups. The tubers are quite palatable, with a potato-like starch and ~16% crude protein (about three times that of potato). Apios is unusual as a "warm-season legume" for its tolerance to winter cold and to flooding. Ongoing work is to characterize, at the at the morphological, molecular, and nutritional levels, diverse lines from wild sources and from a breeding effort in the 1980s, for use as a potential new crop.

Apios is an attractive candidate for a new crop for several reasons. Being native to the Eastern half of the U.S., it is well adapted to this area. Wild populations are primarily clonal, and seem to suffer from few diseases (though powdery mildew, root-knot nematodes, several viruses have been reported; Blackmon and Reynolds, 1986; Valverde, 1990). Yields can be high, with per-plant tuber yields above 3 kg (Blackmon and Reynolds, 1986; Cannon and Belamkar, unpublished). Apios also tolerates wet or waterlogged soil. Nutritionally, Apios is unusual as a starchy tuber, in that it also produces significant quantities of high-quality protein. The protein quality is similar to that in bean seeds, with deficiencies primarily cysteine and methionine (Wilson et al., 1986). *Apios tubers also contain abundant amounts (106.2 to 352.5 mg/100 g) of the 7-O-glucosylglucoside of genistein, which is predominant in the cancer preventive activity of soybeans (Kazuhiro et al., 2010).*

Disadvantages in Apios as a crop are its vining habit (requiring either trellising or allowing trailing growth on the ground); its small tuber size for most genotypes (typically smaller than 50 grams – though some average around 100 g); and the arrangement of tubers like "beads on a string" along stolons that may extend a meter or more from the shoot – making harvesting difficult. Some initial breeding objectives, therefore, include large tubers, high yields, short internodes in stolons, and probably dwarfing in the above-ground habit.

Additional challenges in Apios breeding are that the plant exhibits at least partial self-incompatibility (Bruneau and Anderson, 1988), and seed set has been rare under manual pollinations (Bruneau and Anderson, 1988; Blackmon, pers. comm.).

A breeding program was initiated for Apios improvement in 1985 by Dr. Bill Blackmon and Mr. Berthal Reynolds, at Louisiana State University (Blackmon and Reynolds 1986). Blackmon and Reynolds collected tubers or seeds from 16 states and from southern Quebec (though primarily from Louisiana), and used this germplasm in a succession of crosses and breeding trial evaluations through 1990.

Open crosses were made among the collected lines and from subsequent generations (Reynolds et al., 1990; Blackmon, pers. comm.). Selections were made primarily on the basis of yield, tuber, stolon characteristics. Of more than 2200 progeny evaluated, 50 of the Blackmon and

Reynolds lines, and five wild genotypes (from central Iowa, Ithaca New York, and Laval Canada) are currently under evaluation by Cannon and Belamkar, in Ames, Iowa. These are being phenotyped for approximately two dozen tuber, stolon, and above-ground characteristics. Two years of variety trials have been conducted in Iowa, at three field locations and in several pot conditions. *Two to four genotypes have consistently performed well under Iowa environmental conditions.*

Characterization of sequence-level diversity and of the transcriptome sequences are also being carried out on the current Apios collection. A transcriptome assembly, consisting of 62,614 contigs (and 16,717 additional splice variants), was generated from more than 5 billion Illumina sequence reads from five diverse tissues from one genotype. Additionally, for diversity characterization, approximately half a billion reads per genotype were sequenced from pooled tissues from seven other genotypes. Sequence variants (single nucleotide polymorphisms (SNPs) and small in-del polymorphisms) have been determined for these genotypes.

Preliminary results from the transcriptome sequencing and diversity analysis indicate high rates of nucleotide diversity. A large proportion of loci are heterozygous, as might be expected in a predominantly outcrossing species and in lines representing early generations of a breeding program.

While the outcrossing habit and the high level of heterozygosity will present challenges for a breeding program, it is hopeful to note that we have available now several strong resources: relatively well characterized breeding material, eight densely genotyped lines, and a high-quality transcriptome reference.

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LEGUME IPMPIPE – OVERVIEW OF 2007–2011 CONTRIBUTIONS TO THE LEGUME INDUSTRY

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INTRODUCTION: The Legume ipmPIPE (Pest Information Platform for Extension and Education) enhances the role of extension specialists in Integrated Pest Management (IPM) by providing near real-time access to observations, model output, pest management information, and diagnostic images at http://legume.ipmpipe.org. The Legume *ipm*PIPE Web site includes menus, maps, reports, illustrations, and management links for topics on:

- Legume Crops
  - Common Beans (dry bean, snap bean, and processing bean)
  - Cool Season Legumes (chickpea, field & fresh pea, and lentil)
  - Warm Season Legumes (lima bean and cowpea or black-eyed pea)
- Diseases and Insect Pests
  - Fungal Diseases: Soybean Rust, Common Rust; White Mold; Ascochyta Leaf Spot
  - Bacterial Diseases: Common Bacterial Blight, Halo Blight, Bacterial Brown Spot, and Bacterial Wilt
  - Viral Diseases: *Alfalfa mosaic, Bean common mosaic, Bean yellow mosaic, Beet curly top,* and *Cucumber mosaic viruses*
  - Insect pests (and virus vectors) such as Soybean Aphid, other aphids, and beetles
- Image Gallery & Wiki – e.g., Common Beans (in cooperation with the Bugwood Network)
  - Representative images of priority diseases and pests are available.
- Other Resources
  - State specialists post commentary and survey map reports on diseases and pests.
  - Links are available to resources such as legume growth stages and management recommendations for priority diseases and pests.
  - A real-time Legume Pricing Tool (Multigrain Intl.) was added in 2011
  - A weather summary and forecast resource (ZedX) was added in 2011

PROJECT IMPACTS: During the last 5 years, the Legume ipmPIPE project has evolved in its scope and interactivity with state, regional, and national stakeholders and organizations involved with production and pest management (emphasizing IPM strategies including selection of disease resistant varieties, planting clean seed, suitable crop rotation, scouting and confirmation of economic threats from disease organisms and insect pests, and timely application of pesticides as needed) and marketing of legumes (emphasizing non-soybean crops). Impacts made by this project and its team of state, federal and private industry participants include the following:

- The legume industry representing the following non-soybean pulse crops (2000-2009 records from USDA-ERS) has been impacted by the Legume ipmPIPE project (estimated cost of $350,000/year) with a conservative return of 5% ($48 million or an annual Return on Investment of nearly 140 to 1) by reducing losses from priority diseases and pests affecting:
  - Common Beans - 1,570,000 Acres @ $461 million value ($23 million return)
  - Snap Beans – 100,720 Acres @ $296 million value ($15 million return)
  - Cowpeas – 33,000 Acres @ $18 million value ($1 million return)
  - Lima Beans – 32,000 Acres @ $29 million value ($1 million return)
  - Chickpeas (Garbanzos) – 97,000 Acres @ $27 million value ($1 million return)

- Lentils – 300,000 Acres @ $61 million value ($3 million return)
- Dry Peas - 560,000 Acres @ $88 million value ($4 million return)

- Additional savings include reduced application of pesticides (e.g., fungicides, insecticides) when insect and disease pressures were below economic or injury thresholds; data unavailable on specific amounts of pesticides applied or not during this reporting period.
- A series of 32 field cards covering legume growth stages, diseases (biotic and abiotic), and insects (pests and vectors) has been printed and delivered to more than 10,000 stakeholders throughout North American pulse-growing regions including 20 states in the U.S.; in addition these cards are available online as pdfs. This series has enhanced the accuracy and efficiency of communications and record-keeping by project personnel and stakeholders.
- Field sampling and diagnostic protocols were developed for project use with priority pests and diseases; resulting in more uniform, timely and accurate identification and enumeration of pests and diseases affecting legume crops.
- Image resources (e.g., interlinked web or wiki format) have been developed for 20 priority diseases and pests of legumes with high quality images provided by project participants and stakeholders; web pages have been accessed more than 5,500 times since 2008. Additional links have been made to other educational resources including extension fact sheets, narrated videos on diseases, pest management guidelines.
- The ipmPIPE web site at http://www.ipmpipe.org/ (includes the Legume ipmPIPE) averaged 69,000 hits/month with more than 100,000 hits/month during the legume growing season (May to September).
- Spot monitoring of legume prices (e.g., pinto bean market class) provides a transparent view of market trends for access by stakeholders including more than 10,000 in northern production states and Canadian provinces.
- Access to weather summaries and forecasts provide an invaluable tool for stakeholders to monitor weather conditions that impact production, pest management and harvest decisions related to their legume crops.

ASSETS AND FUTURE PLANS: The greatest asset of the Legume ipmPIPE has always been and remains the outstanding extension specialists, researchers, coordinators, diagnosticians, stakeholders, field workers and others who each year provide "the eyes and feet on the ground" to make this project happen. They are dedicated to the service of the Legume industry, producers, and general public and have been unflagging in their time and devotion. This has forged new linkages with Legume stakeholders and industry enabling the Legume ipmPIPE to be responsive to their needs in the last 5 years, and our ability to meet their future needs depends on continued funding resources.

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REDUCING RISK ASSOCIATED WITH ORGANIC SNAP BEAN PRODUCTION IN WISCONSIN

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INTRODUCTION

In Wisconsin, snap beans (*Phaseolus vulgaris*) are an important processing vegetable crop occupying nearly 80,000 acres of land; less than 2,000 of which are organically managed. Organic snap bean production for processing currently meets less than one-third of the present market demand. Despite price premiums as incentive, it is especially difficult for processors to contract sufficient acres to meet demand due to the high risk and low yields associated with larger-scale organic production. The principal limiting factors effecting organic production in the upper Midwest are: 1) root rot disease (*Pythium ultimum* and *Aphanomyces euteiches* f. sp. *phaseoli*), 2) nitrogen management, and 3) seed corn maggot (*Delia platura*).

In conventional production, snap bean seed is generally treated with a cocktail of insecticides, fungicides and antibiotics to minimize damage to seeds and seedlings due to root and seed rots and seed corn maggot. Dow AgroSciences, LLC has identified a spinosad-based, Organic Material Review Institute (OMRI)-approved insecticide that is effective against seed corn maggot. We have developed a snap bean cultivar resistant to root rot pathogens.

MATERIALS AND METHODS

The objective of this research is to integrate technologies, strategies and experience to determine the optimal genotype, fertilizer type, fertilizer rate, seed treatment and seed source to increase benefits and reduce risk associated with organic snap bean production in Wisconsin. The best management practices are validated with certified-organic, commercial grower cooperators. The experiment was a 2^5 factorial with two contrasting levels for each factor, with 3 reps per location.

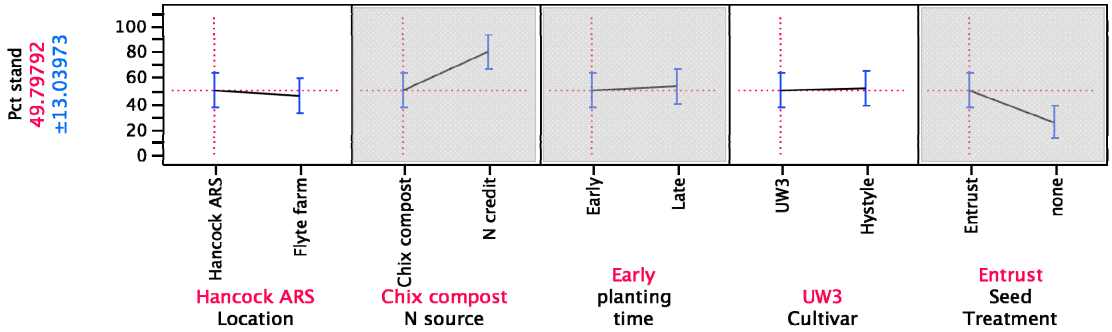
Table 1. Outline of 2^5factorial experimental design

Factor	Levels	Comments
Location	Hancock ARS	Flyte Family Farm is a commercial scale, certified organic producer
	Flyte Family Farm	
Nitrogen source	Chicken Guano	Nitrogen credit at Flyte Family Farm from clover. Nitrogen sources at Hancock ARS from cow manure.
	Prior year nitrogen credit	
Planting time	Early (mid-May)	Flights of ovipositing adult seed corn maggots vary over the season
	Late (mid-June)	
Cultivar	UW3	UW3 is root rot resistant and Hystyle is susceptible
	Hystyle	
Seed treatment	Entrust (0.50 mg ai/seed)	Seed was treated only with Entrust.
	None (naked)	

RESULTS AND DISCUSSION

The ANOVA for plant stand included all main effects, as well as, all first order and higher order interactions. The significant main effects and interactions from the ANOVA for plant stand are illustrated in Figures 1 and 2 below.

Fig. 1. Graphic display of main effects. Significant main effects are illustrated by gray shading.



Significant Main Effects

- Nitrogen source - Plant stands were lower in composted chicken guano (74.7%) compared to fields with a prior year legume as a nitrogen credit (89.3%).
- Planting time – Early planting (mid-May) had a lower plant stand (76.8%) compared to later planting (mid-June) (87.2%). This likely corresponded to flights and oviposition of the seed corn maggot.
- Entrust – Seed treated with 0.50 mg ai/seed Entrust had significantly higher plant stands (91.6%) compared to non-treated, naked seeds (72.3%).

There was no significant difference in plant stand between the two cultivars suggesting that plant stands were not affected by root rot at either location. The first order interactions for percent plant stand among locations (Hancock vs. Flyte), planting time (early vs. late) and seed treatment (Entrust vs. naked) all suggest that pressure of seed corn maggot was not consistent over locations and planting times. Entrust seed treatment was only effective if seed corn maggots were present.

CONCLUSIONS

The prior year nitrogen credit resulted in a higher plant stand and yield as compared to fields dressed with composted chicken guano. This difference could be due to the variation in rates of volatilization of the N source. Seed treated with Entrust had a higher plant stand (27%) compared to naked seed. The positive effects of Entrust are likely due to a deduction in seed damage by seed corn maggot.

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DETERMINING THE GENOMIC STRUCTURE OF A QTL LINKED TO ANGULAR LEAF SPOT RESISTANCE

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INTRODUCTION: Angular leaf spot (ALS) caused by the fungus *Pseudocercospora griseola* (Sacc.) Crous & Braun *(sin. Phaeoisariopsis griseola* (Sacc.) Ferraris*)* is one of the most devastating diseases of common bean (*Phaseolus vulgaris* L.), leading to yield losses of 80% in bean fields around the world [1]. As disease control is most effective by using resistant varieties, it is important to understand the mechanisms of host-pathogen interaction and develop tools that could be applied on breeding programs. Our group has previously identified a major QTL on the linkage group B10 that explain 22% of the ALS resistance under field and greenhouse conditions [2], using the UC (IAC-UNA x CAL 143) mapping population [3]. To further characterize this QTL, we develop a physical map in that region by screening a BAC library with tightly linked molecular markers and determining the gene content within the QTL.

MATERIAL AND METHODS: A BAC library that covers 5-7 times the bean genome, with 30,720 clones and an average insert size of 125 Kb [4], was screened with 11 molecular makers using polymerase chain reaction (PCR). Bulks of 32 clones were co-cultured in 500 µl of Luria Bertani (LB) medium (10 g of Bacto tryptone, 5 g of yeast extract, and 5 g of NaCl in 1 liter of distilled water) supplemented with 12.5 µg/ml chloramphenicol. Cultures were incubated in 96 deep well plates with shaking at 240 rpm, at 37^{o}C for 16 hours. Liquid cultures were grown on solid LB medium and used for colony PCR reactions performed in 25 µL reactions with 0.8 mM dNTPs, 1.5 mM $MgCl_2$, 1X PCR buffer, and 1 unit of Taq polymerase (GoTaq®, Promega). BAC DNA was isolated using standard lysis protocol [5]. BAC-end sequencing (BES) was performed at the Genome Core Facility at UTA (http://gcf.uta.edu/Core_Facility) using the T7 and SP6 primers that border the insert of the pBeloBAC11 vector. New primers for chromosome walking were designing based on the BES using the PRIMER3 (v. 0.4.0) software (http://frodo.wi.mit.edu/primer3).

RESULTS AND DISCUSSION: Six BAC clones were identified with the colony PCR screening; four clones contain only one marker, while the other two carry two markers (Fig. 1A). These six clones span the entire QTL, resulting in a reasonable coverage of the region (Fig. 1A). The insert size of the clones P02H22 and P10A12 containing the same two markers (Pvm13 and IAC137) were estimated by BAC DNA digestion with the *Sac*I restriction enzyme (Fig. 1B). Both BAC inserts have approximately 100 Kb (Fig. 1B). Considering that the genetic distance between the two markers was estimated to be 1.6 cM [3], the kilobase (Kb) per centimorgan (cM) ratio in this QTL region is approximately 62 Kb/cM and does not coincide with the estimated genome average of 120 Kb/cM [6]. BES analysis revealed that the P10A12 insert lies within the P02H22 clone. BLASTN analysis of these sequences revealed strong similarities with retrotransposon (RT) sequences (E-value < 10^{-30}; Fig. 1A). As RT have repeated conversed

regions [7], it is possible that their presence in the QTL could be the cause of increased recombination frequency in this locus that could explain low Kb/cM ratio observed. Sequence analysis also indicated the presence of regions with strong similarities with a chitinase (E-value = $9e^{-120}$) and a TIR-NBS-LRR (E-value = $6e^{-20}$) genes, which are known to play a role in plant resistance against pathogens [8]. Outward chromosome walking is underway with the goal of closing the gaps between the six BAC identified. We expect to identify candidate genes involved in common bean resistance against ALS for future transgenic technologies and develop robust molecular markers to facilitate the development of resistance cultivars using MAS.

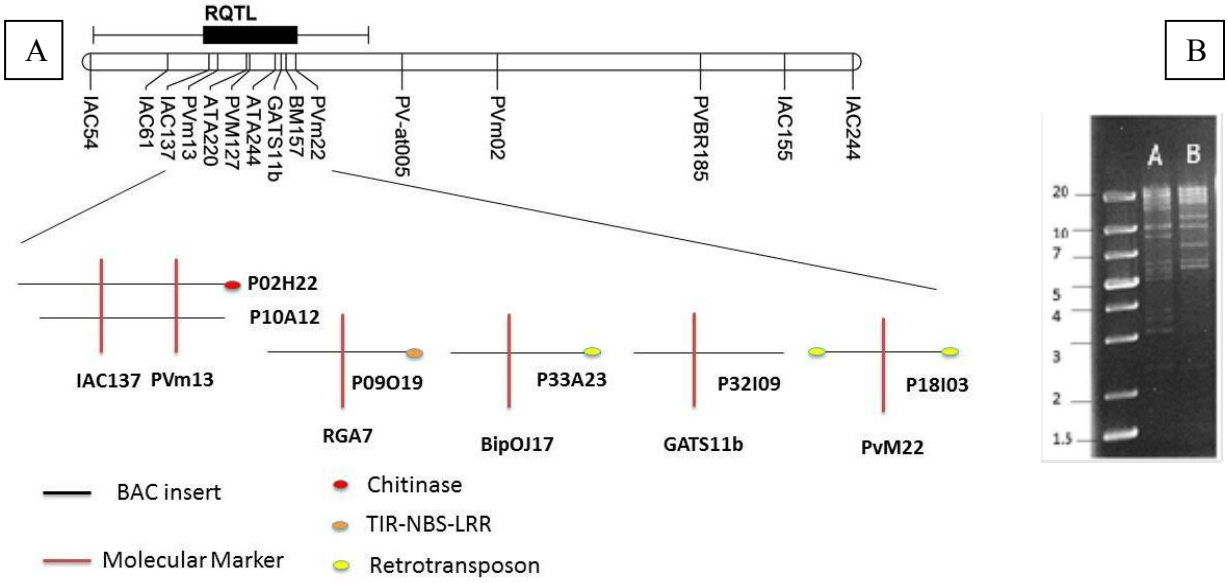

Figure 1. Current genome structure of the ALS resistance QTL. **(A)** Six BAC clones spanning the QTL were identified. P02H22 and P10A12 shared two markers (PvM13 and IAC137) and are overlapping. **(B)** P02H22 (lane A) and P10A12 (lane B) DNA digestion with the *Sac*I restriction enzyme. DNA banding patterns indicates that those clones have differences in DNA sequences. First lane shows DNA Ladder (1Kb Plus, Fermentas®) and the numbers on the left indicate fragment size in Kb.

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VARIABILITY AMONG POPULATIONS OF THE WEB BLIGHT PATHOGEN FROM BEAN FIELDS

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Web blight (WB) in the Americas is mostly caused by aerial isolates of subgroups AG-1-IE and AG-1-IF of *Rhizoctonia solani* (*Thanatephorus cucumeris)*. The disease causes millions of dollars in yield losses due to destruction of leaves or seed blemish that reduces its value. Under field conditions isolates of AG-1-IE and -IF produce abundant mycelia, sclerotia and occasionally basidiospores that could contribute to the spread of isolates to distant locations through soil, water and contaminated symptomless seed (2). Lack of understanding of the genetic population dynamics of the pathogen has limited progress in identification of WB resistance. We have hypothesized that isolates of AG-1-IE and AG-1-IF from different locations are mostly clonal since they appear morphologically and culturally indistinguishable with similar virulence pattern and ITS rDNA sequence (3) The genetic structure of populations of *R. solani* within subgroups AG-1-IE and AG-1-IF was studied in isolates collected from Honduras, Puerto Rico and the Dominican Republic over a 13 year period using DNA fingerprinting and mycelia compatibility.

The 37, 15 and 40 isolates of *R. solani* collected in Honduras, Dominican Republic, and Puerto Rico respectively were grown on V-8 liquid medium to collect mycelia before sclerotia development. DNA was extracted from fresh mycelia with UltraClean Plant DNA isolation kit by MO BIO Laboratories (Solana Beach, CA). PCR amplifications with URP2R, URP6R, ISSR10, and (GACA)4 generated informative fingerprints. Data of 101 loci from the four markers were combined based on high positive correlation obtained by Mantell Correlation analysis. Cluster analysis using the UPGMA algorithm was analyzed with the software PAST (PAleontological STatistics Version 2.01). A UPGMA tree was constructed using iTOL (4). Analysis of molecular variance (AMOVA) was performed to determine genetic variability among populations with GenAlEx 6.3 (5). Mycelia compatibility tests are conducted by pairing isolates within each subgroup against each other on petri plates containing Potato Dextrose Agar and incubated at 21 $\pm$ 1C for 2 weeks. Interactions were scored as compatible or incompatible. Clones were defined as isolates sharing both somatic compatibility and molecular marker phenotype.

The UPGMA tree showed two main clusters separating subgroups that were based on geographical origin and year of sampling for AG-1-IE and geographical origin for isolates of AG-1-IF (Fig.1). AMOVA revealed significant genetic variation among and within populations for each of the subgroups. The AG-1-IE subgroup showed variation of 28, 35 and 37% corresponding to among regions, among populations and within populations, respectively. AG-1-IF subgroups showed 27% of the total variation was among populations of two different locations and 73% was within populations. Φ values were highly significantly in all cases. Most mycelia interactions were scored as incompatible. Only two isolates, collected in the same year and location were clones. Therefore most field isolates of AG-1-IE and AG-1-IF are unique phenotypes suggesting the occurrence of recombination events in the life cycle over time. Genetic variation in AG-1-IE and AG-1-IF *R.solani* populations, due to factors such as

reproduction strategies, mating system, gene flow and geographical isolation may explain the lack of durable resistance to WB reported in dry beans.

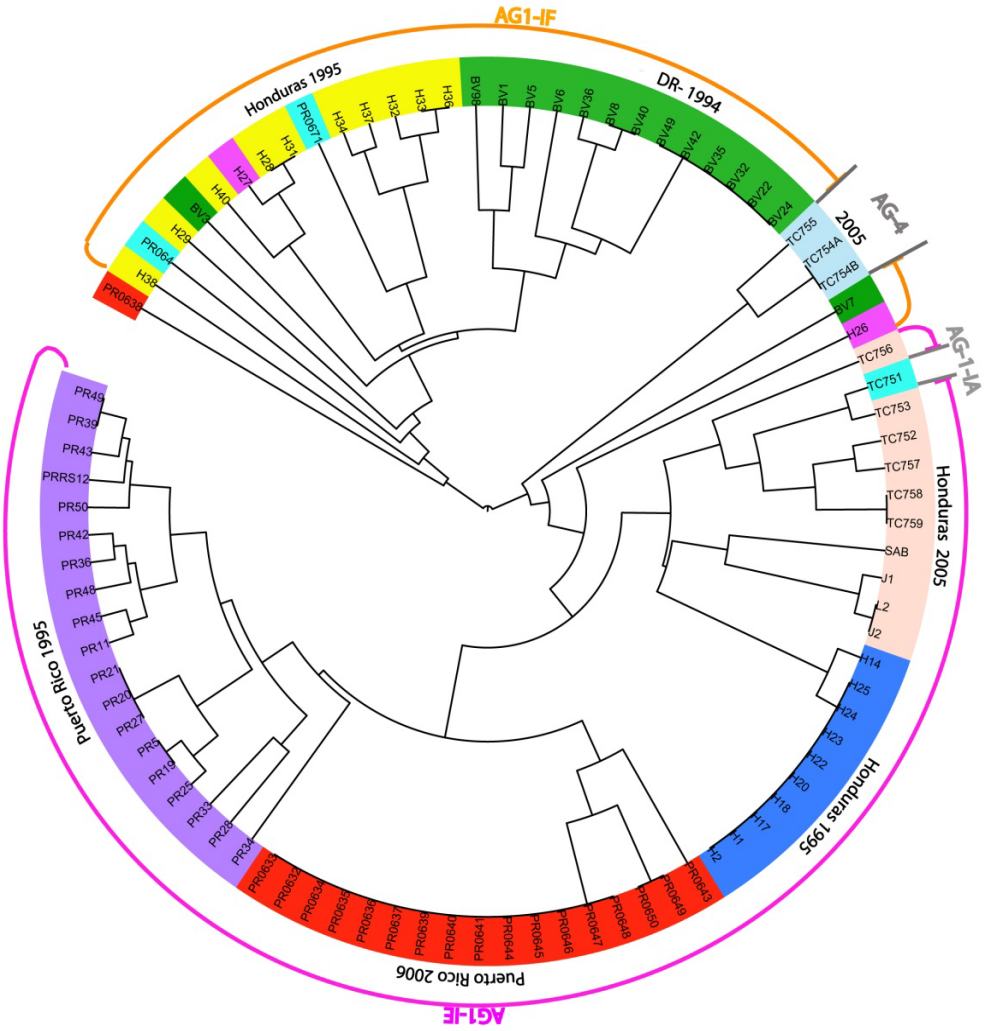

Fig.1 Population structure defined by geographical location and year of sampling (AG-1-IE) or by geographical location (AG-1-IF).

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TOLERANCE TO ABIOTIC AND BIOTIC STRESS IN *PHASEOLUS*

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CIAT has the largest collection of Phaseolus in the world and the genetic variability found in this collection represents an unexplored source of genes that could be used in developing bean varieties that are capable to tolerate both biotic and abiotic stress factors.

In this project we evaluated a Phaseolus mini collection composed of 70 different genotypes belonging to *P. vulgaris, P. lunatus, P. accutifolius, P. parvifolius, P. angustissimus, P. costaricensis, P. carteri, P. filiformis,* and *P. rotundatus*. The accessions were evaluated for resistance to two root rot pathogens, Macrophomina and Sclerotium, using drought and well watered soil conditions. For the combined stress conditions of Macrophomina and drought, we found only 4 accessions that were consistently resistant, 2 from *P. vulgaris* (G12871 and G12947), and 2 from *P. acutifolius* (G40071 and G40180). In the case of combined stress conditions of Sclerotium and drought we found only one accession G23585 (*P. vulgaris*), out of 70, that was able to tolerate both pathogen infection and drought stress conditions. We found that although this disease has not been a major priority for breeders or pathologists, it has a big potential to reduce bean production if the environmental conditions favor the disease development. This preliminary screening forms a scientific basis for further research aimed to identify added value to the genetic diversity found at CIAT with its *Phaseolus* germplasm collection. We would like to use the same strategy to conduct further research in order to find sources of resistance to other pathogens that are favored by high soil humidity such as *Rhizoctonia solani*. Based on phenotypic differences in shoot and root traits under individual drought stress and combined root rot (Macrophomina) and drought stress, we identified two genotypes from *P. acutifolius* (G40001 and G40047) as drought resistant and two genotypes from *P. acutifolius* (G40001, G 40071) and one genotype from *P. vulgaris* (G12875) as resistant to the combined stress conditions of root rot and drought. For root rot of Sclerotium the promising genotypes were G12949 (*P. vulgaris*) and G40001 (*P. acutifolius*) for individual drought stress and G22837 and G12947 (*P. vulgaris*) for the combined stress of root rot and drought stress. These genotypes can serve as parental material for the on-going bean breeding programs.

Based on numerous studies, seems to be a negative correlation between drought and resistance to *M. phaeolina* infection in common beans (*Phaseolus vulgaris*). When we compare the 61 *Phaseolus* accessions (*vulgaris, acutifolius, angustissimus, carteri, fiiformis, lunatus,* and *parvifolius)* this correlation was not fully observed under the conditions tested here, although very narrow differences were obtained. We were expecting that accessions resistant on 80% field capacity would be remarkably more susceptible on 50% field capacity treatment. Although increased disease incidence under water stress is well documented, maybe this response differs among species. The fact that accessions that showed resistance under water stress were not always resistant on 80% field capacity demonstrate that low moisture in soil is not the factor that renders *Phaseolus* plants to the disease infection, at least not as a general process. It seems that

the response depends merely on genotype-environment interaction. That is, disease resistance is observed in specific genotypes under specific soil moisture conditions.

The results from *Sclerotium* assays demonstrate the big potential of this pathogen to affect Phaseolus, independently of the soil moisture. Indeed more research should be done and more accessions should be tested to find potential sources of tolerance. Additionally the fact that a single fungal strain was used opens the possibility that we chose the most aggressive one. Complementary studies should be conducted using more strains. The data showed here represent valuable information on new sources of resistance present on wild species of *Phaseolus* that can be used on breeding programs to diminish the effect of *M. phaseolina* on cultivated beans in areas under drought risk.

CHARACTERIZING *SCLEROTINIA SCLEROTIORUM* POPULATIONS ACROSS BEAN GROWING REGIONS FOR IMPROVED RESISTANCE SCREENING

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Isolates of *Sclerotinia sclerotiorum* collected over the past six years from nine bean production regions in the USA, as well as regions in Mexico and France, have been characterized using mycelial compatibility groups (MCGs). A total of 87 MCGs were formed from the USA isolates. The Idaho greenhouse isolate, all the California isolates and, with only one exception, all Mexico and France isolates (data notshown) were unique (Table 1). Clonality varied from 8 clones out of 62 Michigan isolates to 27 clones out of 59 Washington isolates. The data in Table 1 also demonstrates that some clones are found in six or seven states across the USA while seven are limited to only one or two states. These same isolates have also been tested for aggressivenes under greenhouse conditions using a straw test with G122 as the host, and they were found to have varying degrees of aggressiveness (2.8 to 7.9) based on a scale of 1 = no disease, to 9 = death of the plant. Aggressiveness variation of the USA collection of characterized bean isolates is illustrated in Table 2 and Figure 1. Differences in isolate aggressiveness were found among the isolates ($P<0.001$) but all isolates that are clones had similar aggressiveness. This data provides evidence that pathogen variation can influence resistance screening results. A summary of MCG and aggressiveness characterization of 157 field and greenhouse screening isolates collected across the major USA bean production regions has been published (Otto-Hanson et al., 2011).

Eighteen polymorphic microsatellite markers from a list of 25 on the Kohn website referenced below, have been identified as informative for analysis of bean isolate populations collected from 2003-2012. A study using four of these microsatellite markers and 239 isolates developed a set of 65 microsatellite haplotypes that were found to associate closely with MCGs developed from the 239 isolates. Isolates in a single MCG all had the same microsatellite haplotype in 64% of the MCGs. An analysis of molecular variance (AMOVA) using these microsatellite haplotypes was conducted. Dividing the USA into three geographic bean growing regions identified in Table 2, we found that 70% of the molecular variation of these isolate haplotype populations was within these regions, 20% of the variation was among populations within the regions and 10% of the variations was associated with differences among regions (Table 3).

These characterized isolates and others that are in the process of characterization will be developed into a database that will provide isolate information to breeders/pathologists. These isolates can be used in screening for white mold resistance to be used in local areas or across regions and selecting for high, moderate or low levels of resistance. Pathogen variability exists in both greenhouse and field screening isolates, but use of multi-sites for field and greenhouse screening improves the resistance validation evidence for putative white mold resistant bean lines. These multi-site screenings have resulted in release of 10 dry and snap bean lines for use by public and private breeders around the world.

Location	Total # isolates	Mycelial Compatibility Groups	
		MCGs per location	Shared MCGs Same color dot = MCG shared between those locations
CO	42	6	
MI	62	8	
MN	11	2	
ND	59	12	
NE	48	8	
NY	1	1	
OR	17	9	
WA	59	27	
WI	2	1	
CA	18	12	
ID	1	1	

Table 1. Summary of *Sclerotinia sclerotiorum* isolates by state location and MCGsformed in each location and shared between locations (only showing USA data).

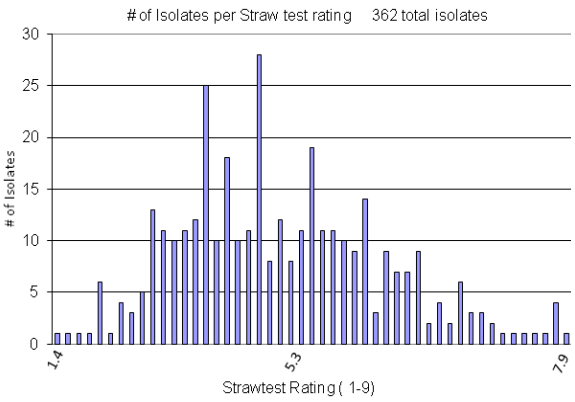

Figure 1. Frequency distribution of 362 *Sclerotinia sclerotiorum* isolates by aggressiveness rating (9 is most aggressive).

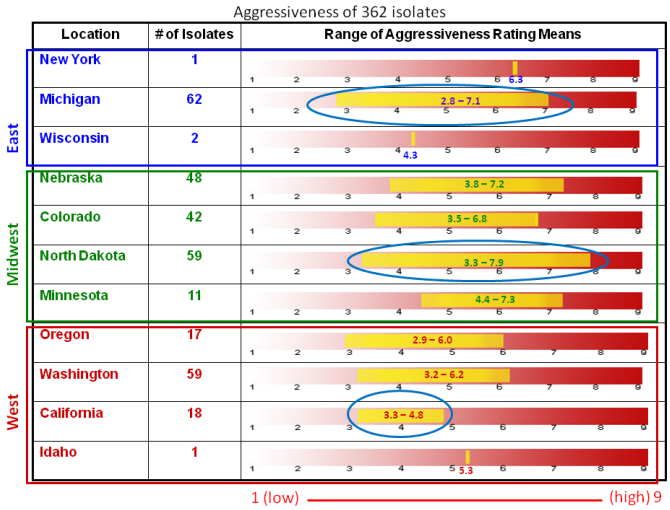

Table 2. Range of *Sclerotinia sclerotiorum* isolate aggressiveness by location using the straw test method and number of isolates per state/region.

Source of variation	df	Sum of squares	Variance components	Percentage of variation
Among populations	5	57.958	0.14541 Va	9.91
Among populations within groups	8	44.335	0.30002 Vb	20.44
Within populations	226	231.061	1.02239 Vc	69.65
Total	239	333.354	1.46783	

Table 3. Analysis of molecular variance for *Sclerotinia sclerotiorum* populations from five geographic areas (Eastern, Central, Western USA, France and Mexico) of based on microsatellite markers.

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USE OF MULTIPLE INOCULATIONS AND EVALUATIONS FOR SELECTING COMMON BEAN WITH HIGH LEVELS OF WHITE MOLD RESISTANCE

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INTRODUCTION

Partial resistance to white mold caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is found in common bean and *Phaseolus* species of the secondary gene pool (Singh et al., 2009a). Resistance to white mold has been introgressed from the secondary gene pool into common bean in recent years (Miklas et al., 1998; Singh et al., 2009a, 2009b). Our objective was to determine the response of a diverse group of common bean genotypes to three inoculations of an aggressive *S. sclerotiorum* isolate ND710 at 7, 14, 21, 28, 35 days post inoculation.

MATERIALS AND METHODS

Common bean genotypes were planted in a randomized complete block design with four replications. Every replicate had three plants sowed in a 10 inch pot, and each plant was inoculated three times at a weekly interval beginning at the fourth or fifth internode of the main stem and branches. The modified cut-stem method (Terán et al., 2006) with three mycelial plugs grown for 48 hours on potato dextrose agar at 28º C was used in each inoculation. Disease severity was recorded on a 1 to 9 scale, where 1= no symptoms and 9= disease passed the third internode or dead plants. Data were analyzed using the SAS (Version 9.1.3) GLM procedure software.

RESULTS AND DISCUSSION

Effects of replicates, genotypes, number of inoculations and number of days evaluations were delayed post inoculations were significant (Table 1). Although the number of inoculations and number of days post inoculation the evaluations were made were confounded because the way common bean plant grows and the main stem and branches are formed it was worth determining their response. In general, white mold disease severity increased with the number of inoculations and the number of days evaluations were delayed post inoculations. Terán and Singh (2009) also concluded that white mold scores increased with delayed evaluations. 'Othello', 'ICA Bunsi', and USPT-WM-1 had a susceptible reaction at all inoculations and evaluation dates (Table 2). Common bean genotypes 92BG-7 and G 122 had an intermediate reaction only at 7 days post 1 inoculation. They had a susceptible response to white mold at all other evaluation days and inoculations. Interspecific breeding line VCW 54 followed by A 195 exhibited the highest levels of white mold resistance at all inoculations and evaluations. Thus, use of multiple inoculations and delayed evaluations may be useful for identifying common bean genotypes with high levels of white mold resistance

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Table 1. Analysis of variance for common bean genotypes to three inoculations of *Sclerotinia sclerotiorum* isolate ND710 at 7, 14, 21, 28, and 35 days post inoculation in the greenhouse in 2011.

		Mean squares
Source	**df**	**White mold disease score**
Replicates	3	78.31*
Genotypes	148	50.56*
No. of days evaluation delayed	4	1212.26*
Genotype x No. d eval. delays	592	1.42
Error	7626	1.83

* Significant at $P \leq 0.05$

Table 2. Response of common bean genotypes to three inoculations of *Sclerotinia sclerotiorum* isolate ND710 at 7, 14, 21, 28, and 35 days post inoculation in the greenhouse in 2011.

	Mean white mold disease score					
	Number of inoculation					
	1	2	3			
Genotype	**7†**	**14**	**21**	**28**	**35**	**Mean**
92BG-7	6.0‡	6.7	7.0	7.1	7.1	6.8
A 195	4.1	4.3	4.5	4.7	5.2	4.6
G 122	6.1	6.5	7.3	7.3	8.2	7.1
ICA Bunsi	7.7	7.9	8.4	8.5	8.6	8.2
NY6020-5	4.4	5.5	6.3	6.6	7.8	6.1
Othello	6.8	8	8.9	9	9	8.3
USPT-WM-1	6.8	8.2	8.5	8.8	8.8	8.2
VCW 54	3.8	4	4.2	4.2	4.2	4.1
Mean	5.7	6.4	6.9	7.0	7.4	6.7
LSD ($P \leq 0.05$)	0.2	0.2	0.3	0.3	0.3	0.3

†Number of days post inoculation white mold response was recorded. ‡White mold scored on a 1 to 9 scale, where 1= no symptoms and 9= disease passed the third internode or dead plants.

LINKAGE MAPPING OF THE *Co-10* AND *Phg-ON* GENES FOR RESISTANCE TO THE ANTHRACNOSE AND ANGULAR LEAF SPOT DISEASES IN COMMON BEAN CULTIVAR OURO NEGRO

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INTRODUCTION

The common bean is an enormously important human food, especially in many countries of the Americas and Eastern and Southern Africa. In these countries, common bean is recognized as a very important source of protein and carbohydrate in the diet of millions of people (Broughton et al. 2003; Gepts et al. 2008). Conversely, the crop is affected by many biological, edaphic, and climatic factors that decrease yield (Schwartz and Pastor-Corrales 1989). Several diseases cause severe yield losses and reduce the quality of dry and snap beans worldwide (Singh and Schwartz 2010). Anthracnose (ANT), caused by *Colletotrichum lindemuthianum* (Sacc. and Magnus) Briosi, and Cavara) and angular leaf spot (ALS), caused by *Pseudocercospora griseola* (Sacc.) Crous and Braun, are among the most widespread and devastating diseases of common bean in the tropics. The Mesoamerican black-seeded common bean cultivar Ouro Negro is highly productive and it has the dominant *Co-10* and *Phg-ON* genes that confer resistance to ANT and ALS. The objective of this study was to identify the association between the ANT *Co-10* and ALS *Phg-ON* disease resistance genes present in cv. Ouro Negro and to identify molecular markers associated with these two genes.

MATERIALS AND METHODS

The cv. Ouro Negro, which possesses the *Co-10* and *Phg-ON* genes that confer resistance to race 73 of *C. lindemuthianum* and race 63-39 of *P. griseola*, was crossed with the female Rudá parent, which is susceptible to both pathogens. For the production of F_2 seeds, the F_1 seeds were sown in a greenhouse in polyethylene pots (48 × 30 × 11 cm) containing a mixture of previously fertilized and sterilized substrate,. A total of 112 F_2 seeds derived from the Ouro Negro × Rudá cross were sown in plastic trays (50 × 30 × 9 cm) containing peat-based substrate, as were 15 seeds from each of the parents Ouro Negro and Rudá. Seedlings were kept in a greenhouse until the first trifoliolate leaves (stage V3) were fully expanded. At that time, the plants were inoculated simultaneously with race 73 of *C. lindemuthianum* and race 63-39 of *P. griseola*, according to Gonçalves-Vidigal et al. (2011).

Twenty-three molecular markers, all mapping on LG Pv04 (PhaseolusGenes database: http://phaseolusgenes.bioinformatics.ucdavis.edu), were chosen for testing. All markers were tested on the parents and on resistant and susceptible bulks. Two contrasting DNA pools (Michelmore et al. 1991) were constructed by combining equal amounts of fluorometrically standardized DNA from five resistant and susceptible plants, respectively, of the Ouro Negro × Rudá F_2 population. Of these 23 markers, two – STSs$g2303^{350}$ and $SF10^{1072}$ – were polymorphic showing contrasting amplification patterns in parental materials and in the resistant vs. susceptible bulks and individuals from the bulks (Fig. 1) and were retained for subsequent

studies. Markers g2303 and SF10 were mapped in the BAT93/Jalo EEP558 (BJ: 71 lines; Freyre et al. 1998) recombinant inbred mapping population and in the F_2 generation from the Ouro Negro × Rudá cross. A goodness-of-fit test for a 1:1 segregation ratio was performed for the segregation of the g2303 and SF10 markers in the BJ population. Linkage analyses were performed using the computer software Mapmaker/EXP 3.0 (Lincoln and Lander 1993).

RESULTS AND DISCUSSION

One-hundred and twelve F_2 plants derived from the Ouro Negro × Rudá cross, and the parents were inoculated with race 73 of *C. lindemuthianum* and race 63-39 of *P. griseola.* A segregation was observed in the F_2 population with 84 resistant and 28 susceptible plants to *C. lindemuthianum* (*P*= 1.0) and 83 resistant and 29 susceptible plants (*P* = 0.83) to *P. griseola*, respectively (Table 1). The data showed the presence of one recombinant among the two disease resistance genes, suggesting a close association between the *Co-10* and the *Phg-ON* resistance genes in Ouro Negro. The molecular marker $g2303^{350}$, previously mapped to LG Pv04, was linked with *Co-10* and *Phg-ON* at 0.9 cM and 1.8 cM, respectively. Similarly, the $SF10^{1072}$ marker was linked with these genes at 7.8 and 8.7 cM, respectively.*Co-10* and *Phg-ON* are inherited together and can be monitored indirectly with $g2303^{350}$ and $SF10^{1072}$.

Table 1 Reaction of F_2 plants from the cross Ouro Negro × Rudá inoculated with races 73 of *Colletotrichum lindemuthianum* and 63-39 of *Pseudocercospora griseola*, respectively, and presence [e](+) or absent (-) of the molecular markers

	Observed numbers of resistant plants to races 73 and 63-39[a]							Linkage distance (cM)[d]	
Generation	$R^{ANT\ b}R^{ALS}$	$R^{ANT\ b}S^{ALS}$	$S^{ANT\ b}R^{ALS}$	$S^{ANT\ b}S^{ALS}$	Expected ratio 3 : 0 : 0 : 1[c]	χ^2	*P* value (3 df)	*Co-10* /	*Phg-ON*
F_2	83	1	0	28	84 : 0 : 0 : 28	0.02	0.92	0.9	
g2303	85 (+)	0	0	27 (-)	84 : 0 : 0 : 28	0.05	0.83	0.9	1.8
SF10	92(+)	0	0	20 (-)	84 : 0 : 0 : 28	3.05	0.09	7.8	8.7

[a]R = Resistant plants; S = Susceptible plants ; [b]ANT= Anthracnose; [c] Two dominant genes in absolute linkage; [d]Linkage distance between the marker and the *Co-10/Phg-ON* genes indicates one resistant plant lacked for the g2303 marker and two susceptible plants presented the SF10 marker; [e] Marker present (+); absent (-)

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MARKER-ASSISTED SELECTION FOR IMPROVING RESISTANCE TO COMMON BACTERIAL BLIGHT IN COMMON BEAN

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INTRODUCTION: The SCAR marker SU91 has been used extensively by the bean (*Phaseolus vulgaris* L.) breeding programs in marker-assisted selection for resistance to common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) (Miklas et al. 2006). The objectives of this study were to investigate the association of SU91 and other SU91 linked markers with CBB resistance, and to estimate the realised gain due to early generation selection using SU91.

MATERIALS AND METHODS: Two different crosses were used in this study. 1) Ninety $F_{4:5}$ recombinant inbred lines (RILs) derived from a cross between susceptible Sanilac and resistant OAC 09-3 were phenotyped in the field disease nursery and in the growth room using a mixture of four *Xap* isolates (Yu et al. 2000). AUDPC (The area under the disease progress curve) was calculated based on three consecutive CBB ratings with one week intervals in the field tests. The RILs were also genotyped using SU91, and two SU91 linked markers (SU91-CG10 and SU91-CG11), which were developed from a BAC clone sequence containing SU91 marker (Shi et al. unpublished; Miklas et al. 2000). 2) Four three-way crosses (TC) were performed, in which the F_1 progeny of the crosses between two sources of CBB resistance (Apex, MBE7 or Rexeter) were top-crossed to a susceptible, early-maturity commercial cultivar (Nautica or AC Compass). Their F_1 progeny were then backcrossed with Nautica or AC Compass to produce four BC_1F_1 families. All F_1 progeny (TCF_1 and BC_1F_1) were phenotyped using a mixture of four *Xap* isolates and genotyped with SU91. The progeny from all F_1 were advanced to F_4 or F_5 by single-seed decent. Four TCF_4 and four BC_1F_5 families with 400 lines from each cross were tested for CBB resistance in an artificially inoculated field nursery in Harrow.

RESULTS: SU91 is a dominant marker, present in OAC 09-3 not in Sanilac. SU91-CG10 is also a dominant marker but in repulsion with SU91. SU91-CG11 is a co-dominant marker, amplifying a 425 bp band for Sanilac and a 464 bp band for OAC 09-3. SU91-CG11 is able to distinguish homozygous and heterozygous RILs (Fig. 1). Genotypic frequency of SU91 was significantly skewed toward the resistant parent (X^2 = 14.55), while SU91-CG11 was less deviated than SU91 (X^2 = 2.88), and SU91-CG10 was segregating following the expected Mendelian ratios (X^2 = 1.36). Both in the field and growth room studies, the new marker SU91-CG11 accounted for a greater proportion of the variation than SU91 (Table 1). The lsmeans of heterozygous genotypes were between homozygous susceptible (A) and resistant (B) genotypes in both field and growth room tests (Table 2). The co-dominant marker SU91-CG11 seems to be a good replacement for SU91.

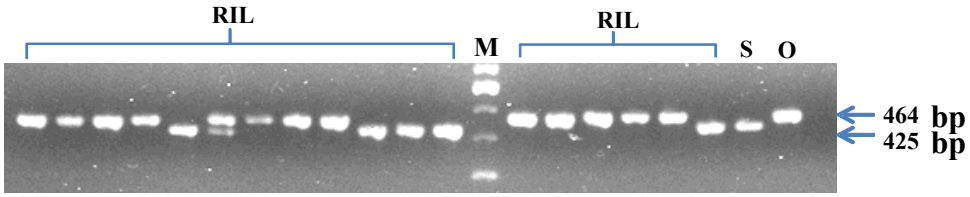

Fig. 1. Co-dominant marker SU91-CG11 genotyping Sanilac (S), OAC 09-3 (O) and their RILs.

Table 1. Single marker QTL analysis

	Field AUDPC		Growth room	
Marker	*p*	R^2	*p*	R^2
SU91	***	0.40	***	0.27
SU91-CG10	***	0.28	***	0.23
SU91-CG11	***	0.42	***	0.36

Table 2. Lsmean of SU91-CG11 in CBB tests

	Field AUDPC			Growth room		
Allele	Lsmean	Std. Error	*p*	Lsmean	Std. Error	*p*
A	39.95	1.45	***	4.30	0.15	***
H	29.81	1.68	***	3.53	0.17	***
B	25.33	1.17	***	2.96	0.12	***

Selection using SU91 marker in an early generation (BC_1F_1) changed the frequency distribution of CBB response of the resulting F_5 population and caused a significant shift in the observed selection gain in the BC_1F_5 progeny as a result of selection in BC_1F_1 of four populations, was estimated as the difference of the selected and unselected populations and ranged between 0.20 to 0.74 units of a 0 to 5 disease severity scale (Fig. 2, Table 3).

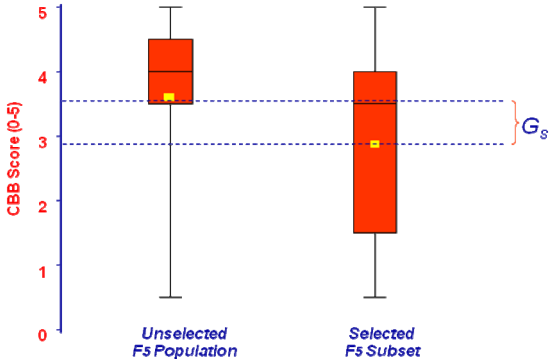

Fig. 2. Boxplots representing the frequency distributions of CBB response of F_5 derivatives of a backcross population after one cycle of SU91 selection in BC_1F_1. Gs represents the realized gain from selection.

Table 3. Realized selection gain in CBB response of F_5 derivatives of BC_1 crossesdue to F_2 enrichment using SU91 selection in BC_1F_1

	No early generation selection				Early generation selection				**Realized gain from selection (CBB score)**
Population	Mean	Min	Max	Sdev	Mean	Min	Max	Sdev	
Nautica *[2]//Apex/MBE7	3.54	0.50	5.00	0.05	3.34	0.50	5.00	0.11	**0.20**
Nautica *[2]//Rexeter /Apex	3.82	0.50	5.00	0.05	3.26	0.50	5.00	0.09	**0.56**
Compass *[2]//Apex/MBE7	4.14	0.50	5.00	0.03	3.74	0.50	5.00	0.08	**0.40**
Compass *[2]//Rexeter /Apex	3.60	0.50	5.00	0.06	2.86	0.50	5.00	0.10	**0.74**

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MAPPING AND EXPRESSION ANALYSIS OF ZINC TRANSPORTER GENES

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Dry beans (*Phaseolus vulgaris* L) are a rich dietary source of zinc, an essential micronutrient that is often limited in the diet. Within *P. vulgaris*, two fold genotypic variability for dry seed zinc levels have been observed ranging from 30-70 μg g^{-1}(Grusak, personal communication). Numerous QTL studies for seed zinc levels have been conducted in Andean and Mesoamerican RIL populations and in inter genepool populations including Dor364 x G19833(Blair et al., 2009). Geneshave been identified in model plant species that are involved in zinc transport throughout the plant andone major family with this function is ZRT-IRT like (ZIP) (Waters et al., 2011). The objective of this study was to place two ZIP genes on the Dor364 X G19833 linkage map. In addition RNA expression data is presented for these two genes in developing pods of two navy bean genotypes with known differences in seed zinc accumulation.

MATERIALS AND METHODS: There are numerous ZIP genes in the *P. vulgaris* genome. ZIP12 and ZIP80a were selected for mapping because based on synteny analysis they belonged on linkage groups Pv03 and Pv06and both linkage groups havemajor QTL for seed zinc concentration in the Dor364 x G19833 mapping population (Blair et al., 2009). The putative ortholog of ZIP12 is AtZIP6_AT2G30080.1 and of ZIP80a is AtZIP2_AT5G59520.1 in *Arabidopsis*. To map these genespolymorphisms in the gene intron sequences in Dor364 and G19833 were identified, primers were designed for the region and used for PCR (amplification conditions: Mg 2.0 mM, dNTP's 0.2 uM, primer 0.3 uM PCR reactions were carried out for 4 min at 95 C, followed by 35 cycles of 45 s at 95 C, 45 s at 50 C, and 1 min at 70 C, and a final period of 5 min at 70 C) and the products were ran on acrylamide gels. Mapdisto (version 1.7 Beta 132) was used to locate the position of the new ZIP genes. Two navy bean varieties, Albion (low seed zinc) and Voyager (higher seed zinc)were greenhouse grown with normal and low zinc fertilizer. Developing pods from each of these lines were harvested at 12-14 days after flowering. RNA was extracted and sequenced from each treatment/variety combination. Differences in gene expression were determined from RNA count data.

RESULTS AND DISCUSSION: Mapping the ZIP genes revealed their location relative to seed Zn QTL (Table1). PvZIP80a mapped near the QTL on Pv03 whereas PvZIP10 did not map near the QTL on Pv06. RNA Seq data on the expression of these two genes in developing pods showed that ZIP10 was more highly expressed that ZIP80. Both genes were uniformly expressed across the genotypes and treatments (Table 2).

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Table1: Location of Zip genes on Dor364 x G19833 linkage groups Pv03 and Pv06 with seed zinc QTL and ZIP genes locations.

Zip Gene	Linkage group	Flanking markers	Distance from nearest seed zinc QTL
PvZIP_scff80A	**3**	**Bng16 and Bmb506**	**12.2 cM**
PvZIP_scff10	**6**	**PvBr163 and Bng46**	**26 cM**

Table 2: Normalized count data for two ZIP genes in pods of Voyager and Albion greenhose grown at normal (+Zn) and low (-Zn).

	Voyager +Zn	Voyager -Zn	Albion +Zn	Albion -Zn
PvZIP10	**163**	**158**	**145**	**154**
PvZIP80	**17**	**21**	**10**	**9**

PATTERNS OF FRUCTOSE, GLUCOSE, AND SUCROSE ACCUMULATION IN SNAP AND DRY BEAN (*PHASEOLUS VULGARIS*) PODS

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INTRODUCTION

Sugars, including fructose, glucose, and sucrose contribute significantly to the flavor and consumer acceptance of vegetable crops (Glanz et al., 1998). In dry beans and edamame, consumers prefer cultivars that are considered sweeter (Mkanda et al., 2007; Wszelaki et al., 2005). Prior studies characterizing the simple sugars of snap bean pods have generally focused on a single cultivar at a single development stage (Muir et al., 2009). In legume crops, including *Phaseolus vulgaris*, the process of simple sugar conversion and complex polysaccharide accumulation in the seed is well understood (Górecki et al., 2001). In contrast, the accumulation of simple sugars in immature ovaries of bean pods is not well understood.

MATERIALS AND METHODS

The objective was to evaluate fructose, glucose, and sucrose levels in several snap, Roma, and dry bean cultivars and landraces with increasing pod diameters. Six diverse common bean genotypes were evaluated in the summer of 2009 and 2010 at the University of Wisconsin Agricultural Research Station at West Madison, including the snap beans 'Hystyle', 'Eagle', 'Black Blue Lake Pole', and 'Ferrari', the Roma-class bean 'Roma II', and the dry bean landrace 'Puebla 152'. To provide a consumer-based evaluation of sugar content relative to maturity, pods were harvested corresponding to sieve sizes 1 through 5 as measured 90° off the suture. Sugars were extracted from freeze-dried samples using a soluble-sugar extraction procedure and analyzed by high-performance liquid chromatography (Sánchez-Mata et al., 2002).

RESULTS AND DISCUSSION

In general, an inverse relationship between fructose and glucose concentration, compared to sucrose concentration, was observed for all genotypes. In all snap and Roma bean cultivars, best-fit regression lines for fructose and glucose concentration showed a significant decrease with increasing pod diameter. In contrast to the snap and Roma bean cultivars, a significant linear increase in fructose and glucose concentration with increasing pod diameter was observed in the dry bean Puebla 152. In contrast to the monosaccharides, the disaccharide sucrose increased in snap and Roma bean cultivars with increasing pod diameter. No significant change in sucrose concentration was observed with increasing pod diameter in the dry bean Puebla 152 (Fig. 1). The rapid decline of fructose and glucose and the simultaneous increase in sucrose in the snap and Roma bean cultivars at large pod diameters likely corresponds to the rapid decrease in acid invertase activity reported by Sung et al. (1994), suggesting that acid invertase may be the primary enzyme responsible for available monosaccharides. The contrasting pattern of sugar development in the dry bean Puebla 152 suggests that the amount or activity of acid invertase may differ between these market classes.

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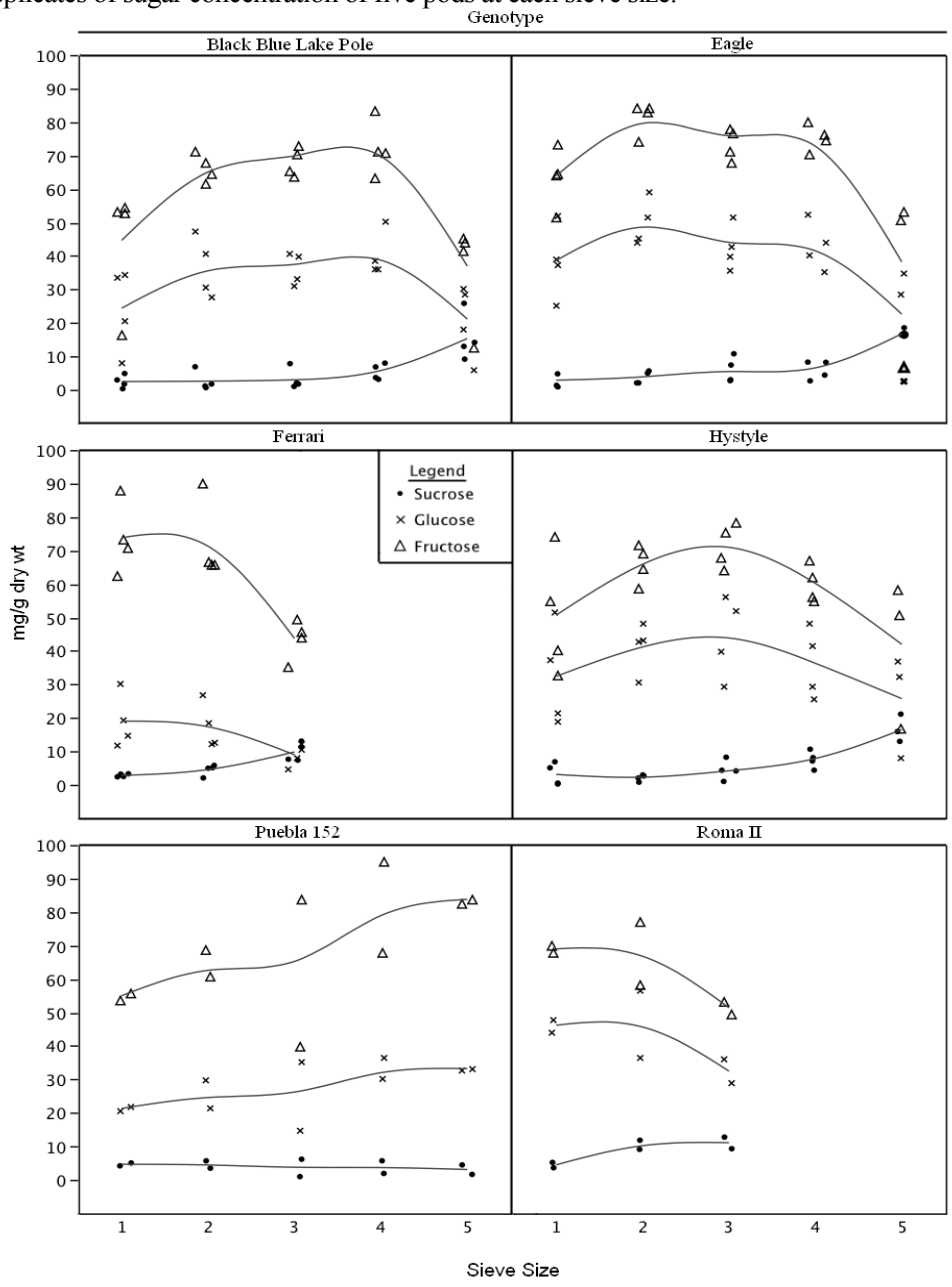
Fig.1. Changes in the concentration of sucrose, glucose, and fructose with increasing pod diameter in six common bean genotypes. Solid lines are for illustration only and connect symbols, which represent arithmetic means of individual replicates of sugar concentration of five pods at each sieve size.



VARIATION IN TOTAL DIETARY FIBER CONTENT IN DRY EDIBLE BEAN CULTIVARS/LINES

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INTRODUCTION

There is currently an increased interest in plant-based dietary components and their effects on human health. One dietary factor that has recently received a great deal of interest is dietary fiber. Dietary fiber is composed of a complex mixture of plant components and was recently defined by the CODEX Alimentarius Commission as: "carbohydrate polymers with ten or more monomeric units, which are not hydrolyzed by the endogenous enzymes of the small intestine of humans (McCleary, 2010)." Dietary fiber consumption has been associated with a positive impact on human health and has been implicated in the prevention of the leading chronic diseases: obesity, type II diabetes, cardiovascular disease, and cancer (Anderson et al., 2009). Results from a large NIH-AARP prospective cohort study reported a significantly lower risk of death in both men and women consuming the highest intake of dietary fiber (Park et al., 2011). However, the oligosaccharide portion of dietary fiber which is present in dry bean is thought to cause digestive problems and contribute to reduced consumer acceptance (Winham and Hutchins, 2011). Therefore, breeding for high fiber and low oligosaccharide content is of interest to increase the health benefits and acceptance of dry beans in the diet while reducing negative digestive aspects. Previously we reported the preliminary results of the dietary fiber content of BeanCAP cultivars (Brick et al., 2011), and this update includes both the fiber and oligosaccharide content, along with the results of the evaluation of additional RIL parents.

MATERIALS AND METHODS

A subset of diverse dry edible bean (*Phaseolus vulgaris L.*) cultivars/lines from the Common Bean Coordinated Agricultural Project (BeanCAP) grown during 2010 in two greenhouse locations in Michigan and Idaho were evaluated for dietary fiber and oligosaccharide content. Additionally, 25 parents of select dry edible bean recombinant inbred line (RIL) populations were also evaluated for dietary fiber and oligosaccharide content.

The samples were prepared for analysis by soaking approximately 1 g of dried bean seed in tubes containing 8 mL of distilled water for 14 hours. After soaking, the tubes were then transferred to an autoclave and cooked for 65 minutes. The cooked samples were then homogenized in the tube, and frozen at -80˚C until analysis. The Integrated Total Dietary Fiber Assay (AOAC Method 2009.01) (McCleary, 2007) was utilized for the analysis of dietary fiber components and oligosaccharide content 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 insoluble, soluble, and total dietary fiber as well as the percentages of raffinose, stachyose, and total oligosaccharide content in the cultivars/lines.

RESULTS AND CONCLUSIONS

The results of the BeanCAP cultivars/lines grown in Michigan and Idaho, and the RIL parents suggest significant variation for dietary fiber and oligosaccharide content (Table 1, 2). For the BeanCAP cultivars grown in Michigan and Idaho, the total dietary fiber ranged from 15.7 to 22.3%. The mean total oligosaccharides ranged from 2.9 to 4.2%. A similar trend was observed for the RIL parents where the total dietary fiber ranged from 17.3 to 22.5%, and the total oligosaccharides ranged from 2.4 to 3.8%. The results suggest that variation exists among dry

bean cultivars/lines for both dietary fiber and oligosaccharide content, and that breeders should be able to modify content using selection.

Table 1: Cultivars/lines with the highest and lowest dietary fiber and oligosaccharide content among BeanCAP entries grown in a greenhouse in Michigan and Idaho.

Entry	Location	% TDF	% IDF	%SDF	Entry	%Oligos	%Raffinose	%Stachyose
Highest					**Highest**			
A801	MI	22.3	16.2	6.1	A801	4.2	0.7	3.5
CDC Rosalee	ID	22.1	14.2	7.9	Midnight	3.7	0.6	3.1
Black Knight	MI	21.5	14.5	7.0	CDC Rosalee	3.7	0.4	3.3
Lowest					**Lowest**			
HY4181	MI	16.8	13.4	3.4	HY4181	3.3	0.5	2.8
HY4181	ID	16.3	10.7	5.6	UCD9623	3.1	0.4	2.7
Black Knight	ID	15.7	11.7	4.0	PK915	2.9	0.4	2.5
Range	NA	15.7 - 22.3	10.7 - 16.2	3.4 - 8.1	Range	2.9-4.2	0.4-0.7	2.5-3.5
$LSD_{(0.05)}$	NA	1.10	0.82	0.88	$LSD_{(0.05)}$	0.55	0.08	0.51

Note: The percentage of total oligosaccharides, raffinose, and stachyose is presented as the mean of the two locations

Table 2: Cultivars/lines with the highest and lowest dietary fiber and oligosaccharides content among a set of parents used to develop RIL populations.

Entry	% TDF	% IDF	% SDF	Entry	% Oligos	% Raffinose	%Stachyose
Highest				**Highest**			
Lassen	22.5	14.8	7.7	P02630	3.8	0.7	3.1
Stampede	21.2	14.8	6.4	TLP19	3.5	0.7	2.8
CDRK	20.8	13.5	7.3	Puebla152	3.5	0.2	3.3
Lowest				**Lowest**			
115M	18.2	14.9	3.3	Yolano	2.6	0.3	2.3
SER22	18.2	13.8	4.4	CDRK	2.4	0.2	2.2
EPM507	17.3	13.9	3.4	Jalo-EEP-558	2.4	0.1	2.3
Range	17.3 - 22.5	12.6 - 14.9	3.3 - 7.7	Range	2.4 - 3.8	0.1 - 0.7	2.2 - 3.3
$LSD_{(0.05)}$	0.54	1.53	0.90	$LSD_{(0.05)}$	0.18	0.05	0.16

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GENETIC VARIABILITY OF MINERAL COMPOSITION IN COMMON BEAN SEED

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ABSTRACT: Common bean genotypes were grown in three different growing sites and analyzed for 17 mineral compositions. The influence of growing sites was observed on all seed mineral contents however, ratio of genotypic variance to genotype x environment variance indicated greater influence and stability of genetic factor on Ca and Sr. It was observed that the Zn concentration is highly correlated with S and Fe and Ca with Sr in common bean seed.

INTRODUCTION: Micronutrient malnutrition is a primary health care issue and currently, by any measure, it is of alarming proportions in many developing nations. Minerals like aluminum (Al), Boron (B), beryllium (Be), calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), potassium (K), sodium (Na), nickel (Ni), phosphorus (P), sulfur (S), strontium (Sr), titanium (Ti), and zinc (Zn) are essential and necessary for normal growth, reproduction, and health (Phan-Thien et al. 2010). There are limited crop breeding program focused on enhancement or improvement mineral composition, with the notable exception of a large CGIAR program aiming to increase only bioavailable Fe, Zn, and carotenoids in a number of staple food crops (Welch and Graham 2004). The common bean (*Phaseolus vulgaris* L.) is a principal grain legume and a valuable source for minerals or micronutrients. However, minerals contents in this crop, like any other plant, dependent on the availability of minerals in soil environment and interaction among soil compositions. The objectives of this study were to 1) estimate the influence of environment, genotype, and their interaction on the composition various minerals in common bean seed, 2) identify variability of micronutrients in common bean seed, and 3) association among the minerals in bean seed.

MATERIALS AND METHODS; *Plant Materials:* Samples were comprised with 11 common bean genotypes involved as parents of several mapping populations. In the Mayville State University green house, common bean genotypes were grown in pots filled with Sunshine mix 1. Seeds of each genotype were also grown in two field locations of North Dakota State University in summer 2010.

Mineral Content Analysis: After harvesting, seeds from each pod of individual plant were mixed thoroughly and 10 seeds were ground in liquid nitrogen and closed acid digestion was performed 250 mg of sample in 5 mL of concentrated nitric acid, and 5 mL of water. Analyses of 17 mineral concentrations were performed on Spectro Genesis Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) using Smart Analyzer Vision software (v. 3.013.0752).

Data Analysis: Seed mineral contents data were analyzed for identifying the influence of genotype, environment, and genotype x environment on mineral content. The simple correlation analysis was performed among different minerals using the SAS 9.2.

RESULTS AND DISCUSSION: Significant influence of genotype (G), environment (E), and GxE were observed on all mineral compositions in common bean seed. A ratio of the variance associated with the environmental effects (σ^2_E) to the genetic effects (σ^2_G) larger than 1.0 indicates the greater influence of environemnt and less than 1.0 indicates greater influence of genetic factors. Ratios of variances between environmental effect and genetic effects (Table 1) indicated highest influence of environment on the variability of Mo concentration (23.30) in common bean seed followed by Cu (8.14), S (3.54), Ni (2.96), Zn (1.51), Mn (1.29), and B (1.28). Greatest influence of genetic factor on the variability of Ca concentration (0.22) was observed in common bean seed followed by Al (0.24), and Ti (0.66). Almost equal influences of environment and genetic factors were observed for variabilty of Fe 0.90, K 0.86, and Sr 1.06.

A variance component ration of $\sigma^2_G/\sigma^2_{GXE}$ (Table 1) larger than 1.0 indicates greater influence and stability of genetic factors relative to the variability associated with the interaction of genotype and environment. The ratio of ($\sigma^2_{E/}\sigma^2_G$) indicated a higher influence of genotype on the concentrations of Ca, Al, and Ti but the ratio of $\sigma^2_G/\sigma^2_{GXE}$ indicated the greater influence and stability of genetic factor on Ca concentration in common bean seed. Although the equal contribution of environment and genotype on the concentration of Sr was indicated but a ration of $\sigma^2_G/\sigma^2_{GXE}$ over 1.0 (1.34) indicated the greater influence and stability of genetic factor in controlling the Sr concentration in common bean seed. To exclude any false positive, we considered correlation with higher R values ($R \geq 0.55$). The highest correlation was observed between Zn and S (R = 0.878) followed Sr and Ca (R = 0.871) and Zn and Fe (R=0.867). The correlation between Zn with P and Fe also reported by Gelin et al. 2007 and between Zn and Fe by Pfeiffer and McClafferty (2007).

Table1. Ratio of variances associated with environment effect to Genetic effect and genetic effect to genetic X environment effect on seed mineral compositions in common bean

	Al	**B**	**Ba**	**Ca**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**Mo**
s^2_E/s^2_G	0.25	1.28	3.37	0.23	8.14	0.90	0.86	8.68	1.29	23.30
s^2_G/s^2_{GxE}	0.13	0.08	0.07	1.91	0.28	0.66	0.07	0.10	0.05	0.22
	Na	**Ni**	**Mo**	**Na**	**Ni**	**P**	**S**	**Sr**	**Ti**	**Zn**
s^2_E/s^2_G	30.14	2.96	23.30	30.14	2.96	4.19	3.54	1.06	0.66	1.51
s^2_G/s^2_{GxE}	0.02	0.52	0.22	0.02	0.52	0.01	0.10	1.34	0.03	0.25

Table 2: Correlations among minerals in common bean seed

	Al	**B**	**Be**	**Ca**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**Mo**	**Na**	**Ni**	**P**	**S**	**Sr**
Al															
B	0.067														
Be	-0.02	0.387													
Ca	0.076	0.407	-0.12												
Cu	0.199	0.290	0.057	0.476											
Fe	0.281	0.050	-0.19	0.210	0.07										
K	0.047	0.184	-0.46	0.526	0.57*	0.34									
Mg	-0.15	0.224	0.104	0.401	0.74*	0.03	0.64*								
Mn	0.186	0.278	0.79*	0.044	-0.05	0.27	0.371	0.06							
Mo	0.250	0.344	-0.33	0.023	-0.51	0.41	-0.07	-0.49	0.032						
Na	0.012	0.332	-0.21	-0.11	-0.31	0.66*	0.170	-0.24	0.180	0.307					
Ni	-0.23	0.059	0.391	0.203	0.70*	-0.25	0.432	0.56*	0.091	-.496	-0.16				
P	0.221	-0.02	-0.26	0.497	0.63*	0.41	0.82*	0.59*	-0.06	-0.20	0.234	0.36			
S	0.073	-0.04	-0.27	0.109	0.026	0.84*	0.484	0.154	0.18	0.29	0.81*	0.02	0.49		
Sr	-0.03	0.484	0.166	0.87*	0.61*	0.04	0.319	0.408	0.167	-0.16	-0.33	0.26	0.34	0.08	
Ti	0.83*	-0.08	0.019	-0.05	-0.18	0.27	-.04	-0.12	0.118	0.100	0.119	-0.1	0.20	0.07	0.13
Zn	0.198	0.024	-0.20	0.195	0.035	0.87*	0.50	0.194	0.196	0.341	0.64*	-.10	0.57*	0.88*	0.03

*Indicate >1% level of probability

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MINING THE *PHASEOLUS* GENUS: HOW MANY SPECIES CAN *YOU* GET INTO A CROSS?

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INTRODUCTION

The *Phaseolus* genus is found in ecological niches ranging from arid deserts to tropical rainforests (Freytag and Debouck, 2002). Under different scenarios of climate change, the common bean (*Phaseolus vulgaris* L.) might confront many of these same extreme conditions, and its close relatives could be useful sources of genetic diversity for the improvement of common bean. The secondary gene pool represented by *Phaseolus coccineus*is characterized by vegetative vigor, an extensive root system, and large biomass and is adapted to environments of excessive rainfall. The tertiary gene pool including tepary bean presents good harvest index, and is adapted to hot, dry environments. In previous years a large number of families were derived from crosses of common bean with tepary bean. Mejia-Jimenez et al. (1994) recovered interspecific progenies with improved fertility, and a large number of interspecific families and lines with significant introgression from tepary bean are available and could be tapped for multiple traits (Muñoz et al. 2004).

MATERIALS AND METHODS

Interspecific crosses combining common bean, *P. coccineus* and *P. acutifolius* have been developed to combine favorable traits of different species. Elite lines were identified from intraspecific and interspecific crosses, with unique traits for drought tolerance:

- RCB 593: derived from the intraspecific cross (NCB 228 x RCB 224) x SXB 244 for drought tolerance, it presents excellent grain filling and good biomass accumulation. It is also relatively tolerant of poor soil fertility.
- ALB 74: derived from the interspecific cross of SER 16 x (SER 16 x *P. coccineus*) which was created for aluminum tolerance (Butare et al., 2011b), it presents deep rooting in soil tubes in greenhouse
- INB 841: Crosses were created among interspecific progenies of *P. vulgaris* and *P. acutifolius* that had presented a superior response to drought in previous years. These were selected under drought in the F2 and F3generations. The line INB 841 was identified in the F5 generation to be promising for drought tolerance, and to present minimal wilting and rapid pod development.

A triple cross of (ALB 74 x INB 841) x RCB 593 was created combining these three parental lines. The F2 population was selected under drought in 2010, the F3 population under anthracnose and heavy rainfall, and the F4 in an acid soil site. F3.5 families were tested again under drought at CIAT, Palmira in the June-to-September planting season in 2011.

RESULTS AND DISCUSSION

Several F3.5 families tested under drought presented traits of the interspecific parent derived from tepary bean, with comparable earliness, but with superior biomass that was derived from the other two parents.More importantly, families expressed little or no wilting while most lines and populations presented midday wilting under intense heat (>32^{o}C). This could be the result of both deep rooting derived from ALB 74, and a contribution from INB 841, which based on greenhouse soil cylinder evaluation, appears not to have a vigorous root system but rather some other mechanism. In a subsequent season, these lines again presented resistance to wilting under severe stress at flowering.

Grain filling of several lines was more accelerated than any of the three parental genotypes. We believe that this represents an enhancement of sink strength, and may result from a contribution from both the *P. vulgaris* lines (from SER 16 and RCB 593) and from INB 841.

We seek to create an ideotype which combines useful traits from several species of *Phaseolus*: good biomass accumulation from *P. vulgaris* and *P. coccineus*, with rapid photosynthate remobilization to grain in the reproductive phase, derived from *P. vulgaris* and *P. acutifolius*. While these preliminary results are based on field observations, we are hopeful that they are an early indication that this is possible.

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NITROGEN USE EFFICIENCY OF DIFFERENT DRY BEAN MARKET CLASSES IN ONTARIO

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INTRODUCTION

Success of the Green Revolution during the second half of the 20th century was in part due to investments in the development of N responsive regionally-adapted crops. Nitrogen fertilizer use exceeded 90 million Mg for the 2005-2006 crop cycle, up from less than 12 million Mg in the early 1960s (Westhoff, 2009). The increased employment of symbiotic nitrogen fixation (SNF) in agriculture world-wide is "only one aspect of the new green revolution, but a critical one" (Emerich & Krishnan, 2009). However, despite this inherent ability of legumes, SNF potential in dry bean is low, compared to other legumes (Isoi & Yoshida, 1991). Nitrogen-responsive high yielding genotypes can be separated from genotypes with stable yield under varied nitrogen regimes, offering opportunities for breeding to reduce the use of this important nutrient.

MATERIALS AND METHODS

Treatments included 64 genotypes across different market classes, with two nitrogen levels applied at planting using a lattice design. Fields for the experiment were nitrogen poor, without any recent nitrogen amendment and planted with cereals harvested as forage for the previous two crop years. In the high nitrogen treatment 100 lb/acre actual N was applied as ammonium nitrate; P and K were applied according to soil test recommendations. Plots were direct harvested at maturity. Experiments were conducted in 2010 and 2011.

RESULTS AND DISCUSSION

In both years the majority of the high-yielding genotypes were nitrogen responsive (i.e. NSI < 1) (Figure 1). Representatives from the navy, cranberry, and black seed classes, as well the sole carioca line (SXB415) were found to be high yielding stably across nitrogen treatments (i.e. NSI ≅ 1). Only SXB415 appears in this area in both years. Response of days to flowering and maturity to nitrogen treatment was as expected with added nitrogen increasing the length of the growth stages (Table 1). Yield was increased with added nitrogen in both years, but only significantly across all genotypes in 2010. Overall yields were decreased in 2011 possibly due to extended drought during July. Cultivars found to be stably high yielding in various market classes may lead to opportunities for breeding nitrogen responsive lines. Notably, these cultivars

come from both gene pools, leading to the expectation future trials will discover valuable parental material for all seed classes.

Table 1. Summary of Agronomic Data in 2010 and 2011

	Days to Flowering	Days to Maturity	100 Seed wt. (g)	Height (cm)	Yield (kg/ha)
2010					
High N	43.79	108.98	36.85	54.95	2623.79
Low N	41.90	104.38	35.29	55.68	2071.02
Reduction (%)	4.30	4.20	4.20	1.30	21.00*
2011					
High N	40.16	102.10	n.d.	48.83	1738.51
Low N	38.20	95.68	n.d.	43.45	1607.28
Reduction (%)	4.86	6.29		11.03	7.55

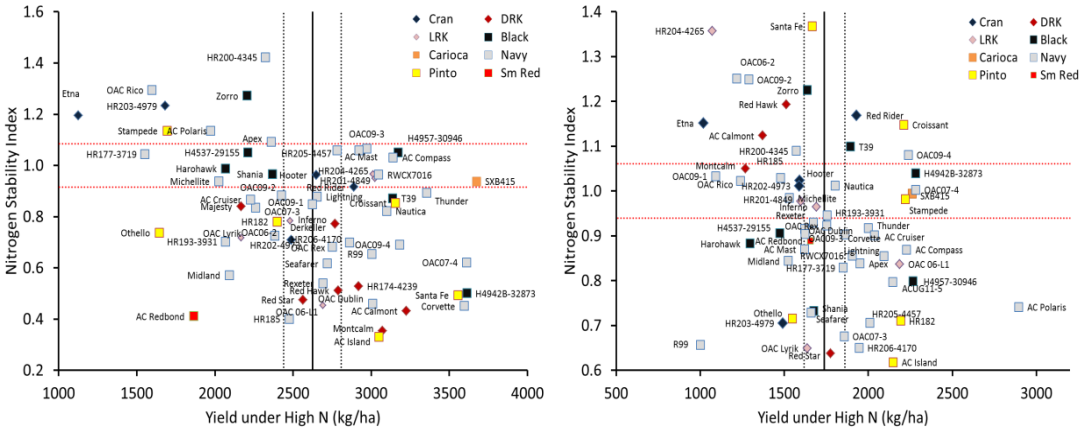

Figure 1. Yield of dry bean lines in high nitrogen conditions versus stability index of yield in 2010 (left) and 2011 (right). The Nitrogen Stability Index is calculated as 1 – ((High N yield – Low N yield)/High N yield) i.e. a line yielding equally in low and high N environments will have a stability index of 1. Also shown: mean yield in High N conditions and 99% confidence limits; and 99% confidence limits around a stability index of 1.

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PLANT ARRANGEMENT ENHANCES DRY BEAN PRODUCTION FOR SOME VARIETIES

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INTRODUCTION:

A two-year study at multiple locations compared the performance of pinto bean varieties with more upright growth habits when grown at closer plant spacing to improve production efficiency and return to growers. Upright type II varieties Croissant and Stampede were compared to more prostrate type III varieties Othello and Montrose grown as 1 or 2 rows (15 cm apart) per 75 cm wide bed at 173,000 or 207,500 plants/hectare under furrow or drip irrigated conditions in Colorado. A 4-bed Mechanical Transplanter system planted 1or 2 rows per 75-cm wide bed at a desired plant population in 2010 and 2011; with 3-4 reps of each 4-bed wide plot by 8 meters in length. The experiment compared a High Yield System (HighYS) at the CSU Research Farm near Fort Collins – furrow irrigated; versus a Low Yield System (LowYS) at the USDA/ARS Research Farm near Greeley – drip irrigated. Standard grower practices were applied for fertilizer, irrigation, weed and insect management; no fungicide treatments were required. Data included plant emergence, node height, biomass, yield as kg/Ha and seed size as 100 seed weight. All data were analyzed statistically with PC SAS combined over years and locations, as well as for individual location and year effects.

RESULTS AND DISCUSSION:

Significant interactions were noted for year, yield system, entry, year*entry, yield system*entry, year*yield system*entry, and rows per bed; interactions were not significant for year*yield system or plant population (Tables 1 and 2). Growing conditions were favorable for plant development with trace infection by plant pathogens and insect pests at both locations. Adequate furrow irrigation water and fertilizer supported plant development and pod set at the High Yield System site. The Low Yield System site with drip irrigation had less favorable growing conditions as a result of soil compaction, moderate fertility, and apparent heat stress. Yields varied from less than 2400 kg to nearly 4800 kg/hectare, depending on the variety and spacing arrangement when data were averaged for 2010 and 2011.

Table 1. Main Effects among Treatments

Treatment	Yield (kg ha^{-1})
Population	
173,000	3474
207,500	3503 NS
Yield System	
Low	2529 b
High	4312 a
Row No. / Bed	
Single	3427 b
Double	3551 a
Pinto Bean Entry	
Montrose	3557 a
Othello	3278 b
Stampede	3644 a
Croissant	3480 a

Table 2. Yield (kg/Ha) at Low (LYS) and High (HYS) Systems; 2 Yr Average

Entry - # rows/bed	LowYS	HighYS
Montrose – single	2540 a	4442 a
Montrose – double	2549 a	4381 a
Othello – single	2535 a	3742 a
Othello – double	2819 a	3866 a
[Type III mean yield diff with 2 rows]	+ 146	+ 31
Stampede – single	2579 a	4443 a
Stampede – double	2464 a	4666 a
Croissant - single	2356 a	4261 b
Croissant - double	2385 a	4716 a
[Type II mean yield diff with 2 rows]	- 44	+ 339
LSD-value $_{0.05}$: = 320		

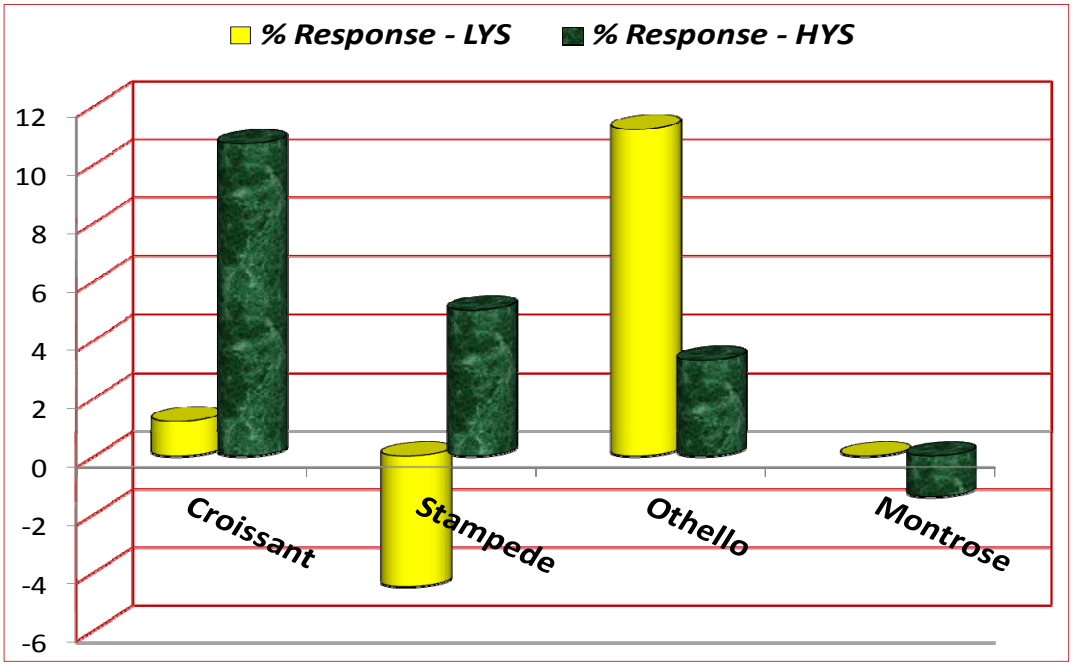

Figure 1. Yield response when 2 lines were compared to 1 line/bed in Low YS and High YS systems; averaged over years and populations.

- Traditional type III varieties (e.g., Montrose, Othello) had higher mean yield with double compared to single rows/bed under both Low (+ 146 kg ha^{-1}) and High (+ 31 kg ha^{-1}) Yield Systems [Table 2, Figure 1].
- Upright type II varieties (e.g., Croissant, Stampede) had lower mean yield with double compared to single rows/bed under Low Yield System (- 44 kg ha^{-1}), and higher mean yield under the High Yield System (+ 339 kg ha^{-1}) [Table 2, Figure 1].
- Within growth habits, there were also interactions with the Yield System and row spacing:
  - Double row increased yield for Croissant in both systems,
  - Double row increased yield for Stampede only in the High Yield System,
  - Double row increased yield for Othello in both systems, whereas Montrose had no response.

Growers must carefully choose varieties with appropriate agronomic and disease resistance characteristics suitable for their environment, production system, and integrated pest management strategy.

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UTILIZING GROWTH POUCHES TO SCREEN BLACK AND NAVY DRY BEAN BREEDING LINES FOR EARLY NODULATION

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Dry beans (*Phaseolus vulgaris*) are capable of biological nitrogen fixation (BNF), however, the amount of nitrogen fixed is generally not sufficient to yield a competitive crop. Production systems, such as organic and low input subsistence farming systems in developing countries, with potentially limited artificial nitrogen application would benefit from increased BNF in dry beans. Additionally, increased BNF could reduce fertilizer application rates under conventional systems providing environmental benefits by reducing run off and release of greenhouse gasses. Previous research at MSU identified reduced productivity as well as reduced BNF in the white seeded navy bean seed class. This research was conducted to evaluate early nodulation characteristics in elite black and navy bean breeding lines from the MSU breeding program.

MATERIALS AND METHODS

Seeds of 18 navy and 18 black seeded dry bean elite breeding lines were germinated on water agar in an incubator at 28 C. Water agar was prepared by dissolving 9 g agar in 1 L water which was then autoclaved and portioned into petri dishes. Seed was sterilized by soaking in 2.5% bleach solution for 2 m followed by 2 rinses, 2 m each, of sterilized water. After 3 days, seedlings were submerged briefly in 3 day old yeast mannitol liquid broth culture of *Rhizobium tropici* UMR 1899. Seedlings were then placed such that roots were inserted through holes cut in the top fold of 21 cm X 28 cm absorbent paper which had been autoclaved and inserted into a standard binder sheet cover. Paper was then moistened with 40 ml nitrogen free Broughton solution (Broughton and Dilworth, 1970). Individual sheets were placed in a 3 ring binder.

Plants were grown under florescent lights at ambient temperatures with 16 h d^{-1}. Nutrient solution was added as needed to maintain proper moisture. Seedlings were monitored daily. Days to first visible nodule, root length at first visible nodule, and root length at 10 d were recorded.

RESULTS AND DISCUSSION

Nodule number ranged from 2 $plant^{-1}$ to 93 $plant^{-1}$. The non-nodulating genotype R99 was included as a check, it did not form nodules over the course of the experiment. Significant differences ($p \leq 0.0002$) were seen between navy bean and black bean genotypes with regard to nodule number. Navy bean genotypes had a mean of 20.9 nodules $plant^{-1}$ while black beans averaged 28.1 nodules $plant^{-1}$ (Figure 1).

Days to first visible nodule ranged from 2.5 d to 6.0 d with an average of 4.7 d for navy and 4.6 d for black. Root length ranged from 6.1 cm to 25.5 cm with an average of 15.1 cm and 15.2 cm for navy and black beans respectively. Root growth after first nodule development ranged from 0.5 cm to 14.8 cm. There were no significant differences between black and navy beans for days to visible nodules, root length, or root growth.

Hungria and Phillips (1993) reported a reduction in nodule number in a white seeded bean genotype when compared to a black seeded line which differed only for seed color at the p locus. They reported a reduced amount of nod gene inducing anthocyanins found in the white seeded genotype compared to the black seeded genotype. This difference resulted in a delay of the symbiosis between bean plant and rhizobia. Flavanoids which are important to early nodule development are lacking or reduced in white seeded lines. This difference may result in reduced BNF ability and productivity in white seeded cultivars.

This research on early nodulation will be combined with information on nodulation and nitrogen fixation at flowering to determine nitrogen harvest by analyzing nitrogen content of the harvested seed. These combined evaluations will help to determine at which phase BNF may be most important and offer the greatest contribution to improving yield. Increasing the time during which BNF occurs by selecting for earlier nodulation, as outlined in this study, may increase the overall amount of nitrogen fixed by common bean.

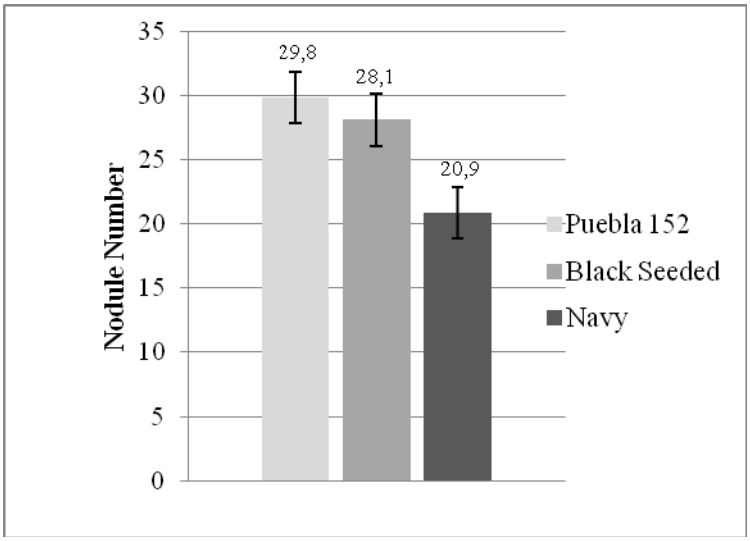

Figure 1. Nodule number by bean seed class.

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INHERITANCE AND QUANTITATIVE TRAIT LOCI ANALYSIS OF FOLATE CONTENT IN DRY BEANS

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INTRODUCTION

Folates are essential vitamins (B_9) needed during rapid cell division that function during DNA, RNA and protein syntheses. Folate deficiency is known to be linked to a number of health problems, including neural tube defects in newborns and many types of cancers in adults. Common beans (*Phaseolus vulgaris* L.) can contain high levels of folate, yet the level of folate may vary significantly among bean genotypes. Naturally occurring forms of folate include terahydrofolate, 5-methylterahydrofolate (5MTHF), 5-formyltetrahydro folate and 10-formyltetrahydrofolate. Among them, 5MTHF accounts for more than 80% of total folate content in common beans (Rebeile et al., 2006). Folates tend to degrade during high temperature processing (Xue et al., 2011). The objectives of this study were to examine the inheritance of folate content in an inter-gene pool F_2 population of dry beans and to identify genomic regions associated with folate content in dry beans.

MATERIALS AND METHODS

Plant material, Field experiment and Phenotyping. Four varieties of common bean were used as parents; 'SVM Taylor Hort', 'Redhawk' and 'AC Elk' from the large-seeded Andean gene-pool and 'Othello' from the Mesoamerican gene-pool. The parents were crossed in a one-way diallel mating design to produce six F_1 hybrids. Parental lines, F_1 crosses and the F_2 populations from each cross were planted in the field in a randomized complete block design with 3 replications. At maturity, three plants from four parental lines, single plants from F_1 hybrids and every single plant from the F_2 populations were hand harvested dried at 30°C, threshed using a single plant thresher and seeds were collected in separate paper envelope. The whole bean seeds were finely ground and the powders were used to measure folate contents. Total folate and 5MTHF content of parental lines, F_1 crosses and one of the F_2 populations was measured using high pressure liquid chromatography (HPLC) with a fluorescence detector, twice after extraction, with a one hour interval at AAFC, Guelph Food Research Center.

SNPs Genotyping. DNA was extracted from leaf tissues collected from Parents, F_1 crosses and F_2 single plants. Based on the significant differences in folate content of parental lines, the F_2 population derived from the cross between Othello and Redhawk was chosen for the QTL mapping study and was genotyped with 116 informative SNP markers (Shi et al. 2011). A linkage map was constructed using JoinMap 4 and was compared with the linkage map reported

by McConell et al. (2010) as a reference map. Single marker QTL analysis was performed to test the association of markers with folate content.

RESULTS AND DISCUSSION

Folate content was significantly different among parents ranging from 147 to 355 µg/100g. The F_2 population of the cross between Othello x Redhawk had continuous variation for folate content but the value of the F_1 deviated from population mean. Transgressive segregation for folate content was observed in both ends of the frequency distribution. Out of 116, sixty seven (58%) SNP markers were polymorphic between the parental lines. Fourty four percent of the markers were aligned with the reference map (McConell et al. 2010). QTL analysis identified a total of eight markers significantly associated with folate content; two each from linkage groups 6 and 9, and one each from 2, 4, 10 and 11 (Table 1). Three markers were significantly associated with folate content in the first injected solution. For the majority of identified QTL, dominance effects appeared to be the major genetic effect. QTL identified for the first injected solution were not effective for the measurements taken after one hour.

Table 1. SNPs markers significantly associated with folate content in a dry bean F_2 population of a cross between Othello and Redhawk.

			First injected solution								Solution injected after one hour							
	Position		5- Methyltetrahydrofolate				Total Folate				5- Methyltetrahydrofolate				Total Folate			
Marker	LG	cM	*P* value	Add.	Dom.	R^2_p	*P* value	Add.	Dom.	R^2_p	P value	Add.	Dom.	R^2_p	*P* value	Add.	Dom.	R^2_p
g457_B	2	112.6	ns	10.26	-2.37	1.2	ns	10.39	-1.43	1.1	ns	0.61	22.9**	4.8	0.04	0.89	25.39**	8.10
g1286	9	62.5	0.03	2.32	28.42**	7.7	0.03	2.71	31.08***	7.9	ns	3.65	-1.21	0.4	ns	4.22	-2.66	0.50
g2498	9	58.6	0.04	1.34	28.91**	7.8	0.04	1.15	30.98**	7.7	ns	0.97	-6.2	0.7	ns	0.07	-8.48	1.10
g2135	11	9.01	0.02	-11.24	24.37*	9.3	0.01	-13.14	27.45*	10.5	ns	-2.15	6.63	0.3	ns	-2.07	8.48	0.40
g1375	4	70.9	ns	0.98	-5.44	0.2	ns	1.32	-5.66	0.2	ns	6.07	18.83*	5	ns	6.13	19.99*	5.60
g2208_B	6	15.5	ns	-11.6	-22.57	6.5	ns	-11.58	-25.68*	7	ns	-0.33	-13.35	2	ns	-0.8	-15.99	3.00
g1436	6	73.5	ns	-8.88	-10.99	2	ns	-9.56	-10.42	2	ns	4.28	-18.98*	6	ns	4.65	-21.24*	6.00
g2260	10	81.8	ns	3.39	-11.28	1.3	ns	5.33	-12.15	1.5	ns	13.94*	-2.02	5	ns	15.14*	-0.57	5.00
Total variance explained by significant markers						23				22				ns				8

Add., Additive effect estimated as half the difference of the two homozygous genotypic groups at each locus; Dom., Dominance effect estimated as the deviation of heterozygous genotypic group from mid parental genotypes at each locus; ns, not significant at p=0.05.

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RESISTANCE TO POWDERY MILDEW IN COMMON BEAN GERMPLASM

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Powdery mildew is an important disease in common bean, causing severe yield losses worldwide. In Asturias (northern Spain), powdery mildew is caused by the fungus *Erysiphe diffusa* (Cooke & Peck) U. Braun & S. Takam, (N. Rispail and D. Rubiales per. com.). Disease produces small, round, greyish or whitish spots on leaves and/or stems which can finally cover the whole upper leaf, even causing plant death. The use of resistant cultivars is the most efficient method to control the disease.

The aim of this work was to investigate the genetic control of resistance using a 0-4 scale to describe the infection types (IT) observed after artificial inoculation.

MATERIAL AND METHODS

Five F_2 populations were evaluated to investigate the inheritance of the resistance (Table 1). In addition, 83 $F_{2:3}$ families derived from the cross between the susceptible cv. Xana and the resistant cv. Porrillo Sintético were analyzed and the number of tested F_3 plants was increased in six families to confirm the mode of inheritance.

A local isolate of powdery mildew obtained from a single spot was used. The inoculation procedure consisted in blowing conidia on seedlings with totally developed primary leaves, in a density of 5-10 spores/cm^2. Inoculations were performed in sets of 25 pots with 4-5 plants placed in a box (80 x 80 x 80 cm). Plants were maintained in greenhouse at moderate temperature (18-24 ºC) and humidity (60-70%).

Plant response was recorded 12 days after inoculation following a 0-4 scale of infection types adapted from Mains and Dietz (1930): IT0, no visible symptoms; IT1, necrotic reaction on the leaves with little or no mycelial development; IT2, necrotic reaction and moderate mycelial development; IT3, moderate mycelial development on the leaves; IT4, abundant mycelial development on the leaves and profuse sporulation.

RESULTS AND DISCUSSION

Observed reactions in five segregating populations fitted to mendelian ratios and different modes of inheritance (Table 1). Reaction of F_2 populations derived from crosses between parents with different IT suggested a qualitative nature of the resistance. Result suggest that resistance in genotypes Cornell 49242 and Porrillo Sintético is conferred by two dominant and independent genes; one gene conferring IT0 (no visible symptoms), and another gene conferring IT3 (limited growth of the pathogen). Both genes show a dominant epistatic relationship. The 9:7 ratio observed in the population derived from Amanda (IT0) x Xana (IT4) suggests that two

complementary genes are involved in the resistance of cultivar Amanda. Finally, IT3 in cultivar X2776 seems to be controlled by one dominant gene, which is also present in Porrillo sintético.

Table 1. Observed segregation for resistance to powdery mildew in five F_2 populations. P1, parent 1; P2, parent 2.

	Parental phenotypes (IT)			Observed segregation					
Parent 1 x Parent 2	P1	P2	Test[a]	IT0	IT3	IT4	Expected ratio	χ^2	*P*
Xana x Cornell 49242	4	0	1	55	13	10	12:3:1	5.78	0.06
			2	51	12	4	12:3:1	0.00	0.98
Xana x Porrillo Sintético	4	0	1	76	23	5	12:3:1	1.18	0.55
			2	96	19	2	12:3:1	5.02	0.08
Amanda x Xana	0	4	1	46	-	36	9:7	0.01	0.92
			2	12	-	13	9:7	0.69	0.40
X2776 x G122	3	4	1	-	91	24	3:1	1.29	0.26
			2	-	73	23	3:1	0.00	0.81
Porrillo Sintético x X2776	0	3	1	65	16	-	3:1	1.19	0.27
			2	80	25	-	3:1	0.11	0.73

[a] Evaluations were carried out in two independent tests

A total of 83 $F_{2:3}$ families obtained from the cross Xana x Porrillo Sintético were also evaluated, and six $F_{2:3}$ families were selected in order to carried out a genetic dissection. Observed segregation fitted to a 3:1 ratio, expected for one dominant gene (Table 2). Results confirmed the presence of two dominant genes in Porrillo Sintético controlling IT0 and IT3, respectively.

Table 2. Observed segregation in six $F_{2:3}$ families derived from the cross Xana x Porrillo Sintético.

	Observed Segregation					
F_3 families	IT0	IT3	IT4	Expected ratio	χ^2	*P*
F_3-59	52	-	15	3:1	0.24	0.62
F_3-63	43	-	16	3:1	0.14	0.71
F_3-22	42	13	-	3:1	0.00	0.81
F_3-71	48	14	-	3:1	0.19	0.66
F_3-37	-	43	15	3:1	0.00	0.87
F_3-95	-	54	17	3:1	0.00	0.89

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EFFECT OF DRY BEAN FLOUR ON DOUGH STRENGTH, EXTRUSION PROPERTIES, AND PASTA QUALITY

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INTRODUCTION

The presence of non-traditional ingredients in pasta can influence dough strength, extrusion properties, and pasta quality. Dry bean flour (non-traditional ingredient) can be added to pasta to improve its nutritional value because it provides to the human diet a good source of protein, carbohydrate, dietary fiber, and some minerals and vitamins. Dough strength is proven to affect mechanical energy and the rate of pasta extrusion, depending on the hydration levels and dough temperature (Yalla and Manthey, 2006). Pasta extrusion is a continuous process and it depends primarily on screw speed and dough strength. These variables influence the residence time distribution, the energy (specific mechanical energy, SME) required, product temperature, the pressure at the die, and product quality target attributes (Owolabi et al., 2008). Preliminary research was conducted to determine the effect of dry bean flour blended with semolina (1:4) on dough strength, pasting properties, extrusion properties, and pasta quality.

MATERIALS AND METHODS

Commercial semolina was obtained from the ND State Mill (Grand Forks, ND). A total of 14 cultivars of dry beans representing four market classes (black, kidney, navy and pinto) were milled into flour using a laboratory scale hammermill. Dry bean flour was blended with semolina (1:4). Dough strength and pasting properties were determined using a mixograph according to AACCI method 54-20.02 and Rapid Visco Analyzer according to AACCI method 76-21, respectively. Semolina and bean flour blends were extruded as spaghetti using a semi-commercial laboratory extruder. Processing data collected included extrusion pressure, extrusion rate and mechanical energy. Specific mechanical energy was determined as Specific Energy/Extrusion rate. Spaghetti was dried using high temperature (70C) drying profile typical of what is used commercially. Cooking quality (optimum cook time, cooked weight, cooked firmness and cooking loss) was determined using AACCI method 65-50. All tests were done in duplicate.

RESULTS AND DISCUSSION

Spaghetti containing navy bean flour had similar color and appearance as spaghetti containing only semolina. Spaghetti containing black bean cultivars showed distinct dark brown appearance. Dry bean flour significantly affected dough properties. In general, dry bean flour increased hydration time and reduced tolerance to overmixing. Dough properties of semolina-bean flour blends varied with market class and with cultivar within market class (Figure 1). Semolina-kidney bean flour blends produced sticky and weak doughs compared to semolina blends containing flour from other market classes. These results might be due to the variability of content and composition of protein of dry beans. As reported by Sathe (2002), protein content in navy, pinto, kidney, and black beans is 22.13, 23.69, 25-29, and 23.50%, respectively. The

albumin:globulin ratio for navy, pinto, kidney, and black beans is 0.97, 0.44, 0.21-0.28, and 1.08%, respectively.

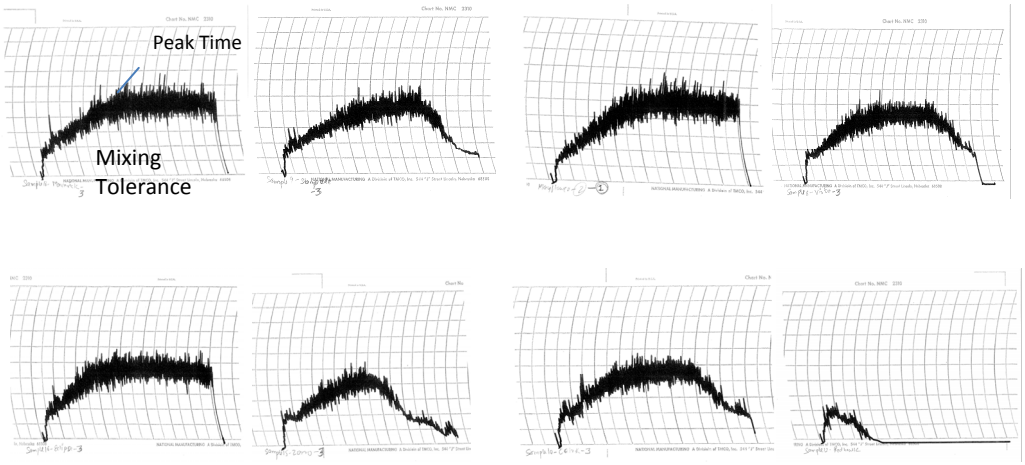

Figure 1. Mixograph of the best and poorest result of some varieties of Pinto, Navy, Black and Kidney beans.

Pasting properties were reduced by the presence of dry bean flour in the semolina. Peak viscosity for semolina alone was over 150 RVU, and for most of market classes was 50-80 RVU. However, semolina blended with flour from Stampede (Pinto) had the highest peak viscosity (146 RVU) when compared to flour from other market classes.

Semolina-dry bean flour blends required higher pressure and more energy to extrude than did spaghetti containing only semolina (control).

Spaghetti containing dry bean flour had shorter cooking time (442-532 sec), greater cooking loss (7.2-8%), and similar cooked firmness (5.0-5.8 gcm) compared to traditional spaghetti (600 sec, 6.2%, and 5.1 gcm, respectively). Cooking quality of spaghetti containing dry bean flour is within the acceptable range.

CONCLUSION

Even tough results varied among market class and among varieties within a market class, the addition of dry bean flour into pasta processing is possible. Further research is needed to determine why these differences occurred and to optimize processing parameters and to assess sensory acceptability.

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ESTABLISHING A STANDARDIZED PROTOCOL FOR DRY BEAN CANNING AT NORTH DAKOTA STATE UNIVERSITY

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INTRODUCTION

The acceptability of canned dry beans by consumers is largely dependent on their quality characteristics. Quality traits are influenced by plant genotype, as well as many processing variables such as soak and blanch time, brine composition, calcium levels in soak water/blanch water/brine, and duration of canning time in the retort (Balasubramanian et al., 2000).

MATERIALS & METHODS

A total of 82 advanced breeding lines including 13 black, 11 navy, 17 great northern, 16 pinto, 14 red/pink, and 11 kidney lines were screened and compared to market standards. Two replications were canned and evaluated from one field location. Seed was cleaned and placed into a humidity chamber at 17°C to increase and equilibrate seed moisture to around 14 to18%. A 90 g solid sub-sample was prepared based on each sample's current moisture content. A standard soak procedure was utilized for each market class; kidney and pinto beans were placed in a 25°C cold soak for 12 to 14 h and navy, great northern, and red/pink beans for 30 min. A 30 min hot soak followed for all market classes. Soaked beans were submersed into cold tap water for 3 to 5 min then drained and transferred to labeled cans. A gently boiling brine solution was added to each can and cans were immediately sealed and placed into a Dixie retort (Model RDSW-3). The retort was heated to 115°C for 45 min followed by a 15 min cooling cycle. Cans were then stored for four weeks at ambient air temperature. After storage, cans were evaluated by a panel of eight people based on Michigan State University's visual scale from 1 to 7 where 1=unacceptable, 2=poor, 3 to 4=average, 5 to 6=above average, and 7=excellent (Michigan State University, 2010).

RESULTS & DISCUSSION

Rating scores of breeding lines did not interact with evaluator except within the pinto group. For all other market classes, differences could be explained by variation among genotypes, not evaluators. *Pinto* (Figure 1): Visual ratings ranged from 2.4 to 4.7. Breeding line significantly interacted with evaluator in the pinto market class. Even so, 5 of 16 breeding lines were rated similar to Lariat (rating 4.7). *Navy*: Visual ratings ranged from 2.9 to 5.5. ND021001 (rating 5.5) was rated better than Vista (rating 4.0). However, ND021001 was similar to ND021006 and ND060514. Only one (02-220-01N) of the 11 breeding lines was rated less than 3.0. *Black*: Visual ratings ranged from 2.5 to 3.2. All breeding lines were similar to the standard; however, the dark brine color suggested the canning protocol may have been too harsh for this market class. *Kidney*: Visual ratings ranged from 3.2 to 5.1. All breeding lines rated less than Pink Panther (rating 5.1). However, 7 of 11 breeding lines were rated similar to Redhawk (rating 4.7). *Great Northern*: Visual ratings ranged from 2.1 to 5.7. ND080486 and NDF09008 rated higher than Matterhorn (rating 4.0). Twelve of 17 breeding lines were rated similar to Matterhorn.

Red/Pink: Visual ratings ranged from 1.4 to 3.8. ND080542 was rated similar to Merlot (rating 3.8) and Sedona (rating 3.4). The remaining 13 breeding lines were rated less than 3.0.

CONCLUSIONS

A protocol has been established to evaluate differences in canning quality based on genotype. All market classes, except pintos and blacks, had a significant breeding line main effect; therefore, differences were based on differences among breeding lines, not evaluators. Future evaluations will involve the incorporation of drain weight and brine score, as well as, the use of multiple locations for seed source in order to estimate genotype-by-environment interactions. Also, further modifications need to be made for a black bean canning protocol.

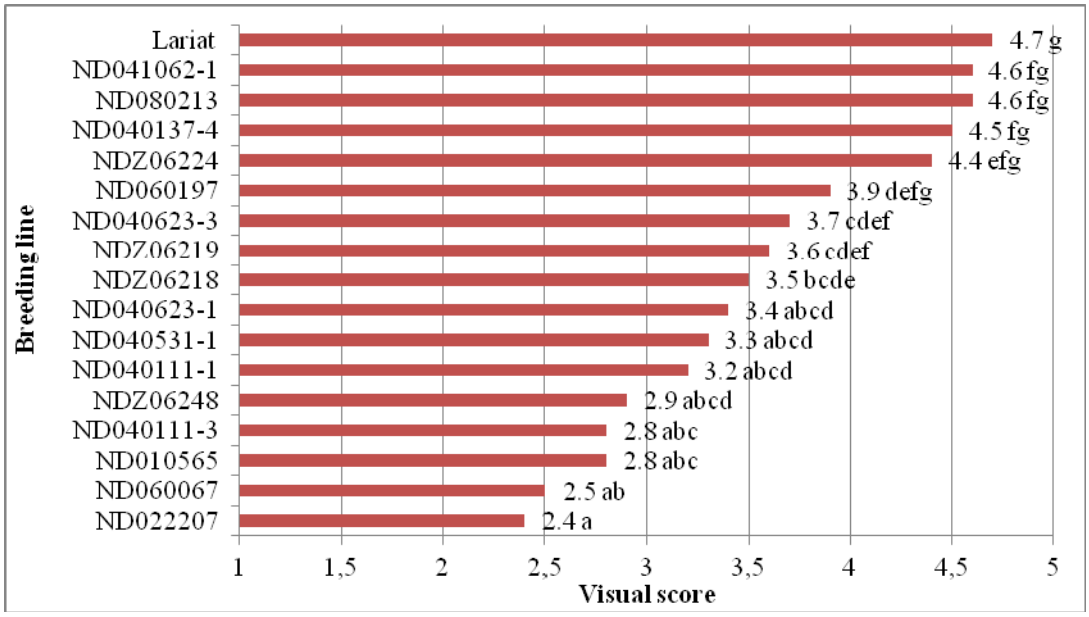

Figure 1. Pinto canning evaluation scores using a visual scale from 1-7; 1=unacceptable, 2=poor, 3 to 4=average, 5 to 6=above average, and 7=excellent.

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GENETIC AND ENVIRONMENTAL EFFECTS ON CANNING QUALITY OF PINTO AND NAVY BEAN CULTIVARS COMMONLY GROWN IN THE CENTRAL U.S.

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Canning quality of dry beans is influenced by several factors such as genotype, environment, and its interaction with the genotype. The canning industry is supplied with dry beans that may come from different growing areas. Each region has specific environmental conditions that will directly affect the quality properties (physical, chemical, and sensory) of the dry beans. From a breeding standpoint, some of these properties are genetically controlled; therefore, it would be possible to identify cultivars that are better suited for the canning process and are less affected by environmental factors. Assuming a standard canning process, this study attempts to understand how much of the variation in canning quality could be attributed to differences in genotype and how much is due to environmental effects and the interactions among these factors.

MATERIALS AND METHODS:

A total of 14 pinto and 8 navy bean cultivars were planted in two field trials in 2010 at 8 locations across central U.S.: Michigan (Wheeler and Richville), Minnesota (Park Rapids), Nebraska (Mitchell and Scottsbluff), and North Dakota (Hatton, Johnstown, and Prosper). Field trials were planted by market class as a randomized complete block design with four replications. Experimental units consisted of 4-row plots, where the two center rows were harvested. Agronomic and yield data were measured and seed samples were bulked for the canning process, where field replications 1 and 2 formed replication 1 for canning and field replications 3 and 4 became replication 2. Canning tests were made at Michigan State University where eight cans per replication were processed. For evaluation, three cans were opened four weeks after canning, three more 15 weeks after, and one can was used for a separate evaluation four weeks after canning, using an independent panel of 14 evaluators. Agronomic and canning data was used in a combined analysis of variance across locations, using a mixed model in which locations and replications were considered random effects and cultivars were considered fixed.

RESULTS AND DISCUSSION:

As seen in other yield trials, agronomic traits showed significant differences among cultivars, locations, and in most cases, a significant (but small) cultivar-by-location interaction. For the canning traits, the two most important factors were location and cultivar with a small number of significant (but small) cultivar-by-location interactions. For pinto beans, there were no significant changes over time for visual score and drain weight, which may be an indication that this market class equilibrated soon after canning or that there was limited starch release within this group. The differences observed in navy beans for brine visual scores between the 4 and 15 week evaluation suggest that this group may release more starch over time compared to pinto

beans; therefore, cultivars with low starch release would be highly desirable. Field trials were repeated during the 2011 growing season, for a total of 16 environments (eight locations, two years), and canning tests are underway.

Table 1. Means of agronomic and canning traits measured across eight locations across central U.S. during 2010.

	FIELD TRIALS							CANNING EVALUATION AFTER 4 WEEKS				CANNING EVALUATION AFTER 15 WEEKS			
CULTIVAR†	SEED YIELD	DAYS TO MATURITY	DAYS TO FLOWERING	PLANT HEIGHT	SEED MOISTURE	100-SEED WEIGHT	SEED PROTEIN	VISUAL SCORE	DRAIN WEIGHT	DAMAGED BEANS	BRINE VISUAL SCORE	VISUAL SCORE	DRAIN WEIGHT	DAMAGED BEANS	BRINE VISUAL SCORE
	Kg Ha^{-1}	d	d	cm	%	g	%	1 to 9	g	n	1 to 9	1 to 9	g	n	1 to 9
PINTO															
ND-307	2222	91	32	46	16.9	38.1	22.3	4.9	223.6	14	4.9	4.9	224.5	17	6.2
LARIAT	2179	93	32	50	17.4	37.5	21.3	4.0	206.7	11	4.7	4.3	206.2	13	6.1
LA PAZ	2127	90	31	48	17.0	35.5	22.0	5.0	209.1	15	4.9	4.6	208.4	17	6.3
WINDBREAKER	2126	88	29	38	16.9	38.8	22.5	4.5	207.4	14	5.0	4.4	208.4	17	6.4
CHASE	2091	88	30	37	16.2	35.7	22.6	4.4	203.9	15	5.3	4.4	204.4	15	6.6
BUSTER	2055	87	28	39	17.0	38.0	21.1	5.5	222.8	23	4.3	5.1	221.4	23	5.9
MAVERICK	2016	87	28	41	16.8	36.6	21.8	4.8	210.7	10	4.7	4.4	211.8	11	5.9
PONCHO	2006	87	27	37	17.1	38.9	19.8	4.7	209.5	12	4.0	4.8	209.6	13	5.7
SANTA FE	1956	89	30	45	17.1	41.8	23.0	6.4	221.9	23	4.9	6.6	219.1	24	6.4
MONTROSE	1893	86	27	36	17.5	36.5	20.6	4.5	204.2	9	5.0	4.6	206.4	9	6.3
STAMPEDE	1869	89	29	46	16.6	36.7	22.3	4.7	215.7	13	5.8	5.2	215.9	13	6.1
BILLZ	1816	88	29	40	14.1	35.1	20.8	4.1	204.7	8	4.6	4.3	205.5	10	6.1
OTHELLO	1778	86	28	37	17.3	36.3	20.6	4.6	211.1	12	5.6	4.7	212.4	11	6.5
BUCKSKIN	1712	86	27	38	17.2	37.2	20.6	5.4	209.9	18	4.1	4.9	210.4	20	5.5
MEAN	1989	88	29	41	16.8	37.3	21.5	4.8	211.5	14	4.8	4.8	211.7	15	6.1
LSD (0.05)	274	2	2	3	2.1	1.8	1.1	1.9	1.9	1	1.9	1.9	1.9	1	1.9
NAVY															
VISTA	2215	98	36	46	17.9	17.9	22.6	4.4	211.4	35	2.1	4.0	209.2	37	4.6
MEDALIST	2193	97	35	47	17.8	16.8	20.4	3.6	209.9	22	2.1	3.1	209.5	29	4.5
AVALANCHE	2183	94	31	45	17.4	19.0	22.8	5.6	210.9	45	2.2	4.6	207.5	50	4.4
T9905	2181	97	35	47	17.5	20.5	21.9	4.5	221.9	35	3.0	4.1	219.9	40	4.6
NAVIGATOR	2067	96	34	51	17.2	18.0	22.8	5.1	207.6	36	2.0	4.0	206.4	40	4.3
SCHOONER	2051	98	36	40	17.6	16.5	21.7	4.6	219.7	37	1.8	4.0	217.6	46	4.1
NORSTAR	1809	94	31	40	18.0	17.7	22.2	5.1	219.7	41	1.7	4.3	217.3	48	4.0
ENSIGN	1793	91	29	41	17.3	19.5	21.4	5.1	205.9	41	2.8	4.3	206.2	43	4.4
MEAN	2062	95	33	45	17.6	18.2	22.0	4.8	213.4	37	2.2	4.0	211.7	42	4.4
LSD (0.05)	261	3	3.6	5	0.8	1.1	1.1	0.6	9.1	7	0.4	0.4	8.6	8	0.3

† Cultivars sorted by seed yield (highest to lowest) within each market class.

FIGURE 1. VISUAL CANNING SCORE FOR 14 PINTO CULTIVARS 15 WEEKS† AFTER CANNING (1-BEST, 9-WORST)

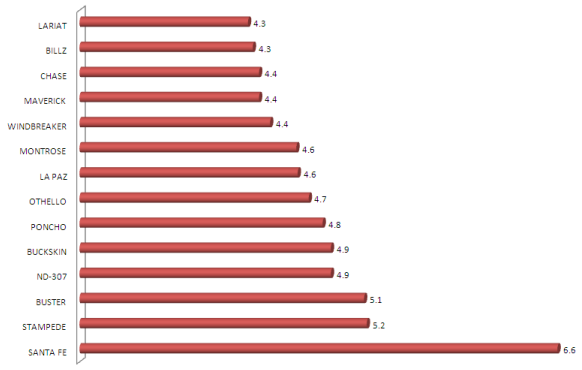
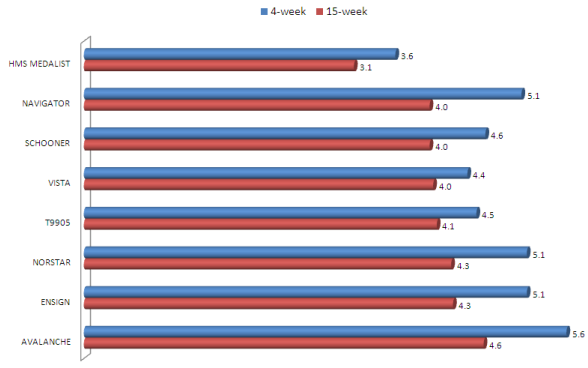


FIGURE 2. VISUAL CANNING SCORE FOR 8 NAVY CULTIVARS 4 AND 15 WEEKS AFTER CANNING (1-BEST, 9-WORST)



† There were no significant differences between the visual scores for 4 and 15 weeks after canning and therefore, only the scores after 15 weeks are shown.

MOLECULAR AND PHENOTYPIC EVIDENCE FOR MULTIPLE ALLELES AT THE RECESSIVE POTYVIRUS RESISTANCE LOCUS *eIF4E*

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The *bc-3* locus is one of three loci that condition isolate-specific recessive resistance to the important potyviruses *Bean common mosaic virus* (BCMV) and *Bean common mosaic necrosis virus* (BCMNV). The *bc-3* resistance allele has also recently been confirmed to condition resistance to several strains of *Clover yellow vein virus* (ClYVV), a component of an important aphid-transmitted virus-disease complex of U.S. Great Lakes snap bean production (Larsen et al., 2008). Recessive resistance to BCMVand BCMNV in common bean germplasm with the *bc-3* resistance allele has been demonstrated to be associated with non-synonymous amino acid substitutions in the eukaryotic translation initiation factor 4E (*eIF4E*) (Naderpour *et al*. 2010). Coding sequence analysis of *eIF4E* in additional potyvirus-resistant common bean germplasm revealed the possibility of allelic variants. Resistance assays and genetic complementation tests were carried out to provide evidence that there are multiple alleles of *eIF4E* that vary in their nucleotide and amino acid sequences and their respective resistance spectra.

MATERIALS AND METHODS

Coding sequence of *eIF4E* was obtained for common bean cultivars and breeding lines listed in Table 1 by employing similar methodology to Naderpour *et al.* (2010). The snap bean breeding line CY-10S is a BC_8S_4 line developed by introgressing the resistance to ClYVV-NY from Clipper into the snap bean cultivar Hystyle. The snap bean breeding line USWKH x H F5 is a BC_2S_4 line developed by introgressing the resistance to ClYVV-NY from USWK6 into the snap cultivar Hystyle. The breeding line B/R RIL105-25 is a recombinant inbred line (RIL) of navy bean seed type derived from a white mold resistance mapping population that was confirmed to carry the *bc-3* resistance allele from Raven. The cultivars and breeding lines were evaluated for virus resistance by standard methodology for mechanical inoculation with the virus isolates ClYVV-NY and BCMV NL-3. Plants that remained asymptomatic 10 days post inoculation (DPI) were re-inoculated to prevent escape and symptoms were evaluated 10, 21, 30, and 45 DPI with reference to the susceptible controls Jade (ClYVV), and Clipper (NL-3). Confirmation of resistance to systemic infection was accomplished by DAS-ELISA using the ClYVV assay from AC Diagnostics, Inc. Table 1 summarizes the differential responses to infection with ClYVV-NY (R=Resistant to Systemic Infection; S= Systemic Infection), the proposed *eIF4E* genotype, and the *eIF4E* sequence features of the cultivars and lines. F1, F2, and F2:F3 Populations were developed by crossing the cvs./lines as listed in Table 2[P1-1 x P2-1 & P1-2 x P2-2]. The cvs./lines were chosen based on their variant *eIF4E* sequence features and their differential reactions to inoculation with ClYVV-NY and BCNV NL-3.

RESULTS

The results of the cDNA and amino acid sequence analysisrevealed a putative novel resistance allele (*PveIF4E*^{3}) characterized by 3 of 4 identical codon changes corresponding to non-synonymous amino acid substitutions previously identified in the *PveIF4E*^{2} resistance allele (Table 1). The results of the virus resistance evaluations for the parents and populations

developed are outlined in Table 2. All parents (n=18), F_1 (n=18), and F_2 (Pop.1: n=160; Pop.2: n=157) individuals showed no symptoms in response to repeated inoculation with ClYVV-NY. DAS-ELISA confirmed the lack of systemic infection by ClYVV in all plants tested as all absorbance values (405 nm) were ~20 fold less than positive controls (data not shown). Parental lines Clipper (*ii, PveIF4E*^{3} *PveIF4E*^{3}) and CY-10S2 (*II, PveIF4E*^{3} *PveIF4E*^{3}) exhibited expected symptoms of systemic infection in response to BCMV NL-3. Parental lines B/R RIL 105-25 (*II, PveIF4E*^{2} *PveIF4E*^{2}) and USWKH x H F5 (*II, PveIF4E*^{2} *PveIF4E*^{2}) showed no symptoms in response to repeated inoculation with NL-3. *PveIF4E* heterozygous (*Ii, PveIF4E*^{2} *PveIF4E*^{3}*)* F_1 plants exhibited expected symptoms of systemic infection in response to NL-3.

Table 1.

Cultivar / Line (Reaction to ClYVV)	Genotype	Nucleotide(nt)/amino acid (aa) positions							
		159/53		194/65		227/76		332/111	
		nt	aa	nt	aa	nt	aa	nt	aa
Dubbele Witte (S)	*ii , PveIF4E*^{3} *PveIF4E*^{3}	C	Asn	T	Phe	C	Ala	A	Asp
Hystyle (S)	*II , PveIF4E*^{2} *PveIF4E*^{2}	C	Asn	T	Phe	C	Ala	A	Asp
Clipper (R)	*ii , PveIF4E*^{3} *PveIF4E*^{3}	A	Lys	A	Tyr	A	Glu	A	Asp
CY-10S (R)	*II , PveIF4E*^{3} *PveIF4E*^{3}	A	Lys	A	Tyr	A	Glu	A	Asp
Imuna (R)	*ii , PveIF4E*^{3} *PveIF4E*^{3}	A	Lys	A	Tyr	A	Glu	A	Asp
Raven (R)	*II , PveIF4E*^{2} *PveIF4E*^{2}	A	Lys	A	Tyr	A	Glu	G	Gly
B/R RIL105-25 (R)	*II , PveIF4E*^{2} *PveIF4E*^{2}	A	Lys	A	Tyr	A	Glu	G	Gly
USWK6 (R)	*II , PveIF4E*^{2} *PveIF4E*^{2}	A	Lys	A	Tyr	A	Glu	G	Gly
USWKH x H F5 (R)	*II , PveIF4E*^{2} *PveIF4E*^{2}	A	Lys	A	Tyr	A	Glu	G	Gly
IVT7214 (R)	*ii , PveIF4E*^{3} *PveIF4E*^{3}	A	Lys	A	Tyr	A	Glu	G	Gly

Table 2.

Cultivar/ Line	Genotype	Symptoms - ClYVV	Symptoms - NL-3*			
			NS	Lc, Ld, M	VN/dTN/D	TN/D
[P1-1] Clipper	*ii , PveIF4E*^{3} *PveIF4E*^{3}	NS - 18/18	0	18/18	0	0
[P2-1] B/R RIL 105-25	*II, PveIF4E*^{2} *PveIF4E*^{2}	NS - 18/18	18/18	0	0	0
[F1-1] Clipper x B/R RIL105-25	*Ii, PveIF4E*^{3} *PveIF4E*^{2}	NS - 18/18	0	0	0	18/18
[F2-1] Clipper x B/R RIL105-25	Segregating	NS - 160/160	NP	NP	NP	NP
[P1-2] CY-10S2	*II, PveIF4E*^{3} *PveIF4E*^{3}	NS - 18/18	0	0	0	18
[P2-2] USWKxH F5	*II, PveIF4E*^{2} *PveIF4E*^{2}	NS - 18/18	18/18	0	0	0
[F1-2] CY-10S2 x USWK xH F5	*Ii, PveIF4E*^{3} *PveIF4E*^{2}	NS - 18/18	0	0	0	18
[F2-2] CY-10S2 x USWK xH F5	Segregating	NS - 157/157	NP	NP	NP	NP

*Symptoms: NS=No Symptoms; Lc, Ld, M=Leaf curl, Leaf distortion, Mosaic; VN=Vein Necrosis; TN=Top Necrois; dTN=delayed Top Necrosis; D=Death of Plant; sS=severe Stunting (NP=Not Phenotyped)

DISCUSSION

There is strong evidence for an additional resistance allele (*PveIF4E*^{3}*)* with a unique resistance spectrum at the *PveIF4E* locus. The resistance spectrum of *PveIF4E*^{3} *(*Clipper allele) is reduced in comparison to the *PveIF4E*^{2} *(*IVT7214 allele). Deployment of *PveIF4E*^{2} should therefore provide the greatest level of protection to damage incited by BCMV, BCMNV, ClYVV-NY, ClYVV-OR, and ClYVV-WI. Future research will evaluate and confirm resistance in the $F_{2:3}$ lines of both populations developed. Efforts will continue the introgression of *PveIF4E*^{2} into snap bean as no cultivars have been identified that carry this important allele. This and future research will assist in continuing to define the gene-for-gene relationship and interactions of the *Phaseolus vulgaris* – potyvirus pathosystem.

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FEASIBILITY OF A 'HOME CANNING' METHOD FOR BEAN QUALITY ASSESSMENT

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INTRODUCTION: Attaining consistent "acceptable processing quality" of dry beans is an essential attribute for suitability in commercial trade and plays an important role in cultivar selection. Wide variability in processing quality attributes (color, hydration, texture and integrity) have been demonstrated among various genetic and environmental factors. Pilot-scale canning procedures suitable for screening dry beans for quality performance have been used in a number of breeding programs (Uebersax and Bedford, 1980; Balasubramanian et al. 2000). Implementing these procedures within a comprehensive breeding program require specialized facilities and significant investment in equipment and personnel. The use of a simple "home canning" protocol could have application in selected areas (Anon. 2009; USDA 2009).

This study was designed to assess the general feasibility of using an established procedure for manually-sealed Ball glass jars and a stove top pressure cooker to assess dry bean processing quality.

MATERIALS AND METHODS: Two classes of beans (navy and pinto) for this study were procured from Bayside Best Beans, Sebewaing, Michigan. Beans (n=3) were presoaked, filled into glass jars, cover with hot water, sealed with lid and security ring, and processed in a stove top pressure cooker. The drained weight of the beans and visual assessment for color, appearance, bean integrity, and texture was conducted. Outline of two methods is given below:

Ball's Bean Canning:	***'MSU 30-30' Bean Canning:***
Ball's: 12 oz (340 g) dry beans per quart/2-pint jars *<we used 170 g/pint jar>*	*(300 x 407 at 90 g solids);* 105 g dry beans/can
▪ Boil beans in water for 2 minutes.	▪ Soak bean in 3x water at 70 F for 30 minutes.
▪ Remove from heat and let soak for 1 hour.	▪ Blanch/cook at 190 °F for 30 minutes.
▪ Drain and add cold water.	▪ Fill cans with hydrated beans equiv. to 90 g solids.
▪ Boil for 30 minutes.	▪ Prepare brine (12 g salt and 15 g sugar per one liter volume), bring to boil.
▪ Fill beans leaving 1-inch head space, add ½ teaspoon of salt (~2.5 g)	▪ Add hot brine (190 °F) to ¼-inch headspace.
▪ Add boiling water, leaving 1-inch headspace.	▪ Seal/double-seam can.
▪ Stir gently to remove air, seal with lid/security ring.	▪ Retort process at 240 °F for 45 minutes.
▪ Process jars for 1 hour 15 minutes at 10-lb pressure	▪ Cool down to < 104 °F.

RESULTS & DISCUSSION: Figure 1 presents the preparatory bean hydration characteristics (hydration ratio=hydrated beans wt./dry beans wt.) and is an indicator of hydration capacity. Drained weight (Figure 2) is a measure of hydrated beans following the canning processing. These measures of navy and pinto beans demonstrate the differences between bean types and process procedures. The weight gain relationships were adequately obtained and demonstrate the extensive hydration preparation used in the Ball procedure. Beans should be adequately hydrated to assure uniform swelling and softening with a minimum of seed breakdown. Further, bean hydration is important to consistent heat penetration during the pressure process. Selection value is derived from observation of the processed bean physical characteristics, particularly, the overall appearance of the beans (Table 1). Quality rating scores were reported as consensus

values of two experienced observers. Beans processed by the Ball and "MSU 30-30" processes were assessed to be acceptable. It is evident that the Ball jar soak procedure and its thermal process (75 mins) is much more extensive than that delivered in the MSU 30-30 method. Thus, it may be viewed as a more aggressive test protocol than commonly used.

It is noted that the Ball method outlines the use of a relatively higher bean fill weight which results in a more solid packed product than typically used. In subsequent work, the authors reduced bean fill weight to 190 g hydrated beans per pint jar (compared to 280 g fill prescribed by Ball and applied identical thermal process conditions with very favorable results. Increased brine to bean ratio resulted in intact beans with excellent integrity.

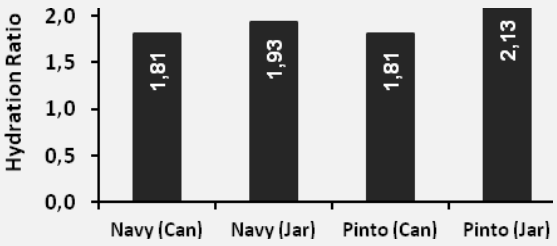

Fig. 1. Hydration ratio of beans in cans and jars

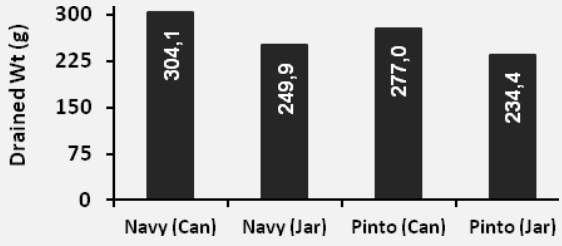

Fig. 2. Drained weight of beans in cans and jars

Table 1. Visual evaluation/rating of beans processed in cans and jars

Sensory Attribute	Cans		Jars	
	Navy	Pinto	Navy	Pinto
Appearance	4.5	4.0	4.5	4.0
Clumps/splits	3.5	3.0	3.5	2.5
Integrity (wholeness)	4.5	4.0	4.5	4.5
Texture	4.0	4.5	4.5	4.0
Color	4.0	4.5	3.5	4.0
1-5 Quality Score (1=Poor; 3=Acceptable; 5=Excellent)				

CONCLUSIONS

Basic considerations for pilot processing quality assessment are well documented; however, screening for quality may also be achieved using home canning jars and a stove top pressure cooker. This limited equipment procedure provides a simple screening protocol that will enable selection for "general commercially acceptable processing quality." The constant cooking process will be sufficient and adequate to screen beans that possess "excessive break-down" and "residual hardness." Processors' use many different procedures and this assessment is not optimized for specific applications, rather it may be used to assure "adequate process acceptability." Further development and optimization is warranted. *Caution*--Improper processing of low acid canned foods is hazardous.

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INFLUENCE OF LEAF COLOR IN A DRY BEAN MAPPING POPULATION ON *EMPOASCA SP.* POPULATIONS AND HOST PLANT RESISTANCE

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Visual cues may be the first line of host plant recognition and an important determining factor when selecting host plants for feeding and oviposition, especially for highly polyphagous insects, such as leafhoppers, which have a broad range of potential host plants (Reeves, J.L. 2011). Temperate *Empoasca fabae* and tropical *E. kraemeri* are two species of leafhoppers that are highly polyphagous. *Phaseolus vulgaris* is among their preferred hosts. *Empoascasp.* feeding can cause serious damage by reducing bean seed yield and quality.
Evaluating *Empoasca* nymph incidence in a dry bean population can be used to assess egg-laying and feeding preferences (antixenosis interactions) (Schaafsma et al., 1998). By correlating nymph incidence with visual traits, such as leaf color, the importance of such traits in determining insect preferences can be inferred. In addition, previous studies have shown that leaf color influences *E. fabae* preferences more so than host odor (Bullas-Appleton et al., 2004).

MATERIALS AND METHODS: *Empoasca sp.* are major insect pests of common bean production throughout the Americas. Temperate *E. fabae* and tropical *E. kraemeri* populations were evaluated on an IBL population of Matterhorn, a susceptible Michigan cultivar, by EMP507, a line developed by CIAT for resistance to the tropical leafhopper *E. kraemeri*. Field studies were conducted at Michigan State University, East Lansing, MI in 2011 and at the Tropical Agriculture Research Station, USDA, Isabella, PR in 2010 and 2011. Correlations between L a b color values and nymph populations were examined to determine whether leaf color plays a role in host preferences and antixenosis-type resistance among *Empoasca sp*.
Plant leaf color was measured using a Kodak Chromameter CR-400 which provides a measurement of color that correlates with human eye-brain perceptions referred to as the Hunter L* a* b* color scale. Each letter represents a specific range along a spectrum from dark to light (L*:0←→100), green to red (a*: -100←→+100), and blue to yellow (b*: -100←→+100). A randomly selected leaf from the upper canopy of 3 plants per plot was analyzed in each replication. Nymphs were counted at 51 DAP by counting nymphs present on 3 trifoliate leaves on each of 3 randomly selected plants per plot. Plants were evaluated for leaf curl (LC) and leaf burn (LC) at 78 and 79 days after planting (DAP) using the damage scale from 0-5. All data were analyzed using SAS Statistical software (SAS Institute, Cary, USA).

RESULTS:COLOR ANALYSIS: Hunter L* a* b* color scale values were normally distributed in each field test but not across locations. Significant correlations between color scale values were determined for each color value both within and across field trials (α=0.05). L* and b* were positively correlated, while a* and b* were negatively correlated. L* and a* were not significantly correlated in this study.

NYMPH – COLOR CORRELATIONS: Nymph counts were found to be normally distributed in each field trial. Mean nymph count values were 4.3±0.16 (MI11), 2.4±0.11 (PR10), and 0.4±0.02 (PR11) with an overall mean of 2.1±0.07. When color scores were analyzed with both *Empoasca*

sp. nymph scores using Spearman rank correlation coefficients, significant correlations were seen with all color spectra across all years and locations (Table 1.). L* (light-dark) and b* (yellow-blue) was negatively correlated with nymph counts, while a* values (green-red) were positively correlated with nymph counts across years and locations. The yellow-blue spectrum (b*) appears to be the most important factor in determining nymph counts (r=-0.4278). Environment was found to play a significant role in leaf color as location was associated with all L* a* b* values (α=0.05), which may be a result of differences in growth due to the particular environments, such as fertility.

Table 1. Correlations between numbers of *Empoasca sp. n*ymphs and L*a*b* values.

	L*	a*	b*
NYMPH COUNTS	-0.14169	0.10915	-0.4278
	<.0001	0.0004	<.0001

DAMAGE - COLOR CORRELATIONS:IBLs were analyzed for correlations between L* a* b* values and LC and LB damage-related indices. LB was found to be significantly correlated with leaf color but LC was only significant with L* values. Correlations of LB with L* a* b* values could be because LB directly affects leaf color, and damage symptoms may have already been present at evaluation.

EMPOASCA DISTRIBUTIONS:L* a* b* values for IBLs where *E. sp.* nymph counts achieved economic threshold levels or greater (N≥1 nymph/trifoliate) where analyzed by species (Table 2.). The distinct ranges for each species suggest the leaf color preferences of each species.

Table 2. L* a* b* leaf color 95% confidence intervals of IBLs where Empoasca species nymph counts reached economic threshold or greater levels (n≥1nymph/trifoliate)

HUNTER COLOR SCALE	*EMPOASCA FABAE*		*EMPOASCA KRAEMERI*	
	MIN (5%)	MAX (95%)	MIN (5%)	MAX (95%)
L*	+35.97	+42.82	+48.82	+56.29
a*	-11.40	-14.40	-10.79	-16.19
b*	+16.60	+24.89	+23.99	+33.62

CONCLUSIONS

Empoasca species appear to have distinct leaf color preferences and therefore, it can also be inferred that there are ranges of leaf colors that they avoid. This would be an example of antixenosis resistance. By selecting against those plants that fall within the insect's preferred ranges, breeders can retain germplasm that may be more resistant to *Empoasca sp.* predation and subsequent damage and yield reductions.

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DEVELOPMENT OF SCREENING METHODS FOR WHITE MOLD DISEASE RESISTANCE IN COMMON BEAN

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White mold caused by *Sclerotinia sclerotiorum* Lib. De Bary represents a major problem in common bean (*Phaseolus vulgaris* L.) production areas. Obtaining resistant cultivars is therefore a priority in bean breeding programs. Reliable resistance tests must be implemented to identify resistant varieties and for this purpose, we developed and compared phenotyping tools in field and in lab conditions.

Lab tests: Eleven bean varieties were evaluated in field and with eight different screening methods in controlled conditions (16 plants X 1 rep in greenhouse / growth chamber) : "cut-stem"; "cut-petiole"; "detached-leaf"; "spray-mycelium"; "drop-mycelium"; "spray-mycelium on flowers"; "spray-mycelium on eight day-old plants" and "oxalic acid".

Table 1. Correlations between lab/field results by Spearman analysis

Méthods	NO	LM	LM+NO	Cut stem	Cut petiole	Detached leaf	Spray mycelium	Drop mycelium	Spray on flowers	Oxalic acid
NO	1	0,65	0,72	0,32	0,29	-0,14	0,41	0,38	0,64	0,41
LM	0,65	1	0,97	0,02	0,02	-0,15	0,26	0,03	0,33	0,4
LM+NO	0,72	0,97	1	0,14	-0,03	-0,07	0,3	0,08	0,31	0,35
Cut stem	0,32	0,02	0,14	1	-0,03	0,2	0,25	0,41	0,08	-0,08
Cut petiole	0,29	0,02	-0,07	0	1	-0,39	-0,13	-0,12	0,68	0,22
Detached leaf	-0,14	-0,15	-0,1	0,2	-0,4	1	0,28	0,38	-0,71	-0,16
Spray mycelium	0,41	0,26	0,3	0,25	-0,1	0,28	1	0,78	-0,16	0,78
Drop Mycelium	0,38	0,03	0,08	0,41	-0,1	0,38	0,78	1	0,01	0,37
Spray on flowers	0,64	0,33	0,31	0,08	0,68	-0,71	-0,16	0,01	1	0,14
Oxalic acid	0,41	0,4	0,35	-0,08	0,22	-0,16	0,78	0,37	0,14	1

No correlation was observed between field and lab conditions (Table 1), however, with the cut stem method, the test of Newman-Keuls identified distinct homogeneous groups.

Also, a correlation study was made between different parameters of the plant architecture and the disease index. Our data indicated that there is a clear correlation between disease index and plant architecture parameters such as flower and pod positions.

Field screening procedures with natural or artificial infection were then tested in two locations in France: La Ménitré (LM) with an artificial infection with sclerotia and mycelium suspension (2006-2009) in a randomized design and Arras (NO) with a natural infection with bloc design. Each plant was rated using a 0 to 3 scale. After 5 years of field assays on 150 lines, a good homogeneity of inoculum has been obtained in the field and is used today to screen our material (Figure 1).

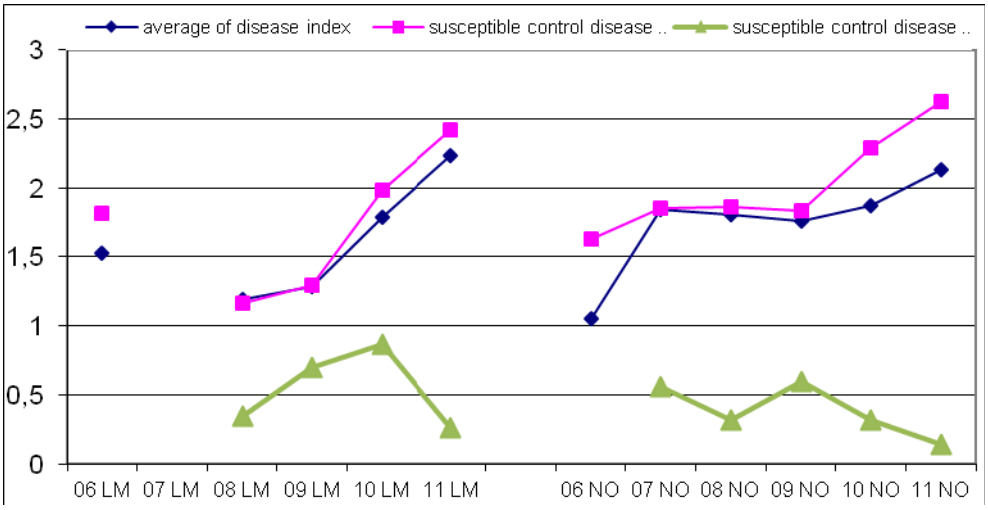

Figure1. Evolution of Disease index at both locations (NO, LM) from 2006 to 2011.

Ultimate final lab test comparing the cut-stem with the excised stem method (ref) in controlled conditions was performed on nine varieties with a known field behavior. No clear distinction between intermediate, resistant or susceptible known varieties could be detected with those methods, even though the resistant control G122 was consistently found as the most resistant variety.

We concluded that lab conditions do not reflect the natural infection process in field. In the future, we will integrate architectural parameters in lab evaluations and make assays with ascospores and as well, pursue efforts on field tests.

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GROWTH RESPONSE OF COMMON BEAN (*PHASEOLUS VULGARIS* L.) LINES TO WATER DEFICIT

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INTRODUCTION

Following a workshop on "Improving Tolerance of Common Bean to Abiotic Stress" held at CIAT in November 2010[1,2], a collaboration was established between the Van Volkenburgh lab at the University of Washington and Steve Beebe and I. Rao at CIAT to determine the physiological basis for drought tolerance in selected lines of *Phaseolus vulgaris*.

A hypothesis driving the work was derived from prior results with maize[3,4] showing that modern hybrids maintain yield despite density stress in part because sensitivity to auxin was reduced during selection. Here, we intend to identify drought-tolerant lines of bean for which this trait can be replicated in greenhouse experiments, and then determine whether tolerance is based on insensitivity to signals normally depressing growth and reproduction in droughted beans.

MATERIALS AND METHODS

Six lines of *P. vulgaris* were obtained from CIAT: parental lines ALB91 and INB841, drought-sensitive lines DOR500 and TC75, and drought-tolerant lines NCB226 and SER118. Plants were grown in a greenhouse in Seattle, WA for ten weeks June-July 2011. Seeds were planted in soil (Sunshine #4, Sungro Horticulture Canada Ltd) in 12 cm^3 plastic pots placed on capillary mats draped in a water bath. For well-watered plants, the bath was level with the bottom of the pots; for the water-deficit treatment, the bath was 30 cm lower than the bottom of the pots. Leaf gas exchange (LiCor 1600 porometer, Lincoln NE), water relations (pressure chamber: Model 600, PMS Instrument Co, Corvallis, OR; osmometer: Advanced Model 3300, Norwood MA), and leaf and pod growth rate (millimeter ruler) were monitored throughout the experiment. Dry biomass of shoots and pods was determined destructively.

RESULTS AND DISCUSSION

Soil water status remained above 80% for well-watered plants and declined steadily from 60% to 10% for plants experiencing water-deficit. Results obtained by imposing water-deficit treatment using raised capillary mats replicated the drought phenotypes reported for these lines in field trials[5].

All lines showed reduced stomatal conductance, transpiration, leaf water and solute potentials under water-deficit conditions. Under drought conditions, TC75 yielded the lowest pod dry weight. Parents (ALB91, INB841) and drought tolerant lines (NCB26, SER118) maintained higher yields. Under both well-watered and drought conditions the solute and water potentials of TC75 were indistinct from other genotypes. Drought-tolerant lines (NCB226, SER118) developed the most negative leaf water potentials, but showed similar osmoregulation suggesting

these leaves had reduced turgor in drought. TC75 showed distinctly lower stomatal conductance than the other genotypes in both well-watered and drought conditions.

In general there was a good correlation between rate of leaf growth rate and pod weight; this was stronger in the well-watered plants. NCB226 maintained the highest leaf elongation rates and pod dry weights in both well-watered and water-deficit treatments. Parental (ALB91, INB842) and drought-tolerant (NCB226, SER118) lines had higher pod and seed weights; the drought-tolerant lines and DOR500 produced the most seeds. TC75 produced the least yield and was strongly affected by drought. All lines increased pod harvest index with drought. Onset of reproduction was earliest in the parental lines, but pod number was higher in drought-tolerant lines.

The results imply that growth and yield maintenance involves some process other than turgor maintenance. Pod weight was correlated with the rate of leaf elongation. The physiological mechanism for maintaining leaf, and possibly pod, growth during drought is key for understanding maintenance of yield in drought. Further experiments are in progress and proposed to test the mechanism of growth-regulation in bean leaves experiencing reduced turgor. We propose that in tolerant lines, an internal signaling mechanism that normally reduces cell expansion rate during water deficit conditions is altered in drought-tolerant lines such that growth of leaves and pods is maintained.

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DEVELOPMENT OF BEAN LINES (*PHASEOLUS VULGARIS* L.) RESISTANT TO BGYMV, BCMNV AND BEAN WEEVIL (*ACANTHOSELIDES OBTECTUS* SAY)

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The common bean weevil (*Acanthoscelides obtectus* Say) is a major seed storage pest (1). BC_3F_3 lines from the cross 'Rojo*3/SMARC2///ICAPijao*2/G40199' were obtained from Dr. James Myers at Oregon State University (OSU). This population was expected to segregate for resistance to the bean weevil (2). Individual plants were selected for local adaptation from a nursery planted at Isabela, Puerto Rico in October, 2010.

A bioassay was developed to screen $BC_3F_{3:4}$ bean lines for resistance to the bean weevil. Plastic cups (150 ml) containing 20 seed were infested with 20 adults of the bean weevil. Date of first emergence was noted and damage to the seed was measured at 35 days after infestation. The lines were evaluated in two trials conducted at Isabela, Puerto Rico during 2011.

Seed of both Andean and Middle American bean cultivars were severely damaged by the bean weevil (Table 1). Three light red kidney lines from the OSU population expressed useful levels of resistance. The date of first emergence of adults of the resistant lines was approximately three weeks later than the susceptible checks. Most of the seed of the resistant lines was undamaged at 90 days after infestation. Seed of resistant lines had ≥ 65% seed without holes. The only other line with a similar level of resistance was RAZ 25 which was developed at CIAT to possess the seed storage protein arcelin 1.

The bean weevil resistant line PR1012-29-3 was crossed with white and black lines and cultivars that have commercial seed type and BGYMV and BCMNV resistance. F_3 and F_4 lines derived from these crosses were planted at Isabela, Puerto Rico in July 2011. Individual plants with local adaptation will be selected and screened in the greenhouse for reaction of the NL3 strain of BCMNV. Resistant lines will be screened for the presence of the *bgm-1* gene using the SR-2 and the *I* gene using the SW13 SCAR markers.

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Table 1. Damage caused by bean weevil (Acanthoselides obtectus) in 20-seed samples of common bean lines of diverse origin.

Identification	Seed type	Total number of holes in the seed	Percent seed without holes	Percent seed weight lost
AO-1012-27-2	Red kidney	9.5	65.0	0.0
AO-1012-29-3	Red kidney	14.5	75.0	0.0
AO-1012-31-4	Red kidney	14.0	65.0	18.8
Badillo	Red kidney	129.0	0.0	45.0
INIAP Fanesquero	White kidney	122.0	0.0	25.0
RAZ 25	Red mottled	13.5	62.5	16.7
INIAP Portillo	Red mottled	180.5	0.0	37.5
INIAP Yungilla	Red mottled	132.5	0.0	37.5
INIAP Concepción	Red mottled	150.0	0.0	37.5
PR9745-232	Red mottled	125.5	0.0	35.8
Catarina	Cranberry	132.0	0.0	37.5
Calembe	Green	143.0	0.0	25.0
Canaria	Yellow	129.5	0.0	25.0
Verano	White	150.0	0.0	25.0
Morales	White	112.5	0.0	25.0
RAZ 75	Small red	23.5	35.0	16.7
INTA Precoz	Small red	120.5	0.0	37.5
DEHORO	Small red	128.0	0.0	33.3
Amadeus 77	Small red	122.5	0.0	33.3
Carrizalito	Small red	106.5	0.0	33.3
CENTA Pupil	Small red	110.0	0.0	25.0
RAZ 50	Black	79.5	10.0	16.7
Aifi Wuriti	Black	140.5	0.0	33.3
DPC 40	Black	103.5	0.0	40.0
ICA Pijao	Black	109.0	0.0	25.0
Mean		93.8	9.0	30.7
LSD(0.05)		18.0	8.9	12.6
CV(%)		9.7	50.1	20.7

EFFICACY OF FOLIAR CONTROLS OF WHITE MOLD IN DRY BEANS

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White mold, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, can cause severe yield and seed quality issues, which dramatically reduces grower returns. White mold control measures include genetic tolerance, foliar control products and management factors such as crop rotation, row width and nitrogen fertilizer use. However, growers frequently use foliar control products to manage this disease. Foliar controls can be divided into three groups; chemical fungicides, alternative products and biological controls. The efficacy of products from each group has been determined in a number of studies, but there is little published work comparing the products from the three groups in a single study.

Four experiments were planted at the Huron Research Station near Exeter ON between 2009 and 2011. White mold disease pressure was enhanced using a susceptible cultivar (Beryl), enhanced fertility and irrigation. An RCB design compared various rates and timings of chemical fungicides (boscalid, flumioxazin, fluopyram and thiophanate-methyl), alternative products (CaCl) and biological products (Bascillus subtilis) +/- boscalid. Parameters measured included disease severity scores (%) 39-50 days after fungicide application and seed yield (kg ha^{-1}). Data from each site was subjected to analysis of variance, and the means separated using Fischer's protected LSD (0.05).

RESULTS AND DISCUSSION:

- Boscalid had less disease severity than the control at all 4 sites, but yield was higher at only 2 sites
- Thiophanate-methyl and *B.subtilis* + boscalid had less disease severity than the control at all 4 sites, and higher yield at 3 sites.
- Fluazinam and fluopyram had less disease severity and higher yield than the control at all sites.
- *B. subtilis* and CaCl were similar to the untreated control for disease severity and yield.

CONCLUSIONS:

- New chemical options (fluazinam and fluopyram) provide more consistent white mold control, compared to boscalid (industry standard)
- Old chemical options (thiophanate-methyl) are renewed because of consistent mold control, recent product price reductions and additional control of other important diseases (e.g. anthracnose)
- Biological (*B.subtilis*) and alternative (CaCl) fungicides are quite ineffective, and are not a reliable option for growers
- There appears to be some additive effect from the combination of *B.subtilis* + boscalid, compared to either product applied alone

- Table 1. White mold disease severity (%) and seed yield (kg ha^{-1}) for dry beans following treatment with chemical, alternative and biological fungicide products at Exeter ON in 2009-2011. Means within a column that share the same letter are not significantly different using Fisher's protected LSD (0.05).

Treatment	Rate (g ai ha^{-1})	Timing[a]	Disease Severity (% infected tissue) 2009a		2009b		2010		2011	
Untreated Control			85	a	87	a	35	A	76	a
Boscalid	392	AB	58	cd	73	bcd	12	bcd	44	c-f
Boscalid	540	A	50	de	73	bcd	12	bcd	41	c-f
Boscalid	540	AB	48	de	67	cde	14	bcd	35	ef
Fluazinam	500	AB	47	de	65	cde	13	bc	44	c-f
B.subtilis	4*	AB	70	bc	77	abc	33	a	65	ab
B.Subtilis + Boscalid	4* + 392	AB	38	e	72	bcd	8	c	44	c-f
Fluopyram	250	A	37	e	67	cde	15	bc	38	def
Fluopyram	150	AB	46	de	56	e	18	bc	30	ef
Fluopyram	250	AB	46	de	61	de	11	bc	31	f
CaCl	874	AB	81	ab	85	ab	16	bc	56	bcd
Thiophanate-methyl	1210	AB	55	d	62	de	16	bc	31	f

[a] – Timing A = early flowering (3 pin pods) and timing B = 10-14 days after first application
* - Rate of L ha^{-1}

Treatment	Rate (g ai ha^{-1})	Timing[a]	Seed Yield (kg ha^{-1}) 2009a		2009b		2010		2011	
Untreated Control			1366	bcd	292	d	1402	c	2133	d
Boscalid	392	AB	1841	abc	394	cd	1902	b	3686	abc
Boscalid	540	A	1874	abc	470	bcd	2085	ab	3680	abc
Boscalid	540	AB	1795	abc	676	a-d	1966	ab	3626	abc
Fluazinam	500	AB	2167	a	810	ab	2141	ab	4025	a
B.subtilis	4*	AB	1288	cd	515	a-d	1346	c	2873	bcd
B.Subtilis + Boscalid	4* + 392	AB	2335	a	529	a-d	2140	ab	3228	abc
Fluopyram	250	A	2141	a	754	abc	2129	ab	3328	abc
Fluopyram	150	AB	1943	ab	891	a	2091	ab	3761	abc
Fluopyram	250	AB	2207	a	838	ab	2318	a	3927	ab
CaCl	874	AB	839	d	320	d	1913	b	2887	bcd
Thiophanate-methyl	1210	AB	2057	a	636	a-d	2242	ab	3704	abc

GENETIC DIVERSITY AND POPULATION STRUCTURE OF COMMON BEAN LANDRACES FROMMATO GROSSO, BRAZIL

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INTRODUCTION: The common bean (*Phaseolus vulgaris* L.) is a traditional food for the Brazilian population and it is cultivated in all the country (Burle et al. 2010). In general in Brazil the common bean crop is explored by smallholders that frequently sowing traditional cultivars. Mato Grosso State, Center Western of Brazil, is an area where high variability can be observed in traditional common bean that are also known as landrace population. This landrace common bean population which presents unknowing structure needs to be characterized, evaluated in relation to the genetic variability. Nowadays studies of population structure and genetic diversity in traditional common bean cultivars using molecular markers are essential to preserve the germplasm and also to assist on genetic breeding programs (Galván et al. 2010). Therefore, this work had as objective to evaluate the genetic diversity and population structure in 61 traditional common bean cultivars from Mato Grosso, Center West of Brazil, using microsatellites markers.

MATERIALS AND METHODS: Genetic relationships among the cultivars were studied using 12 microsatellite markers distributed over the entire bean genome. Population structure analysis was based on Bayesian model, implemented with software *Structure2.3.3.* (Pritchard et al. 2000). For this analysis a preliminary step was utilized. Thus, ten independent runs were performed using the admixture model, a length of burning period of 10000 and 100000 MCMC (Monte Carlo Markov Chain) replicates after burning. A neighbor-joining tree was constructed using software Power Marker (Liu and Muse 2005).

RESULTS AND DISCUSSION: Genomic DNA of 61 traditional common bean cultivars from Mato Grosso state was extracted according to the methodology proposed by Afanador et al. (1993) with modification. Molecular analysis of the 12 microsatellite through Neighbor-Joining method allowed allocating the 61 traditional common bean cultivars in two populations according to Mesoamerican and Andean gene pools (Figure 1).

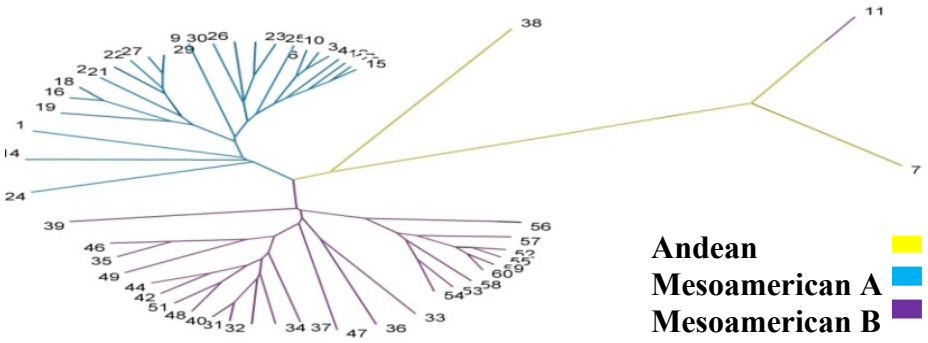

Figure 1. Neighbor-joining tree for the Brazilian traditional cultivars based on the C.S. Chord distances and twelve polymorphic microsatellite markers. Branches are colored according to the Structure simulation for K = 3 groups.

Within the Mesoamerican there was evidence for two subgroups: the first of which corresponded to the Mesoamerican (blue color) and the second to the Jalisco (purple color). Meanwhile, the results with simulation of Delta value (K=2 to K =10) demonstrated that value of K = 3 has adjusted to data shown by Neighbor-Joining method (Figure 1 and 2). Therefore, it can infer that traditional common bean cultivars investigated in the present study are based on the Mesoamerican and Andean gene pools (Figure 1). However, value of K = 3 provided the clustering of these cultivars in three groups (Figure 1): Andean, Mesoamerican A (Race Mesoamerican) and Mesoamerican B (race Jalisco). Delta K indicates that the ideal number of populations in this set of accessions is three. Additionally, among them the most divergent accessions we could point out BGMT 7 and BGMT 11.

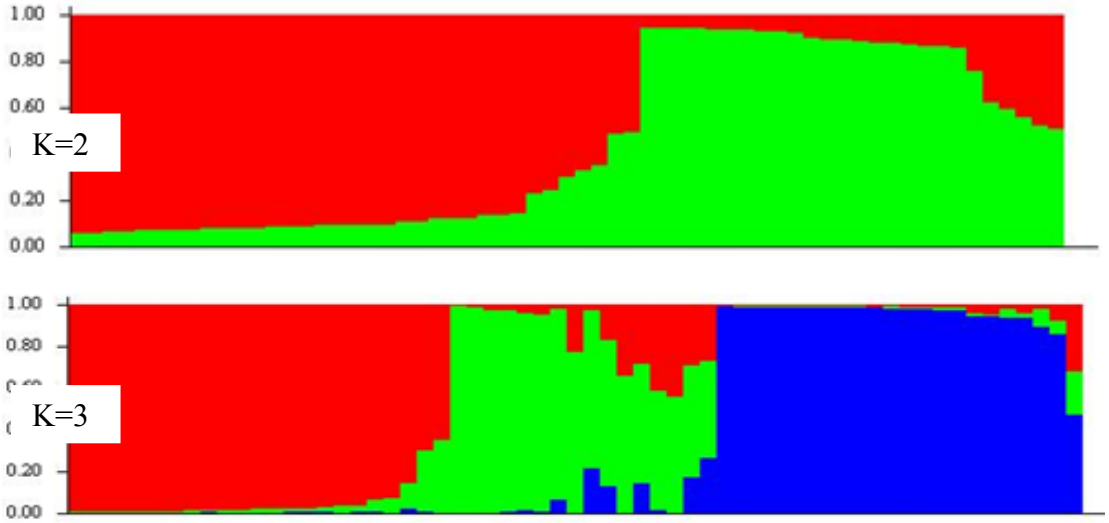

Figure 2. Hierarchical organization of genetic relatedness of 61 common bean traditional cultivars based on 12 microsatellite markers and analyzed by the Structure prog. for K=2 to 10.

CONCLUSION: Microsatellite markers were effective on genetic diversity and population structure analyses of 61 traditional common bean cultivars evaluated, providing important information about population dynamics.

Acknowledgements: This research was supported by FAPEMAT, CNPq and Capes.

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COMMON BACTERIAL BLIGHT RESISTANCE IN COMMON DRY BEAN (*PHASEOLUS VULGARIS*) VARIETY OAC REX – TOWARDS IDENTIFYING A MAJOR RESISTANCE GENE

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Common bacterial blight (CBB), caused by bacterial pathogen *Xanthomonas axonopodis* pv. *phaseoli* and *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* (*Xap*) is an important disease that affects common bean production worldwide. The first variety of common bean registered in Canada for resistance to CBB (OAC Rex) was developed from an interspecific cross, with CBB resistant *Phaseolus accutifolius* (Michaels et al, 2006). At least one major CBB resistance QTL (PV-ctt001) has been reported to be present in this variety on the B4 linkage group (Tar'an et al, 2001; Perry, 2010), and another (SU91) was shown to be located on the B8 linkage group in other varieties, but its location is unconfirmed in OAC Rex. The aim of this study was to identify BiBAC clones with homology to SU91and to identify and characterize potential CBB resistance genes in bacterial artificial chromosome (BAC) clones selected with the PV-ctt001 marker from the OAC Rex binary BAC (BiBAC) library (Perry, 2010).

MATERIALS AND METHODS: SU91 associated library clones were identified by hybridizing OAC Rex library clone membranes with SU91 DIG-labeled probes. Plasmid DNA of high signal clones was isolated and the presence of the SU91 marker was confirmed by amplification and sequencing of the amplified fragments from plasmid DNA.
BiBAC clones selected with PV-ctt001 were previously sequenced using the Roche 454 platform with a unidirectional 400 bp read length and assembled using CLC Genomics Workbench 4.1 using the default assembly protocol. Contigs from the assemblies of PV-ctt001 selected clones that contained LRR homologs were identified by BLAST analysis with a consensus sequence derived from an alignment of known NBS-LRR gene sequences. Two candidate genes were identified in contig 1701 and their protein sequences and secondary structures were predicted. The candidate genes were amplified, isolated and cloned into a TOPO cloning vector for sequencing.

RESULTS AND DISCUSSION: Membrane hybridizations with SU91 markers identified several clones with high hybridization signals. Plasmid DNA was isolated from thirty of these clones, and six clones contained the SU91 marker (Figure 1).

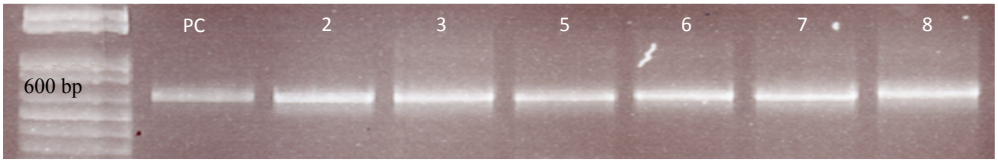

Figure 1. Amplification of SU91 marker from library clones 2, 3, 5, 6, 7 and 8; selected by southern blot analysis using SU91DIG-labelled probes and from genomic OAC Rex DNA (PC).

Contig 1701 from the assembly of PV-ctt001 selected clone 21 contained a cluster of LRR homologs (Figure 2). The predicted proteins coded by two open reading frames (1701-2 and 1701-3) were very similar, rich in leucine, and contained a conserved motif (LxxxLxLxLxxL) and a possible N terminus signal. The conserved leucine rich motifs of the predicted proteins were structurally similar to the previously characterized *P. vulgaris* polygalacturonase inhibiting protein (PGIP), which is involved in resistance to gray mold (Di Matteo et al 2003, Sicilia et al. 2005). The signal peptides they contain indicate that they may be extracellular proteins and may act as pathogen associated molecular pattern (PAMP) recognition receptors (PRR).

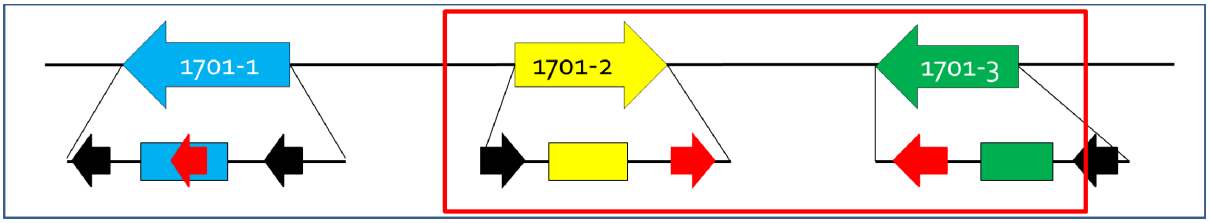

Figure 1. Cluster of open reading frames with homology to leucine rich repeats in contig 1701 from BiBAC clone 21 selected from the OAC Rex BiBAC library (Perry, 2010) with the PV-ctt001 marker. Arrows represent identified promoter (black) and poly A (red) sequences, and coloured boxes represent identified NBS-LRR homologous regions.

Work to further characterize the candidate resistance genes is continuing and their possible roles in CBB resistance will be explored by examining their expression in resistant and susceptible bean germplasm.

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REACTION OF COMMON BEAN CULTIVARS INOCULATED SIMULTANEOUSLY WITH *COLLETOTRICHUM LINDEMUTHIANUM* AND *PSEUDOCERCOSPORA GRISEOLA*

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INTRODUCTION

Angular leaf spot (ALS) caused by *Pseudocercospora griseola* (Sacc.) and anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. and Magnus) Briosi and Cavara, are the most widespread, recurrent, and devastating diseases of the common bean in Latin America and Africa (Correa-Victoria et al 1989; Pastor-Corrales and Tu 1989). In recent years, ALS has become one of the most important constraints to bean production in Brazil and Africa (Liebenberg and Pretorius 1997; Aggarwal et al. 2004; Sartorato 2004). Both the ALS and anthracnose pathogens are seed-borne (Correa-Victoria et al. 1989; Pastor-Corrales and Tu 1989). When infected seeds are planted under environmental conditions favoring these diseases, yield losses caused by the ALS pathogen can reach 70% (Correa-Victoria et al. 1989; Sartorato 2004); losses caused by the anthracnose may be extremely high, up to 100% (Pastor-Corrales and Tu 1989). In common bean genetic breeding programs, simultaneously inoculation of distinct pathogens can provide reduction in the process of obtaining lines / cultivars with resistance. The objective of this work was to evaluate the reaction of common bean cultivars inoculated simultaneously with race 73 of *Colletotrichum lindemuthianum* and race 63-23 of *Pseudocercospora griseola*.

MATERIALS AND METHODS

The experiment was composed by three groups of common bean cultivars, inoculated with *C. lindemuthianum* and *P. griseola*. Group 1 was inoculated only with race 73 of *C. lindemuthianum*; Group 2 with the race 63-23 of *P. griseola*, while cultivars from Group 3 were inoculated with both pathogens. In the three groups, ten plants from each cultivar were inoculated and evaluated. Inoculum preparation of *C. lindemuthianum* and *P. griseola* followed the methodology described by Cárdenas et al. (1964) and Sanglard et al. (2009), respectively. Inoculations were conducted with a brush in abbatial and ad axial sides of first trifoliate leaf (Groups 1, 2 and 3). For inoculations in Group 3, it was used the methodology proposed by Gonçalves-Vidigal et al. (2001). In sequence, plastic trays containing plants were incubated in a mist chamber for 96 h, with 20 ± 2ºC temperature, controlling luminosity and relative humidity. Ten days after inoculation, the plants were evaluated for reaction to anthracnose and angular leaf spot using the scale for diseases from 1 to 9, according to the methodology proposed by Van Schoonhoven and Pastor-Corrales (1987) and Inglis et al. (1988).

RESULTS AND DISCUSSION

The results of common bean cultivars reactions inoculated with race 73 of *C. lindemuthianum* and race 63-23 of *P. griseola* in the three groups of inoculation are showed in Table 1. In the

Group 3, when simultaneous inoculations were made with *P. griseola* and *C. lindemuthianum* in the cultivars: Ouro Negro (resistant to anthracnose and susceptible to angular leaf spot); Mexico 54 (susceptible to ANT and resistant to ALS), and Rudá (susceptible to both pathogens) all cultivars exhibited reaction similar to when they were inoculated with only one pathogen in the Groups 1 and 2. This fact, permit to conclude that no cross protection it was observed. These results allow deducing that in common bean genetic breeding programs aiming anthracnose and angular leaf spot resistance, simultaneous inoculation of distinct pathogens may be accomplished, reducing time and cost in identifying resistant lines/ cultivars.

In this study it was observed that there was no incompatibility interaction of common bean cultivars inoculated simultaneously with *C. lindemuthianum* and *P. griseola*. Considering the complexity of host defense mechanisms and the virulence of evaluated pathogens, additional studies must be accomplished.

Table 1. Reactions of common bean cultivars Ouro Negro, Rudá, Mexico 54 and Cornell 49-242, inoculated with race 73 of *C. lindemuthianum* andrace 63-23 of *P. griseola*

Cultivar	**Group 1** Reaction to race 73		**Group 2** Reaction to race 63-23		**Group 3** Reaction to race 73		Reaction to race 63-23	
Ouro Negro	R	1	S	7	R	1	S	5
Rudá	S	9	S	5	S	8	S	5
Mexico 54	S	7	R	1	S	7	R	1
Cornell 49-242	S	9	R	1	S	9	R	1

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THERMOTHERAPY TO CONTROL SEED-BOURNE DISEASE IN DRY BEAN

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Common bacterial blight (CBB) (*Xanthomonas axonopodis pv. phaseoli*), halo blight (*Pseudomonas syringae* pv. *phaseolicola*) and anthracnose (*Colletotrichum lindemuthianum*) are serious seed-borne diseases affecting the commercial and seed production of dry bean (*Phaseolus vulgaris*) in Canada.

The use of 'disease free' seed is the primary control measure for CBB, although cultivars with genetic resistance may provide some promise in the future (3). Halo blight is a special concern in western Canada as few resistant varieties are available, resistance is controlled by various genes (7) and the pathogen can quickly form new races. Anthracnose is a difficult disease to manage, particularly in Ontario (4), despite the regular use of cultural, chemical and genetic controls. Therefore, scientists need to investigate the potential of new control methods for all three diseases.

Numerous studies have demonstrated that thermotherapy can effectively reduce or eliminate seed-borne pathogens in legumes (5). The effect of dry heat treatment on bacterial pathogens at low temperature levels was tested, however disease eradication was not achieved (6). Hot water treatments at temperatures above 90ºC usually had a lethal effect on the bean seeds, but temperatures between 60-80ºC for various durations did not adversely affect germination (1). However, legume seeds can quickly imbibe water at high temperatures, swell and slough off their seed coats (8). This can result in decreased germination as well as unusual growth characteristics in seedling plants.

A literature review of hot water or hot air treatments to eliminate bacterial pathogens from bean indicated that thermotherapy generally was less effective for large-seeded legume species because of its adverse effect on seed germination (5). Heating bean seed in cooking oil instead of water resolved this problem (5) and reduced the early incidence of CBB (2).

Microwave treatment (9) has been shown to be effective against various fungal diseases, but its effect on CBB, halo blight, and anthracnose is not known. For dry beans, microwave treatment appears to have less impact on seed germination and vigour (9), compared to other thermotherapy methods. Fungal spores differ in their reactions to microwave radiation, with single-celled spore fungi (e.g. anthracnose) being affected more than multi-celled spore fungi (9). Therefore further testing is required to determine the effect of microwave radiation on anthracnose transmission, and to see if this treatment is efficacious on seed-borne bacterial pathogens of dry bean.

The objective of this experiment is to determine if microwave treatment can be effective in managing seed-borne CBB, halo blight, and anthracnose, and will it provide practical disease control in the field.

The various treatment factors tested will include: three market classes of dry beans (navy, kidney and pinto) at three seed moisture contents (10, 15, & 20%) evaluated at varying microwave exposure timings (0, 15, 30, 45, 60, 90, & 120s). This study will consist of a laboratory component and a field component. In the lab component the various treatments will be evaluated for differences in germination, based on the Canadian Grains Commission standard warm germination test, seedling vigor and disease control determined by disease plating method, in which Potato Dextrose Agar will be used for *C. lindemuthianum & X. axonopodis* and King's Medium B for *P. syringae.* The field component will utilize the successful treatments from the lab component, which will be evaluated with and without the aid of a chemical seed treatment. These experiments will be planted near Exeter ON and Morden MB in 2012 and 2013 in a randomized complete block design with four replicates. Ratings will include seedling emergence and vigor, disease severity (leaf, stem, pod and seed), yield and potential economic returns.

Using microwave treatment we hope to manage seed-borne pathogens in dry bean while causing minimal impact to the bean seed health. The secondary implications of this study are to eliminate deep seated pathogens, reduce amount of 'disease free' imported seed, create a new market to cater to organic growers and to determine the treatments potential for large scale use in the agricultural industry.

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PHENOTYPIC EVALUATION OF ROOT ROT RESISTANCE IN AN INTRA-MESOAMERICAN PHASEOLUS VULGARIS RECOMBINANT INBRED POPULATION

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ABSTRACT: Root rot diseases of bean (*Phaseolus vulgaris* L.) are a problem wherever beans are grown, and can reduce yields by half. We crossed the highly resistant line RR6950 (small black seeded indeterminate accession) with OSU5446, a highly susceptible determinate blue lake green bean four-sieve breeding line to produce an Intra-Mesoamerican RIL population. We examined this population in the $F_{6:7}$ generation for root rot resistance (*Fusarium* and *Aphenomyces* spp.) and other processing traits.

INTRODUCTION: Root rot is one of the primary yield limitations of snap bean production in the US, especially within the top three bean producing states: Wisconsin, Oregon and New York (Navarro et al., 2008). When beans are first planted, root rot pathogens soon follow, particularly in intensively managed production systems where irrigation is present, short rotations are used, and bean crops occupy the ground year after year (Schneider et al., 1997; Navarro et al., 2008). The decline in yield can be relatively slow so that growers might not notice or appreciate the hidden yield cost associated with root rot disease. Chemicals and soil fumigation treatments have proven ineffective in controlling the root rot complex (Hoch and Hagedorn, 1974; Rand and Stevenson, 1999).

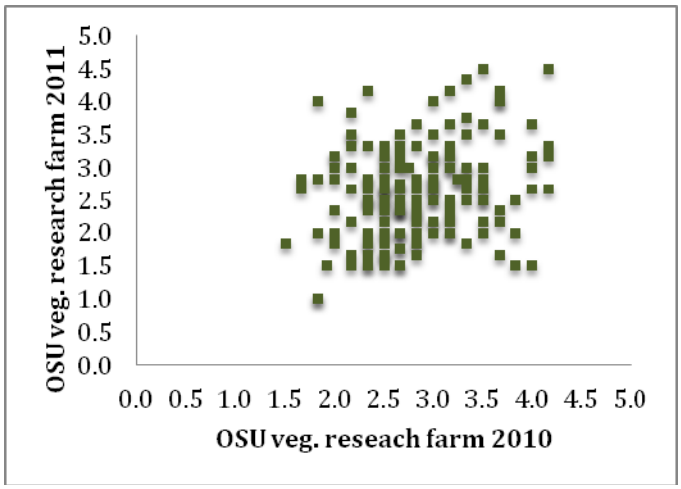
MATERIALS AND METHODS

Study Sites – OR & WI 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. Both sites have been planted in beans for many years, providing uniform disease pressure. OR root rot tends to be predominantly *Fusariuum,* while WI root rot is predominantly *Aphenomyces.*

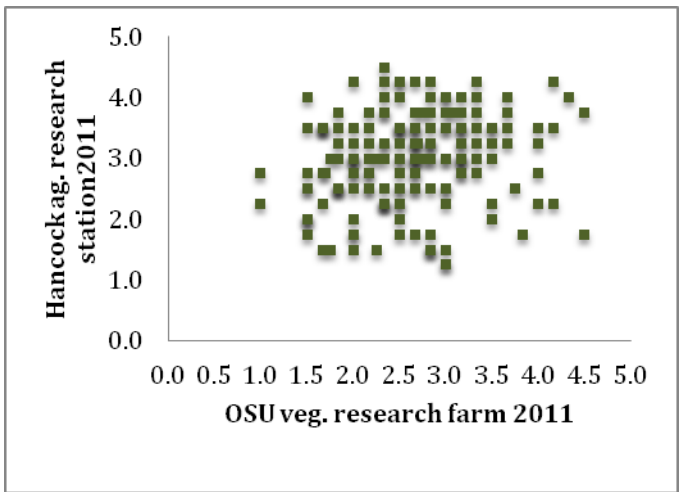
Population development - In 2003, we crossed the highly resistant line RR6950 (small black seeded viny accession) with OSU5446, a highly susceptible determinate blue lake four-sieve breeding line (Table 2). The F2 was advanced to the F3 generation by single plant selection and only one determinate plant from each F2-derived family was advanced to the next generation. From the F3 to F5, generations were advanced by randomly selecting one single plant from each family. In 2008, plants within each F5 family were bulked to develop lines for replicated testing. Two populations (RR6950/OSU5446 with 173 families and the reciprocal with 177 families) are available for mapping and genetic analysis. Both parents are Mesoamerican in origin.

Field Protocol - All genotypes along with parents and checks were replicated three times in a randomized block design. Five-ten plant samples per plot were rated for root rot symptoms on a one – five scale (1=clean, 5=totally infected). Families were also evaluated for flower color, and pod traits (wall thickness, fiber, strings, cross-sectional shape, length, and color).

Results



Correlation between root rot scores at OSU Veg. Research Farm 2010 and 2011 field seasons. (r=0.26***)



Correlation between root rot scores at the OSU Veg. Farm 2011 & Hancock Ag. Research Station 2011 field season. (r= 0.19***)

CONCLUSION; Results suggest a large amount of environmental variation year to year at the OSU site. This winter we will supplement field data with a greenhouse study to reduce amount of variation. There appears to be a potential correlation between *F. solani* and *Aphenomyces* resistance at the OR and WI sites.

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EXPRESSION PROFILING OF WRKY TRANSCRIPTION FACTORS IN COMMON BEAN DURING RUST FUNGAL INFECTION.

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INTRODUCTION

Common bean (*Phaseolus vulgaris*) is a warm season, annual legume that consists of various types of beans and is an essential source of protein. *Uromyces appendiculatus* is a fungal pathogen that produces spores that have a rust-like appearance on common bean crops. Rust is responsible for anywhere between 18-100% yield losses in common bean. Transcription Factors (TFs), which are sequence-specific DNA-binding elements, are proteins that bind to a specific DNA sequence, thus facilitating the transcription of genetic information from DNA to mRNA. WRKY proteins are a family of TFs that are found only in the plant kingdom. Previous studies indicate that a large family of WRKY TFs in *Arabidopsis thaliana* are involved in responses to stress and pathogen attack. It is possible that WRKY TFs play a vital role in pathogen-triggered signal transduction cascades in common bean. In this study, we are focusing on four transcription factors that belong to one super family of 100 genes. We observe the gene expression patterns of WRKY 23, WRKY 27, WRKY 35 and WRKY 54 in the rust resistant cultivar "Sierra" and susceptible cultivar "Olathe" using Real- Time quantitative Polymerase Chain Reaction (RTq-PCR).

MATERIALS AND METHODS

The common bean cultivar Sierra is rust resistant and shows hypersensitive resistance (HR) to the fungus *Uromyces appendiculatus* (rust race 53) while the cultivar Olathe is susceptible to rust race 53 developing rusty spots on the infected leaves. From each genotype, 30 plants were inoculated with spores of rust race 53 on 10 days old seedlings (Protocol obtained from Dr. Pastor-Corrales, USDA-ARS, Beltsville, MD). Total RNA was isolated from 0, 12, 24 and 36 hours after inoculation (hai) from leaf tissue. Purified total RNA was reverse transcribed to obtain cDNA. Real Time PCR primers were designed from bean contigs derived through Roche 454 sequencing (Kalavacharla et al. 2011) as in Table 1. Semi quantitative Real-Time PCR was performed using SYBR green dye (Applied Biosystems, Carlsbad, CA 92008) and

454 Contig Number	Functional Annotation	Primer Sequences
32781	WRKY 23	F: 5'-GCA TGT TGC TGT CAG GGT CAA TGT-3' R: 5'-TGG TGC TGA AGC TGA AAG TGT TGC-3'
30958	WRKY 27	F: 5'- ACG GAA ACT CTG AGA GCA GCT CAA-3' R: 5'-TGC TTC CGT CCT CAC GTA AAC TCT-3'
08830	WRKY 35	F: 5' – TCA GCC TTG ACC TTG GTA TGG GAA- 3' R: 5'- TTG CTG GTA TGA GCT TGG CTG TCA-3'
30192	WRKY54	F: 5'- CAA CAC ACA CAT CCA AGC CCA GTT-3' R: 5'- TGG TTC TGC TGC TGC TGA TAC TGT-3'

Table 1: WRKY Transcription Factors and their primer sequences. Primers were designed from the common bean contig sequencesthat matches with the above TFs. Bean contigs were developed through 454 sequencing.

gene expression was calculated using $2^{-\Delta\Delta CT}$ method. The endogenous gene *cons7* was used as reference gene and Olathe 0 hai as reference time point to calculate expression values.

RESULTS AND DISCUSSION

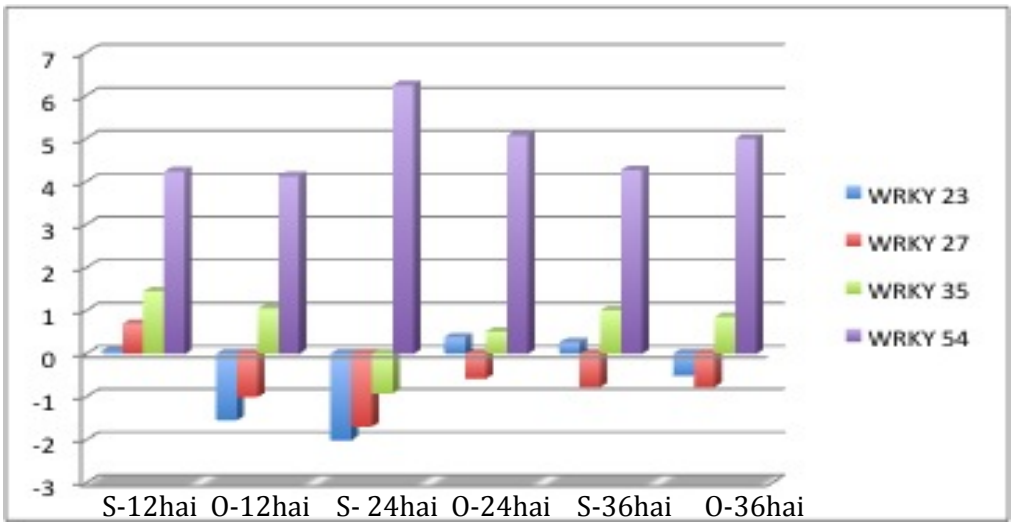

Figure 1. Gene Expression Analysis with Real Time PCR. X-axis: Time intervals when the gene expression was observed. Y- axis: Expression values of TFs in $\log_2$ ratios. S is the genotype Sierra and O is the genotype Olathe. Color code represents the corresponding TF.

All of the four selected WRKY TFs are expressed in common bean based on PCR amplification using cDNA (data not shown due to space limitations). The TF WRKY-54 was up regulated in all the time points in both the genotypes. TFs 23 and 27 are down regulated in the susceptible genotype Olathe at 12 hai. Interestingly these TFs were down regulated in Sierra at 24 hai. TF-WRKY 35 was up regulated in the resistant genotype Sierra at 12hai and down regulated at 24hai in contrast to WRKY 23 and 27(Figure 1). At 36 hai, all the TFs expressed were statistically ($2^{-\Delta\Delta CT}$ value is $\leq 1 \geq$) neutral in both the genotypes except WRKY54. From our findings these TFs showed differential expression in resistant and susceptible genotypes at 12 and 24 hai. Previous findings from *Brassica napus* also showed negative and positive regulation of different WRKY TFs under pathogen stress (Yang et al. 2009). This is one of the first steps in understanding regulation of these plant specific TFs in common bean for pathogen related stress.

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INSECTICIDE EFFICACY AND TIMING STUDIES FOR THE CONTROL OF WESTERN BEAN CUTWORM IN DRY BEANS

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INTRODUCTION

The invasion of the western bean cutworm (WBC) *Striacosta albicosta* (Smith) (Lepidoptera: Noctuidae) into Ontario corn (*Zea mays* L.) and dry bean (*Phaseolus vulgaris* L.) fields is a very recent problem, with the first moths being captured in traps in 2008[1]. In dry beans, the eggs are laid on the underside of leaves by the adult female moths. Newly hatched larvae feed on leaf tissue and flower petals. Once the larvae reach the third instar stage of growth, they begin to feed directly on the developing bean pods which can negatively affect both yield and quality at harvest[4]. These insects are not cannibalistic, which allows for multiple larvae to feed in the same area, and they have the ability to move up to 1.5 metres from their original egg location[2].

In Ontario, cyhalothrin-lambda is registered to control WBC in dry beans, and chlorantraniliprole was approved in 2011. However, little is understood about the efficacy and timing of insecticides, for the control of WBC in dry beans.

OBJECTIVE

Does the insecticide product or application timing influence WBC larval control, bean yield and seed quality?

METHODS

- Experiment 1: Efficacy of 4 insecticides applied 8 days after egg hatch (cyhalothrin-lambda, chlorantraniliprole, dimethoate and flubendiamide) compared to an untreated control.
- Experiment 2: Efficacy of 2 insecticides (cyhalothrin-lambda and chlorantraniliprole) applied at different timing intervals after egg hatch compared to an untreated control. The 5 application timings were: plots sprayed once at 4 days, 11 days, 18 days or 25 days after egg hatch, or sprayed at all 4 timing intervals.
- RCB design at two sites with 4 replicates per experiment at the Plattsville location and 3 replicates per experiment at the Ridgetown location.
- Plots consisted of 4 rows of *Phaseolus vulgaris* cv. T9905, 6 m long with 76 cm row spacing. Each plot was infested with 4 egg masses/row.
- Egg hatch was monitored daily for one week after pinning egg masses to leaves, and larval damage was assessed on 10 plants per plot at weekly intervals.

RESULTS AND DISCUSSION

- Ridgetown site – greater than 90% egg hatch, but less than 1% larval feeding damage.
- Plattsville site – less than 5% egg hatch (possibly due to parasitism by *Trichogramma ostriniae*) and less than 1% larval feeding damage
    - Both sites were abandoned prior to harvest.

Study changes proposed for 2012:

- Inoculate studies twice (one week apart) in order to increase the probability of larval damage occurring.
    - Although a yield reduction up to 7bu/acre can be seen in corn with one WBC larvae per plant[3], no larval damage was observed in 2011 bean trials.
- Collect eggs from non-GMO corn fields in order to procure higher numbers of masses.
    - Although WBC is reportedly not highly susceptible to BT corn hybrids[4], WBC overwintering populations may be higher in non-BT than BT corn fields. Collecting masses for 2012 trials from non-BT fields may yield greater numbers with which to inoculate trials.
- Plant dry beans earlier in the season to match bean flowering with early WBC egg laying in field corn in order to ensure maximum availability of egg masses during susceptible bean stages.

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A MAPPING POPULATION FOR THE EVALUATION OF DROUGHT TOLERANCE IN DRY BEAN

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Development of drought adapted bean varieties is an important strategy to minimize crop failure and improve food security in bean growing regions. However, genetic improvement for drought tolerance has been slow due to the lack of reliable techniques, as well as the inability to create repeatable screening environments. Also, dry bean production is being pushed out towards marginal lands, where groundwater is limited, resulting in pumping restrictions and poor seed yield (Urrea et al., 2009).An alternative approach to breeding for drought tolerance is the use of marker assisted selection; however, few genes have been reported in the literature.

MATERIALS AND METHODS

A recombinant inbred line population was developed from a cross between SER 22, a drought tolerant small red line from CIAT, and 'Buster', a drought susceptible pinto, to identify drought tolerance QTLs. This population is composed of 337 $F_{5:7}$ RILs that show high levels of phenotypic variation in the field. A field trial was planted at Mitchell, NE in 2011 using adjacent non-stressed (NS) and drought stressed plots (DS) with furrow irrigation. An alpha-lattice design, 15 x 23, with genotypes assigned as experimental units and with two replications in each environment was employed. Each plot consisted of one 4.6 m row spaced 0.6 m apart. Beryl-R, Buckskin, Buster, Matterhorn, Morales, Raven, SER 22, and UI 114 were used as reference checks. Both NS and DS plots were irrigated until flowering to ensure good plant establishment and normal vegetative growth. Then water was applied to NS plots only.

To evaluate plant response to water stress, seed yield (kg ha^{-1}), 100-seed weight (g), the number of days to flowering and to maturity, and pod harvest index (%) were determined. A Watermark Soil Moisture probe was placed in the parental line plots and soil water content was measured to a depth of 0.23, 0.46, and 0.76 m. Leaf temperature and stomatal conductance were assessed using a FLUKE Precision Infrared Thermometer Model 572 and a Decagon Devices Leaf Porometer, respectively. To quantify drought severity, the drought intensity index (DII), geometric mean (GM), drought susceptibility index (DSI), and percent yield reduction (PR) were determined under DS and ND conditions. All tests were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Drought stress was severe (DII=0.77) with significant precipitation of 201.2 mm occurring at 42 d after planting for a total amount of water (precipitation + irrigation) of 212.2 and 390 mm for DS and NS, respectively, with an average of 31^0C and 13^0C, maximum and minimum temperature, respectively at Mitchell, NE. Yield under NS and DS ranged from 103 to 2298 kg/ha and from 0 to 1247 kg ha^{-1}, respectively. Under DS conditions, seed yield and 100-seed weight were reduced an average of 60% (SER 22 56%, Buster 66%, checks 51% and the RILs 61%) and 3%, respectively, in comparison to NS conditions.

Using pod harvest index as the major selection index, RILs 215, 274, 179 and 164 were identified for superior seed yield and superior pod harvest index. These lines showed excellent adaptation to drought stress conditions and transgressive segregation in comparison to the parents. RILs 215, 274, 179, 164, 218, and 126 combined superior seed yield with lower pod wall biomass under drought stress conditions. RIL 229 was best suited to both NS and DS conditions in terms of mean seed yield among genotypes and parental lines. Matterhorn also fit as best suited for both environments.

Additional field trial evaluations will be conducted to obtain a better estimate of the variation due to environmental factors. Also, a genetic map based on 6,000 SNPs will be developed to identify QTL regions associated with drought tolerance.

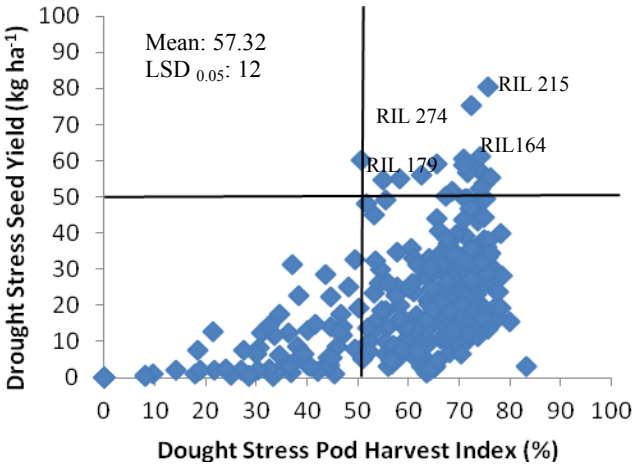

Fig.1. Identification of genotypes that combine superior yield with superior pod harvest index under drought stress conditions.

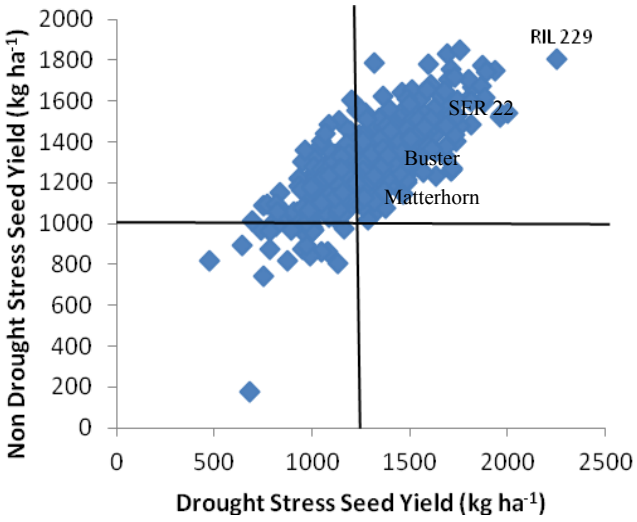

Fig.2. Identification of genotypes that were efficient in both drought and non-drought stress environments.

ACKNOWLEDGEMENTS

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GENETIC MAPPING OF THE *CO-5²* ALLELE FOR RESISTANCE TO *COLLETOTRICHUM LINDEMUTHIANUM* IN MSU 7-1 LINE

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INTRODUCTION: Anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib. is a major disease of common bean (*Phaseolus vulgaris*), which under favorable conditions is able to result in large reductions not only in yield, but also in seed quality. One of the most effective and economical measures to control anthracnose is based on using resistant cultivars, since it implies low cost and less environmental damage (Pastor-Corrales et al. 1995). Additionally, the use of molecular markers, associated with anthracnose resistant genes of common bean, are a powerful tool to assist breeding programs during the process of developing new cultivars with broad and durable resistance spectrum. Previous studies conducted by Vallejo and Kelly (2009) revealed that MSU 7-1 line derived from the cross Black Magic × SEL 111 possessed the*Co-5²*and *Co-7* genes and also exhibits resistance spectrum to races 7, 9, 23, 55, 64, 65, 73, 89, 448 and 453 of *C. lindemuthianum* (Gonçalves-Vidigal et al. 2009). Therefore, the objective of this study was to elucidate the basis of anthracnose resistance in MSU 7-1 and to evaluate new markers linked to the *Co-5* locus.

MATERIALS AND METHODS: The genetic and molecular analyses were carried out in an F_2 population derived from the cross between MSU 7-1 (resistant parent) × Mexico 222 (susceptible parent) inoculated with race 64 of *C. lindemuthianum*. Six STS markers (g1175, g1233, g1378, g2416, g2459 and g2531), previously mapped on linkage group Pv07 (McClean et al. 2010) were assayed for linkage with the *Co-5²*allele using the BSA (Bulked Segregant Analysis) approach (Michelmore et al. 1991). Two contrasting bulks of DNA were developed, one composed of DNA from five F_2 resistant homozygous plants (RB), and the other from five susceptible homozygous plants (SB). Only the g1233 marker was polymorphism among bulks and corresponding parents was tested on individuals of each bulk. This marker was tested in the 90 F_2 individuals from MSU 7-1 × Mexico 222 cross and in the RILs BAT 93/Jalo EEP 558 (B/J: 74 lines; Freyre et al. 1998).

Segregation analysis of the disease reactions of 90 F_2 plants from the MSU 7-1 × Mexico 222 cross was performed with the chi-squared (χ^2). A goodness-of-fit test for a 1:1 segregation ratio was performed for the segregation of the g1233 marker in the BJ population. Linkage analyses were performed using the computer software MAPMAKER/EXP 3.0 (Lincoln and Lander 1993). Linkage group nomenclature followed Pedrosa-Harand et al. (2008) and the map was drawn using the computer software MapChart (Voorrips 2002).

RESULTS AND DISCUSSION: The observed segregation ratio of 3R:1S, in F_2 population from the cross MSU 7-1 × Mexico 222 cross inoculated with race 64 of *C. lindemuthianum*, resulting in asegregation of 69 resistant plants and 21 susceptible plants (χ^2= 0.133; p = 0.71). Also, Figure 1 shows the presence of the STS marker $g1233_{3250}$, in G 2333 cultivar and in MSU 7-1, but it is absent in the Mexico 222, TU, H1 line, PI 207262 and Michigan Dark Red Kidney cultivars. Molecular analyses in the F_2 population with the $g1233_{3250}$ marker showed 3:1 ratio (χ^2

= 0.0148; p = 0.90) that co-segregated with resistanceindicating linkage with the *Co-5*^{2} allele (*Co-5*locus) in coupling phase at a distance of 1.2 cM on the Pv07 linkage group (Figure 2). The segregation of the BAT 93/Jalo EEP 558 (BJ) RI population assayed with the g1233 resulted in a ratio of 1:1 (p = 0.64) for a goodness-of-fit to a 1:1 ratio. The results demonstrated that the monogenic resistance in MSU 7-1 to race 64 of *C. lindemuthianum* is probably conferred by the same *Co-5*^{2}allele identified in G 2333 cultivar. The linkage between the g1233$_{3250}$marker and *Co-5*^{2}allele identified in MSU 7-1 will be of important for the marker-assisted introgression of this allele into commercial cultivars and elite lines, in order to expand the resistance spectrum offuturecommon bean cultivars.

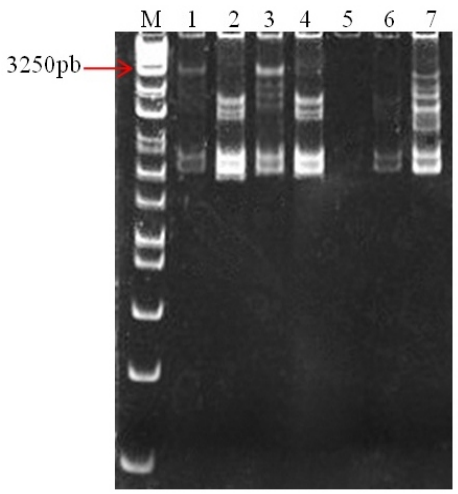

Figure 1. Electrophoresis analyses of amplifications products of marker g1233. M, 1 Kb Plus DNA Ladder; 1, MSU 7-1; 2, Mexico 222; 3, G 2333; 4, TU; 5, Line H1; 6, PI 207262; 7, Michigan Dark Red Kidney. The arrow indicates the DNA band of 3,250 bp linked to resistance allele *Co-5*^{2}.

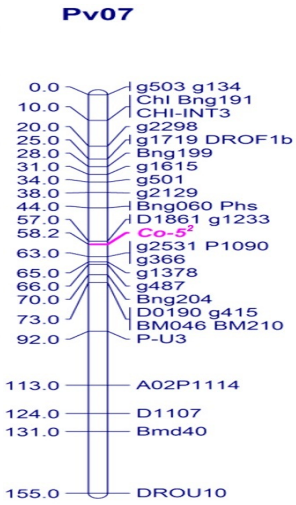

Figure 2. Genetic distance and location of the locus *Co-5*^{2} for resistance to common bean anthracnose, and the molecular marker g1233 on the linkage group Pv07 of *Phaseolus vulgaris* L., using the populations from the cross MSU 7-1× Mexico 222.Map was drawn with MapChart (Voorrips 2002).

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PHENOTYPIC EVALUATION OF DRY BEAN RIL POPULATION FOR DROUGHT RESISTANCE IN RWANDA

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L) is the world's most important food legume. It constitutes an important staple in Rwanda where per capita consummation is among the world's highest (60 kg). As other crops such as maize are becoming important in Rwandan agriculture, beans are being pushed to marginal lands characterized by drought prone infertile soils. The limited irrigation available is reserved to specialty crops such as rice, fruits and vegetables. As the competition from other crops for production areas and resources is increasing, beans need to be resistant to both biotic and abiotic stresses to be productive in Rwanda cropping systems. The present study was conducted to evaluate a Recombinant Inbred Line (RIL) population from a cross of SEA5 with CAL 96 for drought resistance in Rwanda.

MATERIALS AND METHODS

Experiments were conducted for two growing seasons in dry-land Karama research station (2° 16´S, 30° 17´ E, 1,347 m elevation). The first growing season was from November 29th, 2010 to March 1st, 2011; and the second growing season was from April 15th to July 15th, 2011. The RIL population consisting of 125 lines, their parents (SEA5 and CAL96), and 5 checks: RWR1668, RWR2245, SER13, SER14, and SER16 were evaluated for drought resistance. SEA5 is a creamy small sized bean line that was developed for drought tolerance (Singh et al., 2001). CAL 96 is a large seeded Calima type that is widely adapted in East Africa. SER 13, SER14, and SER 16 are small reds that have been selected for drought resistance (Beebe et al., 2008) and released in Rwanda. RWR 2245 and RWR 1668 are Andean bean cultivars widely grown in Rwanda. A 12 x 11 rectangular lattice design with two replicates was used. Two water levels representing irrigated and drought stress treatments were applied. The experimental unit consisted of 1 row of 1.5 m and 0.5 m wide. Experiments were sprinkler irrigated until flowering when irrigation in drought stressed plots was stopped. Irrigated plots continued to receive 25 mm of supplemental irrigation every 2 days.

Drought Intensity Index (DII) was calculated to quantify the severity of drought for each experiment. To determine response to water stress, yield (kg/ha), Harvest Index (HI), number of pods/plant, number of seeds/pod, number of days to physiological maturity, and 100 seed weight were measured for each RIL. The variable number of plants harvested/plot, due to low germination or disease problems, was recorded. Geometric mean (GM) and Drought Severity Index (DSI) were calculated for each RIL. Data were analyzed using PROC MIXED (SAS 9.3, SAS Institute, Cary, NC). Analysis of Covariance (UNCOVA) was performed to adjust plot yields using number of plants as a covariate. Water treatment and genotype were analyzed as fixed effects while season, replication and block were analyzed as random effects.

RESULTS AND DISCUSSION

DII was 0.34 for the first experiment and 0.20 for the second experiment; both exhibiting moderate drought exposure. Seed yields ranged from 383 to 2280 kg/ha under drought conditions while the range was from 356 to 4125 kg/ ha under irrigation. Overall seed yield reduction was 30% under drought stress. Under drought stress, no RIL out yielded SER14 and SER 13 checks. Seed yield of SEA 5 did not differ from seed yield of CAL 96 under irrigation conditions although they were significantly different under drought conditions (Table 1). Drought stress significantly reduced the number of pods/plant and seed/pod. A range of seed size from 22.3 to 44.2 g/100 seed was observed but there was no significant effect of water treatment on seed size. This was probably due to the moderate drought that was experienced in both trials. Under irrigation conditions, CAL 96 consistently had a higher HI than SEA 5. Progenies having higher HI than the parents and checks were observed. The number of days to maturity was influenced by both genotype and water treatment ($p<0.0001$). Average maturity days ranged from 71 to 89 days which is desirable for this location since acceptable maturity for bush beans in Rwanda is between 75 to 80 days.

Table1. Main characteristics of the best and worse yielding RILs grown for two seasons at Karama

	Yield (kg/ha)						
Genotype	GM	Stress	non stress	% reduction	Harvest Index	Days Maturity	100-seed Wt (g)
L41	2342	1329	4125	67.8	0.32	85	41.7
L24	2150	1355	3412	60.3	0.41	89	30.2
SEA5	2140	1924	2379	19.1	0.44	83	30.1
L85	2058	2046	2069	1.1	0.46	88	32.2
L91	1919	1642	2243	26.8	0.50	85	42.2
CAL96	1784	1343	2370	43.3	0.54	82	54.4
L87	1742	1429	2124	32.7	0.42	83	40.1
L81	1742	1514	2005	24.5	0.36	85	36.4
L114	1612	1222	2125	42.5	0.48	83	35.0
L127	1592	1387	1826	24.0	0.40	85	36.7
L53	825	536	1271	57.8	0.29	88	36.1
L26	623	556	697	20.2	0.23	85	36.4
L119	565	383	834	54.1	0.29	85	34.6
L96	428	515	356	-44.7	0.22	86	28.9
Overall mean	1068	934	1027	9.1	0.37	83	26.6

CONCLUSION: Water stress significantly reduced yield although the level of drought exposure was moderate. Few lines out-yielded the resistant parent SEA5 under these drought stress conditions. Additional experiments are being conducted to select lines with enhanced drought resistant and acceptable seed.

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CONTRIBUTION OF GENETIC IMPROVEMENT IN YIELD INCREASE AND DISEASE RESISTANCE IN NAVY BEANS RELEASED IN CENTRAL CANADA SINCE 1930's

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) breeding in Canada has a history of more than 120 years. Early breeding efforts included testing of dry and garden bean introductions, which started soon after the establishment of the Central Experimental Farm (CEF) in Ottawa in 1886 (Park and Buzzell 1995). Long-term dry bean production data (Stats Canada, 2008) indicates that average dry bean yield in Canada has increased by 63% from an average of 1.1 *t ha-1* during 1910s and 1920s (20 year average) to 1.8 *t ha-1* in 1990s and 2000s (18 year average). While improved agronomic practices during the past century have no doubt been an important factor, it is generally accepted that the development of well-adapted high yielding varieties has played a significant role in increasing common bean yield in Canada. In addition to considerable gain in disease resistance, improved canopy structure, suitable for direct combining, which appeared mainly in the navy beans starting in the 1960s, is known to have revolutionized the production of common bean in North America (Kelly 2000) and elsewhere.

The objective of this study was to examine the contribution of genetic improvement in increased yield potential and disease resistance of navy beans released in Canada over the last 80 years and to study the association of yield gain with agronomic traits.

MATERIALS AND METHODS

A collection of 29 navy beans released in Central Canada since 1930s were selected for this study. Agronomic performances of the varieties were tested in 2010 and 2011. In 2010, a field trial was planted in a farmer's field near St. Thomas Ontario. In 2011, field trials were conducted in 3 locations; St Thomas, Woodstock, and Thorndale, Ontario, all in a Randomized Complete Block Design with 4 replications each. Common bacterial blight (caused by *Xanthomonas axonopodis* pv. *Phaseoli* (*Xap*) responses of the genotypes were tested under artificial infection in disease nurseries in Harrow Ontario in replicated trials in 2010 and 2011. Anthracnose response of the genotypes were tested in a disease nursery planted in 2011 at the University of Guelph Elora Research Station, where disease was initiated in the nursery by planting infected seed in spreader rows, planted next to the experimental lines. The anthracnose race in the nursery was race 73, which is virulent on *Co11*, *Co2*, and *Co3*.

Annual yield gain was estimated in each location year and for combined data as the slope of a linear regression line, computed to predict varieties' yield as a function of its year of release. The relative genetic gain was then estimated as the ratio of the slope of the regression line over the average yield of the experiment.

RESULTS

Resistance to common bacterial blight in Canadian navy bean was observed only for varieties released after 2000. Resistance has been achieved over the years through inter-specific crosses to two distinct sources of *P. acutifolius*. Most varieties were found susceptible to race 73 of anthracnose. Historically, anthracnose resistance in Canadian dry bean was provided by the gene *Co2* (formerly known as the *Are* gene). Race 73 was first reported in 2003 in Ontario. The high anthracnose resistance of OAC Seaforth, however, was notable. OAC Seaforth is derived from the cross Seafarer/PI326418.

Significant differences were found among the cultivars for yield in all location years. The slope of the regression line of yield over year of release was significant and indicated an annual gain of 18 kg ha-1 or a relative genetic gain of 0.5 % per year (Fig. 1). Increase in yield potential of navy beans in Central Canada seems to be significantly associated with increased canopy height (Fig. 2) and improved canopy architecture, which is more suitable for direct harvest.

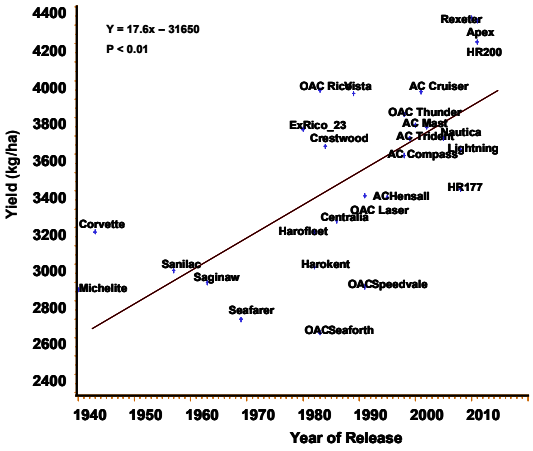

Fig 1. Average yield, combined over years, of navy bean varieties released in Central Canada since 1930s.

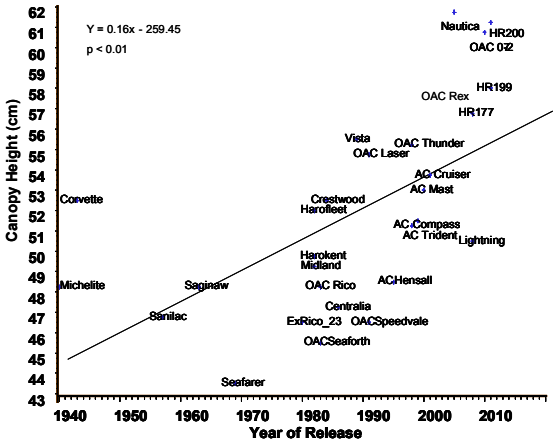

Fig 2. Canopy Height of navy bean varieties released in Central Canada since 1930s.

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QUANTITATIVE VARIATION IN NODULATION POTENTIAL IN AN INBRED LINE POPULATION OF DRY BEAN

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INTRODUCTION

Despite its inherent N_2-fixing ability, the common bean (*Phaseolous vulgaris* L.) is a weak N_2-fixer. Identification of superior genotypes with higher N_2-fixation ability (Bliss, 1993; Bliss et al. 1989, Farid et al. 2011) supports the idea that SNF in common bean can be improved through breeding. Even though noticeable diversity has been reported among different genotypes, only a few studies have reported improvement in SNF ability in common beans. The ultimate goal of this research is to identify QTL associated with SNF in a recombinant inbred line (RIL) population that is segregating for SNF-related traits.

MATERIALS AND METHODS

An $F_{4:5}$ RIL population (n = 140) of a cross between navy bean genotypes Sanilac and ACUG 10-6 along with the parental lines and two checks were studied for nodulation traits in growth pouch assays in a greenhouse at the University of Guelph and in field trials.Germinated seeds of each RIL were inoculated using a commercial peat-based inoculum (*Rhizobium leguminosarum* bv. *Phaseoli;* Becker Underwood, Saskatoon, Saskatchewan, Canada). Germinated seeds were placed in growth pouches (Mega International, St. Paul, MN, United States) in an N-free nutrient solution. The position of the seedling root tip was marked on the growth pouch at the beginning of the experiment. Number of nodules above the root tip marks was counted after 14 days. The RIL population, the parental lines and checks (Table 1) were also planted in a field near Rockwood, Ontario, Canada in two N treatments (0 and 100 Kg N ha^{-1}). In each N treatment, the population and the checks were arranged in a 2-replication 12 x 12 unbalanced square lattice design.

RESULTS

Parental lines and the RILs were different for nodule numbers above the root-tip mark ($P < 0.0001$). In the field, RILs were also significantly different ($p < 0.05$) for nodule number and nodule weight at flowering and shoot dry weight at maturity. Frequency distributions were continuous with presence of transgressive segregation (Figure1). N_2 fixation efficiency, estimated as the ratio of shoot dry weight of the genotypes (RILs and parental lines) to the shoot dry wt. of the non nodulating mutant (R99) was significantly different for the parental lines and the RILs between N = 0 and N = 100 treatments (Figure 2). While the parental lines had similar N_2 fixation efficiency in N = 100, in N = 0 were significantly different.

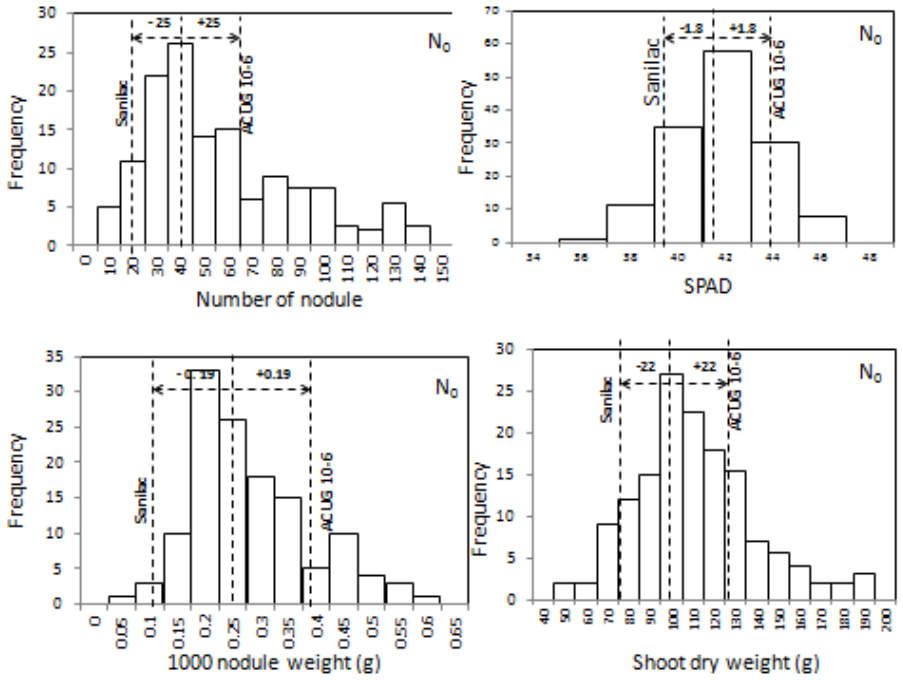

Figure 1.Frequency distribution of Nodule numbers and wt, SPAD reading, and shoot wt. in a RIL population of Sanilac x ACUG 10-6 cross in N = 0 treatment. Vertical Lines represent the mean ± standard error.

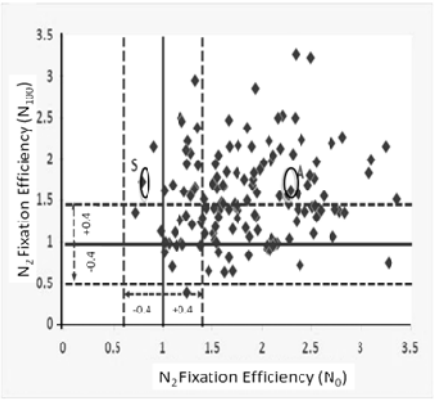

Figure 2. Scatter plot of the N_2 fixation efficiency of the RILs in N = 0 and N = 100 treatments. Vertical and horizontal lines represent unity ± standard error, while A and S are the parental lines, ACUG 10-6 and Sanilac, respectively.

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THE COMMON BEAN GROWTH HABIT GENE PVTFL1Y IS A FUNCTIONAL HOMOLOG OF ARABIDOPSIS TFL1

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INTRODUCTION: The naturally-occurring determinate growth habit in common bean is controlled by a single recessive allele, *fin* (Norton 1915, Koinange et al. 1996). A QTL for growth habit in common bean has also been identified on chromosome 01 (Koinange et al. 1996; Poncet et al. 2004). Near this growth habit QTL, a candidate gene, *PvTFL1y*, homologous to *Arabidopsis Terminal Flowering 1* (*TFL1*), was recently identified and found to co-segregate (Kwak et al. 2008) with the phenotypic locus for determinate growth habit, *fin*. Furthermore, quantitative PCR studies have shown that expression is greatly reduced at *PvTFL1y* in determinate types (Repinski et al. 2012). In this study, we have used *Agrobacterium*-mediated transformation to show that *PvTFL1y* can rescue mutant *tfl1-1* determinate *Arabidopsis* plants. These findings support the hypothesis that *PvTFL1y* is the gene underlying the phenotypic determinate growth locus, *fin*, in common bean.Identification of the locus underlying determinate growth will lead to a better understanding of the domestication process and will allow for further, and faster, manipulation of growth habit and flowering time in future breeding efforts of common bean.

MATERIALS AND METHODS: The *Phaseolus vulgaris* accession BAT93 (indeterminate) and the *A. thaliana* accessions CS39005 (indeterminate; *TFL1*), and CS6167 (determinate; *tfl1-1*) were used in this study. RNA was isolated from BAT93 using the CARTAGEN Total RNA Isolation kit. cDNA was isolated using Invitrogen SuperScript® III First-Strand Synthesis SuperMix with gene-specific primers for *PvTFL1y* (PvTFL1y Forward: 5'GCGGAATTCATGGCAAGAATGCCTTTAGAA and PvTFL1y Reverse: 5'CGCCTCGAGCTAGCGTCTTCTTGCAGCTGT). A full-length coding sequence, amplified from BAT93,was inserted into cloning vector pBSSK (Stratagene, Santa Clara, CA). The PCR amplification consisted of 3 min at 95 °C, 35 cycles of 30 sec at 95 °C, 1 min at 55 °C and then 1 min at 72 °C, followed by a 5 min extension at 72 °C, run with a C1000 thermocycler (Bio-Rad, Hercules, CA). Next, the construct was sub-cloned into a plant expression binary, pB5, which is a modified pBIN19 (Clontech, Mountain View, CA) derivative with a double CaMV 35S promoter and TMV omega translational enhancer for strong constitutive expression in plants. Subsequently, the construct was sub-cloned into *Agrobacterium* strain GV3101, Rif (chromosomal), Gent and Kan (Ti plasmid),and then introduced into *Arabidopsistfl1-1* and *TFL1* plants by the floral dip procedure (Zhang et al. 2006). The presence of the *PvTFL1y* construct was confirmed by sequencing the PCR fragment obtained after amplification with primers PvTFL1y Forward and PvTFL1y Reverse. Additionally, presence or absence of the *Arabidopsistfl1-1* allele, characterized by a single nucleotide substitution G to A at the 5'-end of the fourth exon leading to a missense mutation Gly to Asp at residue 105, was confirmed by PCR (TFL1 Forward: 5'GCCATTGATAATGGGGAGAG and TFL1 Reverse: 5'CGGATTCAACTCATCCTTTGG) and sequencing. The PCR conditions were the same as above. Plants were evaluated for indeterminate or determinate growth habit after the onset of flowering.

RESULTS: Transformed *tfl1-1* and *TFL1* plants are shown alongside non-transformed *tfl1-1* and *TFL1* plants at 20 Days After Planting (DAP) and 40 DAP in Figures 1a and 1b. Nineteen transgenic *tfl1-1* and 34 transgenic *TFL1* lines were produced. Seventeen of the 19 transformed *tfl1-1* mutants had fully restored indeterminate growth and all 34 *TFL1* wild-type plants remained indeterminate. The conversion of the mutant to wild-type phenotype corroborates that the *PvTFL1y* transgene fully complements the function of *TFL1* in *Arabidopsis*. The presence of the *PvTFL1y* transgene was confirmed by PCR amplification and sequencing. All transformed plants were correspondingly genotyped for the presence of the *Arabidopsistfl1-1* (A) or *TFL1* (G) allele.

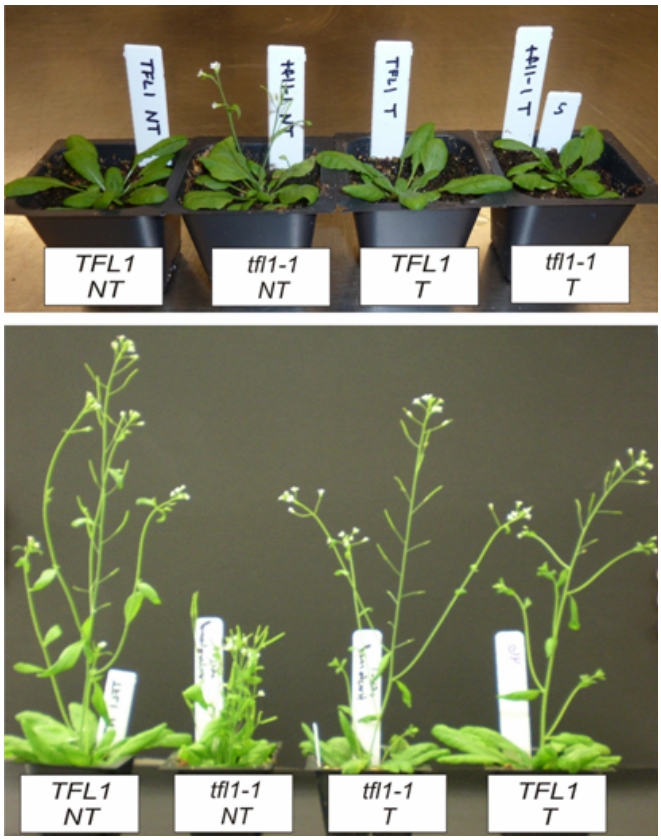

Figure 3. a. Transformed (T) and non-transformed (determinate) plants twenty days after planting. b. Transformed (T) and non-transformed (NT) TFL1 (indeterminate) and tfl1-1 (determinate) plants forty days after planting.

DISCUSSION: This study identified the common bean *PvTFL1y* gene is a functional homolog of the *Arabidopsis TFL1* gene through complementation. Interestingly, several different mutant alleles exist at the *PvTFL1y* locus (Kwak 2008). Future studies should analyze the different *PvTFL1y* haplotypes and their correlated effects on other plant characteristics, such as photoperiod sensitivity, plant height or internode length. *fin* is tightly linked to the photoperiodgene, *ppd*, on chromosome 01(Kelly 2001). Exploring the interaction and linkage of these two loci may expand the geographic locations in which novel adaptation traits can be evaluated. This information can be obtained through backcrossing the different *PvTFL1y* haplotypes into a common genetic background or by association analysis in a sample with known flowering phenotypes. Finally, knowing the molecular basis for determinate growth habit will facilitate the establishment of different breeding strategies, involving determinacy in particular, for the different gene pools of common bean.

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RIBOTYPE CHARACTERIZATION OF *XANTHOMONAS AXONOPODIS* PV. *PHASEOLI*, PATHOGENIC RACE XAPV1

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Xanthomonas axonopodis pv. *phaseoli (Xap)*, is the causal agent of common bacterial blight in common bean, *Phaseolus vulgaris* L. Studies conducted with different Xap strains collected in Central America and Puerto Rico have shown few strains causing a differential reaction on common bean genotypes indicating the presence of pathogenic races. Using one of the proposed pathogenic races and one genotype that showed high resistance, a study of the inheritance of resistance was conducted. It was found that the resistance was controlled by a single dominant gene and the corresponding pathogenic race was designated as **XapV1**. In this study, the genetic information of **XapV1** (Puerto Rico 3353) was compared to other proposed pathogenic races such as Xap 484a (Puerto Rico), Xap 1930 (Nicaragua) and Xap 1934 (Costa Rica) using Riboprints patterns of rDNA and the sequence of the 16S rDNA fragment. Riboprints patterns of rDNA digested with *Eco*RI showed no difference between the Xap strains. In contrast, with *Pvu*II differences were observed between the ribopatterns of the strains from Puerto Rico and those from Costa Rica and Nicaragua. The sequence of the 16S rDNA fragment of XapV1 showed a confidence level to species with matches with *X. axonopodis*, *X. perforans* and *X. vasicola* with 0.00% alignment. Ribotyping resulted to be more informative than sequencing for discrimination of the variable regions of the Xap strains.

MATERIALS AND METHODS

Strains: Four strains were selected representing three geographic locations: Costa Rica, Nicaragua and Puerto Rico (Fig.1). Strains were isolated from: Leaves (Costa Rica, Nicaragua and Puerto Rico XapV1) and seeds (Puerto Rico 484a).

- ***Sequencing:*** The target region was the ribosomal 16SDNA.
- ***Ribotyping:*** Procedures used were as recommended for the Riboprinter TM Microbial Characterization System. DNA was digested with *Eco*RI and *Pvu*II. Strains were considered to have the same pattern if the similarity coefficient between their pattern was >0.93.

RESULTS AND DISCUSSION

- Sequencing of the 16S rDNA was not able to discriminate between the species *axonopodis, perforans* and *vasicola* (Fig.1).

- Using the first pathogenic race designated as XapV1 it was possible to start a genetic comparison of rDNA ribopatterns with other potential pathogenic Xap races of different origins.

- When *Eco*RI was used all Xap strains were grouped within the same ribotype pattern. (Fig 2).

- *Pvu*II discriminated better the variable regions than *Eco*RI. *Pvu*II grouped the Costa Rica and Nicaragua strains within the same ribotype and separated them from the Puerto Rico strains (Fig 3). Puerto Rico strains were different from each other.
- Diversity among the proposed pathogenic strains was observed using *Pvu*II but not with *Eco*RI. Data suggest the use of *Eco*RI to group the strains within the same genus and species. In contrast, the *Pvu*II enzyme was useful to differentiate other variable regions within the species *axonopodis* and pathovar *phaseoli*.
- Similarities of the ribopatterns obtained with *Pvu*II correlates with the similarities observed in the differential reaction induced on *P. vulgaris* when leaf inoculated with the strains from Costa Rica and Nicaragua which is contrasting with XapV1. This fact opens new possibilities for separating pathogenic races of Xap, especially when working with isolates of different geographical origins, that cannot be discriminated using sequencing of the 16S rDNA or other means. This method appears to be an accurate and practical tool to investigate the genetic relationships between XapV1 and other potential pathogenic races of Xap.

Fig. 1. Sequence alignment of the pathogenic race XapV1 (Xap 3353) with other *Xanthomonas* species using 16S rDNA*

0.00% 530 *Xanthomonas axonopodis*
0.00% 530 *Xanthomonas perforans*
0.00% 530 *Xanthomonas vasicola*

0.19% 530 *Xanthomonas citri*
0.19% 530 *Xanthomonas arboricola*
0.19% 530 *Xanthomonas theicola*
0.19% 530 *Xanthomonascampestris*
0.19% 530 *Xanthomonas cynarae*
0.19% 530 *Xanthomonas hortorum*
0.28% 530 *Xanthomonas pisi*

*Confidence level: species

Fig. 2 Riboprint patterns using ***EcoRI*** with rDNA of pathogenic races of *Xanthomonas axonopodis* pv. *phaseoli*

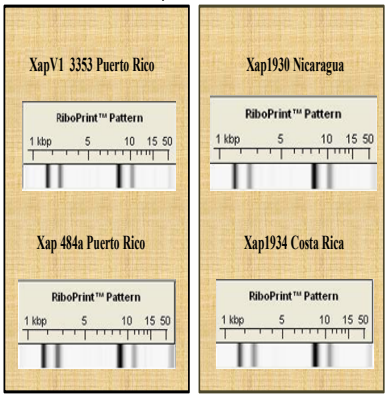
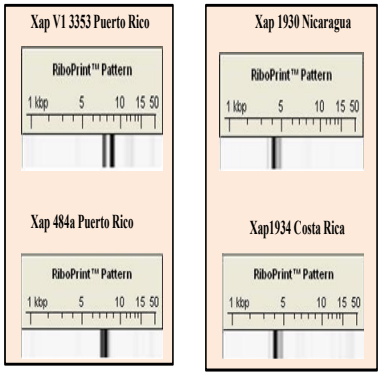


Fig. 3 Riboprint patterns using ***PvuII*** with rDNA of pathogenic races of *Xanthomonas axonopodis* pv. *phaseoli*



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GENERATION OF PROMISING LINES OF COMMON BEAN THROUGH INDUCED MUTATIONS IN THE EMBRZONIC AXES TO INCREASE WEB BLIGHT RESISTANCE AND OVERALL YIELD

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Web blight (WB) is one of the most destructive foliage diseases of common bean in Latin America and the Caribbean (Godoy-Lutz et al. 2008). WB not only reduces yield but also discolors the seed, which lowers market value (Godoy-Lutz et al. 1996a). Every year, significant economic losses around the world are reported for this disease. For this reason, the main objective of this project is to generate promising bean lines and transfer this technology to the agricultural sector, to increase the competitiveness of the national bean production in Costa Rica and, thus, promote a better quality of life for farmers and strengthen national food security. This research is divided into four segments: 1) generation of genetic diversity through mutagenesis and in vitro selection for tolerance to the fungal toxin, 2) increase of promising lines and evaluation for web blight in the greenhouse, 3) morphological evaluation in the field; and 4) and selection using molecular markers.

In the first stage, embryos were extracted from seeds, cultured *in vitro*, and then irradiated with cobalt-60. Tests were conducted with elite bean varieties Bribrí and Brunca, recommended by expert plant breeders (PITTA-Bean). The plantlets were treated with 15 Grays and 15% of *R. solani* extract. Embryos were subcultured periodically to eliminate genetic chimeras and to stabilize possible mutations. Irradiated material was simultaneously preselected for resistance to fungal toxins by culturing in media containing fungal extract. Fungal extract was prepared by growing the fungus in liquid media for 72 hours to release toxins into the culture media. Thus, the plants generated from irradiated embryos were grown under constant selection pressure. During this stage of the project, protocols were developed for the induction of mutations using the *in vitro* culture technique.

After plantlets had been subcultured three times and reached a stage of considerable growth they were transplanted in the greenhouse. Plants were evaluated for characteristics such as survival, height, number of leaves, pods, and seeds per pod, in order to identify lines with promising characteristics. After plant evaluation seeds were collected.

Next, seeds were planted for seed increase. A total of 143 bean lines were obtained and are currently being evaluated in the greenhouse for morphological and agronomic characteristics. Tolerance to the fungal disease web blight was evaluated using the detached leaf technique (Galindo et al., 1982) for the majority of the lines. These results are being corroborated in field tests, in collaboration with the National Institute for Transfer of Technology (INTA).

In 2010, 90 lines were evaluated in the field for agronomic value by the Bean Program for Agricultural Research and Transfer of Technology (PITTA-Bean). Testing is conducted in farmer plots in the Huetar Norte and Brunca Regions and is conducted twice each year, during the periods of bean production in Costa Rica. At present, bean lines have been selected for important agronomic traits such as growth habit, limited guide formation, and number of pods/plant.

In the final stage of this project, mutant bean lines are being characterized using a next-generation DNA sequencing approach called restriction-site-associated DNA (RAD) sequencing, which allows us to generate thousands of sequences adjacent to restriction sites across the genomes of multiple individuals (Baird et al. 2008). Specifically, this work will produce random genome-wide sequence, using an Illumina Hi Seq 2000, for two libraries, each containing 96 barcoded entries (192 total accessions). The sample includes 143 mutant lines and 49 germplasm accessions (wild beans and land races from Costa Rica). Sequences from the 192 lines will then be analyzed for sequence polymorphisms with the goal of finding interesting mutations that appear to co-segregate with desired phenotypes. Additionally, lines will be evaluated for overall sequence diversity.

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DEVELOPMENT OF IMPROVED TEPARY BEAN (PHASEOLUS ACUTIFOLIUS A. GRAY) GERMPLASM

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Heat and drought are major constraints to grain legume production worldwide, while use of tepary bean (*Phaseolus acutifolius* A. Gray.), a desert native species of the U.S. Southwest and Mexico with elevated levels of abiotic stress tolerance, remains limited partly due to seed quality, production, and cooking characteristics. In an effort to generate tepary germplasm with wider adoptability, breeding lines are being developed with improved seed size and growth habit, while maintaining common bacterial blight resistance, and drought and heat tolerance.

Broadly adapted, larger-seeded tepary bean lines were selected from germplasm in the USDA and CIAT core collections in drought tolerance trials conducted in Puerto Rico and Nebraska in 2007 and 2008 (Urrea and Porch, 2009). Single crosses with elite tepary bean lines including Neb-T-1-s and PI 502217-s, previously identified as superior germplasm (Miklas et al., 1994), and the F_1 generation advance, were completed in 2009 in a screenhouse at TARS in Mayaguez, PR. Single plant selections were made under drought stress in Juana Diaz, PR in the winter of 2009-10. The F_3 families were selected for common bacterial blight (CBB) resistance and heat tolerance in a glass house at TARS in 2010, and single plant selections of CBB resistant F_4 lines were conducted in Juana Diaz under drought stress in the winter of 2010-11. The selected lines were evaluated for response to bean common mosaic virus (BCMV) with the NL3 strain in a glass house at the University of Puerto Rico, and yield trials of superior F_4-derived lines were conducted under non-stress conditions, and under concurrent high ambient temperature and excess water stress conditions, in 2011 in Juana Diaz, Puerto Rico. Elemental analysis on seed samples from individual lines were conducted at TARS using the dry ash method (Perkin-Elmer, 1994) and inductively coupled plasma optical emission spectrometry (ICP-OES).

The seed yield performance of the tepary bean lines was similar to the common bean control 'Verano' under non-stress conditions, while the tepary lines were superior under high temperature/ excessive water conditions (Table 1). The majority of the lines showed a semi-erect habit (2-3 architecture ranking) versus a more prostrate habit (4-5) of most tepary beans. The 100-seed weight of the lines ranged from 12.9 to 16.8 g under non-stress conditions, with higher seed size than typical landraces. Compared to tepary beans in previous studies (e.g. Miklas et al., 1994), the lines showed a shorter maturity, with days to harvest ranging from 59 to 62 days after planting, because the material was harvested at physiological maturity in order to avoid a reduction in seed quality due to rain at dry-down. All of the tepary lines showed CBB resistance superior to the CBB resistant common bean control 'Verano', while none showed resistance to BCMV (data not shown).

In a preliminary study of seed elemental composition, protein content of a subset of the tepary bean lines averaged 28.5%, which was higher than the common bean control 'Morales' (24%), and falls within the range of 15-32% published previously (data not shown). 'Morales' showed higher iron, zinc, phosphorus and calcium concentrations than the tepary beans. Replicated evaluations will be needed to confirm these seed elemental concentration results.

The lines are currently being tested to confirm photoperiod insensitivity and broad adaptation to high temperature-arid temperate and high temperature-humid tropical conditions. Additional traits, such as rust, root rot resistance and BNF capacity are available within the tepary bean germplasm pool, while resistance to bean golden yellow mosaic virus (BGYMV) and bean common mosaic virus (BCMV) can be transferred from common bean.

Table 1. Performance of tepary lines and a common bean control in non-stress and high temperature/excess moisture stress trials in Juana Diaz, PR in 2011

Line		Non-Stress					Stress			GM
	Color	Arch[1]	DTH[2]	100SdWt	CBB[3]	kg/Ha	DTH	100Sd Wt	kg/Ha	kg/Ha
PI-440801	yellow	3.8	59	14.3	2.5	1540	52	12.5	221	583
PI-440799	yellow	3	59	15.8	2.3	1903	54	14.3	286	738
Neb-T-1-s	white	4	61	12.4	2.0	1304	53	11.3	590	877
PI-502217-s	white	2.8	59	14.4	2.3	1941	54	11.5	389	869
Verano[4]	white	1.3	66	15.3	3.0	1219	67	10.0	4	70
Tep 15	white	2.5	60	12.7	2.0	1759	54	11.3	458	898
Tep 19	yellow	4	60	16.8	2.3	1691	54	15.8	626	1029
Mean		3.3	59.9	13.5	2.3	1661				
LSD		0.9	2	1.6	0.6	441				
Results from lines in non-replicated (white) trial in Non-stress and replicated (grey) under stress conditions										
Tep 22	white	3	59	13	2.0	2303	54	12.3	599	1175
Tep 23	black	3	62	13.8	2.0	2336	54	12.8	707	1285
Tep 29	white	3	62	16.8	3.0	2063	54	13	597	1110
Tep 32	yellow	3.8	61	15.2	2.0	1739	54	15.8	609	1029
Mean							54.2	12.4	399	
LSD							2.2	2.5	278	

[1]Arch, plant architecture rated on scale of 1 (upright) to 5 (prostrate); [2]DTH- Days to harvest; [3]CBB, Reaction to common bacterial blight under natural conditions on a 1-9 scale; 1= no symptoms and 9= systemic infection causing complete infection of the leaf (van Schoonhoven and Pastor-Corrales, 1987); [4]Verano, common bean control.

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DEVELOPMENT OF MICRONUTRIENT DENSE DRY BEAN VARIETIES WITH IMPROVED DISEASE RESISTANCE

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INTRODUCTION

Improving the micronutrient content of edible crops may combat deficiencies in humans especially resource poor woman and children across Africa. The development of zinc and iron bio-fortified dry bean varieties with improved disease resistance is, therefore, an important research objective of the Southern African Bean Research Network (SABRN). During a Bean Breeders' meeting held in Kampala, Uganda it was proposed that South Africa improve the nutritional value of red speckled sugar (RSS) beans and combine it with resistance to rust, angular leaf spot (ALS) and common bacterial blight (CBB).

MATERIAL AND METHODS

Crosses were made in the greenhouse (October 2006) at Potchefstroom, South Africa, between RSS lines, developed at ARC-GCI with resistance to one or more diseases (CBB+BCMV, Rust+ALS+BCMV) and lines with high Fe and Zn content (AND 620, NUA 45, SEA 5, SEA 15 and SER 16), as recommended by CIAT. One hundred and twenty three F_1 plants, generated from crosses using CBB resistant material were artificially inoculated in the greenhouse using the multiple needle puncture method (Andrus *et al*., 1948) and rated on a 1-9 scale with 1 being highly resistant and 9 being highly susceptible. Lines AND 620 and NUA 56 plants were inoculated as susceptible controls. Susceptible plants were discarded (plants rated >3-9) and single resistant plants (rated 1-3) retained and harvested for further evaluation. Two hundred and thirty nine F_1 seeds derived from crosses using ALS and rust resistance were planted in the greenhouse and harvested as single plants. Fifty seven selected CBB resistant $F_{1:2}$ progenies were included in single row nurseries at Potchefstroom (North West Province) and evaluated for CBB resistance in artificially inoculated plots during the 2007/08 season. Parental lines were planted as checks every tenth row throughout all the trials. Similarly 220 $F_{1:2}$ progenies selected for resistance to rust and ALS were included in single row nurseries at Cedara Research Station (in the mist belt of KwaZulu-Natal) and evaluated for resistance under natural infection. A total of 508 single CBB and rust + ALS resistant plants ($F_{2:3}$) were selected from Potchefstroom and Cedara, respectively for evaluation at the Makhathini Flats (frost free locality in northern KwaZulu-Natal) during winter, 2008. Grain samples consisting of 40 seeds (± 10-15g) were selected from the $F_{2:3}$ progenies (including parental lines and local cultivars) for Fe and Zn analyses at the ARC-Soil, Climate and Water Institute (ISCW), Pretoria, South Africa (data not shown) to use as additional selection criteria. Two hundred and thirty two CBB resistant lines and 585 rust and ALS resistant lines ($F_{3:4}$) selected at Makhathini were evaluated in single progeny rows at Potchefstroom and Cedara, respectively during the 2008/09 season. From these 812 $F_{4:5}$ lines were selected for evaluation at Makhathini during winter, 2009. A total of 976 lines ($F_{4:6}$) were selected on the basis of disease resistance, yield and general adaptation and entered into un-replicated check row trials with the local cultivar, OPS-RS4 as check/standard. These trials were evaluated at 3 localities (Potchefstroom, Harrismith and Cedara) in the main bean producing areas of SA.

Seed of 50 promising lines selected were multiplied at Makhathini during winter 2009 for inclusion in replicated preliminary yield trials (PYT) during the 2010/11 season. Fe and Zn analyses were performed on high yielding lines selected from the checkrow trials and used as additional criteria in final selection of lines to be entered in PYT. Twenty eight high yielding lines with high mineral content (Fe > 90 ppm and Zn > 35ppm) were selected and included in preliminary yield trials (PYT) (randomized block design with three replicates), that were planted at four localities (Potchefstroom, Middelburg, Harrismith and Cedara). Evaluations were made for important diseases, and for agronomic characteristics such as growth habit, general adaptation, seed quality and yield. Data of the PYT were subjected to an analysis of variance (not shown), and selections were only made from those localities where the analysis indicated that results were reliable. The ranking of the mean yield over all reliable localities was used for selections. All lines with inferior disease resistance were eliminated. The computer programme @Maxiplan@ (statistical package of ARC - GCI) was used for these analyses.

RESULTS AND DISCUSSION

Results from the PYT are shown in Table 1. Only data of seven of the highest yielding lines with acceptable levels of disease resistance and mineral content (Fe > 90 ppm and Zn > 35ppm) are included in the table. OPS-RS4 was used throughout the study as standard commercial cultivar and is also included. Seed of these lines were multiplied at Makhathini during the winter of 2011 and will be included in advanced yield trials (AYT) which will be planted at 6 localities during the 2011/12 season. The best performing line(s) will be released. All the lines included in Table 1 are available for distribution to interested SABRN countries.

Table 1: The pedigree, mean yield and characteristics of advanced disease resistant lines with high micronutrient content developed at ARC-Grain Crops Institute compared to local cultivar OPS-RS4

Line/ Cultivar	Pedigree		Growth Habit	Seed Type	Yield (Kg/Ha)	Mean Disease Rating			Micro-nutrient Content (Mg/Kg^{-1})	
	Female	Male				CBB	Rust	ALS	Fe	Zn
PC3870-4	F6BC3 DAVIS OLD/PC229-BC1-P1		II	RSS	2414	1.5	2	2	90.5	41.1
PC3816-1	PC 2536-BC2 (14)	NUA 56	II	RSS	2100	1	1	1	100.4	41.5
PC3816-2	PC 2536-BC2 (14)	NUA 56	II	RSS	2579	1.2	3	2	92.7	52
PC3818-1	PC 2536-BC2 (14)	SEA15	II	RSS	2404	1.2	2	1	103.2	47.1
PC3818-2	PC 2536-BC2 (14)	SEA15	II	RSS	2020	1.2	2	1	113.9	45.9
PC3830-1	PC 3049-BC1 1	NUA 56	II	RSS	2221	1.5	1	1	114.1	48.7
PC3830-2	PC 3049-BC1 1	NUA 56	II	Calima	1891	1.2	1	1	93.2	37.1
OPS-RS 4			II	RSS	2323	2	1	1	124.2	35.2

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DEVELOPMENT OF CANDIDATE GENE MARKERS ASSOCIATED TO COMMON BACTERIAL BLIGHT RESISTANCE IN COMMON BEAN

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INTRODUCTION: Common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*), is a major yield-limiting factor of common bean (*Phaseolus vulgaris* L.) production around the world. Host genetic resistance is the most effective and environmentally-sound approach to control CBB, as it requires no additional chemical or biological measures to function. Two major CBB resistance QTL markers, BC420 and SU91, are located on Chromosome 6 and 8, respectively. However, both markers are dominant markers. Moreover, genetic linkage between random DNA markers and a target locus allele, established by QTL studies, can be broken by genetic recombination. By contrast, CGMs (Candidate Gene Markers), are markers derived from polymorphisms within genes and have complete linkage with target loci (Andersen and Lubberstedt, 2003). Physical mapping enables the efficient generation of new CGMs useful for co-dominant marker development. In addition, it provides a glimpse of local gene content useful for searching candidate genes underlying respective QTLs. The objectives of our study were to 1) identify a target BAC clone harbouring BC420 and SU91 marker, respectively, 2) sequence the BAC clones and assemble them combining three different assembly algorithms, 3) structurally and functionally annotate BAC sequences, 4) develop and screen CGMs using contrasting NILs, and 5) apply association study to evaluate CGMs and validate candidate genes.

MATERIALS & METHODS: Plant DNA was automatically extracted using an AutoGen 850 Alpha system following the manufacturer's directions (AutoGen Inc.). The PCR program was optimized and consisted of initial denaturation at 94°C for 3 min, 20 cycles of 30 s at 94°C, 45 s from 56°C to a "touchdown" (the annealing temperature was lowered 1°C after every two subsequent cycle) at 47°C for primer annealing, 1 min at 72°C for primer extension, followed by 21 cycles with a 47°C annealing temperature, and ended with 1 cycle of 10 min at 72°C. . A population of 392 dry bean lines of different market classes representing plant materials routinely developed in a bean breeding program were used for association study. Association study analyses were carried out with TASSEL 2.1 software, available at http://www.maizegenetics.net/index.php?option=com_content&task=view&id=89&Itemid=119.

RESULTS: Advanced by map-based cloning, four BACs from BC420-QTL locus and one BAC containing SU91 were sequenced by Roche 454 technique and subsequently assembled using merged assemblies from three different programs. Taking account of assembly quality, only the sequences of BAC 32H6 and 4K7 were used for CGM (Gene-Targeted Marker) development and candidate gene selection. For the BC420-QTL locus, 21 novel genes were predicted *in silico*

by FGENESH using *Medicago* gene model, whereas, 16 genes were identified in the SU91-QTL locus. For each putative gene, one or more primer pairs were designed and tested in the contrasting NILs (Near Isogenic Lines). Overall, six and nine polymorphic markers were found in SU91- and BC420-QTL loci, respectively. Afterwards, association mapping was applied in a breeding population of 392 dry bean lines to discover marker-trait associations. Two CGMs per each locus show better association to CBB resistance than BC420 and SU91, including BC420-CG10B and BC420-CG14for BC420_QTL locus, and SU91-CG10 and SU91-CG11 for SU91_QTL locus (Table 1).

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

Marker	Target gene	Forward (5'-3')	Reverse (5'-3')	Size (bp)	14 DAI		21 DAI	
					p[a]	R^2_marker[b]	p	R^2_marker
BC420-QTL locus								
BC420		GCAGGGTTCGAAGACACACTGG	GCAGGGTTCGCCCAATAACG	896	n.s.	0.0051	n.s.	0.0014
BC420-CG3	3	GGACTTAGCGTACGGTTGGA	TGTGGTCGATGAGAACAAGG	558/540	n.s.	0.0081	*	0.0200
BC420-CG4	4	ACCATCCCTCTGCCTTTTCT	TCATCTTCTGATCGGCCTTT	590	n.s.	0.0077	**	0.0196
BC420-CG9	9	AAGCAAACCCTTCCATTCC	TCCCAAACACCAATGGAAAT	415/375	n.s.	0.0104	*	0.0184
BC420-CG10A	10	AAGGCTGCAAAGATTGGAGA	TTGATGAAGCCTTTGGAACC	486	n.s.	0.0049	*	0.0124
BC420-CG10B	10	CCACCTGCCACATAGACCTT	TCTCGAGAAGGGCAGAGGTA	459	*	0.0136	**	0.0256
BC420-CG11	11	GTGTCCATCTCTGGGTGCTT	GGATGCAAAGAAGAGGCAAA	227	n.s.	0.0077	**	0.0196
BC420-CG14	14	CGAGACTCGTGTGCTCTCTG	ACGAAGGTTGATTCCCAGTG	519/425	*	0.0180	**	0.0197
BC420-CG15	15	GATCCCAAGAAAATGGCAGA	CAAGTCGTGGGATTCTGTGA	486	n.s.	0.0046	*	0.0132
BC420-CG17	17	AGCCAGAATGTATCGAATTG	TATGCAACCAAAACCAAAGG	500/510	n.s.	0.0070	*	0.0177
SU91-QTL locus								
SU91		CCACATCGGTTAACATGAGT	CCACATCGGTGTCAACGTGA	628	***	0.1253	***	0.0953
SU91-CG3	3	GCAGAAGATGCCAAGAGGTC	CTCTATTCACCGCCAGCTTC	215	n.s.	0.0001	n.s.	0.0024
SU91-CG9A	9	AGCTGTTATTGGTCATTCATTTG	GATCTCCCCTTATCGTCTTCG	383	***	0.0532	***	0.0469
SU91-CG9B	9	CCCGAGTTAGAAGTAGGTGGAG	TGTTGAAAACAAACTATCGTGAG	501	***	0.0557	***	0.0475
SU91-CG10	10	ATGGTGGAGACGAGATGACC	TCCGACATTGAAACCAGTTG	425/350	***	0.2735	***	0.2554
SU91-CG11	11	GGCGACGGCTTCTTTGAC	TCCAAAGACCAAAGGGTGAG	464/425	***	0.2257	***	0.2193
SU91-CG12	12	ACGAAACACCATACCCCAAA	CGGTCAGCAGTTTCTTCCTC	179	n.s.	0.0011	n.s.	0.0000

[a] n.s., not statistically significant; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.
[b] R^2_marker was calculated as the proportion of sum square due to marker after accounting for all other effects in model.

CONCLUSIONS

Association study shows SU91 was significant associated to CBB resistance at both dates, but not BC420 (Table 1). This finding is consistent with a recessive epistatic model of inheritance between two loci, i.e. the SU91_QTL locus is essential for CBB resistance and the greatest gain in resistance to CBB by selecting breeding materials that are fixed for both QTLs (Vandemark et al. 2008). Co-dominant CGMs, BC420-CG14 and SU91-CG10/SU91-CG11, are recommended to replace BC420 and SU91 for marker-assisted selection. Meanwhile, it indirectly provides gene function proof for gene 11 and 14 from BC420_QTL locus and gene 10 and 11 from SU91_QTL locus.

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ASSESSMENT OF GENETIC DIVERSITY AMONG LIMA BEAN LANDRACES FROM THE DOMINICAN REPUBLIC, HAITI AND PUERTO RICO

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INTRODUCTION

Protection of crop genetic resources is a key approach for securing food sources for the future generations. Crop diversity in part is maintained by farmers who share plant material, introduce new varieties and even select stronger and better genotypes. Also the farmers keep and, in some cases without knowingly, protect the wild relatives of many different species. The establishment of genetic relations within a crop is an important component in crop improvement programs. It helps by providing information about genetic diversity and is a starting point for stratified sampling of breeding populations. Correct assessment of genetic diversity is invaluable in any crops' conservation and for diverse applications including the identification of new combinations with maximum genetic variability for further selection and introgression of desirable genes from diverse germplasm into the available genetic base (Smith, 1984; Cox *et al*., 1986; Mohammandi and Prasanna, 2003).

In this study, we report the first genetic diversity assessment of lima beans (*Phaseolus lunatus* L.) in the Caribbean. Fifty-five landraces collected from the Dominican Republic, Haiti and Puerto Rico were subjected to SSR (Simple Sequence Repeat) marker analysis on polyacrylamide gels (detected using a fluorescence-based technique and an automated DNA sequencer). SSR markers consist of mono-, di-, tri- or tetra-tandem repeat motifs that usually consist of 1 to 6 base pairs of nucleotides. These markers are equally distributed throughout the plant genome, highly polymorphic and reproducible, and very importantly, co-dominant, SSRs can be found between conserved areas in the genomes which helps in the development of primers in order to amplify these regions.

MATERIAL AND METHODS

DNA extraction was performed, from approximately 0.5g of young leaf tissue, according to Dellaporta et al. (1983) protocol with some modifications. Other than the accessions collected, DNA was also isolated from lima bean variety 'Sieva' (Middle-American) and 'Christmas' (Andean) and used as checks in the analysis. A total of 24 SSR markers were used in order to access the genetic diversity of the lima beans accessions (Gaiton-Solis et al., 2002). PCR amplifications were made using the M13-tailed primer fluorescence method and electrophoresed on 6.5 % polyacrylamide gels, using an automated 4300 DNA analyzer (LI-COR, Lincoln, NE, USA) for high-throughput microsatellite data collection.

The bands were scored as present or absent. The molecular weight of each band was assessed by running a 50–350 base pair molecular size ladder in each gel. Scoring of the bands was performed using the Saga GT software (LI-COR). Genetic diversity within and among accession groups was estimated using the software GENSURVEY (Vekemans and Lefevre, 1997). A similarity matrix was then generated using the association index of Jaccard in

NTSYSpc software (Rohlf, 1993) for Principal Coordinate analysis and further clustering analysis based on UPGMA method.

RESULTS AND DISCUSSION

The number of alleles per loci ranged between 1 and 8 with the percentage of polymorphic loci being 74.17 ± 12.64. A high number of alleles were found per locus, at an average of 2.67 ± 1.46. The percentage of polymorphic loci was highest in accessions from PR (75%) followed by DR (70.8%) and Haiti (62.5%). The average proportion of observed heterozygous individuals (H_o) was higher than expected in all three countries.

The overall heterozygosity (H_t) in all accessions was 0.4110 ± 0.1964 with approximately 40% of the diversity due to within country variation (H_s= 0.3969 ± 0.1916) and only 3.65 % (G_{st} = 0.0365 ± 0.0512) due to differentiation among samples. The results reveal a low level of differentiation between country samples.

The UPGMA analysis showed that all samples clustered with the known-check variety (Sieva) of Middle-American descent. Interestingly, all samples from Haiti grouped in a cluster that did not contain any samples from Puerto Rico.

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A STUDY OF N_2 FIXATION ABILITY OF DIFFERENT DRY BEAN GENOTYPES

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INTRODUCTION

Breeding for increased symbiotic N_2 fixation (SNF) can improve legume crops that are normally dependent on N fertilizer for significant yields and will promote development of low-input cropping systems. Despite its inherent N_2-fixing ability, the common bean (*Phaseolous vulgaris* L.) is a weak N_2-fixer, compared to other legumes. However, noticeable diversity has been reported among different common bean genotypes (Bliss, 1993; Bliss et al., 1989, Farid et al. 2011). The availability of superior genotypes with higher N_2-fixation ability (Bliss, 1993; Bliss et al., 1989) supports the idea that SNF in common bean can be improved through breeding. The objective of this study was to assess the potential genetic diversity in some of the Canadian common bean genotypes for N_2-fixation related traits and to characterize the parental lines of the existing mapping populations for N_2 fixation related traits, so that the populations can be used in genetic studies of SNF and related traits.

MATERIALS AND METHODS

Twelve bean genotypes were compared for some SNF-related traits. In the greenhouse, seeds were grown in an N-free MVP turface (Profile products LLC, Buffalo Grove, IL 60089) following inoculation with a commercial peat-based inoculum of *Rhizobiumleguminosarum* bv. *Phaseoli* (Becker Underwood, Saskatoon, Saskatchewan, Canada). Plants were kept water replete at all times by a subsurface drip irrigation system. The pots were arranged in a randomized complete block design with 6 replications. Soil surface was covered by perlate to keep the soil cool. An N-free nutrient solution was used to supply plant water and nutrients. In the field, seeds were inoculated with the same inoculant used in the greenhouse trials before planting. Plants were grown in two locations (Belwood and Rockwood, Ontario, Canada) under low nitrogen, in the summer of 2011. The non-nodulating mutant (R99; Park and Buttery, 1988) and its wild type genotype, OAC Rico, were used as checks. The experimental design was a randomized complete block design (RCBD) with 4 replications in each location.

RESULTS AND CONCLUSIONS

In the greenhouse, the twelve common bean lines differed significantly ($P < 0.0001$) for nodule number and nodule dry weight, shoot dry weight , and SPAD reading (chlorophyll content of the second last fully expanded leaf) (Table 1). Both paternal genotypes of the mapping populations (ACUG 10-6 and OAC 09-3) showed substantial differences with the maternal parent (Sanilac) for nodule weight and number (Table 2). The high and low nodulating genotypes, ACUG 10-6 and Sanilac, had 90% difference in their SPAD reading. In the field, there was significant difference for nodule dry weight and number and shoot dry weight in both field locations. Significant differences were observed between the paternal genotypes of the mapping populations (ACUG 10-6 and OAC 09-3) and the maternal genotype (Sanilac) in both field trials (Table 2). Andean beans tested here generally had higher total nodule dry weight compared to the Mesoamerican beans.

Table 1. Comparing genotypes for SNF traits in the greenhouse.

Entry	Nodule No.	Nodule dwt. (g)	Shoot dwt (g)	SPAD
		Mesoamerican		
Sanilac	770	0.03	3.74	21.30
ACUG 10-6	1092	0.23	6.65	40.66
OAC 09-3	1076	0.22	6.47	34.34
OAC Thunder	647	0.07	3.77	33.87
Zorro	539	0.07	3.78	33.96
SXB 415	518	0.04	4.65	37.34
AC Compass	724	0.06	4.40	39.74
R32 (Nodulating check)	747	0.04	2.18	37.78
R99 (Non-nodulating check)	0	0.00	0.30	41.65
		Andean		
Chinook 2000	1497	0.11	8.56	39.06
Red Rider	819	0.27	6.48	35.79
Majesty	1288	0.26	10.33	34.15
Se	164.4	0.045	1.525	2.068
P value	<0.0001	<0.0001	<0.0001	<0.0001

Table 2. Comparing genotypes for SNF traits in the field (Belwood, ON).

Entry	Nodule No.	Nodule dwt. (g)	Shoot dwt (g)	SPAD	Maturity
			Mesoamerican		
Sanilac	172	0.11	96.46	32.51	138.75
ACUG 10-6	123	0.12	101.10	38.18	141.75
OAC 09-3	207	0.17	113.80	37.73	140.50
OAC Thunder	142	0.13	71.29	37.07	133.00
Zorro	107	0.07	143.36	35.08	141.00
SXB 415	46	0.07	128.04	34.26	143.25
AC Compass	80	0.10	130.80	33.26	132.75
OAC Rico (check)	145	0.29	82.34	38.09	136.00
R99 (Non-nodulating check)	0	0.00	118.01	35.89	139.50
			Andean		
Chinook 2000	253	0.30	55.74	32.73	141.00
Red Rider	174	0.22	117.27	32.02	142.00
Majesty	260	0.37	114.79	32.88	141.25
Se	48.3	0.077	21.328	1.530	1.195
P value	0.0136	0.0153	0.0306	0.0074	<0.0001

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EFFECT OF SOIL COMPACTION AND IRRIGATION MANAGEMENT ON ANTIOXIDANTS IN DRY BEAN PRODUCTION

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INTRODUCTION

Soil compaction not only restricts root function, but also promotes root diseases and increases herbicide injury as well as yield reduction in dry beans. Delaying the initiation of irrigation by one and two weeks delayed maturity by 3 and 6 days, and reduced yield by 5 and 15%, respectively. Dry beans are rich source of phytochemicals such as flavonoids and phenolics that possesses antioxidant activities. The main characteristic of an antioxidant is its ability to trap free radicals. Free radicals damage cellular proteins, lipids, and DNA and have been linked to cancer, heart disease, obesity related problems, and arthritis. Antioxidants reduce risk of chronic diseases including cancer and heart disease. The objective was to study the effect of soil compaction and irrigation management on antioxidants in dry bean production.

MATERIALS AND METHODS

Plots were established at Scottsbluff, NE in 2009 that included combinations of variety, water stress, and soil compaction. A strip-split plot design was used to test the treatments. The strip corresponded to levels of compaction [non-compacted, and heavily compacted (driving a tandem axel truck weighing 56,000 lbs)]. Soil was plowed, roller harrowed, compacted, and a tillage finish was applied. Herbicide was incorporated and soil was leveled off with a tillage finish implement. Two irrigation treatments were assigned to subplots, including full irrigation (100%), and no supplemental irrigation (0%) after flowering. Nine varieties, six great northern Marquis, Matterhorn, 99-131, Emerson, Orion, Tara, Beryl-R, and 2 pinks Roza, and UI-537 were assigned to the sub-plots. Plots were uniformly irrigated through beginning of flowering to avoid early plant loss due to the combination of soil compaction and water stress.

To estimate phenols, flavonoids, and antioxidant capacity ground raw beans (1 g) were suspended in 5 ml of methanol 1:1 (w/v) ratio, 1.2 N. Each suspension was mixed for a minimum of 2 hours and then centrifuged for 15-30 minutes. The supernatant was collected while the pellet was subjected to another extraction process. The supernatants were collected and analyzed for total phenols and flavonoids (Makkar and Slinkard). Total phenols and flavonoids were assessed by the Folin – Ciocalteu method and the polyvinlypolypyrrodlidone method, respectively. Extracts were also analyzed for their antioxidant capacity with the oxygen radical absorbance capacity method described by Cao et al. (1993).

RESULTS AND CONCLUSIONS

Yield was significantly affected by soil compaction and irrigation scheduling. Yield was reduced by 67% and 39% when the soil was heavily compacted and none irrigated, respectively. Zone tillage and broadcast ripping tillage are recommended for alleviating soil compaction.

Total phenols, flavonoids and ORAC were not affected by soil compaction and irrigation scheduling.

Antioxidant capacity of the dry beans was affected by the market class. Roza and UI-537, pink beans, possess highly significant antioxidants compared with the great northern tested in this study.

Figure 1. Phenols, flavonoids, ORAC, and yield of the nine cultivars tested at Mitchell, NE during 2009.

	Market Class	Phenols	Flavonoids	ORAC	Yield
		mg g^{-1}	mg g^{-1}	µmole Trolox 100 g^{-1}	kg ha^{-1}
Marquis	**great northern**	1.09	0.21	2686	1346
Matterhorn	**great northern**	1.16	0.23	2895	1483
Gemini	**great northern**	0.96	0.21	3198	1522
Emerson	**great northern**	1.16	0.22	2571	1360
Orion	**great northern**	1.21	0.26	3100	1353
Tara	**great northern**	1.11	0.24	3094	1588
UI 537	**pink**	2.73*	0.79*	4495*	1778
Beryl-R	**great northern**	1.12	0.22	2800	1518
Roza	**pink**	3.15*	0.90*	4817*	1298

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RECURRENT SELECTION AS A METHOD TO INCREASE RESISTANCE TO ANGULAR SPOT AND GRAIN YIELD ON COMMON BEAN

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INTRODUCTION

Regarding the common bean crop, beyond grain yield, the resistance to diseases is always considered by breeders to develop cultivars. Currently, the angular spot (*Pseudocercospora griseola*) is considered one of the main diseases that reduce yield (Singh e Schwartz, 2010). The symptoms may be observed mainly on leaves and pods, and there are evidences that the resistance to the fungus at those plant organs is a quantitative character controlled by different genes (Borel et al., 2011).

For the common bean breeding aiming diseases resistance and increase in grain yield, one of the recommended methods is the recurrent selection. Thus, the objective of this study was to check the efficiency of this method on increasing grain yield and resistance to the angular spot on leaves and pods.

MATERIALS AND METHODS

It was used five of the best lines from the seven first cycles of a recurrent selection program aiming resistance to angular spot, which began on 1998 (Amaro et al., 2007). The 35 lines were sowed on the dry season of 2011 at UFLA's Experimental Farm located at Lavras - Minas Gerais state, Brazil. A randomized complete blocks design with three replications was used. The following characters were evaluated: grain yield (Kg/ha), angular spot severity on pods and severity on leaves. The evaluation on pods were made by the time of the harvest using a diagrammatic scale that ranges from 1 (resistant) to 9 (susceptible) proposed by Borel et al. (2011). Yet, the evaluation on leaves were made 21, 28, 33 and 41 days after the flowering, in order to calculate the area under the disease progress curve (AUDPC), using a diagrammatic scale that ranges from 1 (resistant) to 9 (susceptible) proposed by Godoy et al. (1997).

Using the mean data of each cycle of recurrent selection for the characters previously mentioned, it was obtained the regression equation between the cycles (independent variable x) and the grades on pods, AUDPC or yield (dependent variable y). The regression b_1 was used to estimate the gain per cycle of recurrent selection. At last, it was estimated the genetic progress (GP) throughout the expression: $GP = (b_1/b_0)*100$.

RESULTS AND DISCUSSION

One of the advantages of using the AUDPC is the possibility to estimate the genetic progress in a more accurate way, since all the assessments seasons are involved on it. For this character and angular spot severity on pods, it is feasible to notice the tendency of reduction of the disease intensity along the cycles (Table 1). The determination coefficients (R^2), which shows the data fitness to the linear regression equation, in spite of not being high, were superior than those found by Amaro et al. (2007). The genetic progress was negative both for AUDPC and severity on pods, which means that the grades reduced in more advanced cycles. In other words, recurrent selection is increasing the resistance to the fungus. For grain yield, there was a raising tendency, with a positive genetic progress. As the angular spot decreases yield, probably the greater

resistance of the more advanced cycles lines is an important factor that reduces the pathogen action over the plant, which contributes for the observed yield gain. These results are in accordance with those found by Amaro et al. (2007), and they ensure that the recurrent selection is efficient to increase angular spot resistance on common beans, and therefore raise grain yield.

Table 1. Means of the seven cycles of recurrent selection for resistance to angular spot, linear regression coefficients (b_0 and b_1) and genetic progress (GP) with the selection on yield, AUDPC and severity on pods.

	AUDPC	PODS	YIELD (Kg/ha)
Cycle I	96.6	5.7	2403
Cycle II	94.7	4.9	2260
Cycle III	83.1	4.0	2178
Cycle IV	78.4	3.9	2463
Cycle V	91.7	4.6	2542
Cycle VI	87.7	3.7	2402
Cycle VII	79.8	4.3	2683
b_0	95.4	5.31	2206
b_1	-1.99	-0.21	53.11
GP(%)	-2.09	-4.03	2.41
R^2	35.4	46.8	46.2

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SOURCES OF RESISTANCE TO *COLLETOTRICHUM LINDEMUTHIANUM* IN CARIOCA COMMON BEAN CLASS

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INTRODUCTION

Many commercial classes of common beans (*Phaseolus vulgaris* L.) are cultivated in Brazil. Carioca class (cream-striped seeds) is the most cultivated, despite the fact that the majority of cultivars released are susceptible to various pathogens (Ramalho & Abreu, 2006). Anthracnose, caused by the fungus *Colletotrichum lindemuthianum*, is an important disease of the common bean. This disease can reduce yield and bean quality due to spots on the grains. The use of resistant cultivars is an important strategy for controlling this disease. However, due to the large pathogen variability, resistant cultivars rapidly become susceptible, leading to a constant search for new sources of resistance to support breeding programs. The aim of this study was identify genotypes of the carioca class resistant to the major *C. lindemuthianum* races that occur in Minas Gerais State, Brazil.

MATERIAL AND METHODS

The reaction of 117 genotypes of the carioca class from the Bean Germplasm Active Bank of the "Universidade Federal de Viçosa" (UFV-BGAB) were evaluated against the races 65, 73, 81, 87, 89 and 453 of *C. lindemuthianum*. These races occur in Minas Gerais State and in other states of Brazil (Rava et al., 1994). The genotypes 'Ruda' (susceptible to all races) and 'Ruda-R' (resistant to all races) were used as controls. 'Ruda-R' has the resistance genes *Co-4*, *Co-6* and *Co-10* (Costa et al., 2010). The inoculum was produced in tubes containing sterile pods partially immersed in BDA (Pio-Ribeiro & Clark, 1975), which were incubated at 23 °C in the dark for 8-10 days. The final inoculum concentration was 1.2 x 10^6 spores/mL. For each *C. lindemuthianum* race, 15 seeds of each genotype were pre-germinated in a germinator using germitex. Soon after primary root had appeared, eight seeds were transferred to trays containing the substrate Topstrato HT©. Ten days later, suspension of each race was applied in both sides of the primary leaves with a DeVilbiss atomizer activated by an electric compressor. The plants were incubated for seven days in a mist chamber (20 ± 1°C and relative humidity greater than 95%) using a 12-hour photoperiod. After this period, the plants were scored as resistant (R) or susceptible (S) as described by Pastor-Corrales (1992), where 1 to 3 = resistant and 4 to 9 = susceptible.

RESULTS AND DISCUSSION

The race 87 was less virulent than the others. Approximately 70% of the genotypes were resistant to this race. The genotypes 'Gen 12-2', 'IAC-Carioca Akytã', 'IAC-Carioca Aruã', 'IAC-Carioca Pyatã', 'Race D', 'UTFB-0022', 'UTF-0029' and 'VC-5' were resistant to the six races (data not shown). Thus, they are promising sources of resistance to *C. lindemuthianum*. Twenty-five genotypes were resistant to five races, all of which were resistant to races 73, 81 and 87 (Table 1). Among them was TO, a known source of resistance to *C. lindemuthianum*, which has the resistance gene Co-4 (Fouilloux, 1976). It is worth mentioning that cultivar 'Pérola', widely grown in Brazil, was susceptible to all races.

Table 1. Reaction of genotypes of carioca class of common bean to *Colletotrichum lindemuthianum.*

Genotypes	Races of *C. lindemuthianum**					
	65	**73**	**81**	**87**	**89**	**453**
UTF 0030	R	R	R	R	R	S
A-805	S	R	R	R	R	R
CNFC 8006	S	R	R	R	R	R
CNFC 9440	S	R	R	R	R	R
CNFC 9444	S	R	R	R	R	R
CNFC 9452	S	R	R	R	R	R
CNFC 9454	S	R	R	R	R	R
CNFC 9455	S	R	R	R	R	R
CNFC 9471	S	R	R	R	R	R
CNFC 9499	S	R	R	R	R	R
CNFC 9506	S	R	R	R	R	R
EMP 250	S	R	R	R	R	R
GEN C 97-2	R	R	R	R	R	S
IAPAR 31	R	R	R	R	S	R
IAPAR 81	R	R	R	R	R	S
LP 98-31	S	R	R	R	R	R
LP 98-76	S	R	R	R	R	R
BRSMG Majestoso	S	R	R	R	R	R
OP-S-78C	S	R	R	R	R	R
BRSMG Pioneiro	S	R	R	R	R	R
Princesa	R	R	R	R	S	R
TO	R	R	R	R	R	S
VC 3	R	R	R	R	S	R
VC 2	R	R	R	R	S	R
VI 4899C	S	R	R	R	R	R
Rudá-R[1]	R	R	R	R	R	R
Rudá [2]	S	S	S	S	S	S

[*]R = Resistant, S = Susceptible; [1]Resistant control; [2]Susceptible control.

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ALTERNATIVE METHOD FOR INOCULATION OF *COLLETOTRICHUM LINDEMUTHIANUM* ON COMMON BEANS

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INTRODUCTION

Colletotrichum lindemuthianum (Sacc. & Magnus) Scribner, causal agent of anthracnose on common beans (*Phaseolus vulgaris*), can provoke serious damages to the crop when the environment conditions are favorable for the fungus (Paula Júnior & Zambolim, 2006). Artificial inoculations are recurrently used in breeding programs aiming resistance to this pathogen. The traditional inoculation method consists in spraying inoculum onto both surfaces of the primary leaves. This process is laborious and time consuming. Therefore, the improvement of the inoculation process with purpose to make it easier and faster is important. Thus, the objective of this study was to evaluate the efficiency of inoculation just onto the adaxial surface of primary leaves under five inoculum concentrations of *C. lindemuthianum*.

MATERIAL AND METHODS

Treatments were arranged as a 3 x 2 x 5 factorial: genotypes (Ouro Negro, Rudá and the line Rudá-R), inoculation methods (inoculum application onto both surfaces of primary leaves or only onto the adaxial surface), and inoculum concentrations (1.2×10^6 as recommended, 1.08×10^6, 0.96×10^6, 0.78×10^6 or 0.6×10^6 conidia/mL). The cultivars were inoculated with the races 65, 89 and 453 of *C. lindemuthianum*. Ouro Negro and Rudá differ in their reaction to the races used and Rudá-R is resistant to the three races. The experimental design used was a completely randomized, with four replications. Each plot consisted of eight plants. The seeds were pre-germinated and sown in trays with 96 cells containing the substrate Topstrato HT©. The trays were maintained in a greenhouse at 24°C and relative humidity of 90%. The plants were inoculated seven days after the sowing, using the following inoculum concentrations. Ten days after the inoculation, the plants were rated for disease severity based on a 1-9 scale (Pastor-Corrales, 1992), in which 1 represents plants without symptoms and 9 dead plants.

RESULTS AND DISCUSSION

Interactions involving genotypes were not significant (Table 1), indicating that reactions of the genotypes to anthracnose were independent of the inoculation method and inoculum concentration. The line Rudá-R showed, as expected, resistance to all races and the cv. Rudá was susceptible to the three races. Ouro Negro was resistant to the races 453 and 89 and susceptible to the race 65. Similar results were obtained by Melo et al. (2008) using the traditional method of the inoculation - on both primary leave surfaces with inoculum concentration of 1.2×10^6 conidia/mL, in which the inoculated plants were kept in a mist chamber (Table 1). The inoculation method was not significant for the races, suggesting that the inoculation can be performed only on the adaxial surface of primary leaves. This allows reducing the amount of inoculum to be produced and, mainly, hasten the process of inoculation, making it less laborious. However, the inoculation should be performed with precision and care, and inoculums must

reaches the stems, because some races of *C. lindemuthianum* cause symptoms mainly in this part of the plants. No significant effects for inoculum concentrations were observed, indicating that the recommended and normally concentration used (1.2 x 10^6 conidia/mL) is much higher than the necessary to cause the maximum symptoms. Our results suggest that the inoculation of *C. lindemuthianum* onto the axial surface is efficient and can be performed in greenhouse with control of temperature and humidity, eliminating the transfer of plants after inoculation by a mist chamber. This facilitates the entire process, reduces work and allows a evaluation of a large number of genotypes at the same time.

Table 1 - Analysis of variance of the reaction of three bean genotypes inoculated with three races of *Colletotrichum lindemuthianum* in five concentrations

Sources	DF	Mean Square		
		Race 65	**Race 89**	**Race 453**
Genotypes (G)	2	841.0617**	824.3045**	822.3652**
Inoculation method (IM)	1	0.0005ns	0.0255 ns	0.0001 ns
Inoculum concentration (C)	4	0.0062 ns	0.6295 ns	0.0153 ns
(B/C)/IM	30	0.0082ns	0.0362 ns	0.0507 ns
G x IM	2	0.0005 ns	0.0255 ns	0.0946 ns
G x C	8	0.0062 ns	0.0630 ns	0.0613 ns
IM x C	4	0.0083 ns	0.0935 ns	0.0309 ns
G x IM x C	8	0.0083 ns	0.0935 ns	0.0343 ns
Error	60	0.0082	0.0362	0.0538
CV (%)		1.44	5.25	6.37
Ouro Negro		8.89 (9.00)[1]	1.00 (1.00)	1.05 (1.00)
Rudá		9.00 (9.00)	8.86 (9.00)	8.88 (9.00)
Rudá-R		1.00 (1.00)	1.00 (1.00)	1.00 (1.00)

ns, ** - not significant and significant by the F test at 1% probability, respectively.
[1] Values between parentheses refer to the results obtained by Melo et al. (2008).

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INVESTIGATING VARIABILITY WITHIN RACE 81 OF *COLLETOTRICHUM LINDEMUTHIANUM* STRAINS FROM BRAZIL

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INTRODUCTION

Colletotrichum lindemuthianum is the causal agent of anthracnose of common bean (*Phaseolus vulgaris*) and the genetic resistance is the best method to control the disease. However, the wide virulence diversity of this pathogen has complicated the breeding for resistance. Studies of race characterization using the standard set of 12 differential cultivars (Pastor Corrales, 1991) have shown the predominance of races 65 and 81 in Brazil (Silva et al., 2007). Previously studies showed that genetic variability within race 65 makes difficult the development of a durable resistance (Davide & Souza, 2009; Ishikawa et al., 2011). Common bean lines able to differentiate the variation within race 65 to complement the results of the standard set of differential cultivars and a new nomenclature to identify the pathogenic variability of race 65 strains was proposed by Ishikawa et al (2011). The objective of this work was evaluate if the same common bean lines can be used to identify variability within race 81 of *C.lindemuthianum.*

MATERIAL AND METHODS

Eight strains from the culture collection of Department of Biology, Universidade Federal de Lavras (UFLA) were used. All the strains were previously tested on the standard set of 12 differential cultivars (Pastor Corrales, 1991)to confirm their classification as race 81. The strains were inoculated on the 12 cultivars from the germplasm collection of UFLA proposed by Ishikawa et al (2011). The strains were inoculated in bean pod culture medium and incubated at 22°C for 10-15 days in the darkness to obtain high sporulation. Eight seeds of each common bean line were sown in a 128 cell polystyrene tray containing Multiplant substrate (Terra do Paraíso, Brazil). Seedlings with fully expanded primary leaves were sprayed with a suspension of 1.2 $x10^6$ conidia/ml. Two replicate trays were used per isolate. The inoculated plants remained in moist chamber at 22°C, photoperiod of 12 h during 3 days. After 7-10 days from inoculation, plants were evaluated using a scale from 1 to 9 (Schoonhoven and Pastor Corrales, 1987). Races were identified as proposed by Ishikawa et al 2011.Pérola cultivar was used as susceptible control.

RESULTS AND DISCUSSION

The cultivars and binary values proposed by Ishikawa et al. (2011) are described on table 1. Following this method, the race 81 strains used in this work were classified on five different patterns of reaction (Table 1, Fig. 1). It was necessary the addition of the cultivar Cometa (2^8) to differentiate the strains previously classified as race 81. Therefore, we suggest the following common bean lines and respective binary numbers to identify the pathogenic variability of strains: Estilo (2^0), Majestoso (2^1), Supremo (2^2), União (2^3), Valente (2^4), Ouro Vermelho (2^5), Madrepérola (2^6), Talismã (2^7) and Cometa (2^8). The adoption of this nomenclature would help the breeder to choose the most virulent strains classified as race 81 in the search of a more durable resistance, allowing the differentiation of regionally important races that could not be discriminated using the standard set of differential cultivars. All of these cultivars are well

adapted to the Brazilian conditions and are commercially available, which facilitates their utilization.

Table 1 – Average score of common bean lines reaction inoculated with different strains of *Colletotrichum lindemuthianum* that belongs to race 81. Average scores < 3 were considered as resistant, whereas plants scoring ≥3 were susceptible.

Cultivars Strains	**A** (2^0)	**B** (2^1)	**C** (2^2)	**D** (2^3)	**E** (2^4)	**F** (2^5)	**G** (2^6)	**H** (2^7)	**I** (2^8)	**J**	**K**	**L**	**Race**[1]
LV 44	1^-	1^-	1^-	$^-$	1^-	1^-	1^-	1.5^-	1^-	1^-	1^-	5.7^+	81.0
LV 127	1^-	1.8^-	1.6^-	7.3^+	6.4^+	6^+	1^-	8.4^+	1^-	1^-	1^-	6.4^+	81.184
LV 132	6.9^+	4^+	4.2^+	1^-	1.4^-	1^-	1^-	1^-	1.33^-	8.6^+	1^-	6.3^+	81.7
LV 153	1^-	1^-	1.9^-	7.2^+	1^-	1^-	1.5^-	1^-	1^-	1^-	1^-	6.8^+	81.8
LV 201	9^+	8.6^+	8.1^+	7.3^+	1^-	1^-	1.3^-	1^-	6.2^+	9^+	1^-	8.9^+	81.271
LV 222	1^-	2.7^-	2.8^-	8.5^+	9^+	8.6^+	1^-	6^+	1^-	1.5^-	1^-	7.8^+	81.184
LV 232	1^-	1^-	1^-	7.4^+	9^+	8.7^+	1.8^-	8^+	1^-	1^-	1^-	7.2^+	81.184
LV 235	7.4^+	3.4^+	4.8^+	1^-	1^-	1^-	1^-	1.8^-	1^-	6.7^+	1^-	6^+	81.7

- Incompatibility reaction (resistent); + Compatibility reaction (susceptiblel) *Cultivars: A – Estilo, B – Majestoso, C – Supremo, D – União, E – Valente, F - Ouro Vermelho, G –Madrepérola, H - Talismã, I - Cometa, J – Ouro Negro, K -Esplendor, L – Pérola (susceptible control).
[1]Race classification using the cultivars and binary number proposed by Ishikawa et al (2011).

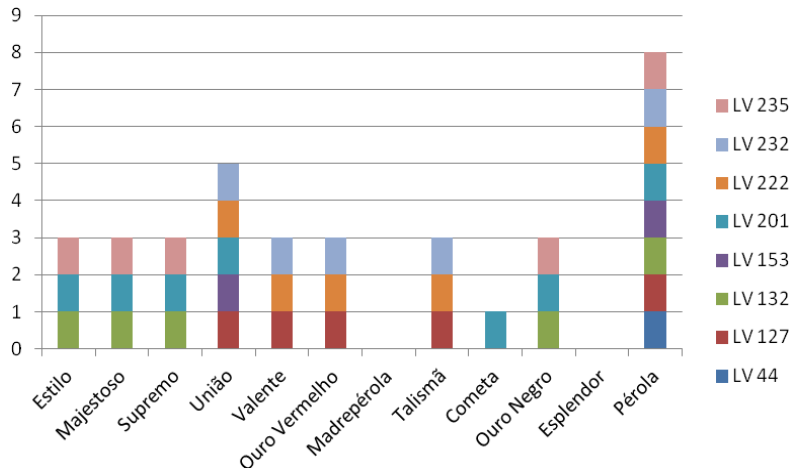

Figure 1 – Reaction of each commercial cultivar inoculated with eight *Colletotrichum lindemuthianum* strains of race 81.

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DETECTION OF SCAR MARKERS LINKED TO RESISTANCE TO COMMON BLIGHT AND ANTHRACNOSE IN THE INIFAP CORE COLLECTION

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INTRODUCTION

The common beans (*Phaseolus vulgaris*L.) are the most consumedlegumeand distributedworldwide,for its food valueand its wideacceptance in thepopulardiet. In Mexico, both common beans and maize are the main sources for the consumption of protein, minerals and vitamins. But the crucial importance of consumption, area and volume of the harvest, the crop is exposed to diseases caused by viruses, bacteria, insect pests of plant and seeds, which reduce the yield and production. In Mexico, the common beans are traditionally sown in monoculture mainly in the semiarid highlands of central and north and for consumption in the south. This has resulted in the dispersal of seed-borne diseases such as common blight [*Xanthomonas campestris* pv. *Phaseoli* (Smith)] and anthracnose [*Colletotrichum lindemuthianum* (Sacc. and Magn.) Scrib].

MATERIALS AND METHODOS

This work monitored the presence of seven SCAR markers, four genes associated with resistance to anthracnose (SAS13, SAB3, SY20 and SZ04) and three Associated with resistance to common blight (SAP6, SU91 and BAC6) in 200 materials of INIFAP bean core collection of Mexico and was included as outgroup 10 commercial cultivars released by INIFAP (Azufrado pimono 78, Bayo zacatecas, Blanco tlaxcala, Canario 101, Flor de junio marcela, Flor de mayo, Garbancillo supremo, Negro jamapa, Pinto villa and Negro michigan. DNA isolation was performed in young leaves of 10 plants per accession based on the method described by Dellaporta. And each SCAR was amplified based on data published by the Bean Improvement Cooperative.

RESULTS AND DISCUSSION

Of the 784 SCARs amplified, was observed in most accessions from Zacatecas (4.5), Chihuahua (4.0), Puebla (4.2) and Oaxaca (4.0). The states of Aguascalientes, Jalisco and Chiapas, but have a high number of accessions, only 3.8 SCARs amplified by accession on average. The commercial varieties only 3.4 SCARs amplified by accession. In most state accessions were amplified under 20 SCARs. In the states of Nuevo Leon and Campeche germplasm only two products were amplified (Table 1). The highest amount of SCARs by the type of seed beans was detected in type 'Vaquita' and gray (4.7) and, conversely, the least amount detected in type 'Cacahuate' (2.5). Although the Bayos beans have the highest number of accessions in the core collection showed only 3.7 SCARs per accession (Table 2). Although the INIFAP common bean core collection contains promising material to serve as a source of resistance to anthracnose and less to common blight, further analysis should be particularly within specific agro-ecological regions and against a wide range of regional pathotypes each pathogen.

Table 1. SCARs amplified products by origin bean germplasm of INIFAP core collection.

State	Na	SCAR							Total	Average
		Anthracnose				Common blight				
		SY20	SAS13	SAB3	SZ04	BAC6	SAP6	SU91		
Chiapas	21	4	14	6	21	2	11	20	78	3.8
Sinaloa	2	2	0	1	2	0	1	2	8	4.0
Jalisco	23	2	12	5	23	9	15	21	87	3.8
Guanajuato	4	0	2	0	4	1	3	4	14	3.5
Aguascalientes	23	6	16	2	23	5	14	22	88	3.8
Coahuila	7	0	4	1	7	4	2	7	25	3.6
Veracruz	7	1	2	1	7	0	4	7	22	3.1
Tamaulipas	7	2	4	0	7	1	4	7	25	3.6
Puebla	16	1	13	4	16	7	12	14	67	4.2
Chihuahua	6	1	5	0	6	2	4	6	24	4.0
BCN	2	0	1	0	2	0	0	2	5	2.5
Estado México	4	2	2	0	4	1	3	4	16	4.0
Durango	8	1	4	0	8	4	4	8	29	3.6
Sonora	4	1	1	1	4	0	2	3	12	3.0
Hidalgo	8	0	6	0	8	2	6	8	30	3.8
Oaxaca	9	3	7	1	9	2	6	8	36	4.0
Zacatecas	13	1	10	2	13	8	12	12	58	4.5
Nuevo León	1	0	0	0	1	0	0	1	2	2.0
Morelos	3	1	1	0	3	0	2	3	10	3.3
Yucatán	1	0	1	1	1	1	1	1	6	6.0
Nayarit	4	0	1	1	4	1	1	4	12	3.0
Querétaro	2	0	2	0	2	1	2	2	9	4.5
Campeche	1	0	0	0	1	0	0	1	2	2.0
Michoacán	7	0	3	1	7	3	4	6	24	3.4
Tlaxcala	3	0	2	0	3	2	2	2	11	3.7
Colima	3	0	0	0	3	1	2	3	9	3.0
Guerrero	6	0	6	0	6	1	3	6	22	3.7
BCS	1	0	1	0	1	1	1	1	5	5.0
SLP	3	0	1	0	3	1	1	3	9	3.0
Quintana Roo	1	0	1	0	1	1	1	1	5	5.0
V. Comercial	10	4	7	4	9	3	2	5	34	3.4
Total/average	210	32	129	31	209	64	125	194	784	3.6

Na=number of accessions for states

Table 2. SCARs amplified products by seed type germplasm of INIFAP core collection

Seed type	Na	SCAR							Total	Average
		Antracnosis				Tizón común				
		SY20	SAS13	SAB3	SZ04	BAC6	SAP6	SU91		
Cacahuate	6	0	1	1	6	0	1	6	15	2.5
Rojo	3	0	2	0	3	1	1	3	10	3.3
Negro	24	3	14	5	24	3	19	24	92	3.8
Jaspeado	18	1	9	0	18	8	14	17	67	3.7
Morado	16	3	13	3	16	3	5	13	56	3.5
Amarillo	12	3	7	3	12	5	9	12	51	4.3
Bayo	51	8	32	5	51	18	26	47	187	3.7
Ojo de Cabra	11	2	7	1	11	2	7	11	41	3.7
Pinto	14	2	8	4	14	4	6	12	50	3.6
Blanco	7	1	4	0	7	4	5	7	28	4.0
Vaquita	6	1	4	1	6	4	6	6	28	4.7
Manzano	5	1	2	2	5	1	4	5	20	4.0
Café	14	2	11	1	14	3	12	13	56	4.0
Gris	3	1	2	1	3	1	3	3	14	4.7
Flor de Junio	3	0	2	0	3	1	2	3	11	3.7
Flor de Mayo	7	0	4	0	7	3	3	7	24	3.4
Commercial V.	10	4	7	4	9	3	2	5	34	3.4
Total/Average	210	32	129	31	209	64	125	194	784	3.7

REFERENCES: Bean Improvement Cooperative (BIC) (2005) SCAR Markers 2005. [http://www.css.msu.edu/bic/Genetics.cfm] [Date: august 22th, 2006].

ANTHRACNOSE AND RUST OF BEAN (*PHASEOLUS VULGARIS*L.) IN SEMI WARM REGIONS OF GUERRERO, MEXICO

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INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is one of the most important food legumes for their medicinal properties as their consumption helps reduce the risk of some diseases (Bennink, 2005). Bean production in Mexico is low because it is a crop that is mainly rainfed that their growth depends on the amount and distribution of rainfall. Add to this inopportune or control pest and disease control in part by ignorance, further limit production. The latter can be reduced from 25% until the totallossof production (Becerra, 1992). . In the state of Guerrero the average yield in 2010 was 0.810 t ha^{-1} (SIAP, 2010). The aim of this study was to identify and evaluate the impact of the pathogens causing the reduction in leaf area and necrosis branches that affect of beans crop in two semiwarm regions of Guerrero state, for that the farmer to make timely appropriate control of these pathogens and thereby achieve a higher bean yield.

MATERIALS AND METHODS

In farmers' fields in the semiwarm region (Tixtla Gro, Mex) located at 1600 m altitude with an average temperature from May to October of 21 ° C to 24 ° C with 1015 mm of rainfall, and semi-arid (less dry ,Chichihualco, Gro, Mex.) temperature between 23 ° C to 26 ° C from May to October with 717 mm of rainfall and 1210 m of altitude. The common beans "Negro Criollo" was sowing planted in row to 80 cm apart and density of 6.25 plants m^{-2}. In a zigzag sampling, we selected four plots with a hundred plants each one. Was recorded the number of diseased plants. To identify the pathogen in leaves with spots, small pieces were cut from the edge of the lesions, were disinfected with sodium hypochlorite 3% for 1 min, washed with sterile distilled water. The plant material was placed in Petri dishes on potato dextrose agar (PDA), incubated the plates for 7 days at 25 ° C. Of colonies were performed macroscopic isolates for identification. Experimental evidence for pathogenicity were healthy bean of one month of age developed in pots. Three plants were used per treatment with their respective control. The inoculation was performed on leaflets of the middle part of the plants and holding them with tape. Similarly we proceeded with the control. The experimental plants were covered with polyethylene bags for 72 h, to avoid rapid drying of the inoculum. The pathogenic microorganisms were isolated and compared with the original (Moreno, 2002). The bean samples were observed ina compound microscope. Taxonomic identification was performed with the help of keys and bibliography (Zamora,1990).

RESULTS AND DISCUSSION

In the plots, we observed the incidence of "Anthracnose" bean caused by *Colletotrichum lindemuthianum* with damage from 10 to 28% in pods and 14 to 36% in leaves of different ages. The disease was observed from 30 days after sowing and was characterized by lesions that initially manifested as small punctuations dark, surrounded by a chlorotic halo thin and scattered over the leaf. It was observed that young tissues are more susceptible, in adult plants showed small patches with little “halo”, caused the rapid deterioration of branches and fruits. Cultures of fungi were developed in 6-7 days and was obtained by isolation of *Colletotrichum lindemuthianum* (Sacc. et Magn.) Scrib., with yellowish-gray mycelium. As a result of artificial inoculation, after 10 days lesions were observed in leaves. This reaction indicated that the inoculated fungus is the causative agent of disease in study. Other disease that was observed in the bean, was rust (*Uromyces phaseoli* var. typica Wint). Symptoms began as yellowish spots on the lower leaf surface. In severe attacks occurred defoliation. The incidence of the disease varied from 20 to 40% and the severity of 10 to 30% damage. This information useful for farmers of the study sites, to implement in a timely measures to prevent and control diseases and thus achieve higher bean yield.

CONCLUSIONS

In the region of study the disease that caused severe damage to bean crop were anthracnose caused by the pathogen *Colletotrichum lindemuthianum* (Sacc. et Magn.) Scrib and rust caused by *Uromyces phaseoli* var. typica (Wint).

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EXCEPTIONAL RUST RESISTANCE IN MESOAMERICAN COMMON BEAN ACCESSION PI 310762

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INTRODUCTION: The common bean rust pathogen, *Uromyces appendiculatus*, is notorious for its enormous virulence diversity and ability to produce new virulent strains.The discovery of highly effective disease resistance genes is a very important step for the development of cultivars with effective rust resistance. PI 310762 is a Mesoamerican, black-seeded bean from Guatemala with exceptional broad-spectrum resistance to many races of the rust pathogen. The objectives of this study were to compare the rust reactions of PI 310762, of bean cultivars with named rust resistance genes, and of other cultivars with broad rust resistancefrom unnamed genes, to several selected rust races, and to determine the inheritance of rust resistance in PI 310762.

MATERIALS AND METHODS: All of the studies reported here were conducted under greenhouse conditionsusing published methodologies (1). To compare the reaction of PI 310762 with those of bean cultivars with named and unnamed rust resistance genes, 10 seedlings of each cultivar were inoculated with 23 racesof the rust pathogen. Twelve of these races belong to Andean gene pool of the bean rust pathogen, while the other 11 races belong to the Mesoamerican gene pool. To determine the inheritance of rust resistance in PI 310762, a cross with Pinto 114 (susceptible) was made, and seedlings of the two parents and of the F_1, F_2, and the two backcross populations were inoculated with two Andean (84 and 105) and two Mesoamerican (67 and 107) rust pathogen races.

RESULTS AND DISCUSSION: In a previous study, PI 310762 was resistant to 89 of 90 races of the rust pathogen maintained at Beltsville. Results from both the previous and the current study show that the PI 310762 is susceptible only to race 85, which is also from Guatemala. This is a significant finding, for it corroboratesthat even bean cultivars with broad resistance to a highly variable pathogen are frequently susceptible to one or a few races isolated from beans of the same gene pool (1). PI 310762 was resistant to all the races that overcome the ten named and mapped rust resistance genes in common bean (*Ur-3*, *Ur-4*, *Ur-5*, *Ur-6*, *Ur-7*, *Ur-9*, *Ur-11*, *Ur-12*, *Ur-13* and *Ur-14*), suggesting the presence of a new gene. PI 310762 was also resistant to the races that infect other common beanswith broad rust resistance, such as Compuesto Negro Chimaltenango (CNC) and PI 260418 (Table1). PI 310762 was resistant to race 108, which is the only known race that overcomes the resistance of the *Ur-11* gene, considered the most effective rust resistance gene known to date.Likewise, PI 310762 was resistant to Andean race 84, the only race in the Beltsville collection that infects Andean bean PI 260418 (Table 1). Race 85, the only one that infects PI 310762, has broad virulence; it overcomes the resistance of *Ur-3*, *Ur-5*, *Ur-7*, *Ur-13*, CNC, and others. Due to spatial constraints we only show partial results in Table 1.

In the inheritance of resistance study, the reaction of PI 310762(resistant parent) was expressed as tiny uredinia (pustules), smaller than 0.3 mm in diameter. The reaction of the susceptible Pinto 114 parent was expressed as large uredinia, larger than 0.5 mm in diameter. All the F_1 plants were resistant. The genetic analysis of the rust resistance in PI 310762 was based on the infection type observed on 157 F_2 plants. A total of 124 F_2 plants were resistant and 23 were susceptible to all four races inoculated. The F_2 population segregation results were

consistent with a 3 resistant: 1 susceptible ratio, indicating monogenic dominant inheritance. The backcross population to the resistant PI 310762 parent was completely resistant while the backcross population to the susceptible Pinto 114 parent showed segregation consistent with a 1 rust resistant: 1 rust susceptible ratio.Taken together, these data indicate that the resistance in PI 310762 to the bean rust pathogen is conferred by a singledominant gene. The discovery of this new gene is a very important contribution for the development of cultivars with effective and broad resistance to the highly virulence-variable bean rust pathogen.

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Table 1. Comparing the reaction of Mesoamerican common bean PI 310762 to 12 selected races of the bean rust pathogen, *Uromycesappendiculatus*,with the reactions of common beans with named rust resistance genes and other beans with unnamed genes but broad rust resistance

Bean Line	Rust Res Gene	Reaction of bean cultivars to 12 selected races of the bean rust pathogen											
		41	44	47	49	53	58	67	73	**84**	85	105	108
Pinto 114	None	4,5	4,5	5,6	4,5	4,5	5,6	4,5	4,5	5,6	5,6	4,5	4,5
Aurora	*Ur-3*	2,f2	4	4,5	4,5	2,f2	5,6	4,5	4,5	2,2+	5,4	2,2+	f2,2
E. Gallatin	*Ur-4*	4	2,f2	5,4	2,f2	4,5	4	4,5	2,f2	6,5	2+,2	5,4	2,f2
Mex. 309	*Ur-5*	3,f2	f2,3	3,2	4,5	f2	5,4	4,5	4,5	2,3	5,4	3,2	4,5
G.G. Wax	*Ur-6*	4,5	f2	2,2+	4,5	4,5	4,5	4,5	f2	3,2	f2,2	4,5	4,5
G.N. 1140	*Ur-7*	4	3,f2	5,4	3,f2	5,4	5,4	3,f2	4,5	5,6	5,4	f2,3	3,f2
P.C. 50	*Ur-9, Ur-12*	4,5	3,f2	5,4	2,2+	4,5	4,5	4	2,2+	f2,3	2,2+	5,6	f2,3
PI 181996	*Ur-11*	f2	f2,3	3,f2	3,f2	3,f2	f2	f2	3,f2	f2	f2	f2	4,5
R. Pioneer	*Ur-13*	4,5	4,5	4,5	4,5	4	4	4,5	4	f2,3	4,6	f2,2	4,5
CNC	Unnamed	f2,3	3,f2	1	3,f2	f2,3	2	4,5	3,f2	2,3	4,5	2,3	4,5
PI 260418	Unnamed	3	3	f2,3	3,f2	3,f2	f2,3	3	3,f2	5,6	f2,3	3,f2	3
PI 310762	Unnamed	1	f2	2,2+	3	3	3,2	f2,3	3,f2	1	4,5	3,f2	3,f2

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

Table 2. Inheritance of rust resistance study showing the reaction of resistant PI 310762 and susceptible Pinto 114 common bean (*Phaseolusvulgaris*) genotypes and derived populations to two Mesoamerican (67 and 108) and two Andean (84 and 105) races of the bean rust pathogen, *Uromyces appendiculatus*

Genotype	No. of plants	Observed number		Expected ratio(R:S)	x^2	p-value
		Resistant*	Susceptible*			
Pinto 114	39	0	39	0 : 1	-	-
PI 310762	38	38	0	1 : 0	-	-
(Pinto114 x PI 310762) F_1	12	12	0	1 : 0	-	-
(Pinto114 x PI 310762) F_2	157	124	33	3 : 1	1.12	>0.20
(Pinto114xPI 310762) x Pinto114	28	12	16	1 : 1	0.32	>0.50
(Pinto114 xPI 310762) x PI310762	16	16	0	1 : 0	-	-

*Rust reactions: Resistant: 1, f2, 2, 3; Susceptible: 4, 5, 6.
See standard bean rust grading scale on Table 1 showing description of all rust reactions.

FIELD AND GREENHOUSE EVALUATION OF LIMA BEAN GERMPLASM FOR RESISTANCE TO *PHYTOPHTHORA CAPSICI*, THE CAUSAL AGENT OF LIMA BEAN POD ROT

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Lima bean (*Phaseoluslunatus* L.) is an important processing vegetable crop in Delaware and the Mid-Atlantic region. There are approximately 5,670 ha of lima beans planted in the state each year, making lima bean Delaware's most widely planted vegetable crop. In recent years lima bean pod rot, caused by *Phytophthora capsici*, has increased in incidence and is becoming more important to lima bean growers and processors in the region.

During the summers of 2010 and 2011, 31 distinct lines of lima bean were evaluated in the field for resistance to *P. capsici*. Plots were established at the University of Delaware's Carvel Research and Education Center, Georgetown DE. Twenty seeds of each line were sown on 6 Jul and flowering began in the second week of September. Inoculum of *P. capsici* was produced in two ways. First, the surface of disinfested cucumbers were inoculated with agar plugs from a lima bean isolate of *P. capsici* and maintained under sterile conditions at approximately 72°F under natural, diffuse daylight on a laboratory bench. After 10-14 days, cucumbers were fully colonized with mycelium and produced an abundance of sporangia. The inoculum was prepared from these cucumbers by submerging them in 1 gallon of tap water for each 10 medium cucumbers, then crushing them manually. The suspension was then sieved to remove most plant material. Alternatively, inoculum was produced from an isolate of *P. capsici* from lima bean growing on V-8 juice agar or potato dextrose agar for up to 3 weeks. Plates were flooded with a small volume of sterile distilled water and gently scraped to remove sporangia and transferred to small misting bottles. Regardless of source of inoculum, pins and flat pods were sprayed to runoff using a backpack sprayer and plants were misted nightly using a low volume/low pressure misting system. Two lines have produced pods and showed no symptoms in both the 2010 and 2011 field screens. They are PI 347826 a landrace collected in California, and PI 477041 a landrace collected in Arizona.

During the winter of 2010/11, a greenhouse evaluation system for identifying lima bean lines with resistance to *P. capsici* was developed. Many lima bean lines are unable to produce flowers and pods in the field in Delaware under long days (more than 12 hours of daylight). This system included the use of a greenhouse room that has computer controlled blackout shades to establish growing environment of 12 hours light and 12 hours dark. As the majority of the plants to be screened in the greenhouse were indeterminate and would be very tall, they were screened in a newly constructed mist chamber within this greenhouse room. The chamber included high intensity lighting and a timer controlled mist system to provide ideal conditions both for plant growth as well as capillary mats to provide sub-irrigation and high humidity in the chamber. In the winter of 2011 greenhouse grown plants of the lima bean cultivar Concentrated Fordhook were successfully infected in our greenhouse chamber using both sporangial spray and agar plugs of *P. capsici*.

Nineteen lines of lima bean known to be photoperiodic and not capable of flowering and producing pods under long day conditions were screened in the greenhouse in winter 2011/12. On 15 Sept 2011 these lines were seeded into 12 inch pots in Promix and grown under 12 hour

light and 12 hour dark conditions in a greenhouse at University of Delaware. After plants produced pods, they were transferred to the inoculation chambers for evaluation. Inoculum was produced from an isolate of *P. capsici* from lima bean growing on V-8 juice agar for up to 3 weeks. Plates were flooded with a small volume of sterile distilled water and gently scraped to remove sporangia and transferred to small misting bottles. Pins and flat pods were sprayed to runoff with inoculum and after inoculation plants were misted each hour for 4 minutes in an enclosed chamber. Plants were evaluated daily starting 5 days after inoculation for signs of infection which includes the formation of mycelium and sporangia and symptoms which include reddening of the pods. Infections are confirmed by microscopic examination of scrapings from potentially infected pods for sporangia diagnostic of *P. capsici.*

Two lines did not show symptoms after being inoculated twice: PI 256405, a landrace from El Salvador, and PI 362772, a landrace from Brazil. These two PIs will be screened in the greenhouse in spring 2012 to confirm resistance. PI 347826 and PI 477041, which were resistant in the field, will be screened in the greenhouse in spring 2012. These two lines have been crossed with susceptible cultivars to generate F_2 populations for field screening in 2012.

Table 1. Field and greenhouse evaluation of lima bean germplasm for reaction to inoculation with *Phytophthora capsici, 2*010-2011.

Line, PI # or Cultivar	Source Location	Screen[1]	Rxn[2]
PI 256814	Ecuador	Field10/11	S
PI 256820	Ecuador	Field10	S
PI 257419	Argentina	Field10	S
PI 347777	CA, USA	Field10	S
PI 347779	AZ, USA	Field10	S
PI 347781	AZ, USA	Field10	S
PI 347786	AZ, USA	Field10/11	S
PI 347787	AZ, USA	Field10	S
PI 347826	CA, USA	Field10/11	**NS**
PI 440807	AZ, USA	Field10	S
PI 440808	AZ, USA	Field10	S
PI 477041	AZ, USA	Field10/11	**NS**
PI 534918	NM, USA	Field10	S
PI 549478	MI, USA	Field10	**S**
PI 549484	LA, USA	Field10	**S**
VA Butterbean	DE,USA	Field10	**S**
W6 17497	Argentina	Field10	**S**
184-85	US cultivar	Field10	**S**
Cypress	US cultivar	Field10	**S**
FH 1072[3]	US cultivar	Field10	**S**
FH 90-1	US cultivar	Field10	**S**
DE0501801A	DE line	Field11	**S**
DE0501805A	DE line	Field11	**S**
DE0402701	DE line	Field11	**S**
DE0407903	DE line	Field11	**S**
DE0407905	DE line	Field11	S
DE0407906	DE line	Field11	S
DE0407907	DE line	Field11	S
DE0505002A	DE line	Field11	S
FH 242	US cultivar	Field11	S
C-elite Select	US cultivar	Field11	S
PI 195339	Guatemala	GH11	S
PI 201478	Mexico	GH11	S
PI 241790	Peru	GH11	S
PI 256384	El Salvador	GH11	S
PI 256405	El Salvador	GH11	**NS**
PI 256804	Colombia	GH11	S
PI 256861	Peru	GH11	S
PI 256890	Peru	GH11	S
PI 256906	Peru	GH11	S
PI 260407	Peru	GH11	S
PI 260417	Bolivia	GH11	S
PI 310620	Guatemala	GH11	S
PI 362772	Brazil	GH11	**NS**
PI 362801	Brazil	GH11	S
PI 362832	Brazil	GH11	S
PI 363023	Brazil	GH11	S
PI 363029	Brazil	GH11	S
PI 195342	Guatemala	GH11	S
PI 347781	USA	GH11	S

[1]GH=greenhouse
[2]Reaction to *P. capsici*: S=susceptible, NS=no symptoms
[3]FH=Fordhook

EFFECT OF HERBICIDES ON THE MYCELIAL GROWTH OF *FUSARIUM OXYSPORUM* F. SP. *PHASEOLI*, *MACROPHOMINA PHASEOLINA* AND *SCLEROTINIA SCLEROTIORUM*

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INTRODUCTION

In Brazil, the herbicides generally applied for weed control on dry beans are metolachlor (Dual 960 CE), fomesafen (Flex), bentazon (Basagran), imazamox (Sweeper), glyphosate (Roundup Ultra), and fluazifop-p-butyl (Fusilade). The former is used as pre-emergent and the others as post-emergent herbicides. Few studies have been carried out on the effects of these herbicides on soil-borne pathogens of dry beans. The objectives of this study were to investigate the effect of concentrations of these herbicides with respect to inhibition of the mycelial growth of *Fusarium oxysporum* f. sp. *phaseoli* (Fop), *Macrophomina phaseolina* (Mp) and *Sclerotinia sclerotiorum* (Ss).

MATERIALS AND METHODS

The isolate of the three fungi were obtained from dry bean plants and maintained on PDA medium at 4 °C. The herbicides were dissolved in dimethyl sulfoxide and added to Petri dishes (9 cm diameter) containing PDA medium after sterilization to produce final concentrations of 1, 10, 100 and 1000 ppm. Mycelial plugs (5 mm diameter) from a 3-day-old culture of Mp and Ss and a 7-day-old culture of Fop were placed onto the center of PDA plates amended with each herbicide. Petri dishes contained only PDA were used as control. A completely randomized design with four replications was used. The radial growth (colony diameter) of mycelia was measured 48 hours after incubation at 23 °C in darkness.

RESULTS AND DISCUSSION

In general,Ss was the most sensible fungus to the herbicides (Fig. 1). None of the fungi were able to grow on PDA amended with metolachlor at 1000 ppm. Fluazifop-p-butyl, bentazon, and fomesafen did not affect Fop growth. Glyphosate at 1000 ppm reduced Fop growth by 40% compared with the control. In a study in vitro, Meriles et al. (2006) found a inhibitory effect of glyphosate at 100 ppm on mycelia growth of *F. solani*. Imazamox decreased slightly the colony diameter of this fungus only at 1000 ppm. In general, colony diameter of Mp decreased slightly as fomesafen and imazamox concentrations increased after 10 ppm. With the herbicide bentazon, colony diameter of Mp decreased slightly after 1 ppm. Fluazifop and glyphosate decreased Mp growth more strongly than these three herbicides as their concentrations increased. Bentazon and imazamox at 1000 ppm reduced Ss growth from 90 mm to 38 mm and 49 mm, respectively. Fluazifop, fomesafen, and glyphosate inhibited Ss growth more effectively at 1000 ppm than those two herbicides. Metolachor showed the highest potential for control of the three fungi since it is a pre-emergent herbicide and it is applied at relatively high rates (2 to 3 L/ha).

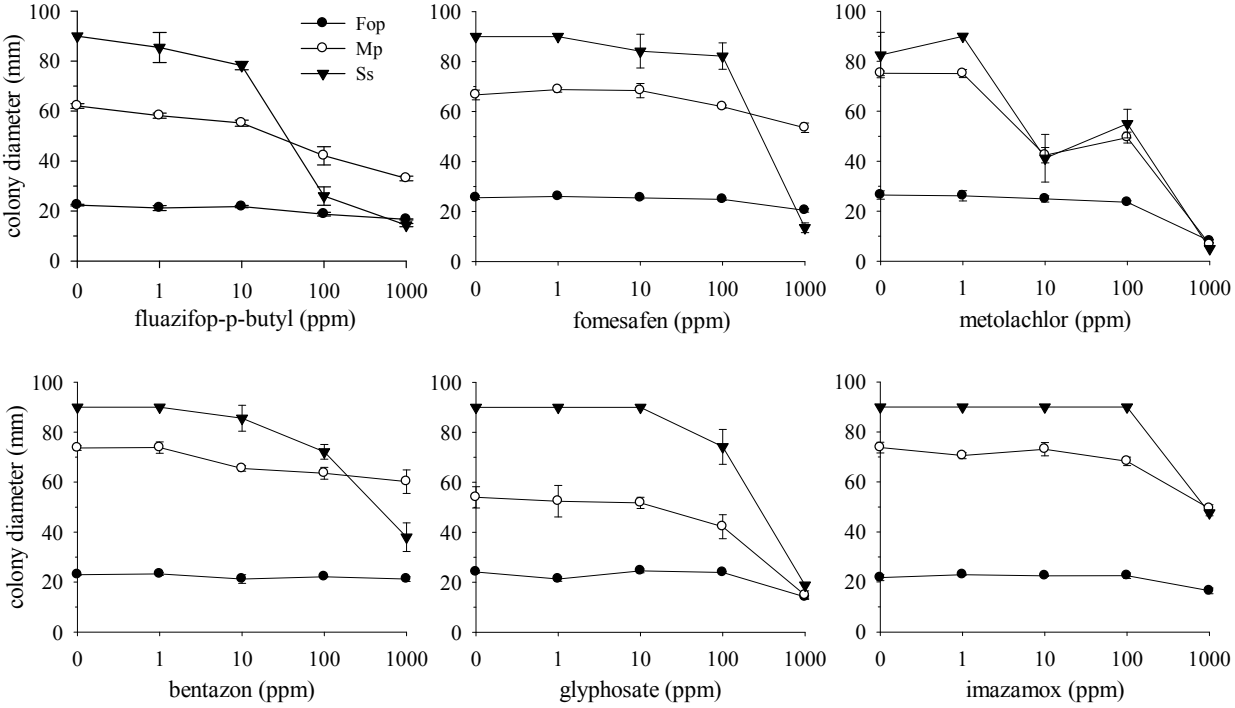

Figure 1 – Effects of concentrations of six herbicides used for weed control on dry beans on the radial growth of *Fusarium oxysporum* f. sp. *phaseoli* (Fop), *Macrophomina phaseolina* (Mp) and *Sclerotinia sclerotiorum* (Ss). Means ± SD. Some bars are hidden by symbols.

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USE OF MUTI SITE SCREENING TO IDENTIFY AND VERIFY PARTIAL RESISTANCE TO WHITE MOLD IN COMMON BEAN IN 2011

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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 partial resistance to WM.

Breeders sent seed of 23 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 field screening methods. Three bean lines were included in both tests as controls: partially resistant G122, Bunsi with field avoidance and susceptible GN Beryl.

The field tests consisted of two rows of each of the 12 entries and one row of a common 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) (Table 1). Nebraska and North Dakota did not have results due to high heat/dry conditions and hail damage, respectively. These problems that resulted in no data were disappointing, but demonstrate the importance of testing in multiple locations. In the field tests, all 9 lines were significantly more resistant than Beryl. In the four fields that reported disease ratings, G122 (the resistant check) was the most resistant, followed closely by P07793. The remaining eight lines were rated from 3.0 (resistant) to 4.1 (intermediate resistance).

The greenhouse tests were conducted on 23 entries, plus 3 controls, using a straw test method of inoculating 21 to 28-day-old plants. The plants were infected 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 a modified Petzoldt and Dickson scale for straw tests (Teran et al, 2006) (Table 2). The greenhouse results indicate that four bean lines, Z0726-9-54, A195, 1144-2 and 1144-1, were more resistant than G122. The actual straw test mean for these lines indicates they have only intermediate resistance; 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. There were six lines that showed intermediate resistance in the field and in the greenhouse test and three lines that demonstrated intermediate resistance in the field, but were rated as susceptible in the greenhouse. These lines exhibited escape or avoidance mechanisms.

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

ENTRY	SEED CLASS	COLLABORATOR	MI	NE[1]	ND[2]	OR	WA	WI	Mean	t Grouping
G122	CRAN	Resistant Check	1	nd	nd	2.4	2.5	4.1	2.5	A
P07793	PINTO	J. Kelly - MI	2	nd	nd	2	3.1	3.4	2.6	A
A195	BAYO	S. Singh - ID	7	nd	nd	1.1	1.5	2.3	3.0	A
P07863	PINTO	J. Kelly - MI	2.3	nd	nd	1.3	4.5	4	3.0	A
37-2	PINTO	P. Miklas - WA	4	nd	nd	1.8	3.5	3.9	3.3	A
Z0726-9-54	PINTO	P. Miklas - WA	4	nd	nd	2	5	3	3.5	A
ND060514	NAVY	J. Osorno - ND	4	nd	nd	1.9	4.8	3.3	3.5	A
50-2	SMALL RED	P. Miklas - WA	2.7	nd	nd	3.1	4	4.4	3.6	A
ND080547	SMALL RED	J. Osorno - ND	4	nd	nd	1.7	6.3	2.7	3.7	A
EX RICO (BUNSI)	NAVY	Intermediate Check	5.3	nd	nd	2.3	4.5	4.4	4.1	A
R08516	SMALL RED	J. Kelly - MI	3.3	nd	nd	2.3	5.8	5.1	4.1	A
BERYL	G. NORTHERN	Susceptible Check	9	nd	nd	4.6	7.5	5.8	6.7	B

*1 = no disease, 9 = plants dead **Alpha = 0.05, LSD = 1.81

[1] = no disease due to high heat and dry conditions, [2] = no disease due to hail damage

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

ENTRY	SEED CLASS	COLLABORATOR	CO	ID	MI	NE	NY	OR	WA	WI	Mean	t Grouping
Z0726-9-54	PINTO	P. Miklas - WA	4.3	5.6	3.75	3.3	.	4.3	5	5.7	4.6	A
A195	BAYO	S. Singh - ID	3.5	3.8	3.5	4.9	.	5.3	6.5	4.5	4.6	A
1144-2	SMALL WHITE	S. Singh - ID	3.1	***4.7***	3.25	3.2	3.4	5	6.6	8.1	4.7	A
1144-1	SMALL WHITE	S. Singh - ID	4.8	***4.3***	2	3.8	3.6	5.3	6.9	6.9	4.7	A
37-2	PINTO	P. Miklas - WA	6.3	4.9	4	3.1	.	4.8	6.3	6.7	5.2	A B
G122	CRAN	Resistant Check	5.2	4.3	4.5	5.8	.	5.7	6.8	4.6	5.3	A B
NE10-11-16	CRAN	C. Urrea-NE	5.5	***6.3***	3.5	5.7	4.3	5.7	7.3	6.2	5.5	A B C
NE10-11-20	CRAN	C. Urrea-NE	6.1	***6.5***	4.75	6.3	4.5	6.1	5.9	6	5.8	A B C D
1144-3	SMALL WHITE	S. Singh - ID	5.6	***5***	2	3.2	3.67	8.3	8.8	8.6	5.6	A B C D E
NE2-10-22	PINTO	C. Urrea-NE	6.2	***8.4***	2	5.7	5.4	5.4	8.8	6.8	6.1	A B C D E
P07793	PINTO	J. Kelly - MI	6.8	6.2	4.25	3.9	.	6	6.7	7.1	5.9	A B C D E
ND060514	NAVY	J. Osorno - ND	6	4.3	3.25	6.6	.	7.5	8.7	5.2	5.9	A B C D E F
NE2-10-19	PINTO	C. Urrea-NE	7.5	***7.7***	3.25	6.5	5.2	7.9	8.8	5.8	6.6	B C D E F G
VRW 32	BEIGE	S. Singh - ID	5.9	5.1	.	6	.	7.5	7.2	7.1	6.5	B C D E F G
1144-5	SMALL RED	S. Singh - ID	5.8	***7.2***	4.75	5.2	6	6.8	8.1	8.7	6.6	B C D E F G
ND080547	SMALL RED	J. Osorno - ND	7.6	6.7	4	4	.	7.8	7.2	8.3	6.5	B C D E F G
R08516	SMALL RED	J. Kelly - MI	7	6.3	5.25	7	.	7.3	7.6	8.3	7.0	C D E F G H
NE14-11-16	RED	C. Urrea-NE	7.1	***5.7***	6	7.2	4.4	7.4	8.7	8	6.8	C D E F G H
P07863	PINTO	J. Kelly - MI	6.4	7.4	6	5.8	.	6.7	8.1	9	7.1	D E F G H
EX RICO (BUNSI)	NAVY	Intermediate Check	6.7	6.7	5.25	6.3	.	8	8.8	8.7	7.2	D E F G H
1144-4	SMALL WHITE	S. Singh - ID	5.9	***6.8***	7	5.9	5.9	8.1	8.8	9	7.2	E F G H
NE1-10-15	G. NORTHERN	C. Urrea-NE	8.1	***8.5***	6	7.5	5.1	8	8.6	9	7.6	F G H
50-2	SMALL RED	P. Miklas - WA	8.1	6.1	5	8	.	8.4	8.9	8.7	7.6	G H
NE1-10-21	G. NORTHERN	C. Urrea-NE	7.9	***8.1***	6.5	8.4	6	8.4	8.9	9	7.9	G H
BERYL	G. NORTHERN	Susceptible Check	8.6	8.3	8	7.4	.	6.1	8.8	8.4	7.9	G H
NE13-11-4	BLACK	C. Urrea-NE	8.7	***5***	6.75	8.7	8.3	8.8	9	9	8.0	H

*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 = 1.56
Numbers in bold were not included in statistical analysis as they were 35 day post evaluation

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LINES FROM BRAZILIAN DRY BEAN BREEDING PROGRAMS WITH WHITE MOLD RESISTANCE

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INTRODUCTION: Approximately 30 % of dry beans harvested in the State of Minas Gerais, Brazil, are produced during the fall-winter season. "Carioca" (cream-striped), black, and red, in this order, are the most important classes of dry bean cultivated in Minas Gerais. The commercial dry bean production system during this season requires irrigation, and the indeterminate growth habit (type III) cultivars used in this production system are prone to outbreaks of white mold (WM) caused by the fungus *Sclerotinia sclerotiorum*. The use of fungicide is common among the farmers, which has been applied successfully up to three times. However, it increases production cost and it is a sourceofenvironmental contamination. The development of bean cultivars combining partial field resistance and architectural avoidance to WM could be a way to reduce disease and improve yield. Dry bean lines developed for Minas Gerais aretested every year at several locations, but generally without WM pressure. The experiments installed to test these lines are called "cultivation and use value" (VCU). The lines tested in the VCU are from the breeding program of Federal University of Lavras, Embrapa Rice and Beans, and Federal University of Viçosa. In each location, generally three experiments are installed: VCU carioca, VCU black, and VCU other colors. Our hypothesis is that among the lines included in the VCUs there are some with levels of WM resistance higher than those of the current cultivars.

MATERIAL AND METHODS: Lines/cultivars tested in the VCUs conducted in 2008, 2009, and 2010 were assessed for their reaction to WM and yield in an area naturally infested with sclerotia, in Oratórios, MG. The experiments were installed between April and July and were sprinkler irrigated. Based on the results obtained in the VCUs, seven lines (VC 17, VP 21, CNFC 10720, CNFC 10722, CNFP 10798, CNFP 11980, CNFC 11965) and the cultivars BRS Vereda and Ouro Branco were selected. In a separate experiment, the reactions of these lines/cultivars to WM were compared with the reactions of the following current cultivars: Pérola (released in 1994), Majestoso (released in 2006), Ouro Negro (released in 1991), and Ouro Vermelho (released in 2005). These four cultivars are widely cultivated in Minas Gerais. The line A 195, which is known for its WM resistance (Singh et al., 2007), was also included for comparison. A randomized complete block design with five replications was used. Plotswere 2 rows x 3 m long, with 0.50 m between rows. Fifteen seeds per meter were sown. Thinning was done at 10 d after emergence leaving 10 plants per meter. White mold intensity (incidence + severity) was evaluated visually, using a 1-to-9 scale (Table 1).

RESULTS AND DISCUSSION: Significant correlations were observed between WM intensity and yield (r = -0.69***), yield and lodging (r = -0.41***), and WM intensity and lodging (r = 0.56***). The lines/cultivars were separated into three groups according to yield (Table 1). Six lines, two of them of type III, were ranked in the group with the highest yield. WM intensity of these lines varied from 4.2 to 5.6. These disease intensities were similar to that verified for the WM resistant line A 195. On the other hand, three current cultivars were ranked in the group

with the lowest yield. WM intensities of these cultivars were higher compared with the other lines/cultivars. Similar results were observed in the VCUs. The cultivar Pérola ranked in the intermediate group and yielded 503 kg/ha more than the cultivar Majestoso. This cultivar was more susceptible to WM than Pérola. This high susceptibility of Majestoso to WM might be an obstacle for its acceptance by the farmers for cultivation during the fall-winter season. The newest cultivar Madrepérola ("carioca" class, released in 2009) was as susceptible to WM as the cultivar Majestoso in the VCUs (results not shown). These results indicate that advanced breeding lines should be tested under WM pressure before being released as a new cultivar. They also suggest that good source of resistance to WM are present in the lines and cultivars of dry bean. Cultivars/lines with resistance to WM might require fewer fungicide applications than current cultivars.

Table 1 – Yield, white mold (WM) intensity, and lodging of current cultivars (in bold) and of lines/cultivars selected for WM resistance in the VCUs. Fall-winter season, Oratórios, Minas Gerais, Brazil, 2011*

Genotype (seed class or color[1])	Growth type[2]	Yield (kg/ha)	WM Intensity[3]	Logding[4]
VC 17 (C)	III	2716±334 A	5.2±0.8 B	3.00±0.35 B
CNFC 10720 (C)	II	2593±604 A	5.6±0.4 B	2.86±0.22 C
CNFP 10798 (Bl)	II	2517±448 A	5.4±1.0 B	2.72±0.22 C
CNFC 10722 (C)	II	2507±567 A	4.4±1.5 B	2.34±0.32 C
CNFP 11980 (Bl)	II	2430±370 A	5.2±0.6 B	2.56±0.13 C
BRS Vereda (R)	III	2210±344 A	4.2±1.3 B	3.20±0.27 B
A 195[5] (Be)	I	1933±590 B	5.1±0.4 B	1.50±0.00 D
Pérola (C)	III	1840±487 B	5.8±1.2 B	3.50±0.41 B
CNFC 11965 (C)	II	1763±340 B	5.3±1.1 B	2.46±0.58 C
VP 21 (B)	II	1707±224 B	5.9±0.4 B	3.02±0.29 B
Ouro Vermelho (R)	III	1403±271 C	6.3±0.7 A	4.06±0.26 A
Majestoso (C)	III	1337±366 C	7.0±0.7 A	3.36±0.54 B
Ouro Branco (W)	I	1227±295 C	5.8±0.8 B	2.40±0.22 C
Ouro Negro (Bl)	III	907±240 C	7.5±0.6 A	4.08±0.23 A
CV (%)		21.0	16.2	11.0

[1] C = carioca, Bl = black, R = red, Be = beige, W = white.
[2] I – determinate growth habit; II – indeterminate growth habit, both main stem and branches strong and upright; III – indeterminate growth habit, branches relatively open and semi-prostrate.
[3] 1 = symptomless or healthy and 9 = severely diseased and eventual plant death.
[4] Determined at maturity on a 1-to-5 scale: 1 - no lodging, and 5 - excessive lodging.
[5] Line resistant to WM (Singh et al., 2007).
* Means followed by the same letters belong to the same group (Scott-Knot test, p = 0.05). Means ± SD.

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IDENTIFICATION AND VALIDATION OF QTL FOR RESISTANCE TO WHITE MOLD IN TWO PINTO BEAN RIL POPULATIONS

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INTRODUCTION

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

MATERIALS AND METHODS

Two RIL populations of 94 $F_{4:7}$ lines derived from a common parent: AN- 37 registered as USPT–WM-1 (Miklas et al., 2006) which has Type IIb growth habit, open canopy, stay-green trait, with unacceptable small pinto seed. AN-37 was developed from a pinto/navy bean cross Aztec/ND88-106-04, (Miklas et al., 2007). P02630 and P02647 are MSU breeding lines with high yield, superior seed quality, upright plant type, but susceptible to white mold. The populations were treated as half-sibs; namely AP630, AP647. The RILs were grown in a naturally infested field in Michigan. Data on canopy height, lodging, days to maturity and white mold incidence was collected at physiological maturity for four years (2007-2010). Greenhouse tests were conducted as described by Petzoldt and Dickson (1996) from 2008-2010. The parents were screened with 440 SSR markers and 80 InDel markers. 114 SSR and 6 indel markers were mapped polymorphic in AP630. 109 of these were screened on the AP02647 population and 34 SSR's were also segregating in AP647. Mapping was conducted using Joinmap 3.0 and MapDisto. QTL analysis was performed by Kruskal-Wallis analysis and composite interval mapping of MapQTL 5.

RESULTS AND DISCUSSION

A QTL for resistance to white mold in the field in 2007 and 2009 was identified near markers BM157 and IAC90 respectively on bean linkage group Pv01. In 2010 the marker IAC90 was significantly associated with field resistance in single marker analysis ($p = 0.05$). In 2008 QTL for resistance were detected on Pv03 and Pv07. Yield QTL were detected on Pv02 and Pv05 accounting for up to 39% of observed variation in yield. These QTL were contributed by alleles from the adapted parent P02630, whereas the QTL for the straw test on Pv02 Pv3 and Pv08 came from the resistant parent AN-37. The QTL on Pv02 was consistent in three separate evaluations while the QTL on Pv03 overlapped with that for field disease resistance in 2008 which could imply that the disease score in 2008 were not severely confounded by environmental conditions. Microsatellite marker screening revealed that 43 polymorphic markers associated with resistance from the first population (AP630) were also segregating in AP647 half-sib population. Analysis

of segregation patterns with significant markers in the AP647 population showed that the AN-37 allele of BMd-34 which was previously unlinked but now mapped near markers on Pv02 increased straw test resistance significantly (Fig. 1). The QTL on Pv02 is likely the same QTL as was previously mapped by other authors (Soule et al; 2011). The favorable alleles of marker BMd-1 in the QTL interval on Pv03 were from the AN-37 parent and contributed to an average of 10% increase in resistance in the greenhouse straw test in three separate tests (Table 1). The QTL on Pv03 could be the same one that was mapped in the Aztec/ND population from which AN-37 parent was originally selected.

Table 1: Cosegregation of linked marker BMd-1 on Pv03 with Straw Test ratings in AP647 population

ENTRY	***STRAW TEST 1***	***STRAW TEST 2***	***STRAW TEST 3***	***MEAN***
P02647 (parent)	4.5	5.0	5.4	4.96
AN-37 (parent)	3.9	3.7	4.0	3.90
AN-37 allele RILs	3.6	4.3	4.3	3.96
P02647 allele RILs	4.7	4.8	4.5	4.67
Test Mean	4.1	4.8	4.8	4.57
% AN-37 alleles in top 10 entries	70 .0	65.0	69.0	68.0
% Increase in Resistance	10.2	9.0	10.8	10.0

Figure 1a and 1b: Loci associated with disease resistance on Pv02 and Pv07 in AP647 population; BMd-34 on Pv02 (Straw Test), and BM209 on Pv07 (field disease incidence) p= 0.0001

Figure 1a – Pv02

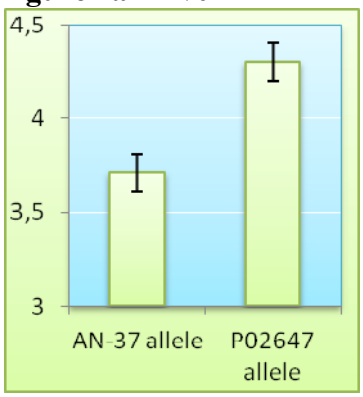
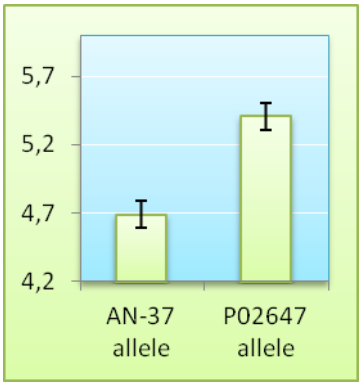


Figure 1b – Pv07



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PROTOCOL OPTIMIZATION TO OBTAIN CARPOGENIC GERMINATION OF *SCLEROTINIA SCLEROTIORUM*

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INTRODUCTION

The white mold disease, caused by *Sclerotinia sclerotiorum,* is one of the most severe diseases on common bean fields in Brazil, especially in the Cerrado region (Ferraz et al., 1999) and irrigated areas during winter production, resulting in high economic losses. In the absence of a susceptible host, this pathogen produces resistance structures such as the sclerotia and may have the carpogenic germination, producing apothecia and ascospores that infect plants. The disease is caused by genetically diverse populations of *S. sclerotiorum* and this pathogen is highly variable within small geographic areas. The lack of knowledge about the pathogen population structure difficult the disease control. In addition, to obtain the sexual stage of the fungus, the conditions must be clearly established, since there are major environmental influences in this process (Hao et al., 2003). So, the goal of this work was to identify effective methodologies to obtain the sexual phase of strains of *S. sclerotiorum.*

MATERIAL AND METHODS

Five methods were evaluated using two strains of *S. sclerotiorum* (UFLA5 and UFLA11) collected in Ijaci, MG. The tests were carried out in two replications for each strain. A replication consisted of a gerbox (11 cm x 11 cm x 3,5 cm) containing the substrate used by each method and 15 sclerotia.

Modified method of Mylchreest e Wheeler (1987)

The method consists in cultivating the sclerotia at20°C in Erlenmeyers flasks of 250 ml on autoclaved beans. After three weeks, the flasks are incubated at 4°C for four weeks. The sclerotia are then removed from the beans and placed at 1 cm deep in Platmax® substrate (Eucatex, Paulina, São Paulo, Brazil) ingerbox with lid. The containers are kept at 10ºC in distilled water spraying regularly. When apothecia began to form, the gerbox are placed in the incubator at 14 hours of light exposure per day at 22ºC.

Modified method of Sun e Yang (2000)

On this method, high intensity of light (120-130 mol m^{-2} s^{-1}), high soil moisture and temperature of 20°C were used. The sclerotia were placed in three layers of sterile paper towel saturated with sterile distilled water and then placed in a sterilized glass petri dish. The plates are incubated at 4,5°C for two months as a pre-conditioning. The sclerotia are then placed on the surface of moistened sterilized sand in a growth chamber with 12hours of light per day. An additional test, without pre-conditioning at 4,5°C was conducted.

Method of Brandão et al. (2008)

The method consists in distributing sclerotia on 250g of soil in a gerbox. The boxes containing soil in the field capacity and sclerotia are incubated at 20°C, with photoperiod of 12 hours light per day.

Method 1 of Paula Júnior (personal communication, 2010)
The sclerotia are arranged on the surface or 0,5 cm deep in a gerbox with sterile sand moisted with water and incubated at room temperature, which was considered to 20^{o}C with exposure to 24 hours of light per day.
Method 2 of Paula Júnior (personal communication, 2010)
This method proposes to put the sclerotia at 0,5 cm deep in sterile and humid sand or soil in gerbox. Boxes should be kept at 18^{o}C with a photoperiod of 12 hours of light per day.

RESULTS AND DISCUSSION

The best methods where the carpogenic germination occurred more quickly and efficiently, were the modified method of Sun e Yang (2000) and the method 2 of Paula Júnior (personal communication, 2010), in which the sclerotia were placed 0,5 cm deep in sand. There was not formation of apothecia when the sclerotia pre-conditioning proposed by Sun e Yang (2000) was carried out. However, when the sclerotia were placed directly on the sand surface, the carpogenic germination occurred after 35 days. Using the second methodology proposed by Paula Júnior (personal communication, 2010) the sclerotia germinated in 35 days when placed 0,5 cm deep in the sand. Carpogenic germination did not occur when soil was used. On the first method of Paula Júnior, the apothecia germination occurred after 50 days when the sclerotia were buried at 0,5 cm deep in the sand. The sclerotia also germinate after three and a half months, when the methodology of Brandão et al. (2008) was used. The assessment of the modified methodology of Mylchreest and Wheeler (1987) did not result in sclerotia formation on autoclaved beans. The results obtained were satisfactory, since they were viable and mature apothecia were obtained over a period of time, sometimes less than that presented by their respective authors.

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STUDY OF CANDIDATE GENES FOR RECESSIVE RESISTANCE AGAINST BCMV AND BCMNV IN *PHASEOLUS VULGARIS* L.

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ABSTRACT: In order to identify the recessive resistance genes against systemic movement of *Bean common mosaic virus* (BCMV) and *Bean common mosaic necrosis virus* (BCMNV) in bean, candidate genes *eIFiso4E, eIFiso4G,* and *eIF4G* were cloned from differential cultivars of bean carrying combinations of *bc* genes. Deduced amino acid sequences of eIFiso4E and eIFiso4G from different genotypes revealed no polymorphism among cultivars. Sequences of *eIF4G* showed two alleles annotated as $eIF4G^1$ and $eIF4G^2$ differing at eight amino acids. Allele specific markers, *eIF4G-ASPs*, were designed on the basis of differences between the two alleles and all genotypes we analyzed for the alleles of this gene. However no correlation was shown among the genotypes concerning the *bc*-genes.

INTRODUCTION: A strain unspecific recessive resistance gene, *bc-u*, and two strain specific loci [*bc-1*($bc\text{-}1^1/bc\text{-}1^2$) and *bc-2* ($bc\text{-}2^1/bc\text{-}2^2$)] mediate resistance to systemic movement of BCMV and BCMNV in *P. vulgaris* (3). Since assigning of these genes in 1978 (1), gene identification in different genotypes of bean have been done on the basis of segregation of resistance/susceptibility to different strains of BCMV and BCMNV, symptomatology and applying molecular markers linked to some of these genes (reviewed in 4). Eukaryotic translation initiation factors, especially the eIF4E and eIF4G families, play important roles in life cycle of potyviruses and some other virus groups (7). Due to the importance of these genes in potyvirus infection in other systems, they were likely candidates for any of the *bc* genes against BCMV and BCMNV in *P. vulgaris*. In the present study we address this hypothesis by cloning these genes from different genotypes of bean and applying a molecular marker.

MATERIALS AND METHODS: Differential cultivars of bean belonging to the host groups (HG) I-XI were obtained from CIAT, Colombia. Additional genotypes with unassigned HGs were received from USDA-ARS, USA. cDNA of *eIF4G*, *eIFiso4G* and *eIFiso4E* were cloned from cultivars using degenerated primers that were designed on the basis of sequences of these genes from *G. max*, *P. sativum*, *M. truncatula*, *L. japonicus*, *N. benthamiana* and *A. thaliana* retrieved from data bases. Allele specific markers were developed to discriminate the *eIF4G* alleles in genotypes of *P. vulgaris*.

RESULTS AND DISCUSSION: Homologues of all three genes *eIF4G*, *eIFiso4G* and *eIFiso4E* were cloned and sequenced at least two times for each genotype. Comparison of the deduced amino acid levels of eIFiso4E and eIFiso4G showed no polymorphism among the genotypes carrying different combination of *bc*-genes. In contrast, the *eIF4G* sequences revealed two alleles annotated as $eIF4G^1$and $eIF4G^2$. Alleles $eIF4G^1$and $eIF4G^2$ were identified in *bc-1* (ITG), *bc-3* (Raven) and susceptible (DW, Widusa), *bc-u* (SGR), $bc\text{-}1^2$ (Amanda), $bc\text{-}1^2$, $bc\text{-}2^2$ (IVT7233), *bc-u, bc-2, bc-3* (IVT7214) cultivars, respectively. The deduced amino acid sequences of alleles $eIF4G^1$and $eIF4G^2$ differed at two amino acids at positions S512T and A513S and deletion of six amino acids (PSGVTS) at positions 514-519 in genotypes carrying $eIF4G^2$allele. Allele

specific markers were developed on the basis of nucleotide deletion between the two alleles and the remaining cultivars were genotyped for *eIF4G* alleles (figure 1). However, comparison of the genotypes based on the electrophoretic patterns of *eIF4G* alleles divided the genotypes in two groups, but without correlation to specific gene combination(s). The results of this research and previous finding of the authors showed that only *eIF4E* of the eukaryotic translation initiation factors but not *eIFiso4E*, *eIFiso4G* and *eIF4G* correlates with recessive resistance to BCMV and BCMNV in *P. vulgaris* (this work, 5). Other host factors such as potyviral VPg interacting proteins, PVIP1 and PVIP2 (2), were also shown previously that have no positive correlation to the known recessive resistance to these viruses in bean (6). Based on the past and present analysis, there is no indication that eIF4G, eIFiso4G and eIFiso4E are candidates for the known recessive resistance except *PVIP2* that shown to affect viral multiplication in some *bc-1* genotypes (6).

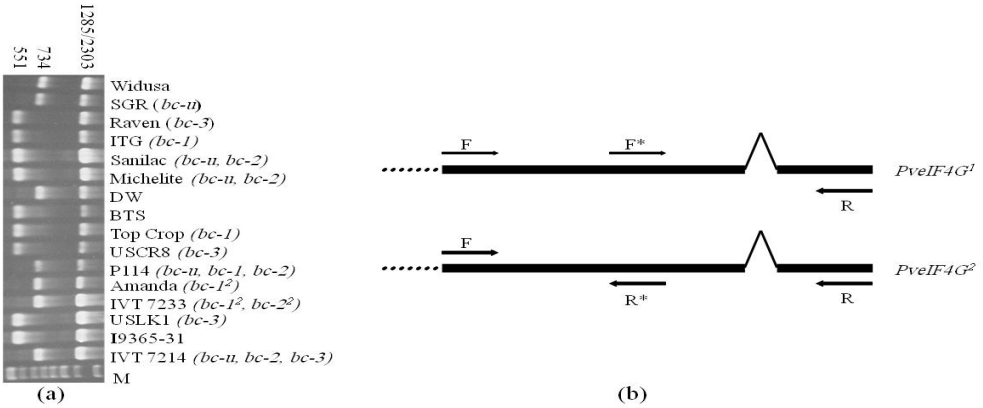

Figure 1) Genotyping of differential cultivars of *P. vulgaris* carrying combinations of *bc*-genes for the *eIF4G* alleles (a) using *eIF4G-ASP* markers that were designed on the basis of sequences of exones 6 and 7 and intron 6 of *PveIF4G* gene (b). The name for each cultivar and the proposed *bc*-gene combination (s) is shown on the top of each lane. M, DNA bands 1303 and 551, 1285 and 734 bp indicate DNA marker, $eIF4G^1$ and $eIF4G^2$alleles, respectively. F* and R* are specific forward and reverse primers differentiating $eIF4G^1$ and $eIF4G^2$alleles, respectively.

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THE NECESSITY FOR RE-ASSIGNING THE RECESSIVE RESISTANCE *BC*-GENES IN *PHASEOLUS VULGARIS* AGAINST *BEAN COMMON MOSAIC VIRUS*

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ABSTRACT
Four *bc* loci confer recessive resistance to strains of the potyviruses *Bean common mosaic* (BCMV) and *Bean common mosaic necrosis* (BCMNV)in *Phaseolus vulgaris*. Our studies on recessive resistance in F_1 and F_2 populations derived from genotypes carrying *bc-3* revealed susceptibility in some plants. Moreover visualization of resistance responses in bean differential cultivars using a GUS-tagged infectious cDNA clone showed some inconsistencies in resistance of some genotypes concerning on the proposed *bc*-genes. Our findings propose genetic and *in situ* histochemical evidences necessitate reassessment of *bc*-genes at least in some genotypes.

INTRODUCTION
The *bc*-genes (namely *bc-u*, *bc-1*, *bc-2* and *bc-3*) confer strain-specific resistance whereas the '*I*' gene mediates extreme resistance to BCMV and hypersensitive resistance to BCMNV and some BCMV strains (1, 7). Assigning these resistance genes to different genotype/cultivars of bean have been done on the basis of segregation of resistance/susceptibility to different strains of these viruses, symptomatology and applying molecular markers linked to some of these genes (1, 3). In the present study we show genetic and *in situ* evidences necessitate re-assigning of *bc*-genes in *P. vulgaris*.

MATERIALS AND METHODS
P. vulgaris differential cultivars belonging to 11 host groups (HG) were received from CIAT, Columbia. Additional cultivars/genotypes belonging to unassigned HGs and the BCMV-RU1 strain were obtained from USDA-ARS, USA. Crossing of the *bc-3* genotypes were done as described elsewhere (4). The GUS-tagged infectious cDNA clone of BCMV-RU1 was constructed and agroinoculated on *P. vulgaris* genotypes as described previously (6). Parental genotypes USCR8 and IVT7214, F_1 hybrids and F_2 populations were also agroinoculated with BCMV cDNA Clone. Inoculated and systemic leaves were analyzed by GUS staining four days, one week and three weeks post inoculation (wpi).

RESULTS AND DISCUSSION
GUS staining of the inoculated and systemic leaves of genotypes USCR8 (*bc-3*) (3) and IVT7214 (*bc-u, bc-2, bc-3*) (1) four days, 1wpi and 3wpi showed complete resistance to BCMV-RU1 infectious cDNA clone as expected (data not shown). GUS staining of the same leaves of the F_1 and F_2 plants derived from crossing these genotypes showed susceptibility or resistance in some plants (figure 1) (5). As *bc-3* confers complete resistance to BCMV-RU1 (1, 2), the F_1

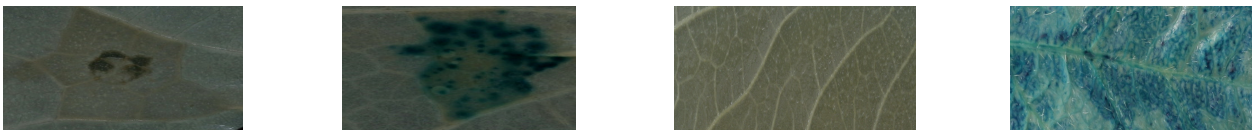
plants supposed to be resistant unless one of the parental plants carry other gene (s) than *bc-3*. However similar inconsistencies have been shown previously (2).

Our studies with BCMV-RU1 infectious clone and differential cultivars of *P. vulgaris* revealed different pattern of GUS staining in the whole susceptible genotypes Dubbele White (DW), Stringless Green Refugee (SGR), Common Red Mexican (CRM), The Prince and Sutter Pink (SP). Genotypes DW and SGR displayed extensive blue staining whereas SP, CRM and The Prince showed weak staining.

Genotypes Improved Tender Green (ITG) and Top Crop (TC) belong to HG9b and both proposed to carry *I, bc-u, bc-1* gene combination. Upon inoculation with BCMV-RU1-GUS, *in situ* histochemical assays displayed sporadic blue staining in genotype ITG whereas TC showed complete resistance.

Genotypes UI-114-8 and Pinto-114 carry *bc-u, bc-1, bc-2* gene combination and belong to HG5. UI-114-8 was previously reported to be resistant to BCMV-RU1 (2). However, we found that Pinto-114, displayed GUS staining in both inoculated and systemic leaves although a few blue foci were visible.

Taken together all these data propose the possible presence of additional resistance factors and also necessitates reassigning of the *bc*-genes in *P. vulgaris*.



(A) (B) (C) (D)

Figure1. GUS staining of the inoculated (A and B) and systemic (C and D) leaves of the F_1 and F_2 plants derived from USCR8 x IVT7214.

ACKNOWLEDGMENT

We are grateful to CIAT and Dr. Richard Larsen, USD-ARS, USA for the bean genotypes and BCMV-RU1 strain.

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RECURRENT SELECTION PROGRAM FOR TOLERANCE TO BEAN GOLDEN MOSAIC VIRUS IN BLACK COMMON BEAN

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Processes as parent selection, maintenance and exploitation of genetic variability, and conduction of the most promising segregating populations are basic steps for efficiency and success of all plant breeding program.Once new challenges are emerging worldwide on food production, the plant breeders, including those who work with autogamous plants, have considered the populational breeding and the breeding for quantitative traits as the main responsible for the yield increasing (Cargnin, 2007). Therefore, the recurrent selection could beconsidered as an important strategy to allow the stacking of favorable alleles associated to the target quantitative traits in each selection cycle, resulting in superior improved lines and populations. As a complementary tool to be used during the conduction of a recurrent selection program, the molecular markers can be extremely useful for parent selection to ensure the availability of initial genetic variability and monitor this variability over the program.

Bean golden mosaic virus (BGMV) is one of the heaviest constraints on bean production in Latin America, causing significant yield losses ranging from 40% to 100% (Morales *et al.*, 2004). In Brazil, it has been estimated that about 200,000 ha became inappropriate for common bean grow during the dry season due the severe incidence of BGMV. This virus is transmitted by the whitefly *Bemisia tabaci* (Gennadius) in a persistent and circulative manner. The disease is characterized by yellow-green mosaic of leaves, stunted growth and distorted pods, which may vary among genotypes. Control practices have focused primarily on controlling the vector by contact or systemic high-toxicity insecticides, with the concomitant problems of development of pesticide-resistant forms, low cost-benefit ratio and environmental concerns.

The main goal of this breeding program is to develop and conduct populations under recurrent selection design in order to obtain common bean black seeded lines tolerant to the BGMV. For this reason, it focuses on the development of base populations, performance testing of segregating progenies, and evaluation of the process efficiency by estimating the genetic progress over the selection cycles. SSR markers will also be used as background markers to assess and monitor the genetic variability over the program.

Based on previous studies on tolerance to BGMV developed by our research group, the following common bean lines were selected as parents for the composition of the original population (C0S0): Pinto 114, A 775, A 429, IAPAR 57, LM 21306-0, Ônix (LM 30630), Red Mexican 35, and Redlands Greenleaf C. Conical crosses were done using all these parents. Firstly, simple hybrids were obtained: Pinto 114 / Redlands Greenleaf C.; Ônix / LM 21306-0; IAPAR 57 / A 429; A 775 / Red Mexican 35. After that, the simple hybrids were crossed to develop double hybrids: (Pinto 114 / Redlands Greenleaf C.) // (A 775 / Red Mexican 35) and (IAPAR 57 / A 429) // (Ônix / LM 21306-0). The double hybrids were then crossed to obtain multiple hybrids from all eight parents. The F_1 generation formed by all multiple hybrids was conducted under shade house condition to obtain F_2 plants (the C_0S_0 generation), with selection

for black seeded plants. The base population (C_0S_0) composed by 4,910 plants was grown in the field and inoculated with BGMV seven days after germination, as described by Melo *et al.* (2005). Disease reaction was scored 50-60 days after inoculation, using the 1-9 degree scale proposed by Costa *et al.* (1990), where the degree 1 is equivalent to the absence of symptoms and 9 represents plants close to collapse or dead. Sixty-three $C_0S_{0:1}$ plants were initially selected as tolerant to the pathogen. Seeds from these plants were sown in the field using a plant-row design, and then inoculated with the virus. Fifty-five $C_0S_{0:2}$ progenies were selected as tolerant. Aiming to develop a new recombination cycle, these 55 tolerant genotypes were crossed following a circulant diallel design, resulting in the base population for the second cycle of selection (C_1S_0). This population was increased and, consequentially, the C_1S_1generation was obtained and inoculated with the pathogen under shade house condition, as described by Melo *et al.* (2005). Disease evaluation was done as previously described. The $C_1S_{1:2}$ progenies identified as most tolerant were selected to be sown and screened in the field. The top 20 $C_1S_{1:3}$ resulting progenies were selected and crossed to develop the third recombination cycle (C_2S_0), using a circulant diallel design. The C_2S_0 population was increased and about 15,000 C_2S_1 seeds were obtained and sown in the field. Out of them, 347 plants were selected based on their tolerance to BGMV. The resulting $C_2S_{1:2}$ progenies were also screened in the field and 201 $C_2S_{1:3}$ families were obtained and are being evaluated during the dry season of 2012 for reaction to BGMV and other diseases as well as for agronomic performance. The top 30 progenies will be selected and screened with a set of 40 SSR markers aiming to determine the allelic diversity present in these lines and identify the presence of genetic structuration in populations obtained by recurrent selection. In order to reduce the number of crosses and still keep the genetic variability, 10 $C_2S_{1:3}$ families tolerant to BGMV and most genetically divergent each other will be used for recombination and, consequently, begin a new cycle of recurrent selection (C_3S_0).

Aiming to estimate the recurrent selection efficiency per cycle, trials will be conducted to compare the mean performance of the best 30 families obtained from different selection cycles in relation to their parents. Despite of the transgenic solution already developed by Embrapa, this recurrent selection program aims also develop regularbreeding solutions for the genetic control of the BGMV to attend farmers that chose to grow conventional cultivars.

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RESPONSE OF COMMON BEAN GENOTYPES TO LESS-AGGRESSIVE AND AGGRESSIVE STRAINS OF *XANTHOMONAS CAMPESTRIS* PV. *PHASEOLI* CAUSING COMMON BACTERIAL BLIGHT

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INTRODUCTION

Common bacterial blight caused by *Xanthomonas campestris* pv. *phaseoli* is an important seed-transmitted disease worldwide. Low levels of resistance occur in common bean landraces from Mexico, and *Phaseolus coccineus* has an intermediate level of resistance. The tepary bean (*P. acutifolius*) and the derived interspecific breeding lines have the highest levels of resistance (Singh and Muñoz, 1999). Lema et al. (2007) reported significant differences among common bean genotypes, and bacterial isolates and densities. Duncan et al. (2011) observed interaction between common bean genotypes and bacterial strains but, without any crossovers. The objectives of this study were to: (i) determine the response of common bean genotypes to less-aggressive and aggressive strains of *X. campestris* pv. *phaseoli*, and (ii) assess the effects of bacterial densities.

MATERIALS AND METHODS

A randomized complete block design with three replications was used. *X. campestris* pv. *phaseoli* strains AGX08 (less-aggressive) and Xcp25 (aggressive) grown on nutrient glucose agar for 48 hours and inoculum densities of 1.7 x 10^8 and 3.2 x 10^8 cfu mL^{-1} were used. Sequential inoculations on the primary and trifoliolate leaves 10 and 23 days after planting were made with both strains, using a sterilize florist frog. Disease severity was recorded 14 (primary leaf) and 21 (trifoliolate leaf) days after inoculation. Disease scores were recorded using 1 to 9 scale, where 1= no visible symptoms; and 9= necrotic lesions extended beyond the inoculated area reaching to the leaf edge. Data was analyzed using the SAS GLM procedure software. Also, the presence or absence of the SCAR markers BC420 and SU91, which are linked with the tepary bean-derived resistance QTL (quantitative trait loci) was determined.

RESULTS AND DISCUSSION

Significant differences ($P \leq 0.05$) among common bean genotypes and bacterial strains occurred (Table 1). But, the effects of bacterial densities were not significant ($P > 0.05$). 'Othello' had a susceptible response to both bacterial strains in primary and trifoliolate leaves (Table 2). 'Montcalm' had a resistance reaction in the primary leaf and an intermediate response in trifoliolate leaf to the less-aggressive strain AGX08. But, Montcalm was as susceptible as Othello to the aggressive strain Xcp25. USDK-CBB-15 and USWK-CBB-16 were susceptible only in the trifoliolate leaf at both densities of Xcp25, and VAX 1 had a similar response only at the high density. On average, Xcp25 was more aggressive than AGX08, and mean disease scores were higher in the trifoliolate leaf than in the primary leaf. VAX 5 and VAX 6 had a resistance reaction to both strains at both densities in both leaves. Othello, Montcalm, and VAX 1 lacked SU91 and BC420 markers; VAX 3, VAX 4, VAX 5, and VAX 6 only had SU91; and only Wilkinson 2 and XAN 159 had both markers. Bacterial strain aggressivity and plant parts inoculated should be considered for breeding for resistance.

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Table 1. Analysis of variance for common bean genotypes evaluated for common bacterial blight against pathogenic strains AGX08 and Xcp 25 in the greenhouse in 2011.

		Mean squares	
Source	**df**		
		Primary leaf	Trifoliolate leaf
Replicates	2	0.05	21.04**
Bacterial strains (B)	1	106.89**	398.70**
Bacterial density (D)	1	0.04	1.14
Genotype (G)	22	96.62**	77.57**
B x D	1	0.01	0.001
B x G	22	18.77**	17.77**
D x G	22	0.28**	3.51**
B x D x G	22	0.26**	4.29**
Error	614	0.17	1.53

** Significant at $P \leq 0.05$

Table 2. Mean disease scores for common bean genotypes at two densities of *Xanthomonas campestris* pv. *phaseoli* strains AGX08 and Xcp25 in the greenhouse in 2011.

		Primary leaf				Trifoliolate leaf				
	SCAR	**AGX08**		**Xcp25**		**AGX08**		**Xcp25**		
Genotype	**marker**	**C1†**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**Mean**
Montcalm	None	1.0‡	1.0	8.3	8.7	4.2	4.9	8.1	8.7	5.6
Othello	None	8.4	8.6	8.9	8.9	8.8	8.2	8.3	8.7	8.6
USDK-CBB-15	SU91	1.0	1.1	3.8	4.4	4.8	3.8	6.6	7.2	4.1
USWK-CBB-16	SU91	1.0	1.0	3.6	2.9	3.3	3.4	6.4	6.8	3.6
VAX 1	None	1.0	1.0	4.6	5.3	1.7	1.1	5.5	7.2	3.4
VAX 3	SU91	1.0	1.0	1.0	1.0	1.1	1.0	4.2	2.9	1.7
VAX 4	SU91	1.0	1.0	1.1	1.0	1.3	1.4	2.0	5.3	1.8
VAX 5	SU91	1.0	1.0	1.0	1.0	1.3	1.0	2.2	2.4	1.4
VAX 6	SU91	1.0	1.0	1.0	1.0	1.1	1.5	2.8	2.3	1.5
Wilkinson 2	BC420, SU91	1.0	1.0	1.0	1.0	3.2	4.3	5.2	4.6	2.7
XAN 159	BC420, SU91	1.0	1.0	1.2	1.5	4.0	5.0	5.3	5.0	3.0
Mean		1.7	1.7	3.2	3.3	3.2	3.2	5.1	5.6	3.4
LSD ($P \leq 0.05$)		0.2	0.2	0.2	0.2	0.6	0.6	0.6	0.6	-

†C1= 1.7 x 10^8 cfu mL^{-1}, and C2= 3.2 x 10^8 cfu mL^{-1}.

‡Disease scores, where 1= no visible symptoms; 3= ≤3 necrotic lesions fused together, 6= fused necrotic lesions covering but limited to the inoculated area, and 9= necrotic lesions extended beyond the inoculated area reaching to the leaf edge.

NEW CBB-TOLERANCE SOURCE SPECIFIC SU91 MARKERS

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There are two main sources of common bacterial blight (CBB) tolerance being used in Canadian bean breeding programs: the HR67 source which derives from Xan159 (Thomas and Waines, 1984), and the OAC 95-4 (OAC Rex) source which derives from an independent interspecific cross with tepary bean (Michaels et al. 2006). The dominant SCAR marker SU91 (Pedraza et al., 1997) works for both sources and has been used in routine breeding operations for several years.

Vandemark et al. (2008) published a method for assaying SU91 using a TaqMan probe. This method eliminates the need to run gels and can be interpreted using a real-time PCR machine. The sequence used to design this assay came from Xan159 and the assay has worked well in progeny from crosses to this source of resistance. When this assay was used in progeny from the OAC 95-4 source however, it took more cycles of PCR before a positive reaction was observed (Figure 1a). This is likely due to sequence differences in the amplified region between Xan159 and OAC 95-4.

Source-specific markers were designed that can be used to screen material for either HR67- or OAC 95-4-derived CBB tolerance. A third marker was designed to target either source. A fluorescent, intercalating dye, such as SYBR green, can be included in assays using these markers so that results can be interpreted using real-time PCR without the need for an expensive TaqMan probe.

MATERIALS AND METHODS

The SU91 SCAR marker primers were used to amplify the 669 bp amplicon from lines HR67 and OAC Rex. Amplicons were gel purified and sequenced. Three pairs of primers were designed; one pair specific for the HR67 source of CBB tolerance, one pair specific for the OAC 95-4 source, and one pair that can be used for both sources. The product sizes were designed to be within the ideal range recommended for real-time PCR.

Real-time PCR was done in 25 µl reactions containing 50 ng genomic DNA, 0.3 µM forward primer, 0.3 µM reverse primer, 12.5 µl of Maxima® SYBR Green/ROX qPCR Master Mix, and sterile water to final volume. PCR thermocycling profiles consisted of an initial denaturation of 15 min at 95°C, followed by 40 cycles of 20 s at 95°C, 40 s at 55°C, and 40 s at 72°C. All PCR was run and analyzed on a StepOnePlus Real-Time PCR System (Applied Biosystems).

RESULTS AND DISCUSSION

Source-specific markers were designed around the several SNPs and single 8 bp indel found between the sequences of the HR67- and OAC Rex-derived SU91 amplicons. Figure 1b and 1c show the amplification curves produced by each of the source-specific markers when tested on DNA from lines HR67, OAC Rex, and negative control line CDC Pintium using real-time PCR. Figure 1d shows the amplification curve of a third primer designed to amplify both sources. All three markers accurately amplify only the predicted targets. Should a real-time PCR machine not be available, the regular PCR products can be run out on a gel, stained and scored as usual.

Using the new markers reported here to screen breeding material should help reduce time (no need for gels) and cost (no need for a specific fluorescent probe like TaqMan). They will also allow one to distinguish between these two different sources of CBB tolerance.

Table 1: Primer sequences of new markers.

Marker	Forward Primer	Reverse Primer	Band Size
SU91-HR67	GGTTGCATAGTGTCCAACAA	GATTACTTCTTCTGCCAACCA	185 bp
SU91-OAC Rex	GTGTGTTTAATATAGGTTGCATACAA	GATTACTTCTTCTGCCAACCA	191 bp
SU91-Universal	CTCTTTTATCCCTCCTTTGTGT	GATTACTTCTTCTGCCAACCA	217 bp

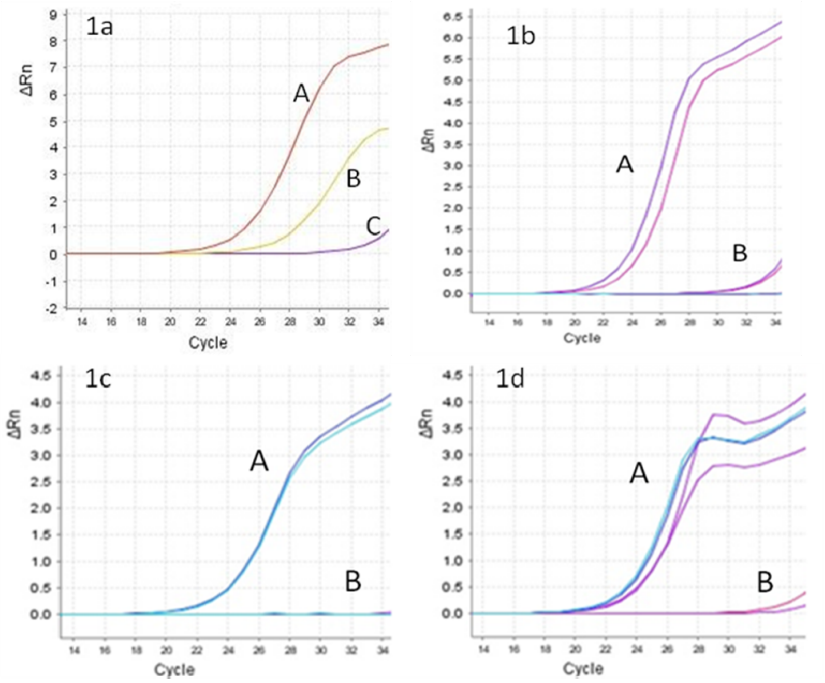

Figure 1: Amplification plots. 1a. SU91 SCAR marker screened against 1 rep of HR67 (A), OAC Rex (B) and CDC Pintium (C). 1b. SU91-HR67 marker screened against 2 reps of lines HR67 (A), OAC Rex and CDC Pintium (B). 1c. SU91-OAC Rex marker screened against 2 reps of lines OAC Rex (A), HR67 and CDC Pintium (B). 1d. SU91-Universal marker screened against 2 reps of lines HR67, OAC Rex (A) and CDC Pintium (B).

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IDENTIFICATION OF QTL FOR RESISTANCE TO BACTERIAL WILT IN DRY BEAN

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INTRODUCTION: Bacterial wilt, caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (*Cff*) is a seed-borne disease of dry bean. It is also a quarantine disease in many countries. A resurgence of bacterial wilt disease in dry bean was observed in some bean growing regions including North Dakota, Colorado, Nebraska, Wyoming and Alberta (Huang et al. 2009). Yellow, orange, purple and pink variants of the bacterial wilt pathogen have been reported (Huang et al. 2006; Harveson and Vidaver 2008), although, yellow variant is most commonly observed in dry bean. Resistance to bacterial wilt has been observed in most market classes of dry bean (Hsieh et al. 2005; Conner et al. 2008). However, few cultivars in the navy bean market class have resistance to bacterial wilt. The inoculation techniques used to identify bacterial wilt resistant lines include inoculating cotyledonary node, petiole and seed. The above disease screening techniques are time consuming (two to four weeks from inoculation to disease rating) and often inconsistent due to the confounding effect of inoculation with seedling growth and subsequent disease ratings. Development of bacterial wilt resistant cultivars is the most effective strategy to control this disease, and identification of molecular markers linked to bacterial wilt resistance will improve the efficiency of the selection process. The objective of this study was to evaluate RILs of two mapping populations to identity QTL for bacterial wilt resistance.

MATERIALS AND METHODS: Dry bean genotypes previously used as parents to develop mapping populations, and disease resistant and susceptible check cultivars were screened for resistance to yellow and orange variants of *Cff* using the hilum injury/seed inoculation method (Hsieh et al. 2005). Twenty seeds per genotype per replication were inoculated with yellow or orange variant of *Cff*. The experiment had three replications over time. At 14 days after inoculation, each seedling was rated for disease severity on a 0 to 5 scale where, 0 = no wilt symptoms, 1 = wilt on one of the primary leaves, 2 = wilt on both primary leaves, 3 = wilt on first trifoliolate, 4 = death of seedling after development of primary leaves and 5 = no seedling or death of seedling before development of primary leaves. Based on the results of the above study, 106 RILs from Raven/I9365-31 (R31) and 85 RILs from Aztec/ND88-106-04 (AN) populations were evaluated for resistance to the yellow variant of *Cff* as noted above. These populations were previously populated with 125 and 137 markers.

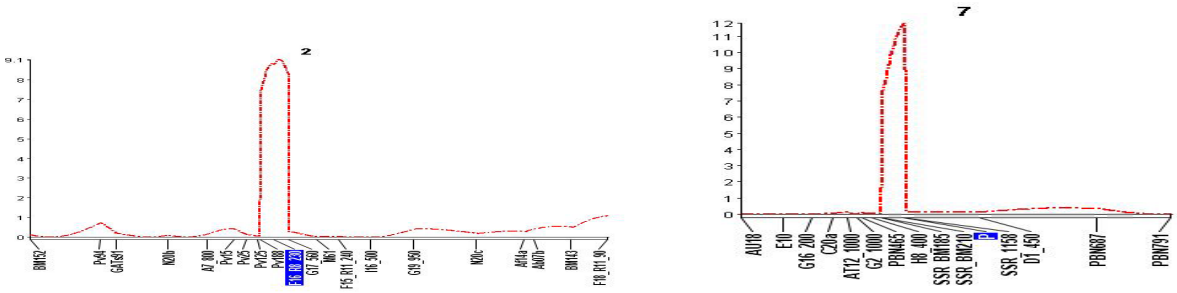
RESULTS AND DISCUSSION: Morden003 check, and Raven and ND88-106-04, parents of the mapping populations were susceptible to both yellow and orange variants of *Cff* (Table 1). Resolute check, and I9365-31 and Aztec parents were resistant to *Cff*. The disease severity of the R31 RILs ranged between 0.3 and 3.7, and that of AN RILs between 0.3 and 5. Although, dry bean genotypes with resistance to *Cff* have been identified, very little is know about the inheritance of resistance to bacterial wilt in dry bean. Earlier investigations indicate the inheritance of resistance to *Cff* in dry bean is either quantitative or qualitative depending on the resistance source (Coyne et al. 1965, 1966). Composite interval mapping revealed two major

QTL for resistance. The QTL in R31 and AN populations each spanned 5cM regions on Pv2 and Pv7, respectively, both near previously identified QTL WM2.2 and WM7.3 for resistance to white mold (Soule et al., 2011). Pv2 explained 30% of the phenotypic variation and Pv7 near the *P* locus for seed color explained 51%. Validation of these QTL is pending.

Table 1. Resistance of select dry bean parental lines and check cultivars to yellow and orange variants of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*.

		Disease Severity (0 to 5)[a]	
Genotypes	Market Class	Yellow Variant	Orange Variant
Morden003 [b]	Navy	2.5	3.1
Resolute [b]	Great northern	0.7	1.2
Raven	Black	2.6	2.7
I9365-31	Black	0.6	1.0
Raven/I9365-31 (RILs)		0.3 to 3.7[c]	Not tested
Aztec	Pinto	0.3	1.3
ND88-106-04	Navy	3.6	4.7
Aztec/ND88-106-04 (RILs)		0.3 to 5.0[c]	Not tested

[a] 0 = no wilt to 5 = no seedlings or death of seedling before development of primary leaves.
[b] Morden003 and Resolute are susceptible and resistant check cultivars, respectively.
[c] Range of disease severity ratings of recombinant inbred lines.



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PHYSIOLOGICAL STUDY OF COMMON BEAN DROUGHT TOLERANCE THROUGH WATER STRESS TRIALS

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INTRODUCTION

In the last years the scarcity of water resources, summed up to global warming, has produced negative consequences upon agriculture activity in the world. In this context, research center worldwide have been developing studies in order to better understand the behavior of species cultivated under these environmental alterations (Stenseth, 2002). In regard to common bean *(Phaseolus vulgaris* L.), water deficiency is considered the second highest common bean yield reducer, being only overcome by disease damaging. In response, breeding programs have been focusing on water stress evaluations, once the sought of cultivars tolerant to drought stress is eminent. Thus, the present work had as objective to evaluate the effect of water stress in photosynthetic pigments of cultivar Pérola and line LP 9728.

MATERIALS AND METHODS

The experiment was composed by the following groups: Pérola/control, Pérola/stress, LP 9728/control and LP 9728/stress). The plants were irrigated by aspersion and submitted to three cycles of water stress. Each cycle lasted 4 full days (96 h), with intervals of 20 days irrigation among cycles. The experimental design used was full randomized with 5 repetitions, being one experimental unit formed by one pot containing one plant. Leaves were collected and foliar tissue was homogenized with 0.1 g $CaCO_3$ + 2 mL cetone 80%. This procedure was conducted under indirect luminosity. After that, this mixture was centrifuged (6,000rpm) for 10 min at 10 °C. The supernatant was collected and measured using a spectrophometer (Lichthenthaler, 1987). The chlorophylls and carotenoids concentration were calculated and expressed in mg g^{-1} MF.

RESULTS AND DISCUSSION

Values observed for control and water stress groups were of 2.24 and 1.80 mg. g MF^{-1}, respectively (Figure 1A). These results revealed that water stress reduced significantly the content of chlorophyll *a* present in Pérola cultivar (19.6%). Whereas, LP 9728 line water stress effects were much milder, demonstrating a less significant influence on the amount of chlorophyll *a*, which values were of 2.43 and 2.28 mg. g MF^{-1} control and water stress groups, respectively. In relation to chlorophyll *b* percentage, water stress propitiated higher reduction in the amount of chlorophyll *b* (Figure 1B) present inPérola cultivar (36.4%). Meanwhile, the reduction of chlorophyll *b* in LP 9728 line showed to be much less intense, 28.2%, resulting in a difference of 8.2% when compared with results obtained in Pérola.

Carotenoids content was also reduced significantly after plants were submitted to water stress (Figure 1C). Pérola cultivar exhibited a carotenoid content of 1.00 and 0.82 mg. g MF^{-1} for the control and stress groups respectively. On the other hand, line LP 9728 showed much smaller losses: 0.73 (control) and 0.48 (stress) mg. g MF^{-1} (Figure 1C). In Figure D, it was observed that water stress condition induced a reduction of 20.35% of Total Chlorophyll Content (TCC) in Pérola cultivar, whereas line LP 9728 demonstrated a TCC reduction of 14.95%.

The reduction of chlorophyll pigments detected in foliar tissue of plants submitted to drought stress (Fig 1A and 1B) is probably related to degradation of carotenoids, since these pigments are indispensable in the mechanism of absorbance and transference of radiant energy, and shield chlorophyll during photooxidation process (Sharma and Hall, 1991).

In conclusion, results demonstrated that line LP 9728 was more tolerant to water stress than Pérola cultivar. This common bean line (LP 9728) could be useful in breeding programs as source of drought stress tolerance.

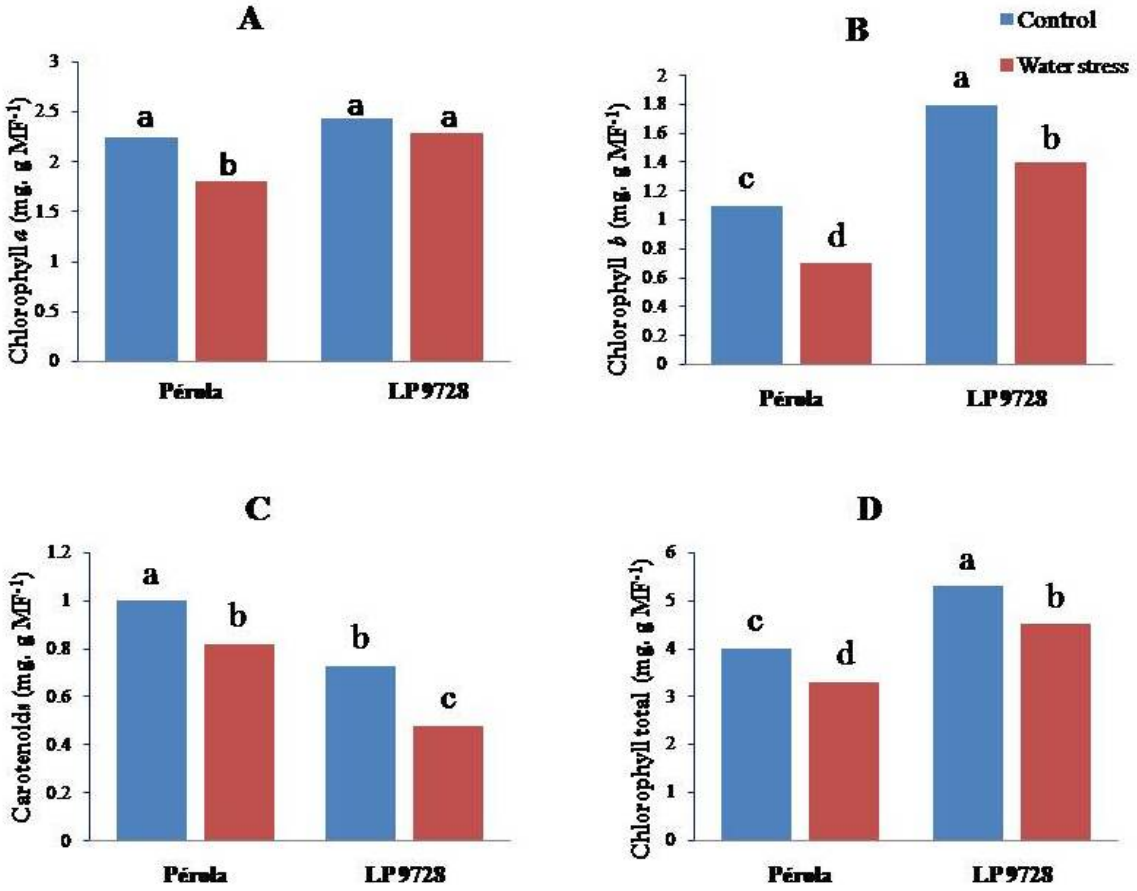

Figure 1. Contents of chlorophylls *a* (**A**), chlorophyll*b* (**B**), carotenoids (**C**) and total chlorophyll (**D**) in plants of *Phaseolus vulgaris* L. (Pérola cultivar and line LP 9728) under **control** condition and **water stress**. Means followed by the same letter do not differ among themselves according to Skott Knott test at 5% probability. The bars represent standard deviations of the means.

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SEQUENCE AND FUNCTIONAL CHARACTERIZATION OF THE DRY BEAN GENE STPP AND ITS RELATIONSHIP TO DROUGHT RESPONSE

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Water is a key element for the growth and development of any plant species, as a reactive in the photosynthesis, as structural element, as transport and as regulator of temperature. During the last years drought stress has become relevant due to a large damage to rainfed crops, superior to any damage caused by other natural meteorological phenomena. On the other hand, a large number of genes related to drought response have been reported in diverse plant species, mainly in *Arabidopsis thaliana*, among them different types of LEA proteins, protein kinases, bZIP proteins, ABA receptors, and transcription factors. In dry bean the gene serin treonin protein phosphatase (PvSTPP) has shown to be a good model due to a strong response under drought stress in field trials.

The induction of PvSTPP was studied in two bean cultivars of contrasting drought response, Pinto Saltillo (PS), tolerant, and Bayo Madero (BM), susceptible; in addition its functional characterization was made by growing the cultivars under salt and heat stress and also by induction with ABA. The intensity of gene PvSTPP expression in response to these stress factors was recorded at different times and along with the speed of expression was considered for the kinetics of the expression (Figure 1). PvSTPP show a clear response under drought stress in roots of Pinto Saltillo, being also stimulated by ABA, high temperature and also when grown in salty soil. Similar results have been reported for protein phosphatases of all kind; for example in rice (*Oryza sativa*) protein phosphatases (OsPPs) have been recorded under drought, salinity and cold stress (Pais *et al*, 2009). Results suggest that PvSTPP could be a regulatory protein induced by osmotic stress and ABA.

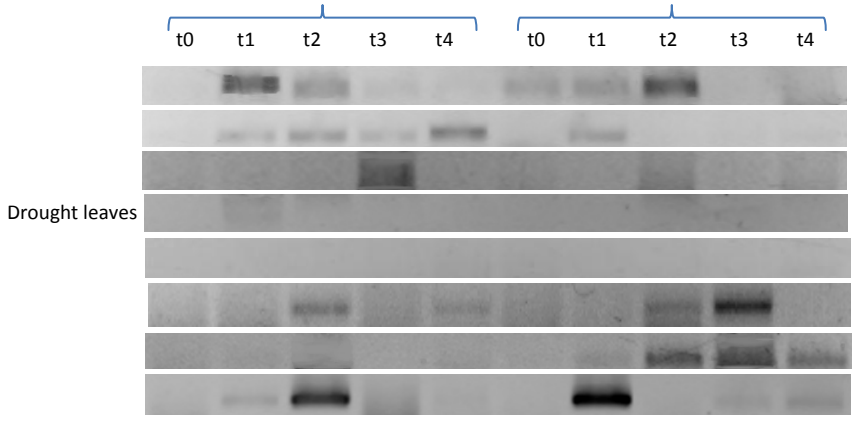

Figure 1. Expression of the gene Serin Treonin Protein Phosphatasa (PvSTPP) in leaves and roots of cultivars Pinto Saltillo (PS) and Bayo Madero (BM).

The DNA sequencing of the gene PvSTPF from the two bean cultivars was carried out by Genome Walking. DNA and cDNA sequences were compared and the results indicated that the cDNA from BM has four modifications with regard to PS, two base changes (SNPs) and two deletions (Figure 2a). One deletion is found within the reading frame and causes a modification in the transcribed protein of BM, thus according to the program PolyPhen the gene function can be damaged. Furthermore, one of the SNPs causes a change from tyrosine to histidine (Figure 2b), whereas the second SNP and the second mutation were of no consequence. The amino acid sequences were aligned with the NCBI database by using the algorithm Megablast. With the program Weblogo the conservation of amino acids in the region corresponding to the modified amino acid was compared with 100 similar sequences, resulting in a rare modification (Pham *et al*, 2010). These results will allow us to develop DNA-based molecular markers to separate Pinto Saltillo from Bayo Madero and to determine its usefulness in selection.

a)

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PvSTPF-BM   1----------------------------------------CCTCTCTCAGCCA-TTCTTCTCGAGCTCCGTGCCCCCATGCGCGTCTGCGGT----51
                                                     ||||||||||||| ||||||||||||||||||||||||||||||||||||||||
PvSTPF-PS   1--GGAGATCCGTCAACTCTGCGTCAATGCCCGCCAAATCTTCCTCTCTCAGCCAATTCTTCTCGAGCTCCGTGCCCCCATGCGCGTCTGCGGT----91

PvSTPF-BM 621----GGATATGAGTTTTTTGCAAAGAGAAGATTAGTCACGATATTTTCTGCTCCGAATCATGGTGGAGAATTTGACAATGCTGG------------701
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
PvSTPF-PS 661----GGATATGAGTTTTTTGCAAAAAGAAGATTAGTCACGATATTTTCTGCTCCGAATTATGGTGGAGAATTTGACAATGCTGG------------741

PvSTPF-BM 946---ATAGTATATTGTAAACTGAACCACCCAGGCAT-CTTCTGTGCCA-----------------------------------------------990
                  ||||||||||||||||||||||||||||||||| |||||||||
PvSTPF-PS 986---ATAGTATATTGTAAACTGAACCACCCAGGCATTCTTCTGTGCAGGAGCAGAATTATTGCTTTATACACCAGTTGGTTGCTTGTGATTGG---1076
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b)

Figure 2. a) Sequence alignment of PS and BM, underlined in red are the differences identified, b) Conserved amino acids. In position 329, pointed out with a red arrow, the exchange of a tyrosine by a histidine is observed, that corresponds to the triplet shown in box a.

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IMPACT OF DROUGHT STRESS ON SOME PHYSIOLOGICAL PARAMETERS OF COMMON BEAN (*PHASEOLUS VULGARIS* L.)

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INTRODUCTION

The phenomenon of drought is very popular in the environment. Especially in summer it is a major factor which reduces the quality of crops and limiting yield (Acosta-Diaz et al., 2009). Common bean (*Phaseolus vulgaris* L.*)* belongs to the plants that need moist soil for proper growth and development. Periodically occurring water deficit in soil often has negative consequences for this plant life processes. Scientific research often move subjects of bean plants resistance to harmful abiotic factors, due to the use of these plants not only in the food industry (because of it's high nutritional value), but also it can be used in medicine, as well as processing (Podleśny 2005).

The aim of the study was to determine the impact of drought stress on certain physiological parameters of common bean cultivars: 'Basta' and 'Erla'.

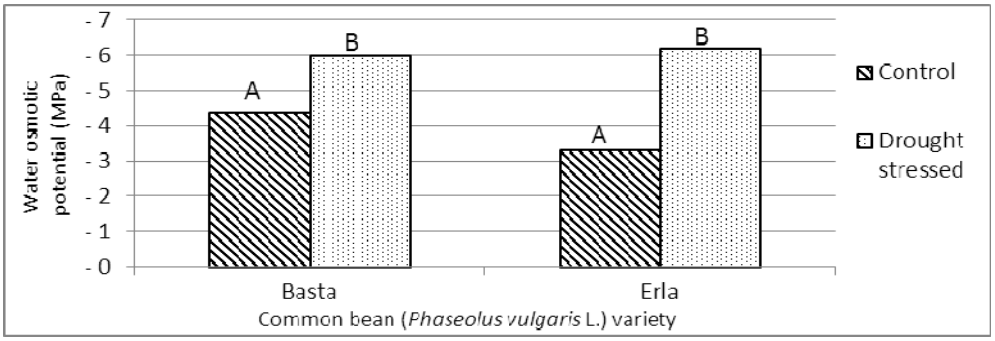
MATERIALS AND METHODS

The experiment was conducted with two (potential drought-tolerant) cultivars of *Phaseolus vulgaris* – 'Basta' and 'Erla'. Plants were grown in pots, arranged in the phytostatic chamber with controlled conditions: light intensity of 300 μmol $m^{-2} \cdot s^{-1}$, temperature 24°C at day, and 18°C at night, photoperiod 14h/10h (day/night) and relative air humidity - 65%. Control plants were grown at soil with watering about 60% of full soil water capacity. Drought stress was introduced by the withholding (after 28 days of sowing) watering plants for a period of 12 days (approximately 20% of full soil water capacity). The measurements were made at the end of stress period on fully matured primary and first trifoliate leaves. Measurements of CO_2 assimilation ($\mu mol \cdot m^{-2} \cdot s^{-1}$), leaf transpiration ($mmol \cdot m^{-2} \cdot s^{-1}$), stomatal conductance ($mol \cdot m^{2} \cdot s^{-1}$) and CO_2 content in the intercellular expanses ($\mu mol\ CO_2 \cdot mol^{-1}$) were carried out using LCA- 4 gas analyzer (ADC company, England). The system was used with the broad leaf chamber PLC4B. The leaf water potential was measured with a pressure chamber EL 540-305 (ELE-International Ltd, England). The results were developed in the program "Statistica 9.1" produced by Statsoft. The Duncan's test was used (at significance level $\alpha = 0.05$) to determine the differences between control and stressed variants.

RESULTS AND DISCUSSION

By analyzing the size of the bean leaf osmotic potential, significant effects of water deficit in the substrate to increase the osmotic potential were observed. There were no significant differences in the values of ratio between the cultivars (Figure 1.). Obtained data is similar to those found in reports, where authors also suggest that, increasing leaf osmotic potential is one of the most important mechanisms of adaptation, for *Phaseolus* plants to survive drought (Yordanov et al. 2000; Zlatev 2005).

Figure 1. Average values of water potential of common bean depending on type of watering (letters above charts = homogenous groups)



Significant influence of the drought stress on the intensity of CO_2 assimilation (photosynthetic rate) of leaves, as well as leaf transpiration rate and stomatal conductance was found. The lowest values of the intensity of the process of assimilation were noted in the stressed plants from cultivar 'Basta' (2,91 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$), while the highest in control from cultivar 'Erla' (Table 1). Significant differences were also observed in the rate of photosynthetic water use (WUE). Plants exposed to drought were characterized by a significantly lower rate. Occurrence of water stress disturbs many life processes of plants, from which most disrupted is water management. With it, in turn, is related nutrient uptake, which leads to the synthesis of cellular organelle dysfunction, as well as damaging the photosynthetic apparatus (Figueirido et al. 2008; Olszewski et al. 2007; Yordanow et al. 2000; Zlatev, Berova 2002).

Table 1. Average values of gas exchange indicators of common bean depending on type of watering (h.g. = homogenous groups)

Cultivar	Watering	Photosynthetic rate (Pn)		Transpiration rate (E)		Stomatal conductance (Gs)		WUE	
		(μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)	h.g.	(mmol $H_2O \cdot m^{-2} \cdot s^{-1}$)	h.g.	(mol$\cdot m^{-2} \cdot s^{-1}$)	h.g.	(Pn/E)	h.g.
Basta	**Control**	9,31	B	0,73	B	0,037	B	12,75	B
	Drought	2,91	A	0,14	A	0,005	A	20,79	A
Erla	**Control**	12,30	C	2,22	C	0,094	C	5,54	C
	Drought	3,09	A	0,10	A	0,002	A	30,90	A

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SCREENING BUSTER/ROZA RILS FOR DROUGHT STRESS RESISTANCE

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Droughtresistance is conditioned by many traits including rooting pattern (Sponchiado et al., 1989; Beebe et al., 2006), capacity to partition a greater proportion of carbohydrate to seed under stress (Rao, 2001), and capacity to set pods and fill seeds under stress (Beebe et al., 2007). Terán & Singh (2002) reported that race Durango germplasm from the semiarid highlands of Mexico possessed the best drought resistance among landrace germplasm, but that even better lines were derived from a double cross combining race Durango and race Mesoamerica. Roza pink bean, derives from a similar interracial cross (Burke 1982), and exhibits drought stress resistance in the Pacific Northwest. Conversely, Buster pinto does poorly under similar drought stress conditions. Both Roza and Buster, performequally well under optimum conditions (Table 1). The objective of the study was to initiate characterization of the differential response to drought between these two parents.

MATERIALS AND METHODS

The Buster/Roza $F_{6:8}$ RILs (140), two parents and two checks, were tested at two locations in 2011: Othello, WA, and Scottsbluff, NE.Each site planted 144 lines in a split-plot lattice design with two replications and two treatments,drought stress (DS) and non-stress (NS). Both treatments were furrow irrigated on a regular watering schedule until flowering; thus, simulating terminal drought stress, as thereafter water was applied to NS treatment only. Soil water content data was collected at seven depths (6 inch intervals):3X during the growing season: at field capacity at flowering, mid-pod fill, and physiological maturity. Yield reduction, geometric mean, drought intensity index, and drought stress index were calculated.Data were analyzed using SAS (Proc Mixed).

RESULTS AND DISCUSSION

Roza expressed greater drought tolerance in WA than in NE, perhaps because of the higher yield potential exhibited in WA (Table 2). Buster had poor performance in both locations. The yield range of the RILs was 899-4513 $kgha^{-1}$ (DS) and 2713-6804 $kgha^{-1}$ (NS) in WA and 248-1368 $kgha^{-1}$ (DS) and 883-2428 $kgha^{-1}$ (NS) in NE. Of the 140 RILs, there were two that exceeded the geometric mean of Roza at both locations, BR-88 and BR-130 (data not shown). Excluding BR-130 in NE, they also had a lower DSI, indicating potential transgressive segregation for enhanced resistance to drought.

The soil water content (Fig. 1) indicated a variety*time*depth interaction under DS($P<.0001$). Roza was more effective than Buster at extracting water from greater depths. Further studies, including soil moisture data on specific lines in addition to the parents, are planned to further phenotype this RIL population to facilitate subsequent QTL analysis for drought response.

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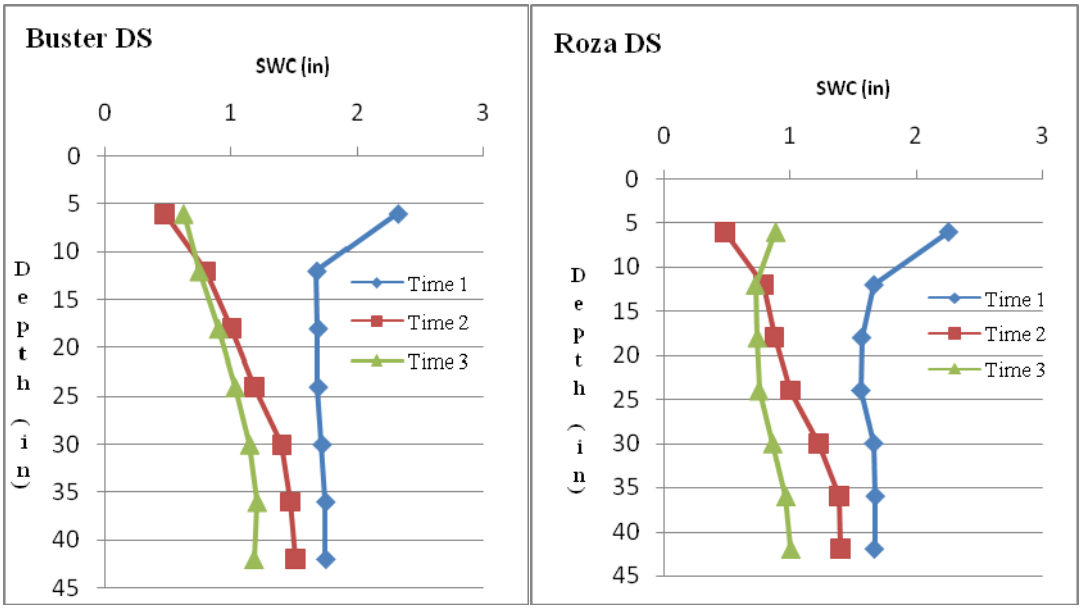
Table 1. Preliminary Data. Mean yield performance (kg ha^{-1}) of Roza pink and Buster pinto under stressed and optimum field conditions for three years in Prosser, WA.

	Drought & low soil fertility			Optimum conditions		
Cultivar	2002	2004	2006	2002	2003	2004
Roza	3490	4390	4000	5060	4125	4470
Buster	550	1790	190	4750	4290	4610

Table 2. Mean yield under drought (DS) and non-stress (NS), geometric , mean (GM), % yield reduction (PR), and drought stress index (DSI) in WA and NE in 2011.

Othello, WA (DII = 0.53)					
Line	NS	DS	GM	PR	DSI
	kg ha^{-1}			%	
Roza	5738	4172	4893	27.3	0.5
Buster	5173	2116	3309	59.1	1.2
Mean of RILs	5197	2773	-	-	-
Scottsbluff, NE (DII = 0.52)					
Roza	2218	1073	1543	51.6	1.0
Buster	1745	753	1147	56.8	1.1
Mean of RILs	1722	818	-	-	-

Figure 1. Soil water content (SWC) for Buster and Roza under drought stress (DS) at 7 depths and 3 time intervals in WA in 2011.



ION SELECTIVE TRANSPORTATION AND THEIR ROLE IN OSMOTIC ADJUSTMENT IN SALINISED *PHASEOLUS* SPECIES

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Osmotic adjustment (OA) is considered to be an important component of salt tolerance mechanisms in plants; it is usually defined as a decrease in the cell sap osmotic potential resulting from an increase in intracellular osmolytes to prevent the loss of cell water (Navarro *et al*., 2007). The intracellular osmolytes include inorganic ions and organic solutes (Hauser and Horie, 2010). The inorganic ions, which mainly include K^+, Na^+, and Cl^- are low-cost (a little energy and carbon), but it will bring ionic toxicity when plants accumulate excess inorganic ions, especially at high NaCl concentration (Hauser and Horie, 2010). The study investigated the accumulation of inorganic osmolytes and their role in osmotic adjustment in NaCl-stressed *Phaseolus* species.

MATERIALS AND METHODS

Two wild and two cultivated species of *Phaseolus* were used in this study: *P. vulgaris* PI325687, a wild type (PvW); *P.acutifolius* G40169 (PaW), a wild type (WS); *P. vulgaris*G04017, a cultivated type (PvC); and *P.acutifolius* G40142, a cultivated type (PaC). Plants were grown in nutrient solution under greenhouse conditions. Seedlings were allowed to grow with no salinity stress until the emergence of the first trifoliate leaf, when several NaCl treatments were added to the solutions (0, 30, 60 and 90 mM). A randomized complete block design with a split-plot arrangement of salt treatments and six replications was used. Predawn water potential (Ψ_w) of a whole leaf was measured with a pressure chamber (Model 3000, Soilmoisture, Santa Barbara, CA) (Scholander *et al.* 1965). Osmotic potential (Ψ_π) was estimated according to the method of Alves and Setter (2004) with 80% water content in the control plants. The osmotic adjustment (OA) was calculated as the difference in osmotic potentials between salt-stresses and control plants (Alves and Setter, 2004). Estimation of the ability of ion selective transportation $S_{K+/Na+,}$was calculated from the following formula: $S_{K+/Na+}$= (K^+/Na^+ in leaf)/ (K^+/Na^+ in root).

RESULTS AND DISCUSSION

In general, the osmotic adjustment can help the NaCl-stressed plant to maintain the water balance and cell growth, alleviate the salt injury, and improve its salt tolerance (Hauser and Horie, 2010). In our work, when compared with the control, osmotic potential decreased (Fig. 1B) and osmotic adjustment was significantly enhanced in leaves at 60 and 90 mM NaCl treatments for 20 days (Fig. 1C). The different effects of OA on the alleviation of salt injury in *Phaseolus* species were found under stresses with various NaCl concentrations. Firstly, the PvW, PvC, and PaC can basically maintain water potential under 30 and 60 mM NaCl treatments (thought to be moderate saline level), but they significantly decreased under 90 mM NaCl (severe stress), except in PaW (Fig. 1A). Secondly, the increase in Na^+ in roots and Cl^- in leaves and decrease in K^+ in roots and leaves of *P. vulgaris* and *P. acutifolius* treated with 90 mM NaCl were the most obvious when compared with those under 60 mM NaCl treatment (See Bayuelo-Jiménez *et al*., 2009). Thus, the plants treated with 90 mM NaCl were most easily subjected to

nutritional deficit, ionic imbalance, and toxicity. Thirdly, the values of $S_{K+/Na+}$describe the ability of remaining Na^+ in roots, K^+ entering into leaves, and their higher values mean the stronger ability of the above selective ion transportation (Pitman, 1984). The values $S_{K+/Na+}$all significantly increased in PvC and PaW under 60 and 90 mM NaCl, but decreased under 90 mM NaCl in PvW and PaC, when compared with the control (Fig. 1D). This imply that the *Phaseolus* species could retain more Na^+ in roots, and selectively transport more K^+ into leaves under moderate salt treatment (60 mM NaCl), but this ability was limited by severe salt stress (90 mM NaCl). The maintenance of higher leaf K^+ concentrations in salt-tolerant *Phaseolus* species could be, by far, one of the most important mechanisms underlying superior salt tolerance as reported in other species (Hauser and Horie, 2010).

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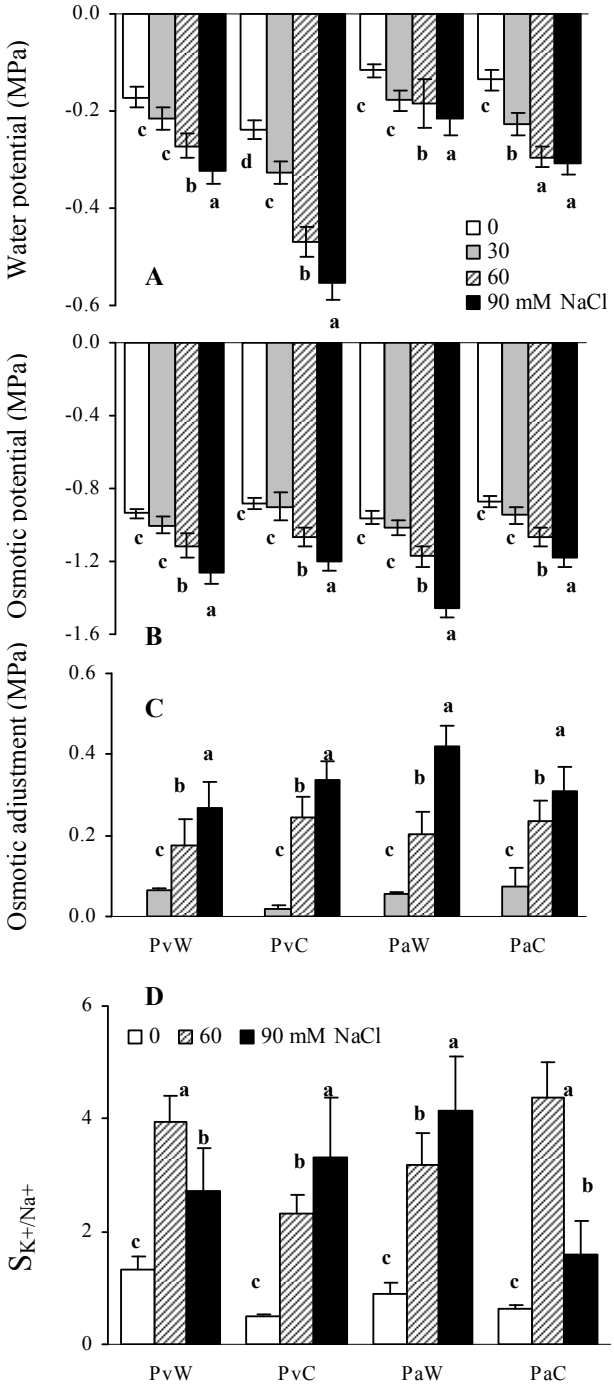

Figure 1. Changes in the water potential, osmotic potential, osmotic adjustment, and the values of $S_{K+/Na+}$of *Phaseolus* species following a 20-day salinization period. Eachvalue represents the mean ± SE of six replicates. Bars with the same letter are not significantly different according to ANOVA post hoc test ($P \leq 0.05$)

QTL FOR DROUGHT AND HEAT STRESS ADAPTATION IN THE A55 X G122 CROSS

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INTRODUCTION. In the tropics, high temperature or drought conditions can occur at various times of the year primarily depending on when the rainy or dry seasons come and on elevation or rain shadow effects. Although beans can support adverse conditions of lower than expected rainfall or high temperatures over short periods, prolonged periods cause irreversible damage and common bean is considered to be a drought susceptible and heat sensitive crop. Meanwhile, bean architecture is in part determined by the determinacy gene *fin* which can affect yield potential and adaptation to various stress environments. The goal of this research was to identify quantitative trait loci (QTL) for architectural, phenological and yield or yield component traits based on the development of a microsatellite (SSR) based genetic map for an inter-genepool population derived from a cross between a Mesoamerican genotype with erect architecture (A55) and an Andean genotype with heat tolerance (G122). The population was evaluated in four experiments conducted in a tropical location across two rainy seasons and across dry season drought stress and dry season irrigated treatments.

MATERIALS AND METHODS. *Plant Material and Field Experiments:* We grew an inter-genepool, F_8 recombinant inbred line (RIL) population derived from the cross A55 x G122. The parental lines were also included in the experiments and differed in that the Andean genotype G122 is a source of heat tolerance according to Shonnard and Gepts (1994) and the Mesoamerican genotype A55 is a source of erect architecture and good yield potential (Singh et al. 2003). The population was planted across four environments in Palmira, Colombia (4 ºS latitude, 1000 m above sea level altitude, 24º C mean annual temperature, 1000 mm annual rainfall) half in rainy seasons and half in drought. All experiments except for the unreplicated first rainy season planting consisted of randomized complete block designs with 3 repetitions each.

Genetic Mapping and QTL analysis: DNA was extracted from RILs and parents to use as templates in screening of 144 microsatellites from various sources. Any polymorphic markers that were found to be polymorphic in the parents were amplified for the entire A55 x G122 population. Phenotypic data from the randomized complete block experiments were analyzed with an analysis of variance conducted with a generalized linear model Genotypic data was processed with MAPMAKER version 3.0. Microsatellite marker placement was according to previous mapping by Blair et al. (2003) and the genetic map was established with minimum LOD of 3.0. QTL analysis was conducted using QTL Cartographer version 2.5, with composite interval mapping (CIM).

RESULTS AND DISCUSSION. *Map construction:* A total of 71 SSR loci and 246 AFLP, RAPD, seed protein or phenotypic markers were integrated together into a genetic map with a total distance of 982.8 cM. A total of 12 linkage groups were identified, 11 of which corresponded to numbered chromosomes from the common bean genome which could be aligned to the reference maps described in Blair et al. (2003) through a comparative analysis of SSR

locus placement. The total distance of the map was smaller than the reference maps presented in that publication, perhaps due to lower recombination or differential marker coverage. One linkage group (ag12) had only AFLP markers and was un-anchored.

QTL identification: A total of 51 QTL were identified based on the evaluation of 10 phenotypic variables related to stress tolerance. Of these, 4 were found for the first rainy season, 13 were found for the second rainy season and 34 were found for the dry season treatments (21 under drought stress and 13 under non-drought conditions). At least one QTL appeared on each linkage group, except for linkage groups b05, b08 and b09. In terms of QTL distribution, one observation was that more QTL were associated with SSR markers than with AFLP markers despite the lower number of the former marker type. This is perhaps due to a better distribution of SSR among the gene-coding regions of the genome, compared to AFLPs which may cluster in non-coding regions of the genome. The A55 parent was high yielding under all conditions, whereas, G122 was found to be resistant to high temperatures, but not to drought. Some QTL for yield and even seed weight were associated with erect growth habit and were located on linkage group b01 near the *fin* locus while other QTL for seed weight were found across the genome (Table 1).

Table 1. Quantitative trait loci associated yield and seed size, in A55 x G122 recombinant inbred line population in the first rainy season (RS1), the second rainy season (RS2), the dry-season, irrigated (DS-I) and the dry-season, drought stress (DS-DS) environments.

Trait	QTL	Env.†	LG	Closest Marker	Source	Effect	Significance‡		
							LR	R2	TR2
Yield (kg/ha)	*Yld1.1*	RS1	1	14U	A55	1.20	20.17	0.24	0.43
	Yld4.1	DS-I	4	4L	A55	124.6	16.62	0.17	0.48
	Yld11.1	RS1	11	15K	A55	165.69	15.9	0.15	0.52
	Yld11.2	DS-I	11	ATA6	A55	127.92	15.63	0.16	0.47
	Yld11.3	DS-DS	11	12K	A55	81.71	13.58	0.13	0.51
100 seed weight	*Sw1.1*	DS-DS	1	2B	A55	1.62	22.71	0.19	0.6
	Sw6.1	RS1	6	BM187	G122	-2.23	35.07	0.26	0.69
	Sw6.2	RS2	6	BM187	G122	-1.99	39.24	0.2	0.86
	Sw6.3	DS-DS	6	BM187	G122	-1.42	21.9	0.16	0.73
	Sw7.1	RS1	7	BM185	G122	-1.85	26.74	0.18	0.69
	Sw7.2	RS2	7	*Phs*	G122	-2.58	47.25	0.35	0.79
	Sw7.3	DS-DS	7	BM210	G122	-1.46	22.35	0.17	0.75
	Sw11.1	RS2	11	4B	G122	-1.74	25.51	0.11	0.81
	Sw12.1	RS2	12	11P	A55	1.43	21.41	0.09	0.81

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MOLECULAR TRANSCRIPTIONAL ACTIVITY OF TREHALOSE-6-PHOSPHATE SYNTHASE REVEALS ITS IMPLICATION ON STRESS TOLERANCE IN COMMON BEANS

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Extreme environmental conditions including salinity and high temperatures strongly affect grain production in common bean (*Phaseolus vulgaris*). Drought is the main factor that affects yields and result in serious economic losses (1). Trehalose is an important disaccharide widely distributed in plants. Several studies have demonstrated that a main biological function is to serve as a signaling molecule in the processes of tolerance to cold, heat and dehydration, osmotic and oxidative stress, etc. In common bean some researchers have shown that trehalose is implicated on stress tolerance, nevertheless the main function and genetic mechanisms in the process remain unknown (2). In order to know the possible role of trehalose on *P. vulgaris* under stress conditions, the main objective of this research was to analyze the molecular transcriptional activity (RT-PCR) of trehalose-6-phosphate-synthase enzyme (T6PS) on tolerant (BAT 477) and susceptible (Pinto UI-114) common bean genotypes grown under controlled conditions.

Two contrasted common bean cultivars (BAT 477, resistant; Pinto UI-114, susceptible) to water deficits were grown under controlled conditions and two humidity levels: irrigated plants and water-stressed plants during vegetative phase. Humidity treatments as well as experimental conditions were previously described (4). The plant material (100 mg) was soaked on nitrogen liquid. RNA was purified using the commercial reactive TRIzol® reagent (Invitrogen) (http://tools.invitrogen.com/content/sfs/manuals/15596018%20pps%20Trizol%20Reagent%20061207.pdf) and after treated with Dnase I amplification grade. For the cDNA synthesis it was used 200 ng of RNA using the commercial kit Scrip II RNase H reverse transcriptase (Invotrogen, USA, 2010) and using a dT-oligo. The reverse transcription was performed under next conditions: initial incubation to 42 ºC for 2 min, addition of 1 µL of reverse transcriptase enzyme, second incubation to 42 ºC by 50 min for polymerization, third incubation to 70 ºC for 15 min to inactivate the enzyme and finally, permanent conditions to 4 ºC. For T6PS gene transcript quantification we used specific primers TPS2F and TPS2R (Table 1). The PCR conditions employed 2 µL of RT reaction; 45 µL of mix reaction containing 5 µL of PCR buffer 10X and 25 µL of $MgCl_2$; 1 µL of dNTP's 10 mM; 2 ng μL^{-1} of each primer; 0.2 µL of Taq DNA polymerase and water. The cycles employed were: 95 °C for 2 min, from 20 a 30 cycles of 95 °C for 30 s, 52 °C for 30 s and 72 °C for 1 min, and finally 72 °C for 7 min. The internal control used was the actin gene which primers sequences were: b-ActF 5'CCA-AGG-CCA-ACC-GCG-GA-AGA-TGA-C 3' b-ActR 5' AGG-GTA-CAT-GGT-GGT-GCC-GCC-AGA-C 3' (human β-actin, Titan One Tube RT-PCR Kit, Roche, USA). The extracted bands were quantified according with the intensity at 260 nm. These values were graphed in order to obtain a numerical value corresponding to the different levels of transcriptional expression at several times of each culture. The data obtained were normalized using the formula (V1-V2/V2) using Excell, where V1 correspond to band quantification on TPS gene and V2 to β-actin gene.

Results showed that in susceptible lines the T6PS expression levels increased when the plant was subjected to drought stress. These results are agreed with previous reports (3, 5). In

contrast, the tolerant line BAT 477 (Fig. 1) showed a considerable decrease in T6PS expression levels under stress conditions. Previous reports suggested that trehalose levels were increased when plants are subjected to stress; nevertheless, these results suggest that tolerance mechanism in BAT 477 is independent of those mediated by trehalose. The knowledge of mechanisms for stress tolerance is a major challenge for agricultural biotechnology. For this reason, future researches will be directed towards to the identification and characterization of molecular mechanisms that underlie drought stress tolerance in common beans.

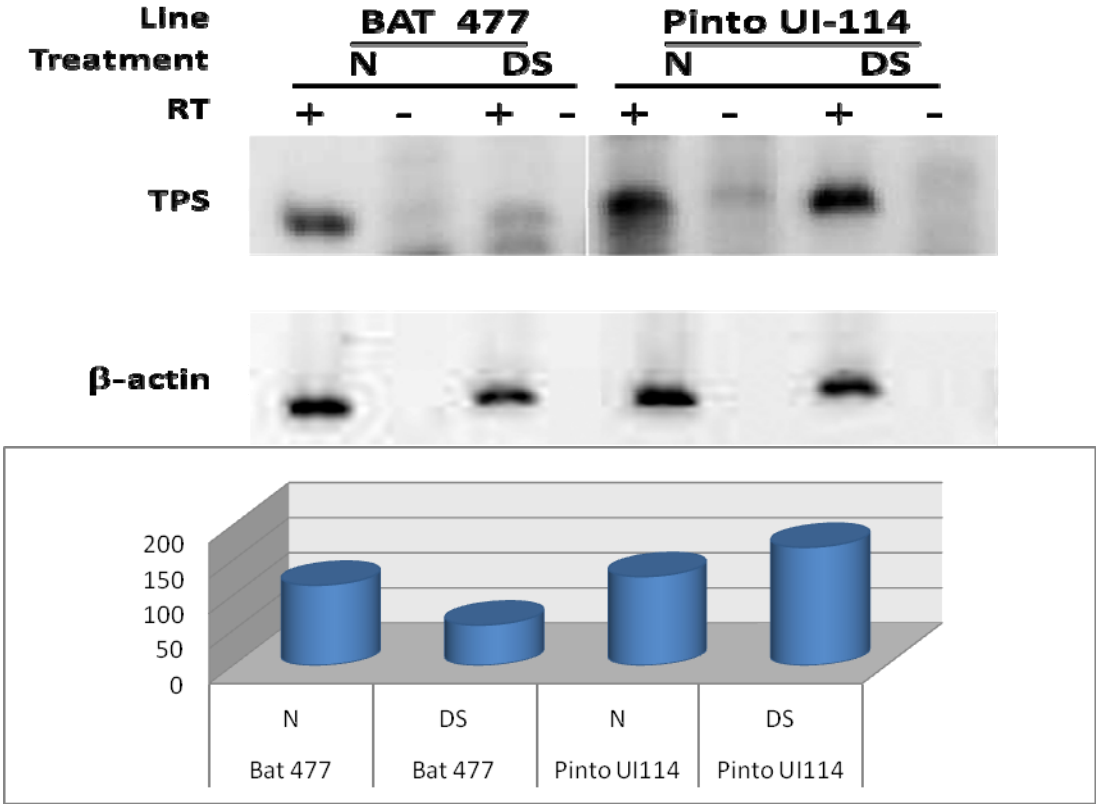

Figure 1. Expression levels of trehalose-6-phosphate synthetase (T6PS) in *P. vulgaris*. N= Irrigated, DS= drought stressed; RT= reverse transcriptase; + or – enzyme presence or absence, respectively. Upper figure: electrophoresis gel; lower figure: normalized level expression values.

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FEEDING PREFERENCE OF *ANTICARSIA GEMMATALIS* (HÜBNER) (LEPIDOPTERA: NOCTUIDAE) IN GREEN BEAN GENOTYPES

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INTRODUCTION

Anticarsia gemmatalis Hübner (Lepidoptera: Noctuidae) is a severe defoliating insect of tropical and subtropical climate widely distributed, occurring from the United States to Argentina (Ford et al., 1975). In Brazil, this lepidopterous is popularly known as velvetbean caterpillar and it may cause economical losses to peanut, alfalfa and bean crops (Gallo et al., 2002).

The aim of this work was to evaluate the non-preference for feeding of *A. gemmatalis* larvae to green bean genotypes, under laboratory conditions.

MATERIALS AND METHODS

The experiment was carried out at Faculdade de Ciências Agrárias e Veterinárias – UNESP, in the Departamento de Fitossanidade, Laboratório de Resistência de Plantas a Insetos, in Jaboticabal, São Paulo State, Brazil, at the temperature of 25 ± 1ºC, RH of 70 ± 10% and photophase of 12h. The following green bean genotypes were used: Favorito, HX10093000, Itatiba II, UEG 19, UEG 11, UEG 15 and UEG 13. Free-choice and non-choice non-preference for feeding tests were performed, where in both, leaflets of the green genotypes plants with approximately 25 days-old were collected in a greenhouse and then leaf discs of 2.5 cm in diameter were prepared.

In free-choice test, leaf discs of the genotypes were placed in Petri dishes of 14.0 cm in diameter with filter paper softly moistened with distilled water at the bottom where one eight days-old caterpillar of *A. gemmatalis* per genotype was released in the center of the plate. For non-choice test, one leaf disc per Petri dish of 9.0 cm in diameter was used where one caterpillar of the same age per plate was released. In both tests the attractiveness of the caterpillars was evaluated at 1, 3, 5, 10, 15 and 30 minutes and at 1, 2, 6, 12, 24 and 45 hours after releasing. Leaf area consumed (LAC) was also assessed after the closure of the tests through an electronic leaf area measurer device, model LI-COR 3100. Randomized blocks design and completely randomized design was used for free-choice and non-choice tests, respectively, both with 10 replications. Data were transformed in $(x + 0.5)^{1/2}$, submitted to the analysis of variance (ANOVA) by F test and the means compared to Tukey test, at 5% probability.

RESULTS AND DISCUSSION

In free-choice and non-choice non-preference for feeding tests of *A. gemmatalis*, no significant differences were observed in the attractiveness of the caterpillars among the genotypes in any of the evaluated times (Tables 1 and 2).

However, leaf area consumed differed significantly among the green bean genotypes in both tests (Tables 1 and 2). In free-choice test, the genotypes Itatiba II (0.31 cm^2) and UEG 15 (0.18 cm^2) showed the least leaf area consumed, differing from Favorito (1.57 cm2) and HX10093000 (1.41 cm^2) (Table 1). In non-choice test, HX10093000 maintained with the highest

leaf area consumed, 2.30 cm^2, differing from the other green bean genotypes, where UEG 15 and UEG 13 stood up numerically with the lowest leaf area consumed (Table 2).

We concluded the genotypes UEG 15 and Itatiba II were the least preferred for feeding in free-choice and non-choice tests, wheareas the genotype HX10093000 was highly susceptible to *A. gemmatalis*.

Table 1. Number of *Anticarsia gemmatalis* larvae attracted to green bean genotypes in different times and leaf area consumed (LAC), in free-choice test. Jaboticabal, SP, Brazil, 2012.

Genotypes (G)	Minutes						Hours						LAC (cm²)
	1	3	5	10	15	30	1	2	6	12	24	45	
Favorito	0,3 a	0,3 a	0,4 a	0,3 a	0,5 a	0,4 a	0,3 a	0,4 a	0,7 a	0,5 a	0,8 a	0,2 a	1,57 c
Hx10093000	0,4 a	0,4 a	0,3 a	0,6 a	0,4 a	0,4 a	0,5 a	0,4 a	0,3 a	0,3 a	0,1 a	0,3 a	1,41 bc
Itatiba II	0,2 a	0,2 a	0,1 a	0,0 a	0,2 a	0,3 a	0,2 a	0,2 a	0,2 a	0,4 a	0,4 a	0,2 a	0,31 a
UEG 19	0,5 a	0,5 a	0,4 a	0,4 a	0,4 a	0,5 a	0,4 a	0,3 a	0,2 a	0,4 a	0,1 a	0,3 a	0,76 abc
UEG 11	1,0 a	0,8 a	0,7 a	0,6 a	0,3 a	0,4 a	0,4 a	0,3 a	0,2 a	0,5 a	0,5 a	0,6 a	0,60 ab
UEG 15	0,5 a	0,5 a	0,5 a	0,5 a	0,7 a	0,6 a	0,6 a	0,3 a	0,5 a	0,3 a	0,5 a	0,3 a	0,18 a
UEG 13	0,2 a	0,2 a	0,2 a	0,2 a	0,2 a	0,2 a	0,3 a	0,4 a	0,5 a	0,2 a	0,6 a	0,4 a	0,68 ab
F (G)	$1,80^{NS}$	$1,05^{NS}$	$0,92^{NS}$	$1,66^{NS}$	$0,80^{NS}$	$0,32^{NS}$	$0,34^{NS}$	$0,16^{NS}$	$1,17^{NS}$	$0,37^{NS}$	$1,62^{NS}$	$0,66^{NS}$	7,08*
C.V. (%)	32,38	32,77	33,19	31,29	32,42	35,71	34,01	32,07	31,57	33,11	33,18	29,36	23,59

[1]Means followed by the same letter in column do not differ significantly by Tukey test, at the level of 5% probability. For analysis, data were transformed in (x + 0.5) ½.

Table 2. Number of *Anticarsia gemmatalis* larvae attracted to green bean genotypes in different times and leaf area consumed (LAC), in non-choice test. Jaboticabal, SP, Brazil, 2012.

Genotypes (G)	Minutes						Hours						LAC (cm²)
	1	3	5	10	15	30	1	2	6	12	24	45	
Favorito	0,3 a	0,3 a	0,0 a	0,2 a	0,2 a	0,1 a	0,3 a	0,3 a	0,2 a	0,4 a	0,1 a	0,2 a	0,83 a
Hx1093000	0,5 a	0,5 a	0,5 a	0,4 a	0,3 a	0,3 a	0,3 a	0,4 a	0,1 a	0,1 a	0,1 a	0,2 a	2,30 b
Itatiba II	0,2 a	0,4 a	0,4 a	0,2 a	0,1 a	0,1 a	0,2 a	0,2 a	0,2 a	0,1 a	0,2 a	0,4 a	1,21 a
UEG 19	0,4 a	0,2 a	0,3 a	0,4 a	0,4 a	0,4 a	0,3 a	0,3 a	0,1 a	0,2 a	0,1 a	0,2 a	0,86 a
UEG 11	0,2 a	0,2 a	0,0 a	0,0 a	0,3 a	0,0 a	0,2 a	0,1 a	0,1 a	0,2 a	0,3 a	0,3 a	0,52 a
UEG 15	0,3 a	0,1 a	0,1 a	0,1 a	0,1 a	0,1 a	0,1 a	0,3 a	0,1 a	0,1 a	0,1 a	0,0 a	0,48 a
UEG 13	0,4 a	0,4 a	0,3 a	0,3 a	0,3 a	0,3 a	0,2 a	0,2 a	0,3 a	0,2 a	0,2 a	0,2 a	0,46 a
F (G)	$0,53^{NS}$	$0,93^{NS}$	$2,46^{NS}$	$1,28^{NS}$	$0,67^{NS}$	$1,28^{NS}$	$0,30^{NS}$	$0,47^{NS}$	$0,44^{NS}$	$0,73^{NS}$	$0,44^{NS}$	$0,85^{NS}$	7,43*
C.V. (%)	28,71	27,79	24,98	26,20	27,24	25,83	27,37	27,77	24,68	25,55	24,68	26,32	24,60

[1]Means followed by the same letter in column do not differ significantly by Tukey test, at the level of 5% probability. For analysis, data were transformed in (x + 0.5) ½.

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FEEDING OF *ANTICARSIA GEMMATALIS* (HÜBNER) (LEPIDOPTERA: NOCTUIDAE) IN BEAN GENOTYPES

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INTRODUCTION

The velvetbean caterpillar *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Noctuidae) is a species of tropical and subtropical climate, occurring from the south of the United States to Argentina (King & Saunders, 1984). This insect is considered the major pest of soybean crops in the United States, Mexico, Colombia, Venezuela, Argentina (Panizzi & Correa-Ferreira, 1997) and Brazil (Di Oliveira et al., 2010), and may also have economical importance to common bean, peanut and alfalfa crops (Gallo et al., 2002).

The aim of this work was to evaluate the non-preference for feeding of *Anticarsia gemmatalis* larvae in bean genotypes, in laboratory.

MATERIALS AND METHODS

The assay was carried out in Laboratório de Resistência de Plantas a Insetos of Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, SP State, Brazil, under controlled conditions. The following bean genotypes were used: Pérola, RAZ 49, BRS Supremo, IAC Carioca-Tybatã, IAC Galante, IAC Diplomata, IAC Harmonia, IAPAR 81, IAC Una e IAC Carioca-Eté. Free-choice and non-choice non-preference for feeding tests were performed. In both tests leaflets of 20 days-old plants of the bean genotypes were collected in a greenhouse and through a punch, leaf discs of 2.5 cm diameter were prepared.

In free-choice test, leaf discs were disposed in Petri dishes of 14.0 cm diameter with filter paper softly moistened with distilled water at the bottom, and then one eight days-old caterpillar of *A. gemmatalis* per genotype was released. For non-choice test one leaf disc per Petri dish of 9.0 cm diameter was used, where one caterpillar of the same age was transferred per plate. In both tests the attractiveness of the insects was evaluated at 1, 3, 5, 10, 15, 30, 60, 120, 360, 720, 1440 and 1880 minutes after releasing. Leaf area consumed (LAC) was also measured through an electronic leaf area measurer device, model LI-COR 3100. Randomized blocks and entirely randomized blocks design were used for free-choice and non-choice tests, respectively, both with 10 replications. Data were transformed in $(x + 0.5)^{1/2}$, submitted to the analyses of variance (ANOVA) and means were separated with Tukey test, at 5% probability.

RESULTS AND DISCUSSION

From the results obtained in free-choice test, there were significant difference in the attractiveness of the larvae among the genotypes in almost all the assessed times, except at 1440 and 1880 minutes after releasing. In general, in these periods of times IAC Harmonia was the most attractive whereas the other genotypes were equally the least attractive to the insects (Table 1). However, the leaf area consumed did not differ statistically among the bean genotypes (Table 1). In non-choice test the genotypes differed significantly among themselves only at three and

five minutes, where IAC Una and BRS Supremo were the most attractive, whereas IAC Galante, IAC Harmonia, IAPAR 81 and IAC Carioca Eté were the least attractive to *A. gemmatalis* (Table 2). Regarding the leaf area consumed the genotype IAC Harmonia and IAPAR 81 showed the lowest and highest values, with 0.45 and 1.80 cm^2, respectively (Table 2).

We concluded IAC Harmonia was the least consumed, whereas the other genotypes were susceptible to *A. gemmatalis*, evidenced in non-choice test.

Table 1. Number of *Anticarsia gemmatalis* larvae attracted to bean genotypes at different minutes and leaf area consumed (LAC), in free-choice test. Jaboticabal, SP, Brazil, 2012.

GENOTYPES	1'	3'	5'	10'	15'	30'	60'	120'	360'	720'	1440'	1800'	LAC (cm^2)
Pérola	0,3a	0,3a	0,1a	0,2a	0,2a	0,1a	0,1a	0,2a	0,1a	0,2a	0,6	0,1	0,78
RAZ 49	0,1a	0,1a	0,2a	0,1a	0,2a	0,4a	0,2a	0,3a	0,2a	0,5ab	0,5	0,3	0,67
BRS Supremo	0,3a	0,2a	0,7ab	0,2a	0,4a	0,4a	0,3a	0,6a	0,5a	0,4ab	0,8	0,0	0,98
IAC Carioca Tybatã	0,4a	0,1a	0,2a	0,2a	0,2a	0,2a	0,2a	0,6a	0,2a	0,1a	0,4	0,6	0,72
IAC Galante	0,4a	0,3a	0,5ab	0,5a	0,5a	0,6a	0,4a	0,4a	0,6a	0,40ab	0,5	0,3	0,95
IAC Diplomata	0,7ab	0,4a	0,4ab	0,5a	0,3a	0,3a	0,5a	0,4a	0,6a	0,6ab	0,3	1,0	0,91
IAC Harmonia	1,4b	1,6b	1,5b	2,0b	1,9b	2,0b	2,6b	2,6b	3,2b	1,4b	1,0a	0,8	0,45
IAPAR 81	0,7ab	0,5a	0,2a	0,3a	0,5a	0,3a	0,2a	0,6a	0,3a	0,6ab	0,3	0,6	0,96
IAC Una	0,6ab	0,9ab	0,6ab	0,5a	0,5a	0,6a	0,3a	0,7a	1,0a	0,5ab	0,3	0,4	1,35
IAC Carioca Eté	0,1a	0,1a	0,1a	0,0a	0,0a	0,0a	0,0a	0,3a	0,1a	0,1a	0,1a	0,1	0,44
F	2,92**	5,41**	2,86**	5,41**	5,58**	5,76**	8,82**	5,74**	10,67**	2,20*	$1,28^{ns}$	$2,08^{ns}$	$1,72^{ns}$

Table 2. Number of *Anticarsia gemmatalis* larvae attracted to bean genotypes at different minutes and leaf area consumed (LAC), in non-choice test. Jaboticabal, SP, Brazil, 2012.

GENOTYPES	1'	3'	5'	10'	15'	30'	60'	120'	360'	720'	1440'	1800'	LAC (cm^2)
Pérola	0,2	0,2ab	0,1a	0,1	0,2	0,1	0,0	0,2	0,2	0,4	0,6	0,7	1,32ab
RAZ 49	0,3	0,5ab	0,5ab	0,4	0,5	0,4	0,3	0,4	0,3	0,4	0,5	0,7	0,92ab
BRS Supremo	0,4	0,4ab	0,7b	0,3	0,2	0,2	0,2	0,3	0,3	0,2	0,2	0,3	0,79ab
IAC Carioca Tybatã	0,2	0,1a	0,2ab	0,4	0,4	0,2	0,0	0,0	0,0	0,6	0,4	0,7	1,43ab
IAC Galante	0,1	0,0a	0,0a	0,0	0,3	0,1	0,3	0,3	0,4	0,3	0,2	0,7	1,14ab
IAC Diplomata	0,4	0,3ab	0,4ab	0,3	0,1	0,3	0,2	0,2	0,5	0,3	0,3	0,4	1,06ab
IAC Harmonia	0,1	0,1a	0,1a	0,1	0,0	0,0	0,1	0,1	0,3	0,4	0,3	0,3	0,45a
IAPAR 81	0,2	0,1a	0,1a	0,1	0,1	0,0	0,1	0,3	0,2	0,3	0,5	0,6	1,80b
IAC Una	0,6	0,7b	0,4ab	0,4	0,3	0,4	0,3	0,5	0,6	0,1	0,0	0,5	1,39ab
IAC Carioca Eté	0,1	0,1a	0,1a	0,1	0,1	0,0	0,0	0,0	0,3	0,0	0,4	0,5	1,17ab
F	$1,45^{ns}$	3,11**	3,18**	$1,44^{ns}$	$1,44^{ns}$	$1,86^{ns}$	$1,28^{ns}$	$1,58^{ns}$	$1,32^{ns}$	$1,41^{ns}$	$1,45^{ns}$	$1,09^{ns}$	1,94*

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EFFECT OF DOSES OF AZADIRACHTIN ON *ANTICARSIA GEMMATALIS* (HÜBNER, 1818) (LEPIDOPTERA: NOCTUIDAE) LARVAE, APPLIED ON *PHASEOLUS VULGARIS*L. LEAVES

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INTRODUCTION

The worldwide increase of organic cultivation due to the crescent demand by healthiest foods enables to small farmers an alternative of gains (Campanhola&Valarini, 2001).

In Brazil, common bean lines (*Phaseolus vulgaris* L.) to the organic cultivations were tested (Carvalho et al., 2006). In common bean crops occurs many phytophagous insects, among them, *Anticarsia gemmatalis* (Hübner, 1818) (Lepidoptera: Noctuidae) (Praça et. al, 2006), and the neem oil have been used as a control practice (Gheller et al., 2007).

This study aimed to verify the impact of six concentrations of an azadirachtin based commercial product (Azamax®) on *A. gemmatalis* larvae.

MATERIALS AND METHODS

The experiment was carried out in the Resistance of Plants to Insects Laboratory, at the Faculdade de Ciências Agrárias e Veterinárias – UNESP Jaboticabal, São Paulo State, Brazil. The experiment was conducted at temperature of 25 ± 2ºC, relative humidity of 70 ±10%, and photophase of 12 hours. Six different concentrations of Azamax® product were evaluated (azadirachtin 1.2 EC): 0.01%; 0.03%; 0.06%; 0.16%; 0.40% and 1% (Bliss, 1934) and two treatments as control, being applied in one only distilled water, and in another a physiologic insecticide of the benzoylureas chemical group (lufenuron - Match®), concentration of 1.5%. The leaves of common bean plants, cultivar Pérola, were dippedinto the emulsions by one minute and next they were exposed in the environment by 30 minutes, in order to be dried, protected of lighting to avoid product degradation. After drying, the leaves were put in Petri dishes (9.0 cm in diameter and 1.5 cm in height), lined with wetted filter paper. In each Petri dish five newly-hatchedlarvaewerereleased.

A three-day period of feeding was chosen in order to assure the product consume by all the larvae, being this period lesser than the degradation of azadirachtin in field (Stokes & Redfern, 1982).After this period the larvae were transferred to leaves without any treatment and exchanged on alternate days. The larval mortality evaluations were performed at three, five, seven and ten days after the beginning of the experiment, being the insect mass (mg) assessed at ten days.

A completely randomized design was used with six repetitions, using 30 larvae per treatment. The data obtained was submitted to the analysis of variance by F test, and their average compared by Tukey test, at 5% probability. The calculation to verify the doses percentage of efficiency (%E) was performed through the Abbott formula (Abbott, 1925).

RESULTS AND DISCUSSION

Three days after the treatment with azadirachtin, the concentration of 0.16% caused the highest mortality of *A. gemmatalis* larvae, being similar to the chemical product and differing significantly of the control (water). This probably occurred due to the fact that concentrations higher than 0.16% of Azamax® caused inhibition on the feeding, occasioning a late effect on the *A gemmatalis* larvae mortality. Assessing at five days, all the treatments differed significantly to the control (water). At seven and ten days after the exposure of the larvae to the treatments, the concentrations higher than 0.06% of Azamax® and the chemical product lufenuron showed the highest index of mortality (Table 1). Independently of the azadirachtin concentrations, there was deleterious effect on the insect, interfering on the insect mass. Under the conditions in which this work was performed, Azamax® showed satisfactory insecticide effect on *A gemmatalis*, being its efficiency similar to the chemical product (Table 1).

Table 1: Average number of dead larvae (N), product efficiency (%E) on the control of *Anticarsia gemmatalis* at three, five, seven and ten days after the treatment on bean leaves and the body mass (M) at ten days. Jaboticabal, SP, Brazil, 2012.

Treatment	Conc.	3		5		7		10		$M^{1(n)}$
	c.p. (%)	N^1	%E	N^1	%E	N^1	%E	N^1	%E	(mg)
1- Water	-	0.00b	-	0.33b	-	8.33c	-	1.67c	-	$34.3a^{(20)}$
2- Azamax®	0.01	0.33ab	6.67	2.67a	53.33	3.17b	63.33	3.50b	40.00	$11.5b^{(09)}$
3- Azamax®	0.03	0.83ab	16.67	2.67a	53.33	4.17ab	83.33	4.50ab	63.33	$8.6b^{(03)}$
4- Azamax®	0.06	0.50ab	10.00	4.00a	80.00	4.83a	96.67	5.00a	60.00	-[2]
5- Azamax®	0.16	2.00a	40.00	4.17a	83.33	5.00a	100.00	5.00a	86.67	-[2]
6- Azamax®	0.40	1.50ab	30.00	3.50a	70.00	5.00a	100.00	5.00a	86.67	-[2]
7- Azamax®	1.00	1.33ab	26.67	2.83a	56.67	4.83a	96.67	5.00a	100.00	-[2]
8- Match®	0.15	2.33a	46.67	5.00a	100.00	5.00a	100.00	5.00a	100.00	-[2]
F (Treatment)		2.80*		7.36**		28.28**		22.78**		11,51**
C. V. (%)		39.29		22.24		9.41		7.52		1,28

[1]Averages followed up by the same letter in the column not differ significantly among them by Tukey test at 5%probability. [n]Number of analyzed larvae.[2]Insuficient number of larvae to the statistical analysis (To the analysis the data were transformed at $(x+0.5)^{1/2}$).

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INFESTATION OF THE BLACK APHID *APHIS CRACCIVORA* KOCH (HEMPITERA: APHIDIDAE) ON BEAN CULTIVARS, IN FIELD CONDITIONS

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INTRODUCTION

Among the pests that infest bean crops limiting their production the black aphid *Aphis craccivora* Koch (Hemiptera: Aphididae) stands out, causing direct injuries due to continuous sap suction in twigs and leaves, deforming buds and leaves and affecting the development of the plants (Gallo et al., 2002). Furthermore, these aphids are transmitters of important viruses that infect agricultural crops such as common bean, cowpea and green beans. Thus, host plant resistance is an important control tactics regarding the philosophical precepts of integrated pest management (IPM), which aims the use of plants with certain genotypic characteristics that benefit them to be less infested or injured by phytophagous insects, resulting in higher yields of better quality (Lara, 1991). There are few reports in literature relating resistant bean cultivars to the black aphid, and therefore, this work aimed to evaluate the infestation of *A. craccivora* on 19 bean cultivars, in field conditions, during the "dry season", in Jaboticabal, São Paulo State, Brazil.

MATERIALS AND METHODS

The assay was carried out in an experimental area of the Departamento de Fitossanidade of Faculdade de Ciências Agrárias e Veterinárias – FCAV/UNESP, located in Jaboticabal, São Paulo State, Brazil. Nineteen bean cultivars were used: IPR Eldorado, IAC Carioca-Tybatá, BRS Cometa, Guará, IAPAR 81, IPR 139, BRS Supremo, IAC Alvorada, IAC Carioca Eté, BRS Pontal, IAC Diplomata, IPR Siriri, IAC Galante, Pérola, IAC Centauro, IAC Una, IAC Formoso, BRS Requinte and IAC Harmonia.

Randomized blocks design was used, with four replications, totaling 76 plots. For each sampling, 10 leaflets per plot were collected at random, packed in paper bags and conducted to the Laboratório de Resistência de Plantas a Insetos, where the number of nymphs and/or adults of *A. craccivora* on the underside of the leaflets were quantified through a stereoscope.

Data of the average number of nymphs and/or adults of *A. craccivora* were transformed in $(x + 0.5)^{1/2}$ for normalization and then they were submitted to the analysis of variance (ANOVA) by F test and means were compared to Tukey test, at 5% probability when they were significant.

RESULTS AND DISCUSSION

There were significant differences of the infestation of *A. craccivora* among the evaluated bean cultivars (Table 1). The cultivar IAC Centauro showed the highest infestation of the insect (5.3), IPR Eldorado showed intermediate infestation (2.9), whereas the other cultivars were the lowest infested, varying from 1.3 to 2.5 aphids on BRS Pontal and IAC Harmonia, respectively (Table 1). Moraes & Bleicher (2007) assessing the non-preference of *A. craccivora* for cowpea (*Vigna*

unguiculata [L.] Walp.) cultivars observed that all studied genotypes were equally preferred to the black aphid in field conditions, whereas in the greenhouse the cultivar Epace-10 was the least preferred by the insect. The same authors verified the cultivars Epace-10 and Patativa were the least preferred by *A. craccivora* in an assay carried out in greenhouse, attributing this fact to a possible resistance mechanism, antixenosis and/or antibiosis.

We concluded that cultivar IAC Centauro shows higher infestation of *A. craccivora*; IPR Eldorado shows intermediate infestation; and the other cultivars are less infested by the black aphid.

Table 1. Average number of *Aphis craccivora* on 10 assessed leaflets from plants of 19 bean cultivars in six periods of evaluation. Jaboticabal, SP, Brazil, 2011.

Cultivars	***Aphis craccivora*** [1]
1- Pérola	1.5 a
2- Guará	1.5 a
3- IPR Eldorado	2.9 ab
4- IPR Siriri	1.4 a
5- IPR 139	1.6 a
6- IAPAR 81	2.3 a
7- BRS Cometa	1.6 a
8- BRS Pontal	1.3 a
9- BRS Requinte	1.9 a
10- BRS Supremo	2.1 a
11- IAC Carioca-Tybatá	2.1 a
12- IAC Formoso	2.3 a
13- IAC Carioca-Eté	1.4 a
14- IAC Galante	1.5 a
15- IAC Alvorada	1.6 a
16- IAC Una	1.5 a
17- IAC Diplomata	2.3 a
18- IAC Centauro	5.3 b
19- IAC Harmonia	2.5 a
F	2.55**
C.V.(%)	20.57

[1]Averages followed by the same letter, in column, do not differ among themselves by Tukey test, at 5% of probability.

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INFESTATION OF *BEMISIA TABACI* B BIOTYPE ON BEAN CULTIVARS

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INTRODUCTION

The major pest of bean crop, the whitefly *Bemisia tabaci* (Genn., 1889) B biotype (Hemiptera: Aleyrodidae) injuries directly the plants due to its feeding in the phloem, weaken them by the suction of nutrients in addition to inject toxins causing physiological problems in bean plants and indirectly through the sugar excretion of honeydew which provides the growth of saprophytic fungi, generally of the genus *Capnodium* (sooty mold), onto the leaves, flowers and fruits, preventing gas changes and photosynthesis, thus reducing the production (Lima, 2001).

However, the most serious damage caused by *B. tabaci* B biotype is the transmission of viruses such as the Bean Golden Mosaic Virus (BGMV), among others. The BGMV is one of the major problems of bean crops in Latin America by causing economical losses ranging from 30 to 100% depending on the cultivar, phenological stadium of the plants, vector population, presence of alternative hosts and environmental conditions (Salguero, 1993).

In this context, host plant resistance may be used as another control tactics within the integrated pest management (IPM) aiming to attenuate the damages caused by *B. tabaci* B biotype since this method reduces the population of the insects to not harmful levels, it does not imbalance the environment, it holds persistent and cumulative effects, it does not overtax the cost of production and it does not demand specific knowledge by the agricultural grower (Lara, 1991). Thus, this work aimed to evaluate the natural infestation of *B. tabaci* B biotype on bean cultivars in the winter season, in Jaboticabal, SP, Brazil.

MATERIALS AND METHODS

The assay was carried out in an experimental field of the Departamento de Fitossanidade at Faculdade de Ciências Agrárias e Veterinárias – FCAV/UNESP, located in Jaboticabal, São Paulo State, Brazil. Ten bean cultivars were used: IAC Centauro, IAC Una, IAC Galante, IAC Diplomata, BRS Supremo, BRS Requinte, IPR Siriri, IPR Eldorado, Guará and Pérola.

The evaluation (quantification) of eggs and nymphs of *B. tabaci* B biotype was performed through a stereoscope in laboratory. Samplings of adults were made in 10 plants per plot, assessing visually 10 leaflets of the upper third of the plants using the turned leaf technique, which consists in holding the leaf by the petiole and turn it slowly and carefully, preventing the flight of the insects.

Collected data of the number of eggs, nymphs and adults of *B. tabaci* B biotype were transformed in $(x + 0.5)^{1/2}$, submitted to the analysis of variance (ANOVA) by F test and means compared by Tukey test at 5% probability.

RESULTS AND DISCUSSION

There were significant differences of the number of eggs of *B. tabaci* B biotype. The cultivar IPR Eldorado showed the least number of eggs (1.33), differing of BRS Supremo which was the most oviposited, holding an average of 4.33 eggs in 10 leaflets of bean plants according to Tukey test

at 5% probability (Table 1). The other cultivars behaved as intermediate to the number of eggs. Regarding the infestation of nymphs, no significant differences were observed in the experimental field (Table 1), ranging from 0.20 (IAC Una) to 0.50 (IAC Centauro and Guará) nymphs in 10 leaflets.

For the infestation of adults of the whitefly, the cultivar IAC Una stood out as the least attacked (0.54) differing significantly of the cultivars IAC Centauro and Pérola, which showed 1.58 and 1.68 adults, respectively (Table 1).

The conclusions obtained in this experiment were that the cultivar IAC Eldorado was the least oviposited and IAC Una was the least attacked by the adults of *B. tabaci* B biotype.

Table 1. Number of eggs, nymphs and adults of the whitefly, *Bemisia tabaci* B biotype, in 10 leaflets obtained from plants of 10 bean cultivars, in six samplings in the winter season. Jaboticabal, SP, Brazil, 2010.

Cultivar (C)	Eggs	Nymphs	Adults
IAC Centauro	3.65 ab	0.50 a	1.58 a
IAC Una	1.67 ab	0.20 a	0.54 c
IAC Galante	3.83 ab	0.33 a	1.10 ab
IAC Diplomata	2.35 ab	0.40 a	1.21 ab
BRS Supremo	4.33 a	0.33 a	1.10 ab
BRS Requinte	3.12 ab	0.41 a	0.71 ab
IPR Siriri	2.44 ab	0.38 a	1.31 ab
IPR Eldorado	1.33 b	0.34 a	0.77 ab
Guará	3.21 ab	0.50 a	1.06 ab
Pérola	3.60 ab	0.30 a	1.62 a
F (C)	2.21*	0.65^{NS}	2.51**
C.V. (%)	58.86	57.89	39.47

Averages followed by the same lowercase letter in column do not differ among themselves by Tukey test at 5% of probability.

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INFLUENCE OF ABIOTIC FACTORS ON THE INFESTATION OF *BEMISIA TABACI* B BIOTYPE ON BEAN CROP IN THREE SOWING SEASONS, IN JABOTICABAL, SP, BRAZIL

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INTRODUCTION

The whitefly *Bemisia tabaci* (Genn., 1889) B biotype (Hemiptera: Aleyrodidae) is one of the major pests of common bean crop, *Phaseolus vulgaris* L. Among the abiotic factors, rainfall, relative humidity and temperature highlight influencing the population fluctuation of the insect (Butler Jr. et al., 1983; Villas Bôas et al., 1977; Leite et al., 2002).

In studies of Leite et al. (2002), authors reported the temperature has direct effect on the period of eggs incubation and on the complete cycle of *B. tabaci*. Higher temperature and lower relative humidity favor its development and dispersion and the whitefly population tend to be higher with shorter biological cycles, resulting in frequent outbreaks during dry months (Butler Jr. et al., 1983), whereas rainfall is pointed as one of the most adverse factors, reducing the pest population especially when it is constant (Villas Bôas et al., 1997).

Thus, we aimed to evaluate the influence of abiotic factors on the infestation of the whitefly *B. tabaci* B biotype on bean crop in three sowing seasons, in Jaboticabal, São Paulo State, Brazil.

MATERIALS AND METHODS

The assay was carried out in an experimental field measuring 760 m^2 of the Departamento de Fitossanidade of Faculdade de Ciências Agrárias e Veterinárias – FCAV/UNESP in Jaboticabal, São Paulo State, Brazil from August 2010 to June 2011, using the common bean cultivar Carioca. No application of any insecticide was sprayed on the field throughout the period of evaluation of *B. tabaci*.

Population assessment of the whitefly was done at 25 days after emergence of the plants in six samplings per sowing season (water, dry and winter), with 10 leaflets evaluated in 76 points of the area. For the evaluation of adults performed in the field, turned leaf technique was used and for nymphs, leafs were collected from bean plants, stored in paper bags, conducted to the laboratory, and its number was counted through a stereoscope. Average monthly climatic data such as rainfall (mm), relative humidity (RU) and average temperature (ºC) in Jaboticabal, SP, Brazil, were obtained from the weather station of FCAV/UNESP, located near the experimental field.

Linear correlation was performed to analyze the infestation of eggs and nymphs of *B. tabaci* B biotype with the abiotic factors rainfall, relative humidity and average temperature.

RESULTS AND DISCUSSION

There was incidence of eggs and nymphs of *B. tabaci* B biotype in all evaluations except in the two last ones, where the presence of eggs was not observed (Table 1). The infestations of the pest ranged from 0 to 233.3 eggs and from 0.2 to 149.0 nymphs. Water season highlighted with

the highest means of either infestations of eggs and nymphs of the whitefly. Similar results were obtained by Jesus et al. (2010) relating the incidence of *B. tabaci* throughout the whole period of evaluation, however, distinctly of this study, those authors reported higher populations from the end of water season to the mid of dry season.

Table 1. Number of eggs and nymphs of *Bemisia tabaci* B biotype and climatic variables (temperature - ^{0}C, relative humidity - % and rainfall – mm) in bean crops. Jaboticabal, SP, Brazil, 2010/2011.

Sampling Dates	Infestation (no.)		Temperature (^{o}C)	Relative Humidity (%)	
	Eggs	Nimphs	Mean	Mean	Rainfall (mm)
			Winter Season		
3/08/2010	46.8	2.34	21.7	50.3	0.0
11/08/2010	66.6	10.9	19.4	54.2	0.0
18/08/2010	29.7	11.5	18.7	50.1	0.0
25/08/2010	34.9	6.3	20.9	34.2	0.0
1/09/2010	76.1	3.4	24.4	33.0	0.0
8/09/2010	45.0	6.5	23.2	48.2	7.7
			Water Season		
10/01/2011	172.5	141.4	23.5	82.8	49.7
17/01/2011	233.3	85.3	23.6	84.7	101.5
24/01/2011	168.9	78.8	25.0	76.2	0.7
31/01/2011	10.1	88.9	25.8	68.1	7.0
7/02/2011	9.7	149.8	24.1	76.6	74.9
14/02/2011	15.8	49.8	24.7	74.0	4.9
			Dry Season		
23/05/2011	3.2	1.1	18.0	65.6	0.0
30/05/2011	3.4	0.2	18.4	62.6	0.0
6/06/2011	2.5	0.2	18.6	59.1	0.0
13/06/2011	0.6	0.6	16.9	73.1	29.4
20/06/2011	0.0	0.4	18.2	61.0	0.0
27/06/2011	0.0	0.4	19.1	65.2	0.0

Positive and significant linear correlations for eggs x average temperature (r=0.4915*, y=11.636x-197.3), eggs x rainfall (r=0.5513.2*, y=0.2358x+3.2797), nymphs x average temperature (r=0.6744*, y=11.645x-213.12), nymphs x relative humidity (r=0.6621**, y=2.2417x-103.92) and nymphs x rainfall (r=0.7014*, y=0.4114x-0.7453) were established.

From the obtained results, we concluded the whitefly was present in practically all samplings and abiotic factors influenced its population fluctuation.

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INFESTATION OF *BEMISIA TABACI* (GENN.) B BIOTYPE ON BEAN CULVITARS AND THE INCIDENCE OF GOLDEN MOSAIC VIRUS

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INTRODUCTION

The whitefly *Bemisia tabaci* (Genn., 1889) B biotype (Hemiptera: Aleyrodidae) is one of the major pests of common bean, *Phaseolus vulgaris* L., causing both direct and indirect injuries to the crop. Among the indirect ones, the transmission of Bean Golden Mosaic Virus (BGMV) stands as the most serious damages caused by the insect (Salgueiro, 1993).

Among the methods adopted to control the whitefly, host plant resistance highlights as an important tactics within the integrated pest management (IPM), aiming the use of plants with certain genotypic features that provide them being less infested or injured by phytophagous insects, resulting in higher yields with better quality (Lara, 1991). Therefore, our work aimed to assess the infestation of adults of the whitefly and the incidence of the golden mosaic virus on plants of bean cultivars in water growing season, in Jaboticabal, São Paulo State, Brazil.

MATERIALS AND METHODS

The study was carried out in an experimental area of the Departamento de Fitossanidade of Faculdade de Ciências Agrárias e Veterinárias – FCAV/UNESP, located in Jaboticabal, São Paulo State, Brazil. Nineteen bean genotypes were used: IPR Eldorado, IAC Carioca Tybatã, BRS Cometa, Guará, IAPAR 81, IPR 139, BRS Supremo, IAC Alvorada, IAC Carioca Eté, BRS Pontal, IAC Diplomata, IPR Siriri, IAC Galante, Pérola, IAC Centauro, IAC Una, IAC Formoso, BRS Requinte and IAC Harmonia. The experiment was set up in a randomized blocks design, with four replications, totaling 76 plots.

Three samplings were performed, weekly, at 25 days after emergence (DAE), quantifying the average number of adults of the whitefly on 10 leaflets at random per plot. In addition, the presence of mosaic virus symptoms was assessed at 50 DAE and damage scores were assigned. For this, the total number of plants with symptoms and healthy ones in each plot were counted (percentage of plants with golden mosaic virus symptoms) and visual damage scores were assigned with scale ranging from 0 (without symptom) to 9 (100% of symptom). Data of the number of adults of *B. tabaci* B biotype, percentage of plants with golden mosaic virus symptoms and damage scores were submitted to the analysis of variance (ANOVA) by F test, and means compared by Tukey test, at 5% probability.

RESULTS AND DISCUSSION

Significant differences of the infestation of adults of *B. tabaci* B biotype in the experimental area were not observed (ranging from 2.38 in IAC Centauro to 4.88 in Guará), however, the percentage of plants with golden mosaic virus symptoms and visual damage scores differed significantly among the genotypes (Table 1). For the evaluation of the percentage of plants with symptoms, bean cultivars Pérola (5.30%), IPR Eldorado (6.90%), IPR Siriri (3.90%), IPR 139

(2.30%), BRS Pontal (4.80%), BRS Requinte (5.30%), BRS Supremo (4.00%), IAC Formoso (1.80%), IAC Diplomata (2.20%) and IAC Centauro (2.38%) highlighted, showing less symptoms of the golden mosaic virus, differing significantly from the cultivar IAC Harmonia which has shown 30.90% of the plants with the virus disease symptoms (Table 1). The other cultivars behaved intermediately (Table 1). Faria & Zimmermann (1988) aiming to identify resistant bean cultivars to the golden mosaic virus transmitted by adults of *B. tabaci* found that cultivars IAPAR 57 and IAPAR MD 820 has shown resistance features to this disease.

Similar behavior was observed for the visual damage score, being the cultivar IAC Harmonia with the highest score (5), differing significantly from IPR Eldorado, IPR 139, BRS Pontal, BRS Requinte, BRS Supremo, IAC Carioca Tybatã, IAC Carioca Eté, IAC Galante, IAC Alvorada, IAC Diplomata and IAC Centauro, which almost did not show symptoms (score 0).

From the obtained results we can highlight there were no differences among the cultivars for the infestation of *B. tabaci* B biotype adults and the cultivar IAC Harmonia showed the highest incidence of the golden mosaic virus as well as the highest visual damage score.

Table 1. Infestation of *Bemisia tabaci* B biotype adults, percentage of plants with golden mosaic virus symptoms and visual damage scores on bean cultivars. Jaboticabal, SP, Brazil.

Cultivars	Adults[1]	Plants with Symptoms (%)[1]	Damage Scores[1]
1- Pérola	4.25 a	5.30 b	1 bc
2- Guará	4.88 a	12.12 ab	1 bc
3- IPR Eldorado	3.25 a	6.92 b	0 c
4- IPR Siriri	3.63 a	3.95 b	1 bc
5- IPR 139	2.75 a	2.31 b	0 c
6- IAPAR 81	4.00 a	10.24 ab	1 bc
7- BRS Cometa	3.38 a	13.76 ab	2 ab
8- BRS Pontal	2.75 a	4.81 b	0 c
9- BRS Requinte	4.63 a	5.30 b	0 c
10- BRS Supremo	3.25 a	4.01 b	0 c
11- IAC Carioca-Tybatá	5.13 a	6.52 ab	0 c
12- IAC Formoso	4.13 a	1.81 b	1 bc
13- IAC Carioca-Eté	3.13 a	4.93 ab	0 c
14- IAC Galante	4.13 a	8.41 ab	0 c
15- IAC Alvorada	3.25 a	5.42 ab	0 c
16- IAC Una	4.00 a	6.73 ab	1 bc
17- IAC Diplomata	2.88 a	2.22 b	0 c
18- IAC Centauro	2.38 a	3.31 b	0 c
19- IAC Harmonia	5.13 a	30.90 a	5 a
F	0.99^{NS}	2.39**	6.04**

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NON PREFERENCE FOR FEEDING OF *CEROTOMA ARCUATA*(OLIVIER, 1791) (COLEOPTERA: CHRYSOMELIDAE) ADULTS BY COMMON BEAN GENOTYPES

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INTRODUCTION

Brazil is the largest worldwide producer of bean with annual average production of 3.5 million tons. Typical feed in Brazil, bean is cultivated by small and big producers in all the regions, being the largest producers Paraná State that harvested 298,000 tons in the agricultural year of 2009/2010, and Minas Gerais State, with the production of 214,000 tons in the same period (Mapa, 2012).

Cerotoma arcuata(Olivier, 1791) (Coleoptera: Chrysomelidae)is known, in the same way the other bettles of Chrysomelidae genus, as "leaf beetles", because it causes damages in the leaves of dry bean (*Phaseolus vulgaris* L.), soybean (*Glycine max* [L.] Merril), cowpea (*Vigna unguiculata* [L.] Walp.) and other legumes (Salas et al., 1999). In the USA, Iowa State, *Cerotoma trifurcate* (Foster) caused a decreased on the soybean production by 2,600 kg ha^{-1} (Smelser; Pedigo 1992).

This study aimed to evaluate the resistance degrees of different genotypes of common bean to the *C. arcuata* adults attack.

MATERIALS AND METHODS

The study was carried out in the Resistance of Plants to Insects Laboratory, at the Faculdade de Ciências Agrárias e Veterinárias – UNESP Jaboticabal, São Paulo State, Brazil. The experiments were conducted at temperature of 25 ± 2ºC, relative humidity of 70 ±10%, and photophase of 12 hours. Nine common bean genotypes were tested in order to evaluate the attractiveness and consumption of leaves by *C. arcuata* adults, in free-choice and no-choice tests. The genotypes used were: Pérola, RAZ 49, BRS Supremo, IAC Galante, IAC Diplomata, IAC Harmonia, IAPAR 81, IAC Una and IAC Carioca-Eté.

In order to perform the free-choice test, glass containers of 26.2 cm in diameter and 5.0 cm in height were used, and the discs (2.54 cm in diameter) of the respective genotypes were distributed on the filter paper, wetted with distilled water, where nine insects were released per replication. In no-choice test, Petri dishes of 9.0 cm in diameter and 1.5 cm in height were used, where one disc of leaf by replication, of each one of the respective genotypes was distributed and put on filter paper wetted with distilled water. One *C. arcuata*adult was introduced in each Petri dish.

The attractiveness was evaluated in many periods of time as well as the consumed area in leaves (CAL) (cm^2) on each genotype, in both tests.

The data obtained at the two tests of non preference for feeding of *C. arcuata* adults were transformed to $(x + 0.5)^{1/2}$ and submitted to the analysis of variance by F test, and their average compared by Tukey test, at 5% probability.

RESULTS AND DISCUSSION

In free-choice test, there were not significant differences in relation to the attractiveness in any of the evaluated periods. However, assessing the consumed area in leaves (CAL) (cm²) values, there were significant differences, being the genotype IAC Carioca-Eté (0.50 cm²) the least consumed, whereas the genotypes BRS Supremo and IAPAR 81 (2.05 cm² in both genotypes) were the most consumed. In no-choice test, there were not significant differences in any period concerning the attractiveness, occurring significant differences only in the consumed area in leaves (CAL), being the genotype IAC Carioca-Eté (0.43 cm²) the least consumed, while the genotype BRS Supremo (1.39 cm²) was the most consumed.

Table 1: Number of *Cerotoma arcuata* adults attracted and consumed area in leaves (CAL) (cm²), in bean genotypes discs of leaves, in free-choice and no-choice tests. Temperature= 25±2 ºC, Relative Humidity= 70±10% and photophase= 12 hours. Jaboticabal, SP, Brazil, 2012.

FREE-CHOICE TEST					
	TIMES				CAL
GENOTYPES	1' to 15'	30' to 120'	360' to 1380'	AVERAGE	
Pérola	0.60 a	0.97 a	0.67 a	0.72 a	1.64 ab
RAZ 49	0.48 a	0.67 a	0.30 a	0.48 a	1.57 ab
BRS Supremo	0.52 a	0.60 a	0.47 a	0.53 a	2.05 b
IAC Galante	0.62 a	0.87 a	0.60 a	0.68 a	1.36 ab
IAC Diplomata	0.34 a	0.57 a	0.47 a	0.44 a	1.41 ab
IAC Harmonia	0.34 a	0.40 a	0.87 a	0.50 a	1.85 ab
IAPAR 81	0.32 a	0.70 a	0.57 a	0.49 a	2.05 b
IAC Una	0.06 a	0.17 a	0.27 a	0.14 a	0.87 ab
IAC Carioca-Eté	0.22 a	0.23 a	0.23 a	0.23 a	0.50 a
F	0.79^{NS}	0.99^{NS}	1.94^{NS}	1.09^{NS}	2.32*
C.V.(%)	33.18	37.07	22.90	27.09	30.27
NO-CHOICE TEST					
	TIMES				CAL
GENOTYPES	1' to 15'	30' to 120'	360' to 1440'	AVERAGE	
Pérola	0.60 a	0.53 a	0.77 a	0.63 a	0.64 ab
RAZ 49	0.24 a	0.47 a	0.43 a	0.35 a	0.68 ab
BRS Supremo	0.38 a	0.40 a	0.67 a	0.46 a	1.39 b
IAC Galante	0.40 a	0.47 a	0.70 a	0.50 a	1.26 ab
IAC Diplomata	0.46 a	0.47 a	0.44 a	0.46 a	0.77 ab
IAC Harmonia	0.46 a	0.50 a	0.57 a	0.50 a	0.94 ab
IAPAR 81	0.48 a	0.73 a	0.67 a	0.60 a	0.67 ab
IAC Una	0.30 a	0.63 a	0.53 a	0.45 a	1.01 ab
IAC Carioca-Eté	0.08 a	0.20 a	0.47 a	0.22 a	0.43 a
F	1.32^{NS}	1.04^{NS}	1.42^{NS}	1.49^{NS}	2.16*
C.V.(%)	24.14	24.50	16.65	17.01	24.19

[1]Averages followed up by the same letter in the column not differ significantly among them by Tukey test at 5%probability. (To the analysis the data were transformed at $(x+0.5)^{1/2}$).

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ATTRACTIVENESS AND LEAF CONSUMPTION OF *CEROTOMA ARCUATA* (OLIVIER, 1791) (COLEOPTERA: CHRYSOMELIDAE) ADULTS FOR SNAP BEAN GENOTYPES

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INTRODUCTION

Cerotoma arcuata (Olivier, 1791) (Coleoptera: Chrysomelidae) is considered pest of agricultural importance because it is polyphagous, causing losses on several legume species, such as bean, cowpea and soybean (Salas et al., 1999). Continuous use of chemical control based only on the insect presence feeding on bean leaves is technically incorrect and may cause imbalance in the pest population and resistance to chemical products (Fazolin; Estrela, 2004). Lara (1991) recommends the use of resistant varieties as ideal tactic of insect control because it shows compatible costs with the culture and does not cause ecologic imbalances such as chemical control.

Therefore, this work aimed to assess the non preference for feeding of *C. arcuata* adults by snap bean genotypes.

MATERIALS AND METHODS

The experiment was carried out in the Resistance of Plants to Insects Laboratory, at the Faculdade de Ciências Agrárias e Veterinárias – UNESP Jaboticabal, São Paulo State, Brazil. The experiments were conducted at temperature of 25 ± 2ºC, relative humidity of 70 ± 10%, and photophase of 12 hours. The following snap bean genotypes were used: Favorito, HX 10093000, Itatiba II, UEG 19, UEG 11, UEG 15 and UEG 13.Tests of non preference for feeding in free-choice and no-choice were performed, and in both of them, plants leaflets of snap bean genotypes with about 30 days of age were collected in greenhouse and next leaf discs of 2.5 cm in diameter were prepared.

In free-choice test, leaf discs of the related genotypes were put in Petri dishes of 14.0 cm in diameter lined with filter paper wetted with distilled water, where one *C. arcuata* adult was released per genotype. In no-choice test, one leaf disc was used by Petri dish of 9.0 cm in diameter, where one *C. arcuata* was released per dish. Attractiveness was evaluated in many periods of time as well as the consumed area in leaves (CAL) (cm²) on each genotype, in both tests. Randomized*blocks design and completely random design were used for the free-choice and no-choice tests, respectively, both of them with 10 replications.*The data obtained were transformed to $(x + 0.5)^{1/2}$ and submitted to the analysis of variance by F test, and their average compared by Tukey test, at 5% probability.

RESULTS AND DISCUSSION

In free-choice test, there was significant difference only at 12 hours, being the genotype Favorito the most attractive differing from UEG 19 and Itatiba II.

Regarding the consumed area in leaves there was no statistical difference among the genotypes, being numerically the least consumed UEG 13 and UEG 19 (Table1).

Table 1: Number of *Cerotoma arcuata* adults attracted for leaf discs of snap bean genotypes in different evaluation times and consumed area in leaves (CAL), in free-choice test. Jaboticabal, SP, Brazil, 2012.

Genotypes	Minutes[1]						Hours[1]				C.A.L. (cm²)[1]
	1'	3'	5'	10'	15'	30'	1h	2h	6h	12h	
Favorito	0.20 a	0.10 a	0.30 a	0.40 a	0.40 a	0.60 a	0.80 a	0.70 a	0.60 a	1.20 b	1.64 a
Hx10093000	0.10 a	0.20 a	0.20 a	0.10 a	0.30 a	0.30 a	0.90 a	0.40 a	1.20 a	0.70 ab	1.94 a
Itatiba II	0.10 a	0.20 a	0.40 a	0.20 a	0.30 a	0.60 a	0.70 a	0.50 a	0.60 a	0.50 a	2.02 a
UEG 19	0.20 a	0.30 a	0.30 a	0.20 a	0.20 a	0.30 a	0.50 a	0.50 a	0.60 a	0.30 a	1.35 a
UEG 11	0.00 a	0.10 a	0.40 a	0.30 a	0.30 a	0.40 a	0.90 a	0.90 a	0.90 a	0.40 ab	1.91 a
UEG 15	0.30 a	0.20 a	0.30 a	0.50 a	0.80 a	0.90 a	1.30 a	1.20 a	0.90 a	0.50 ab	1.96 a
UEG 13	0.40 a	0.30 a	0.40 a	0.30 a	0.90 a	1.00 a	0.70 a	0.80 a	0.90 a	0.40 ab	1.48 a
F	1.22^{NS}	0.40^{NS}	0.20^{NS}	0.57^{NS}	2.00^{NS}	1.72^{NS}	0.64^{NS}	1.32^{NS}	0.81^{NS}	2.20*	0.50^{NS}
C.V. (%)	25.02	26.44	31.01	31.21	30.76	30.66	35.13	32.20	32.84	29.90	25.66

[1]Averages followed up by the same letter in the column not differ significantly among them by Tukey test at 5%probability. (To the analysis the data were transformed at $(x+0.5)^{½}$).

In no-choice test there was significant difference at the attractiveness of *C. arcuata* adults by the genotypes only at 30 minutes, being the genotypes UEG 19 and Favorito the most attractive, differing from Itatiba II. Regarding the consumed area in leaves there was significant difference among the genotypes, being UEG 13 (0.79 cm²) and UEG 19 (0.99 cm²) the genotypes which showed the least consumption, differing from Favorito (2.40 cm²) (Table 2).

Table 2: Number of *Cerotoma arcuata* adults attracted for leaf discs of snap bean genotypes in different evaluation times and consumed area in leaves (CAL), in no-choice test. Jaboticabal, SP, Brazil, 2012.

Genotypes	Minutes[1]						Hours[1]				C.A.L. (cm²)[1]
	1'	3'	5'	10'	15'	30'	1h	2h	6h	12h	
Favorito	0.10 a	0.10 a	0.20 a	0.60 a	0.80 a	0.90 b	1.00 a	0.80 a	0.80 a	0.80 a	2.40 b
Hx10093000	0.40 a	0.40 a	0.50 a	0.70 a	0.80 a	0.80 ab	0.90 a	1.00 a	0.80 a	0.70 a	1.5 ab
Itatiba II	0.10 a	0.10 a	0.20 a	0.40 a	0.50 a	0.30 a	0.60 a	0.60 a	0.70 a	0.50 a	1.26 ab
UEG 19	0.20 a	0.20 a	0.20 a	0.70 a	0.50 a	0.90 b	0.80 a	0.90 a	1.00 a	0.90 a	0.99 a
UEG 11	0.10 a	0.10 a	0.30 a	0.40 a	0.50 a	0.60 ab	0.80 a	0.90 a	0.80 a	0.80 a	1.12 ab
UEG 15	0.00 a	0.20 a	0.30 a	0.50 a	0.60 a	0.80 ab	0.80 a	0.80 a	0.90 a	0.60 a	1.62 ab
UEG 13	0.20 a	0.20 a	0.30 a	0.60 a	0.50 a	0.70 ab	0.80 a	0.90 a	0.90 a	0.90 a	0.79 a
F	1.23^{NS}	0.73^{NS}	0.53^{NS}	0.63^{NS}	0.81^{NS}	2.43*	0.96^{NS}	1.23^{NS}	0.69^{NS}	1.20^{NS}	3.24*
C.V. (%)	23.83	25.55	28.13	26.45	25.31	20.63	18.00	16.43	16.32	20.70	26.56

[1]Averages followed up by the same letter in the column not differ significantly among them by Tukey test at 5%probability. (To the analysis the data were transformed at $(x+0.5)^{½}$).

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NON PREFERNCE FOR FEEDING OF *DIABROTICA SPECIOSA* (GERMAR, 1824) (COLEOPTERA: CHRYSOMELIDAE) ADULTS BY SNAP BEAN GENOTYPES

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INTRODUCTION

Snap bean belongs to botanical species *Phaseolus vulgaris* L. which comprises different types of bean, used as green pod or dried grains (Swiader et al., 1992). One of the main problems in snap bean crops is the presence of defoliator insects. Among them, *Diabrotica speciosa* (Germar) stands out, because it is a polyphagous pest, widespread in the states of Brazil. The adults feed on buds and leaves, which may cause leaf area reduction and a decrease on the photosynthetic capacity of plants (Machado et al., 2007).

This work aimed to evaluate the feeding preference of *D. speciosa* adults by snap bean genotypes.

MATERIALS AND METHODS

The research was performed in the Resistance of Plants to Insects Laboratory, at the Faculdade de Ciências Agrárias e Veterinárias – UNESP Jaboticabal, São Paulo State, Brazil. The experiments were conducted at temperature of 25 ± 2ºC, relative humidity of 70 ±10%, and photophase of 12 hours. The snap bean genotypes used were: Favorito, HX 10093000, Itatiba II, UEG 19, UEG 11, UEG 15 e UEG 13. Experiments of feeding preference in free-choice and no-choice tests were carried out, and in both of them, plants leaflets of snap bean genotypes with about 25 days of age were collected in greenhouse and then were prepared leaf discs of 2.5 cm in diameter.In free-choice test, leaf discs of the respective genotypes were put on Petri dishes of 14.0 cm in diameter, lined with filter paper wetted with distilled water, where one adult of *D. speciosa* was released by genotype. In no-choice test, one leaf disc was used per Petri dish of 9.0 cm in diameter, where one *D. speciosa* adult was released per dish. The attractiveness was evaluated in many periods of time as well as the consumed area in leaves (CAL) (cm²) on each genotype, in both tests. Randomized*blocks design and completely random design were used for the free-choice and no-choice tests, respectively, both of them with 10 replications.*The data obtained were transformed to $(x + 0.5)^{1/2}$ and submitted to the analysis of variance by F test, and their average compared by Tukey test, at 5% probability.

RESULTS AND DISCUSSION

Analyzing the results obtained in free-choice test, there was significant difference for attractiveness of *D. speciosa* adults by genotypes only at 3 minutes and 24 hours. Initially, the genotype UEG 11 was the least attractive, whereas Itatiba II was the most attractive. However, at the last evaluation the genotype UEG 11 was the most attractive, differing statistically in comparison to the genotypes HX10093000 and UEG19. Regarding the consumed area in leaves, UEG 11 stands out with the highest consumption (1.11 cm²), differing from UEG 19 (0.11 cm²) (Table 1).

In no-choice test there was no significant difference, thereby neither to attractiveness of *D. speciosa* adults by genotypes nor to consumed area in leaves. Nevertheless, the genotype UEG 19 stands out numerically with the lowest consumed area in leaves (0.2 cm²) (Table 2).

Concluding, UEG 19 shows*resistance*of the*non-preference*-for-feeding*type, in free-choice test.*

Table 1: Number of *Diabrotica speciosa* adults attracted and consumed area in leaves (CAL) (cm²), in discs of leaves of snap bean genotypes, in free-choice test. Temperature= 25±2 ºC, Relative Humidity= 70±10% and photophase= 12 hours. Jaboticabal, SP, Brazil, 2012.

Genotypes (G)	Minutes						Hours					CAL (cm²)
	1	3	5	10	15	30	1	2	6	12	24	
Favorito	0.3 a	0.3 ab	0.4 a	0.5 a	0.6 a	0.5 a	0.3 a	0.6 a	0.7 a	0.5 a	0.6 ab	0.81 ab
Hx10093000	0.1 a	0.1 ab	0.2 a	0.2 a	0.1 a	0.2 a	0.2 a	0.3 a	0.3 a	0.4 a	0.1 a	0.63 ab
Itatiba II	0.6 a	0.7 b	0.5 a	0.6 a	0.7 a	0.6 a	0.5 a	1.0 a	0.6 a	1.0 a	1.1 ab	0.48 ab
UEG 19	0.1 a	0.1 ab	0.2 a	0.2 a	0.3 a	0.2 a	0.2 a	0.1 a	0.5 a	0.2 a	0.1 a	0.11 a
UEG 11	0.0 a	0.0 a	0.3 a	0.1 a	0.2 a	0.1 a	0.3 a	0.6 a	1.1 a	0.9 a	1.4 b	1.11 b
UEG 15	0.6 a	0.7 ab	0.5 a	0.7 a	0.7 a	1.0 a	1.0 a	0.7 a	0.3 a	0.2 a	0.5 ab	0.58 ab
UEG 13	0.2 a	0.3 ab	0.4 a	0.2 a	0.2 a	0.2 a	0.3 a	0.3 a	0.4 a	0.5 a	0.6 ab	0.55 ab
F (G)	2.60^{NS}	2.98^{*}	0.53^{NS}	1.63^{NS}	$2,14^{NS}$	2.25^{NS}	1.77^{NS}	1.93^{NS}	1.40^{NS}	2.28^{NS}	3.39^{*}	3.19^{*}
C.V. (%)	27.60	28.84	30.61	31.00	29.73	32.64	32.73	32.07	34.30	32.55	35.32	30.86

[1]Averages followed up by the same letter in the column not differ significantly among them by Tukey test at 5%probability. (To the analysis the data were transformed at $(x+0.5)^{½}$).

Table 2: Number of *Diabrotica speciosa* adults attracted and consumed area in leaves (CAL) (cm²), in discs of leaves of snap bean genotypes, in no-choice test. Temperature= 25±2 ºC, Relative Humidity= 70±10% and photophase= 12 hours. Jaboticabal, SP, Brazil, 2012.

Genotypes (G)	Minutes						Hours						CAL (cm²)
	1	3	5	10	15	30	1	2h	6	12	24	36	
Favorito	0.5 a	0.5 a	0.4 a	0.3 a	0.3 a	0.3 a	0.4 a	0.4 a	0.6 a	0.4 a	0.5 a	0.5 a	0.5 a
Hx10093000	0.6 a	0.6 a	0.5 a	0.6 a	0.7 a	0.7 a	0.9 a	1.0 a	0.5 a	0.7 a	0.7 a	0.6 a	0.6 a
Itatiba II	0.1 a	0.1 a	0.1 a	0.1 a	0.3 a	0.3 a	0.3 a	0.6 a	0.7 a	0.5 a	0.5 a	0.4 a	0.4 a
UEG 19	0.5 a	0.5 a	0.5 a	0.6 a	0.7 a	0.6 a	0.8 a	0.7 a	0.1 a	0.2 a	0.2 a	0.2 a	0.2 a
UEG 11	0.4 a	0.4 a	0.4 a	0.6 a	0.6 a	0.5 a	0.8 a	0.9 a	0.5 a	0.6 a	0.7 a	0.6 a	0.6 a
UEG 15	0.1 a	0.1 a	0.1 a	0.2 a	0.4 a	0.6 a	0.5 a	0.6 a	0.3 a	0.2 a	0.3 a	0.3 a	0.3 a
UEG 13	0.3 a	0.3 a	0.3 a	0.3 a	0.5 a	0.6 a	0.5 a	0.7 a	0.4 a	0.3 a	0.2 a	0.8 a	0.8 a
F (G)	1.82^{NS}	1.82^{NS}	1.34^{NS}	2.03^{NS}	1.20^{NS}	0.98^{NS}	2.47^{NS}	2.05^{NS}	1.67^{NS}	1.63^{NS}	2.01^{NS}	1.70^{NS}	3.57^{NS}
C.V. (%)	27.06	27.06	27.52	26.81	26.75	26.80	23.63	21.39	26.89	27.13	26.52	26.35	29.71

[1]Averages followed up by the same letter in the column not differ significantly among them by Tukey test at 5%probability. (To the analysis the data were transformed at $(x+0.5)^{½}$).

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NON PREFERENCE FOR FEEDING OF *DIABROTICA SPECIOSA* (GERMAR, 1824) (COLEOPTERA: CHRYSOMELIDAE) ADULTS BY COMMON BEAN GENOTYPES

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INTRODUCTION

Common bean is one of the agricultural products of major socioeconomical importance in Brazil (Ferreira et al., 2006) constituting an important source of protein (Ribeiro et al., 2005, 2007), carbohydrates, and iron (Ferreira et al., 2006).

Among the problems that affect common bean crops, the pests stand out, and in the last years the damages caused by *Diabrotica speciosa* (Germar, 1824)have deserved attention. According to studies carried out by Hohmann and Carvalho (1989), the average consumption of bean leaves by *D. speciosa* is 0.70 cm^2 per day. Data indicate that, in the initial phase of the culture (one week after the plants emergence), two beetles per plant are capable to eat about 16% of the leaves, in 24 hours of feeding (Silva et al., 2003).

This research aimed to evaluate the resistance degrees when fed by leaves of different common bean genotypes to *D. speciosa* adults attack.

MATERIALS AND METHODS

The study was carried out in the Resistance of Plants to Insects Laboratory, at the Faculdade de Ciências Agrárias e Veterinárias – UNESP Jaboticabal, São Paulo State, Brazil. The experiments were conducted at temperature of 25 ± 2ºC, relative humidity of 70 ±10%, and photophase of 12 hours. Nine common bean genotypes were tested in order to evaluate the attractiveness and consumption of leaves by *D. speciosa* adults, in free-choice and no-choice tests. The genotypes used were: Pérola, RAZ 49, BRS Supremo, IAC Galante, IAC Diplomata, IAC Harmonia, IAPAR 81, IAC Una e IAC Carioca-Eté.

In order to perform the free-choice test, glass containers of 26.2 cm of diameter and 5.0 cm in height were used, and the discs (2.54 cm in diameter) of the respective genotypes were distributed on the filter paper, wetted with distilled water, where nine insects were released per replication. On the other hand, in no-choice test, Petri dishes of 9.0 cm in diameter and 1.5 cm in height were used, where one disc of leaf by replication, of each one of the respective genotypes was distributed and put on filter paper wetted with distilled water. One *D. speciosa* adult was introduced in each Petri dish.

The attractiveness was evaluated in many periods of time as well as the consumed area in leaves (CAL) (cm^2) on each genotype, in both tests.

The data obtained at the two tests of non preference for feeding of *D. speciosa* adults were transformed $(x + 0.5)^{1/2}$ and submitted to the analysis of variance by F test, and their average compared by Tukey test, at 5% probability.

RESULTS AND DISCUSSION

The values that refer to the attractiveness by *D. speciosa* adults in free and no-choice tests were significant only on the overall average, at this, in free-choice test the genotypes Pérola and IAPAR 81 were the most attractive (0.56 and 0.52 insects, respectively), whereas IAC Galante (0.19) was the least attractive. On the other hand, in no-choice test IAC Diplomata was the least attractive (0.26), not differing of IAC Carioca-Eté (0.42), but it differed significantly in comparison to the other genotypes. For consumed area in leaves (CAL), there was significant difference only in no-choice test, where IAPAR 81 (1.79 cm²) was the most consumed genotype, whereas IAC Diplomata (0.36 cm²) was the least consumed.

Table 1: Number of *Diabrotica speciosa* adults attracted and consumed area in leaves (CAL) (cm²), by bean genotypes discs of leaves, in free-choice and no-choice tests. Temperature= 25±2 ºC, Relative Humidity= 70±10% and photophase= 12 hours. Jaboticabal, SP, Brazil, 2012.

FREE-CHOICE TEST					
	TIMES				CAL
GENOTYPES	1' to 15'	30' to 120'	360' to 1440'	AVERAGE	
Pérola	0.32 a	0.93 a	0.60 a	0.56 c	1.15 a
RAZ 49	0.30 a	0.53 a	0.60 a	0.45 bc	1.55 a
BRS Supremo	0.24 a	0.57 a	0.43 a	0.38 abc	1.31 a
IAC Galante	0.04 a	0.27 a	0.37 a	0.19 a	1.18 a
IAC Diplomata	0.22 a	0.17 a	0.50 a	0.28 ab	1.25 a
IAC Harmonia	0.20 a	0.27 a	0.37 a	0.26 ab	0.86 a
IAPAR 81	0.38 a	0.67 a	0.60 a	0.52 c	1.50 a
IAC Una	0.16 a	0.33 a	0.40 a	0.27 ab	0.72 a
IAC Carioca-Eté	0.10 a	0.23 a	0.43 a	0.23 ab	0.78 a
F	0.82^{NS}	2.05^{NS}	0.58^{NS}	6.52**	1.18^{NS}
C.V.(%)	24.12	27.52	19.40	29.38	27.31
NO-CHOICE TEST					
	TIMES				CAL
GENOTYPES	1' to 15'	30' to 120'	360' to 2880'	AVERAGE	
Pérola	0.50 a	0.57 a	0.56 a	0.54 b	0.88 ab
RAZ 49	0.42 a	0.43 a	0.60 a	0.49 b	1.54 ab
BRS Supremo	0.44 a	0.50 a	0.66 a	0.54 b	1.25 ab
IAC Galante	0.52 a	0.44 a	0.66 a	0.55 b	1.42 ab
IAC Diplomata	0.12 a	0.20 a	0.44 a	0.26 a	0.36 a
IAC Harmonia	0.44 a	0.70 a	0.36 a	0.47 b	0.81 ab
IAPAR 81	0.38 a	0.50 a	0.62 a	0.50 b	1.79 b
IAC Una	0.50 a	0.57 a	0.48 a	0.51 b	1.09 ab
IAC Carioca-Eté	0.36 a	0.50 a	0.42 a	0.42 ab	1.25 ab
F	0.76^{NS}	0.89^{NS}	2.34^{NS}	4.36**	2.68*
C.V.(%)	24.45	23.28	11.91	26.83	25.53

[1]Averages followed up by the same letter in the column not differ significantly among them by Tukey test at 5%probability. (To the analysis the data were transformed at $(x+0.5)^{1/2}$).

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FEEDING OF *SPODOPTERA FRUGIPERDA* (SMITH) (LEPIDOPTERA: NOCTUIDAE) IN GREEN BEAN GENOTYPES

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INTRODUCTION

The fall armyworm *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) is a polyphagous pest widely distributed in all regions of Brazil due to the high availability and diversity of food (Cruz, 1995). The fall armyworm feeds not only on grass crops such as maize, millet, wheat, sorghum and sugarcane but also on other grown plants, e.g., cotton, bean, peanut and potato (Gallo et al., 2002).

Thus, the aim of this work was to evaluate the non-preference for feeding of *S. frugiperda* larvae in green bean genotypes, under laboratory conditions.

MATERIALS AND METHODS

The experiment was carried out at Faculdade de Ciências Agrárias e Veterinárias – UNESP, in the Departamento de Fitossanidade, Laboratório de Resistência de Plantas a Insetos, in Jaboticabal, São Paulo State, Brazil, under temperature of 25 ± 1ºC, UR of 70 ± 10% and photophase of 12h. The following green bean genotypes were used: Favorito, HX 10093000, Itatiba II, UEG 19, UEG 11, UEG 15 and UEG 13. Free-choice and non-choice non-preference for feeding tests were performed, where in both, leaflets of approximately 25 days-old plants of bean genotypes were collected in greenhouse and then leaf discs of 2.5 diameter were prepared.

In free-choice test leaf discs of the green bean genotypes were placed in Petri dishes of 14.0 in diameter with paper filter softly moistened with distilled water at the bottom where one eight days-old caterpillar of *S. frugiperda* per genotype was released in the center of the plate. For non-choice test one leaf disc per Petri dish of 9.0 cm in diameter was also used where one caterpillar of the same age was released. In both tests the attractiveness of the caterpillars was evaluated at 1, 3, 5, 10, 15 and 30 minutes and at 1, 2, 6, 12 and 24 hours after releasing. Leaf area consumed (LAC) was also assessed through an electronic leaf area measurer device, model LI-COR 3100. Randomized blocks design and completely randomized blocks design were used for free-choice and non-choice tests, respectively, both with 10 repetitions. The obtained data were transformed in $(x + 0.5)^{1/2}$, submitted to the analysis of variance (ANOVA) by F test and means compared by Tukey test at 5% probability.

RESULTS AND DISCUSSION

In free-choice test the genotype Favorito showed the lowest attractiveness of the caterpillars and the genotype UEG 15 the highest until 6 hours, being significant differences observed at 3, 5, 10, 15 and 30 minutes and at 2 and 12 hours between the genotypes Favorito and UEG 15 (Table 1). However, the leaf area consumed did not differ significantly among the genotypes (Table 1).

For non-choice test, there were significant differences between the genotypes UEG 15 and Hx 10093000 only after 6 and 12 hours of evaluation (Table 2). Regarding the leaf area consumed, differences were observed between the genotype UEG 19, with the highest average of consumption, and Hx 10093000, with the lowest average (Table 2).

We concluded the genotype Hx 10093000 was the most consumed by *S. frugiperda* and the genotype UEG 19 was the least attacked by the insect.

Table 1. Number of *Spodoptera frugiperda* larvae attracted to green bean genotypes in different times of evaluation and leaf area consumed (LAC), in free-choice test. Jaboticabal, SP, Brazil, 2012.

Genotypes	Minutes						Hours					LAC (cm²)
	1	3	5	10	15	30	1	2	6	12	24	
Favorito	0.2 a	0.1 a	0.1 a	0.1 a	0.1 a	0.1 a	0.2 a	0.1 a	0.2 a	0.3 ab	0.6 a	0.81 a
Hx 10093000	0.9 a	0.8 ab	0.8 ab	0.8 ab	0.8 ab	1.1 ab	0.7 a	0.9 ab	0.7 a	1.2 c	0.9 a	3.03 b
Itatiba II	0.8 a	0.5 ab	0.5 ab	0.5 ab	0.6 ab	0.6 ab	0.6 a	0.7 ab	0.8 a	0.4 abc	0.4 a	1.28 a
UEG 19	0.3 a	0.3 ab	0.4 a	0.4 a	0.4 ab	0.4 ab	0.4 a	0.4 ab	0.4 a	0.4 abc	0.3 a	0.61 a
UEG 11	0.8 a	0.5 ab	0.4 a	0.4 a	0.6 ab	0.7 ab	0.5 a	0.5 ab	0.4 a	0.4 abc	0.4 a	0.48 a
UEG 15	1.1 a	1.1 b	1.3 b	1.3 b	1.3 b	1.3 b	1.0 a	1.2 b	1.0 a	1.0 bc	0.4 a	0.83 a
UEG 13	0.6 a	0.6 ab	0.7 ab	0.7 ab	0.7 ab	0.6 ab	0.7 a	0.6 ab	0.6 a	0.1 a	0.5 a	1.21 a
F (G)	2.30^{NS}	3.11^{**}	3.08^{**}	3.76^{**}	3.31^{**}	2.93^{**}	1.26^{NS}	2.88^{**}	1.53^{NS}	4.65^{**}	0.90^{NS}	5.7^{NS}
C.V. (%)	30.60	28.83	28.83	28.83	29.11	31.91	32.38	29.90	33.29	27.56	34.14	31.29

[1]Means followed by the same letter in the column do not differ by Tukey test at 5% probability. For analysis, data were transformed in $(x + 0,5)^{½}$.

Table 2. Number of *Spodoptera frugiperda* larvae attracted to green bean genotypes in different times of evaluation and leaf area consumed (LAC), in non-choice test. Jaboticabal, SP, Brazil, 2012.

Genotypes	Minutes						Hours					LAC (cm²)
	1	3	5	10	15	30	1	2	6	12	24	
Favorito	0.3 a	0.2 a	0.3 a	0.3 a	0.3 a	0.3 a	0.3 a	0.3 a	0.3 ab	0.7 b	0.6 a	1.49 ab
Hx 10093000	0.2 a	0.2 a	0.1 a	0.1 a	0.1 a	0.1 a	0.1 a	0.1 a	0.1 a	0.8 b	0.7 a	2.79 b
Itatiba II	0.2 a	0.2 a	0.2 a	0.2 a	0.2 a	0.2 a	0.2 a	0.2 a	0.2 ab	0.7 b	0.7 a	1.95 ab
UEG 19	0.2 a	0.2 a	0.3 a	0.3 a	0.3 a	0.3 a	0.2 a	0.2 a	0.2 ab	0.7 b	0.5 a	1.00 a
UEG 11	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.5 ab	0.0 a	0.8 a	1.81 ab
UEG 15	0.0 a	0.0 a	0.1 a	0.2 a	0.2 a	0.1 a	0.1 a	0.1 a	0.8 b	0.0 a	0.7 a	1.67 ab
UEG 13	0.4 a	0.4 a	0.4 a	0.4 a	0.3 a	0.3 a	0.3 a	0.4 a	0.4 ab	0.8 b	0.6 a	1.37 a
F (G)	1.45^{NS}	1.36^{NS}	1.26^{NS}	1.07^{NS}	0.81^{NS}	0.96^{NS}	0.80^{NS}	1.20^{NS}	2.79^{*}	8.78^{**}	0.40^{NS}	3.74^{**}
C.V. (%)	24.76	24.31	25.44	26.08	25.94	25.29	24.86	25.02	26.06	20.50	24.28	19.76

[1]Means followed by the same letter in the column do not differ by Tukey test at 5% probability. For analysis, data were transformed in $(x + 0,5)^{½}$.

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NON-PREFERENCE FOR FEEDING OF *SPODOPTERA FRUGIPERDA* (SMITH) (LEPIDOPTERA: NOCTUIDAE) IN BEAN GENOTYPES

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INTRODUCTION

The fall armyworm *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) is a phytophagous, polyphagous and cosmopolitan insect originated from the tropical and subtropical zones of American continent and widely spread in these regions (Cruz, 1995). Among the hosts of this pest, crops of maize, wheat, sorghum, rice, sugarcane, soybean, common bean, cotton, peanut, tomato, cabbage and kale highlight (Silva et al., 1968; Ali et al., 1989; Cruz, 1995).

The aim of this work was to evaluate the non-preference for feeding of *S. frugiperda* larvae in bean genotypes, in laboratory.

MATERIALS AND METHODS

The assay was carried out in Laboratório de Resistência de Plantas a Insetos of Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, SP State, Brazil, under controlled conditions. The following bean genotypes were used: Pérola, RAZ 49, BRS Supremo, IAC Carioca-Tybatã, IAC Galante, IAC Diplomata, IAC Harmonia, IAPAR 81, IAC Una and IAC Carioca-Eté.Free-choice and non-choice non-preference for feeding tests were performed, where in both, leaflets of 20 days-old plants of the bean genotypes were collected in a greenhouse and through a punch leaf discs of 2.5 cm diameter were prepared.

In free-choice test, leaf discs belonging to the bean genotypes were disposed in Petri dishes of 14.0 cm diameter with filter paper softly moistened with distilled water at the bottom, and then one eight days-old caterpillar of *S. frugiperda* per genotype was released. For non-choice test one leaf disc per Petri dish of 9.0 cm diameter was used, where one caterpillar of the same age was transferred per plate. In both tests the attractiveness of the insects was evaluated at 1, 3, 5, 10, 15, 30, 60, 120, 360, 720, 1440 and 1800 minutes after releasing. Leaf area consumed (LAC) was also measured through an electronic leaf area measurer device, model LI-COR 3100. Randomized blocks and entirely randomized blocks design were used for free-choice and non-choice tests, respectively, both with 10 replications. Data were transformed in $(x + 0.5)^{1/2}$, submitted to the analyses of variance (ANOVA) and means were separated with Tukey test, at 5% probability.

RESULTS AND DISCUSSION

From the obtained data in free-choice test, no significant differences were found in the attractiveness of *S. frugiperda* larvae in relation to the leaf discs of bean genotypes in any evaluated time (Table 1). However, the genotype IAC Harmonia was the least consumed, with 0.28 cm^2, differing significantly from IAPAR 81, IAC Carioca Tybatã and RAZ 49, which showed the highest values for consumption, 1.62, 1.45 and 1.39 cm^2, respectively (Table 1). Similarly to the former test, the attractiveness of larvae did not differ among bean genotypes in non-choice test either, whereas the leaf area consumed was significantly lower in IAC Harmonia,

with 1.14 cm^2 (Table 2). The genotypes IAPARA 81 (3.44 cm^2), IAC Carioca Tybatã (3.13 cm^2), Pérola (2.90 cm^2), BRS Supremo (2.89 cm^2), RAZ 49 (2.67 cm^2) and IAC Carioca Eté (2.64 cm^2) were the most consumed by *S. frugiperda* in non-choice test (Table 2).

In general, we concluded that genotype IAC Harmonia stood as the least consumed by the insect, whereas the other genotypes were susceptible to *S. frugiperda*.

Table 1. Number of *Spodoptera frugiperda* larvae attracted to bean genotypes at different minutes and leaf area consumed (LAC), in free-choice test. Jaboticabal, SP, Brazil, 2012.

GENOTYPES	1'	3'	5'	10'	15'	30'	60'	120'	360'	720'	1440'	LAC(cm^2)
Pérola	0.7	0.8	0.9	1.0	0.8	0.4	0.5	0.8	0.6	0.4	0.6	1.12ab
RAZ 49	0.5	0.7	0.7	0.8	0.8	0.5	0.3	0.6	0.3	0.4	0.7	1.39b
BRS Supremo	0.7	0.7	0.7	0.8	0.9	1.0	0.4	0.3	0.0	0.6	0.4	0.79ab
IAC Carioca Tybatã	0.8	0.7	0.9	0.8	0.8	0.6	0.5	0.7	0.6	0.6	0.4	1.45b
IAC Galante	0.8	1.1	1.0	1.2	0.8	1.0	0.6	0.6	0.6	0.6	0.2	0.87ab
IAC Diplomata	0.8	0.8	0.6	0.9	0.7	0.6	0.5	0.8	0.4	0.4	0.5	0.73ab
IAC Harmonia	0.1	0.2	0.2	0.2	0.2	0.0	0.2	0.4	0.6	0.5	0.5	0.28a
IAPAR 81	0.8	0.9	0.9	0.8	1.2	0.9	0.7	0.6	0.6	0.7	0.7	1.68b
IAC Una	0.5	0.4	0.4	0.7	0.5	0.7	0.3	0.3	0.3	0.7	0.4	0.75ab
IAC Carioca Eté	1.2	1.1	1.2	0.9	1.1	1.0	0.8	0.8	0.6	0.6	0.4	1.33ab
F	1.25^{ns}	1.12^{ns}	1.19^{ns}	0.93^{ns}	1.05^{ns}	1.60^{ns}	0.69^{ns}	0.82^{ns}	1.07^{ns}	0.37^{ns}	0.68^{ns}	3.71**

Table 2. Number of *Spodoptera frugiperda* larvae attracted to bean genotypes at different minutes and leaf area consumed (LAC), in non-choice test. Jaboticabal, SP, Brazil, 2012.

GENOTYPES	1'	3'	5'	10'	15'	30'	60'	120'	360'	720'	1440'	1800'	LAC(cm^2)
Pérola	0.7	0.8	0.9	0.6	0.8	0.7	0.6	0.9	0.5	0.2	0.5	0.6	2.90b
RAZ 49	0.9	0.8	1.0	0.9	0.9	0.8	0.9	0.9	0.4	0.2	0.7	0.5	2.67b
BRS Supremo	0.9	0.8	0.9	0.7	0.9	0.7	0.8	1.0	0.4	0.1	0.5	0.6	2.89b
IAC Carioca Tybatã	0.7	0.7	0.8	0.7	0.7	1.0	0.5	0.6	0.9	0.2	0.4	0.8	3.13b
IAC Galante	0.7	0.6	0.6	0.6	0.7	1.0	0.8	0.7	0.9	0.2	0.5	0.6	2.17ab
IAC Diplomata	0.8	0.9	0.7	0.4	0.7	0.7	0.9	0.5	0.9	0.3	0.3	0.5	2.02ab
IAC Harmonia	0.4	0.5	0.6	0.5	0.5	0.6	0.4	0.5	0.5	0.3	0.3	0.5	1.14a
IAPAR 81	0.7	0.8	0.9	0.7	0.5	0.6	0.4	0.8	0.6	0.1	0.2	0.6	3.44b
IAC Una	0.8	0.9	0.8	0.5	0.6	0.8	0.8	0.8	0.7	0.0	0.5	0.6	2.59ab
IAC Carioca Eté	0.9	0.9	0.8	0.8	1.0	0.8	0.9	0.9	0.5	0.3	0.4	0.7	2.64b
F	1.23^{ns}	1.00^{ns}	1.11^{ns}	0.97^{ns}	1.53^{ns}	1.14^{ns}	2.21^{ns}	1.84^{ns}	1.95^{ns}	0.61^{ns}	0.80^{ns}	0.35^{ns}	4.43**

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EFFICIENCY OF AZADIRACHTIN 1.2 EC ON THE CONTROL OF *SPODOPTERA FRUGIPERDA* (SMITH) (LEPIDOPTERA: NOCTUIDAE) ON *PHASEOLUS VULGARIS* L. LEAVES

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INTRODUCTION

Brazil stands out among the countries with the highest bean production (Faostat, 2009). The legumesare source of vegetable protein used in the human feeding, being the common bean, *Phaseolus vulgaris* L., one of main protein foods of Brazilian people (Roston, 1990).

Spodoptera frugiperda (Smith) (Lepidoptera: Noctuidae) can occur in bean crops, causing damages in plants and considerable reductions on the production (Quintela, 2002). In order to control this pest, the use of terpenes with insecticide activity is an alternative (Viegas Júnior, 2003). The limonoids present in *Azadirachta indica* (A. Juss.) are the most active compound and azadirachtin is the main compound with insecticide effect (Schmutterer, 1990).

This work aimed to evaluate the efficiency and the mortality of *S. frugiperda* using different Azamax® concentrations, commercial product with 1.2% of azadirachtin.

MATERIALS AND METHODS

The work was performed in the Resistance of Plants to Insects Laboratory, at the Faculdade de Ciências Agrárias e Veterinárias – UNESP Jaboticabal, São Paulo State, Brazil. The experiments were conducted at temperature of 25 ± 2ºC, relative humidity of 70 ±10%, and photophase of 12 hours.

Six different concentrations of Azamax® were assessed (Azadirachtin 1.2 EC): 0.01%; 0.03%; 0.06%; 0.16%; 0.40% and 1% (Bliss, 1934) and two treatments as control, being applied in one only distilled water, and in another was used one physiologic insecticide of the benzoylureas chemical group (lufenuron - Match®), concentration of 1.5%. The leaves of common bean plants, cultivar Pérola, were dippedinto the emulsions by one minute and next they were exposed in the environment by 30 minutes, in order to dry, protected of lighting to avoid product degradation. After drying, the leaves were put in Petri dishes (9.0 cm in diameter and 1.5 cm in height), lined with wetted filter paper. In each Petri dish five newly-hatchedlarvaewerereleased.

A three-day period of feeding was chosen in order to assure the product consume by all the larvae, being this period lesser than the degradation of azadirachtin in field (Stokes & Redfern, 1982).After this period the larvae were transferred to leaves without any treatment and exchanged on alternate days. Larval mortality evaluations were performed at three, five, seven and ten days after the beginning of the experiment, being the insect mass (mg) assessed at ten days.

A completely randomized design was used with six repetitions, using 30 larvae per treatment. The data obtained was submitted to the analysis of variance by F test, and their average compared by Tukey test, at 5% probability. The calculation to verify the doses percentage of efficiency (%E) was performed through the Abbott formula (Abbott, 1925).

RESULTS AND DISCUSSION

It was verified that at three days after the treatment with azadirachtin, the concentration of 0.01% caused the least *S. frugiperda* larvae mortality, in comparison to the other treatments, not differing, although, of the control (water). The same occurred at five and seven days after the application of the treatments. The concentrations of 0.16%, 0.40% and 1.00% of Azamax® showed efficiency higher than 80% on the larval mortality at seven days after the application, being that the number of dead larvae had no difference in comparison to the lufenuron treatment. At ten days, the concentration of 1.00% performed the best control of the pest, showing efficiency of 100%, similar index in relation to the obtained with the chemical insecticide (Table 1). Independently of the azadirachtin concentrations, there was deleterious effect on the insect, interfering on the insect mass. Under the conditions in which this work was performed, Azamax® showed satisfactory insecticide effect on *S. frugiperda*, being its efficiency similar to the chemical product (Table 1).

Table 1: Average number of dead larvae (N), product efficiency (%E) on the control of *Spodoptera frugiperda* at three, five, seven and ten days after the treatment on bean leaves and the body mass (M) at ten days. Jaboticabal, SP, Brazil, 2012.

Treatment	Conc.	3		5		7		10		$M^{1(n)}$
	c.p. (%)	N^1	%E	N^1	%E	N^1	%E	N^1	%E	(mg)
1- Water	-	0.00c	-	0.83c	-	1.17b	-	1.33d	-	$42.9^{(22)}$
2- Azamax	0.01	0.50bc	10.00	1.33bc	26.67	1.67b	33.33	2.00cd	40.00	$6.6b^{(18)}$
3- Azamax	0.03	1.00abc	20.00	2.17abc	43.33	2.83ab	56.67	3.17abc	63.33	$7.6b^{(08)}$
4- Azamax	0.06	0.83abc	16.67	1.83abc	36.67	2.67ab	53.33	3.00bc	60.00	$4.5b^{(11)}$
5- Azamax	0.16	1.33abc	26.67	3.67a	73.33	4.17a	83.33	4.33ab	86.67	$4.6b^{(04)}$
6- Azamax	0.40	1.83ab	36.67	3.33a	66.67	4.33a	86.67	4.33ab	86.67	$2.0b^{(04)}$
7- Azamax	1.00	1.50ab	30.00	2.50ab	50.00	4.50a	90.00	5.00a	100.00	-[2]
8- Match	0.15	2.33a	46.67	3.83a	76.67	4.33a	86.66	5.00a	100.00	-[2]
F (Treatment)		4.55**		7.23**		9.37**		12.16**		13.22**
C.V.(%)		28.37		19.21		16.13		13.47		1.69

[1]Averages followed up by the same letter in the column not differ significantly among them by Tukey test at 5%probability. [n]Number of analyzed larvae.[2]Insuficient number of larvae to the statistical analysis (To the analysis the data were transformed at $(x+0.5)^{1/2}$).

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LEAF REMOVAL AND GROWTH IN AN EARLY STAGE OF BEAN (*PHASEOLUS VULGARIS* L.)

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INTRODUCTION.Plant shoot, and especially its leaves, are the *source* of photosynthates. These are transported to non-photosynthetic parts (*sinks*) such as roots, especially important during the initial stage of development where the photosynthates are used for energy and growth. Thus, a dependence between shoot and root is established. This dependence is involved in the biomass or dry weight partition following the source-sink physiological concept (Taiz and Zeiger, 2010). Therefore, the removal of leaves, as in the case of damage by leaf-eating insects or hailstorms which decreases the size of the source, influences the further growth of the plant. The objective of the present work was to explore the growth of the bean plant when some leaves are removed in an early stage of development.

MATERIAL AND METHODS.Bean cv. Bayo Madero, type III was employed. The plants were grown under greenhouse conditions. The experimental design was a completely randomized with five replications and four treatments: 1) control (intact plant), 2) removal of the first compound leaf (FCL), 3) removal of the two simple leaves (SL) and 4) removal of the first compound leaf plus the two simple leaves (FCL+SL). Seeds were sowed (27/09/2011) in plastic pots, filled with 1189 g of red "tezontle" (inert volcanic cinder substrate). The experimental unit consisted of a pot with one plant. The plants were watered with a complete nutrient solution (Steiner, 1984). When 80 percent of the plant population had just displayed the first compound leaf, the treatments were applied. At this time the cotyledons were either shriveled and abscised or about to abscise. Fourteen days later, the plants were sampled and their growth determined. The plants were separated into their component organs. The root volume was determined by water displacement. All the plant organs were dried in a forced-air oven at 70 ^{o}C for 72 hours to determine their dry weight. Besides, the root:shoot ratio was calculated. Statistical analysis of data was performed by using the SAS program.

RESULTS AND DISCUSSION. **Root Volume**. Root volume for all the treatments was lower than the control, but the two lowest were SL and FCL+SL, in which the simple leaves were removed. These results point out the capital role of the simple leaves in the root growth in the early development of the plant (Table 1). **Root Dry Weight**. FCL+SL showed the lowest dry weight and was different from the control and FCL (Fig. 1A). Root dry weight differences were due to the root *new growth* that took place from the day when treatments were given to the day of sampling. However, it must be pointed out that the photosynthates generated in that period were involved not only in the root growth, but also in the shoot *new growth*. **Shoot Dry Weight**.Dry weight differences of the shoot were due to the biomass synthesized *after* the treatments. In relation to the treatments SL and FCL+SL, it is evident the effect due to the excision of the simple leaves compared to the effect of the excision of the first compound leaf (just displayed) in FCL treatment (Fig. 1B). **Dry weight partition and root: shoot ratio**. The

plant response to leaf removal was evident in the differential dry weight allocated to root and shoot (Table 1).

Table 1. Root volume, dry weight partition and root:shoot ratio of bean subjected to removal of leaves in the early growth stage.

Treatment	Root volume (cm^3 pl^{-1})	Dry weight partition (%)		Root:shoot ratio (%)
		Root	Shoot	
Control	17.0 a*	33	67	38
First compound leaf (FCL)	11.4 b	26	74	35
Simple leaves (SL)	5.6 c	27	73	49
First compound leaf +Simple leaves (FCL+SL)	4.1 c	28	72	37

*Different letters within column indicate statistical difference.

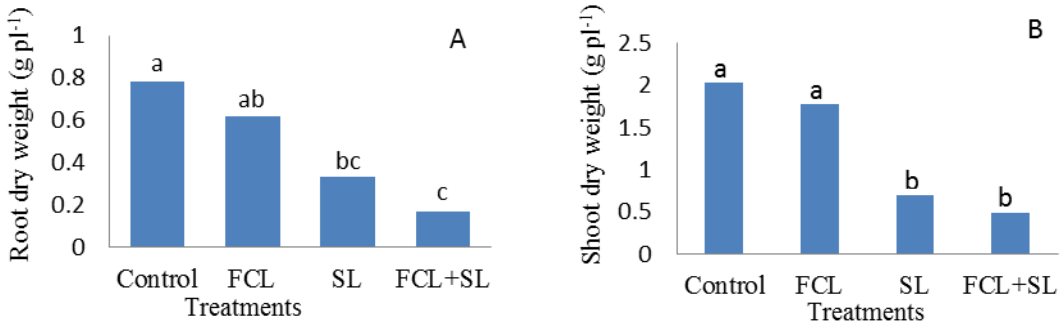

Figure 1. Dry weight of root (A) and shoot (B) after fourteen days of treatments application. Treatments: FCL=removal of the first compound leaf; SL= removal of simple leaves; FCL+SL=removal of the first compound leaf plus simple leaves. pl=plant. Different letters above the columns indicate statistical differences.

CONCLUSIONS. In an early stage of the vegetative period of bean (when the first compound leaf had just displayed) the removal of the simple leaves had a capital importance for the growth of the root and shoot. This might be critical for the establishment of the plant. Simple leaves derive their biomass in part from the cotyledons reserves. In the case of FCL treatment, the results suggest that the contribution of photosynthates from the simple leaves (already developed when the first compound leaf was excised), might be important in the *new growth* of the plant.

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EFFICIENCY OF DIFFERENT THERMAL PROCESSES TO REDUCE ANTINUTRITIONAL FACTORS IN COMMON BEAN CULTIVARS

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INTRODUCTION: Common bean (*Phaseolus vulgaris* L.) consumption has been decreasing in México due to few fast food options prepared with this legume. An increase in the consumption of common bean has been associated with a reduction of clinical cases for diabetes, heart attacks, cancer and other diseases related to obesity. Common bean seeds contain hemagglutinins and trypsin inhibitors, considered as important antinutritional factors (Reynoso *et al*., 2006). Influence of traditional cooking and other thermal processes need to be validated in order to reduce antinutritional factors contained in common bean raw seeds. Industry need low cost technologies to use bean seed and grain wastes to produce healthy and high value added processed foods. The objective of the study was to measure the efficiency of two thermal processes to reduce seed antinutritional factors contained in four common bean cultivars.

MATERIALS AND METHODS: Common bean seed flour was obtained by grinding the grains of Pinto Saltillo, Negro San Luis, Flor de Mayo Media Oreja and Canario Magón cultivars in a hammer mill (Thomas-Wiley Miller Lab, Model 4). Traditional cooking time for each cultivar was determined using Mattson cooker apparatus with 25 (90 ± 1 g) plungers, heat resistant glasses of 2000 mL, distilled water, and an electric stove. After determining the cooking time using the Mattson method, 2 kg of each cultivar were cooked according to the appropriate time for each cultivar (Pinto Saltillo 76 min, Negro San Luis 63 min, Flor de Mayo Media Oreja 96 min y Canario Magón 59 min). Seeds were also cooked using different autoclaving time process (0, 10, 20, 30, 40, 50 min), starting when 121 °C and 15 lbf were reached. After cooking the seeds, they were dehydrated in a forced air oven (50 °C; 72 h) and then grounded to obtain common bean flour. Raw seeds flour was also included for each cultivar as a reference for determining Trypsin Inhibitors Units (TIU) (Official Method Ba 12-75) (AOCS, 1993) and hemagglutinins content (hemaglutinant activity modified method) (Jaffé, 1980). A completely randomized design in factorial arrangement with two replications was used. The analysis of variance and means comparison test (least significant difference) were performed using MSTAC ver. 3.1.

RESULTS AND DISCUSSION: Highly significant ($p<0.01$) differences were observed for TIU and hemagglutinins content among cultivars and treatments (Tables 1 and 2). Highest TIU values were registered in raw seeds of cultivars Flor de Mayo Media Oreja (27.2 TIU mg^{-1}). Significant lower values for TIU were observed in raw seeds of Negro San Luis (6.0 TIU mg^{-1}) and Canario Magón (7.5 TIU mg^{-1}). Flor de Mayo Media Oreja showed highest values for hemagglutinins activity 191,716 U while Negro San Luis registered the lowest hemagglutinins activity 38,760 U considering cultivar averages. Irregular response was observed among treatments and cultivars for hemagglutinins content and highest value was observed in Flor de Mayo Media Oreja in 30 min autoclaving treatment. Traditional grain cooking seems to be an efficient method to reduce hemagglutinins content in Pinto Saltillo (3,967 U), Negro San Luis (4,156 U), Flor de Mayo Media Oreja (32,150 U) and Canario Magón (16,368 U). Similar results were observed only for

Canario Magón in 50 min autoclaving process (16,342 U). In other cultivars (Pinto Saltillo and Negro San Luis) lower values in hemagglutinins content was observed from 30 min. Hemagglutinins in Flor de Mayo Media Oreja showed persistence after 50 min of autoclaving process.

CONCLUSIONS: Traditional cooking and autoclaving resulted efficient methods to reduce TIU in all the evaluated cultivars. Flor de Mayo Media Oreja common bean cultivar showed the highest values for trypsin inhibitors and hemagglutinins content. Traditional cooking showed better efficiency compared to autoclaving for reducing hemagglutinins in common beans. Differential response was observed among cultivars and autoclaving time for reducing hemagglutinins content.

Table 1. Trypsin inhibitors content in four common bean cultivars with eight cooking treatments.

Treatments	Pinto Saltillo	Negro San Luis	Flor Mayo Media Oreja	Canario Magón
	(TIU mg^{-1})*			
1. Raw seeds	9.5^{c}	6.0^{de}	27.2^{a}	7.5^{d}
2. Traditional Cooking	2.0^{hijkl}	2.0^{hijkl}	3.0^{gh}	4.7^{ef}
3. Autoclaving (0 min)	3.8^{fg}	0.6^{klm}	16.9^{b}	3.7^{fg}
4. Autoclaving (10 min)	1.2^{ijklm}	0.5^{lm}	5.0^{ef}	4.7^{ef}
5. Autoclaving (20 min)	2.3^{ghij}	0.5^{lm}	1.1^{ijklm}	2.1^{hijk}
6. Autoclaving (30 min)	1.6^{hijklm}	1.3^{ijklm}	0.8^{jklm}	2.6^{ghi}
7. Autoclaving (40 min)	1.2^{ijklm}	1.3^{ijklm}	0.4^{m}	1.3^{ijklm}
8. Autoclaving (50 min)	1.1^{ijklm}	5.4^{e}	0.3^{m}	1.7^{hijklm}
Mean**	2.8^{b}	2.2^{b}	6.8^{a}	3.5^{b}
CV (%)	19.4			

*Trypsin inhibitors unit; **[a-b]Differences among cultivars; [a-m]Differences among cultivars and treatments.

Table 2. Hemagglutination activity performed in four common bean cultivars witheight cooking treatments.

Treatments	Pinto Saltillo	Negro San Luis	Flor Mayo Media Oreja	Canario Magón
	(Units)*			
1. Raw seeds	$226{,}904^{d}$	$123{,}828^{f}$	$239{,}322^{c}$	$103{,}618^{h}$
2. Traditional Cooking	$3{,}967^{m}$	$4{,}156^{m}$	$3{,}2150^{j}$	$16{,}368^{l}$
3. Autoclaving (0 min)	$123{,}945^{f}$	$65{,}704^{i}$	$248{,}011^{b}$	$116{,}762^{g}$
4. Autoclaving (10 min)	$65{,}337^{i}$	$33{,}497^{j}$	$250{,}332^{b}$	$124{,}262^{f}$
5. Autoclaving (20 min)	$65{,}894^{i}$	$32{,}406^{j}$	$251{,}635^{b}$	$65{,}663^{i}$
6. Autoclaving (30 min)	$15{,}913^{l}$	$16{,}726^{l}$	$256{,}780^{a}$	$64{,}709^{i}$
7. Autoclaving (40 min)	$16{,}285^{l}$	$16{,}858^{l}$	$129{,}793^{e}$	$28{,}284^{k}$
8. Autoclaving (50 min)	$16{,}413^{l}$	$16{,}875^{l}$	$125{,}703^{f}$	$16{,}342^{l}$
Mean**	$66{,}830^{b}$	$38{,}760^{c}$	$191{,}716^{a}$	$67{,}000^{b}$
CV (%)	2.1			

*Hemagglutins units; **[a-c]Differences among cultivars; [a-m]Differences among cultivars and treatments.

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ASTRAGALINE LEVELS IN CREOLE AND BREEDING LINES OF COMMON BEAN FROM RIO GRANDE DO SUL STATE, BRAZIL

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INTRODUCTION

The common bean isoneof the mainfoods in the dietof Brazilians, being able to supplyan important part ofmanyimportant substancesin maintaining healthsuch as the antioxidants.

Besides being a source of essential nutrients, the beans have chemical constituents that are considered with action functional components such as flavonoids, including flavonols, glycosylated or not, anthocyanidins, proanthocyanidins and isoflavones and phenolic acids. Present in large amounts of vegetables, phenolic compounds are associated as chemical mediators between plants and microorganisms. Among these compounds, flavonoids are the most interesting because they are related to various functions such as protection, resistance to various types of stress, nitrogen fixation, among others (GONZALEZ de MEJIA et al., 2005).

The common beanhas someundesirableattributes, such asphytates, factorsflatulent, phenolic compounds, enzyme inhibitors, hemaglutinin(lectin) and allergenswhich mustbe eliminated forits effectiveuse as food(GUPTA, 1987). Thehighest concentration of polyphenolsis found in colored seed coats, and the peelwhite seedsand otheranatomical partsof the seedhave lowerconcentrations (BRESSANI et al., 1991).
Theobjective of this studywas to investigatethe behavior oflandraces andimprovedcultivarsof common beansfrom RioGrande do Sulfor the levels ofastragalina in whole grains.

MATERIAL AND METHODS

The field experimentwas conductedat the Cascata Experimental Stationof EmbrapaTemperate Climate,inPelotas, Rio Grande do Sul State in the year2009/2010, using300 kgha-1NPK fertilizer.The seeds were sownin October 2009and harvestingin February 2010. The seeds were driedand placedin cotton bagsduring thestorage period. Levelsof antioxidantastragalinalandraces andimprovedcultivarsof beans withdifferent colorsof the grain were analyzed, according Correia et al. (2006)..

The cultivarswere divided intotwo groups:thefirst group formed by thelandraces;the second by cultivars and breeding lines from differentbreeding programs.Data wasanalyzed using thestandard deviation; are consideredsuperior those genotypes whoobtaineda value exceedingthe mean plusonestandard deviation.

RESULTS AND DISCUSSION

The distributionof phenolic compoundswas quite variable, where thecultivarswith red andyellow seed coatspresented higher levelswhen compared to those with black seed coats,with the exception ofFTNobre andTB02-11.The cultivarFTNobreisrecognized as of a good nutrition quality, and TB02-11 is a breeding line. In this study the genotypesTB02-24 (10.632 mg/100 g), TB02-26 (11.550 mg/100 g), TB 02-20(5.000 mg/100 g) and Amarelinho Iolanda(3.791 mg/100 g), presented the highest astragaline content.
Study withfourdifferentcolorsof beans, using the technique ofHPLC/MSshowed thatthe highest level ofastragalinawasobserved in seeds ofnon-staining black(Hu etal., 2006). The landraces had mean values slightly higher than cultivars from breeding programs.
The methodfor quantification ofAstragalinawas validatedaccording to current regulations, and wasjustified inits purposes.

Table 1 – Astragaline levels in creole and breeding lines of common bean from Rio Grande do Sul State, Brazil. 2010.

Creole cultivar	Seed color	Astragaline (mg%)	Breeding line	Seed color	Astragaline (mg%)
Mouro Tavares 187	Purple	0,216	03 FPJ CF 29-1	Black	0,134
Mato Grosso	Black	0,799	FT Nobre	Black	11,050
Roxo Redondo	Purple	0,209	BRS Expedito	Black	0,142
Amarelo Iolanda	Yellow	3,791	BRS Valente	Black	0,094
AM 5	Black	0,015	Minuano	Black	0,100
Guabiju Brilhante	Black	0,079	Guapo Brilhante	Black	0,216
Rosinha Precoce	Black	2,581	Macanudo	Black	0,897
Preto Ibérico	Black	0,061	Macotaço	Black	0,076
Biriva 264	Black	0,221	BRS Pampeano	Black	0,027
TB 0220	Black	5,000	BRS Guerreiro	Black	0,165
TB 0221	Black	0,032	TB 0201	Black	0,025
TB 0222	Black	3,791	TB 0203	Black	0,665
TB 0223	Black	0,070	TB 0207	Black	0,059
TB 0224	Red	10,632	TB 0210	Black	0,045
TB 0225	Black	0,015	TB 0211	Black	10,502
TB 0226	Red	11,550	TB 0212	Black	0,294
TB 0301	Black	0,015	TB 0213	Bege	2,423
TB 0302	Black	0,048	TB 0219	Black	0,128
TB 0303	Black	0,067	Iraí	Cream	0,024
TB 0304	Cream	0,015	Carioca	Cream	0,058
TB 0305	Black	2,581			
TB 0306	Black	0,015			
TB 0307	Red	0,043			
TB 0308	Black	0,058			
TB 0309	Brown	0,392			
TB 0310	Red	0,068			
Media		1,571	Media		1,380
Standart deviation		3,087	Standart deviation		3,360

CONCLUSION: Among landraces, TB 02-26, TB 02-24, TB 02-20 and Amarelo Iolanda, and from cultivars and breeding lines, FT Nobre and TB 02-11 were those with higher levels of astragaline. The highest concentrations of astragaline were found in seeds with no black seed coats, although some black bean cultivars showed high levels.

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SEED PHYTATE QTL IN COMMON BEAN AND ASSOCIATION WITH CANDIDATE GENES

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INTRODUCTION

Common bean is an important, high-quality staple food that provides large amounts of protein and mineral micronutrients to the diets of people in many countries. Phytates are a storage form of organic phosphorus which is used by the plant in various stages of growth and development but can have certain anti-nutritive properties due to chelation of minerals such as iron and zinc. At the same time, phytates provides certain health benefits and therefore are the subject of both mutagenesis and breeding program objectives for functional food properties. The objective of this study was to evaluate the quantitative trait loci associated with seed phytate concentration and content on a per seed basis and to develop functional molecular markers for genes from the phytic acid synthesis pathway.

MATERIALS AND METHODS

Plant Material and Field Experiments: We used a well-characterized mapping population, DOR364 x G19833 in three field experiments across two seasons with three repetitions each and two levels of soil phosphorus fertilization in the second season. Plantings were in Darien, Colombia (1,400 m above sea level; 20 ºC average yearly temperature, 1,650 mm annual rainfall, Udand soil type, pH 5.6) where native soil phosphorus (P) levels are around 10 ppm. Fertilization with superphosphate at a rate of 60 kg / ha P was applied in row to obtain commercial level production in 2007a. In 2007b, two trials were conducted: one under the full rate of high P fertilization (HP) and one trial with medium levels of P fertilization (MP) based on 30 kg / ha P. Randomized complete block designs with 3 repetitions each were used in all three experiments. At harvest, the seeds were weighed for 100 seed weight in grams and stored at 20 °C and 30% relative humidity until grinding. Before grinding, a sample of 10 seed from each plot was hand cleaned with de-ionized water and then freeze dried for two days in a freeze drier. This seed was ground in teflon chambers with zirconium grinding balls in a modified Retsch mill.

Phytate extraction: Phytate extraction used a similar method as Blair et al. (2009) based on 0.5 g of ground seed powder and acid digestion with 20 mL of 0.65M HCl. Phytates were purified from raw extracts by diluting to 0.2 X in de-ionized water and loading on an AG1-X8 solid-phase columns connected to a VisiprepTM Solid Phase Extraction Vaccum Manifold at 5 mmHg pressure. Column-bound phytates were washed once with 10 mL of 0.07M NaCl to eliminate free phosphates and other impurities. Elution of the phytates from the columns was then conducted with 10 mL of 0.7M NaCl. Following extraction, phytates were measured with the Wade reagent on a UV-1601 spectrophotometer.

RESULTS AND DISCUSSION

Candidate Genes: A total of 11 genetic markers were tested on the parents of the recombinant inbred line (RIL) population. Of these, 6 were based on the gene sequences produced by Fileppi et al. (2009) and 5 were newly designed markers for the same genes analyzed by this previous study but targeted to introns that might be more polymorphic based on common bean-soybean gene structure comparisons. The newly designed markers were developed based on soybean and common bean sequences for PvIPK1, PvIPK2, PvITPKα (also known as PvIPK3), PvITPKβ, PvMIK, PvMIPSv and PvMIPSs. These genes encode myo-inositol (3)P1 synthase, myo-inositol kinase and various inositol kinases. Agarose gel evaluation or CAPS digestions were as described in Fileppi et al. (2009) while single strand conformation polymorphism (SSCP) gels were as described in Galeano et al. (2009b). We also conducted a synteny analysis based on common bean versus soybean genome comparisons for all the phytic acid pathway genes. The candidate genes were genetically mapped so that they can be useful for marker assisted selection.

QTL Identification: Seed phytate concentrations were calculated and expressed as percentage of phytic acid in total seed, based on a molecular weight of 660. Analysis of variance was carried out and the averages used to detect quantitative trait loci (QTL) with single point analysis conducted with the program qGENE to discover the determination coefficients (R2) of each marker associated with phytate concentration. Table 1 shows that MIPSs, one of two paralogs of the myo-inositol (3)P1 synthase gene family, located on linkage group B01 and expressed in common bean seed rather than in vegetative tissues was significant for association with seed phytate levels in the population.

Table 1. Markers for the phytic acid biosynthesis pathway, their map position on the DOR364 x G19833 common bean (*Phaseolus vulgaris*) mapping population and in segments of the soybean (*Glycine max*) genome and their association (R2 value) with phytate concentration.

Gene	GenBank entry	LG in D x G (*P.vulg*)[1]	Syntenic chrom. (*G. max*)[2]	Phytate R^2
IPK1	AM491806, FN356966	na	**Gm06** /Gm04	na
IPK2	FN356968	b11	Gm11/**Gm12**	ns
IPK3	AM887977	b03	**Gm05Gm17**	ns
MIPSv	AM048843	b02	Gm05 **Gm08**	ns
MIPSs	New entry	b01	**Gm18-**	0.14
ITPKb	New entry	b02	NA	ns

ns = represents non-significant in the R2 columns for phytate and phosphorus concentration. Significance values shown for MIPSs. [1]/ linkage group (LG).in common bean [2]/ Synteny location according to blastx of the common bean sequence to the soybean (Gm) genome with the bolded being the higher hit.

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QTL FOR IRON REDUCTASE ACTIVITY IN COMMON BEAN ROOTS AND ASSOCIATION WITH NUTRITIONAL QUALITY

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INTRODUCTION

Plant iron uptake is a key element of plant nutrition and helps determine crop nutritional quality. Strategy I plants, such as common bean take up iron through a process that involves an iron reduction mechanism in their roots. This reduction is required to convert ferric iron to ferrous iron, prior to the uptake of the latter form. Root absorbed iron is critical for the iron nutrition of the plant, and for the delivery of iron to the shoot and ultimately the seeds. Legume seeds, including those of common beans, are good sources of iron because they are generally rich in this element. The objectives of this study were to determine the inheritance of iron reductase activity in a low x high seed iron cross (DOR364 x G19833), to identify quantitative trait loci (QTL) for this trait, and to assess possible associations with seed iron levels.

MATERIALS AND METHODS

Iron Reductase Experiments: Two growth conditions (1 and 15 µM concentration, respectively, iron limited and iron sufficient) were selected as treatments for evaluating the DOR364 x G19833 recombinant inbred lines. For each experiment, seeds were germinated for 4-6 days on germination paper and then planted and assayed from hydroponic media in a growth chamber with excised, entire root systems using 12-day old plants which were also weighed to determine fresh weight *per* methods described in Grusak et al. (1990). Rates of iron reduction were expressed as µmol Fe reduced g FW-1 h-1. Values used in QTL determinations were the averages of four root systems.

Marker Development: Two techniques were used for marker development. First, a search was made of FRO-like sequences in the bean EST database, with one simple sequence repeat marker made (Forward 5'-CCACAGCTTTGATCTCTA GC-3' and Reverse 5'-CACAGAAAACTGAGCATTCA-3'). Second, primers for PvFRO (For-5'-GAGGCCTACGTTACCAGAGAAAA--3', Rev 5'-CGGTGTTGGAACTTCCACATTC-3') were designed from FRO mRNA sequences cloned by degenerate primers and used in a cleaved amplified PCR sequence (CAPS) reaction with the enzyme *Hind*III. The CAPS markers was evaluated on 1.5% agarose gels run in 0.5X TBE; while the microsatellite marker was run on 4% polyacrylamide silver stained gels. Genetic mapping of successful markers was performed with Mapmaker v. 3.0 and a minimum LOD of 3.0 using the genetic map from Blair et al. (2009).

QTL analysis: QTL were detected first with composite interval mapping (CIM) analysis that was carried out using the software program QTL Cartographer v. 2.5 and the genetic map for the DOR364 x G19833 used in Blair et al. (2009) along with the markers for iron reductase genes

described above. In the CIM analysis, determination coefficients were calculated for each interval separately (R2) and for each interval given the background markers (TR2). QTL were reported for LOD>2.5. Single point regression analysis was conducted for the successful FRO markers.

RESULTS AND DISCUSSION

A single major QTL was found for iron reductase activity under iron-limited conditions (1 µM Fe) on linkage group b02 and another major QTL was found under iron sufficient conditions (15 µM Fe) on linkage group b11. Additionally, markers for bean iron reductase (FRO) homologues were found on linkage groups b06 (for the SSR marker) and b07 (for the CAPS marker), with *in silico* mapping based on common bean synteny with soybean and Medicago confirming that the b07 locus is homologous with loci on Gm10 and Mt01.

While none of the loci aligned with the QTL for iron reductase activity, genes conditioning iron reductase activity in iron sufficient bean plants appear to be associated with genes contributing to seed iron accumulation (Table 1). Namely, associations between the b07 and b11 QTL were found with several QTL for seed iron. The fact that the QTL for iron reductase activity aligned with seed iron accumulation QTL made suggests the underlying gene may be homologs of genes controlling iron homeostasis.

In conclusion, the QTL for iron reductase activity under iron limited conditions may be useful in environments where beans or other legumes are grown under iron deficiency in alkaline soils, while the QTL for iron reductase under sufficiency conditions may be useful for selecting for enhanced seed nutritional quality.

Table 1. Association of FRO loci and flanking markers with iron reductase activity (IRA) under iron limited (1 µM Fe) and iron sufficient (15 µM Fe) growth conditions and with seed iron concentration.

		IRA (1 uM)		IRA (15 uM)		Fe-ICP[1]		Fe-AAS[1]	
Locus	**Chr.**	**LOD**	**R2**	**LOD**	**R2**	**LOD**	**R2**	**LOD**	**R2**
FRO – SSR	6	0.33	1.20	0.04	0.18	1.23	3.21	0.10	0.35
Bng009	6	0.39	1.44	0.05	4.74	0.95	2.62	0.11	0.6
Bng027	6	0.02	0.05	1.08	0.23	1.15	2.89	0.21	0.4
FRO – CAPS (*Hind*III)	7	0.97	1.70	0.05	2.90	0.06	0.17	2.31	6.2
Bng060	7	0.14	0.44	0.63	2.66	1.02	1.02	**3.85***	**10.5***
Phs	7	0.12	0.40	0.23	1.84	1.46	1.46	**3.30***	**10.6***

1/ Fe-ICP and Fe-AAS as described in materials and methods and Blair et al. (2009), respectively.
* indicates significance at LOD threshold of 2.5 in single point regression analysis.

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MAPPING HEALTH RELATED TRAITS IN THE COMMON BEAN GENOME

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ABSTRACT: We have analyzed segregation of 51 SSRs markers in a RILs population and identified eight linkage groups of the common bean genome corresponding to core linkage groups. A putative QTL for anthocyanin content was identified linked to three markers and mapped to linkage group 2 of the common bean genome.

INTRODUCTION: The common bean (*Phaseolus vulgaris* L.) is among the five domesticated *Phaseolus* species that are native to the Americas. It's has high nutritional value helps to manage diabetes, reduces cholesterol, and cancer risks. Common bean (2n = 2x = 22) represents 50% of the grain legumes consumed worldwide. The genome size for common bean is estimated to be about 450 to 650 million base pairs (Mb)/haploid (Bennett and Leitch 1995) and is considered to be one of the smallest genomes among major crop species. DNA-marker-based genetic linkage maps have been developed and exploited to identify, tag, and map disease resistance genes and QTLs (Quantitative Trait Loci) for several traits in common bean, which led to improved breeding strategies and implementation of marker-assisted selection. The ultimate goal of our research was to enhance common bean with micronutrients using genomic approaches. Over the last few years, we have been studying genetic diversity among common bean genotypes, mineral and antioxidant contents, and environmental influence on mineral composition in common bean seed. The objectives of this study were to 1) genotype a RILs population with molecular markers, 2) to develop genetic linkage map of the common bean genome, and 3) to exploit the intervals of this map to identify QTLs for health related traits.

MATERIALS AND METHODS: ***Plant Materials****:* A recombinant inbred line (RILs) population (AG) consisted of 54 lines derived from a cross between genotype A55 and G122 were grown in three replications at Mayville State University green house during December, 2010 to April, 2011.

Anthocyanin Assay: After harvesting, 2-3 seeds from each line of each replication was ground using mortar and pestle. Three hundred microliter of methanol with 1% HCl was added to 400 mg ground sample and mixed thoroughly. The mixture was incubated overnight at 4 °C in a dark container. After incubation, the mixture was diluted by adding 400 µl distilled water followed by adding 500 µl of chloroform. The mixture was centrifuged at high speed for 2-5 min. The supernatant was transferred to fresh centrifuge tube and the volume was adjusted to 800 ml by adding 60% methanol and 1% HCl. The absorbance of the extracted solution was recorded at 530 and 657 nm wavelength using spectrophotometer. The anthocyanin content was estimated by using the formula A_{530}-(0.25).A_{657} proposed by Rabino and Mancilleni, 1986.

DNA Preparation: Total genomic DNA was extracted and purified from leaf tissue of 2- to 3-wk-old greenhouse-grown plants using the CTAB method as described by Doyle and Doyle (1987). The DNA sample of each line including two parents was diluted to 50ng/µl with water and 150 µl of each sample was pipetted into 96-well PCR plate.

Selection of primers and PCR amplification: One hundred- twenty eight SSRs primers mapped to eleven linkage groups (http://www.css.msu.edu/bic/PDF/Bean_SSR_Primers_2007.pdf) of common bean core maps were selected. The screening of the primers for segregation was performed using a PCR program consisting of one cycle of 95ºC for 3 min; 35 cycles of 95ºC for

1 min, from 52 to 60ºC for 1 min, and 72ºC for 2 min; and one cycle of 72ºC for 10 min. The amplified PCR products were separated using 6% non-denaturing polyacrylamide gel electrophoresis (PGE) system for two hours at 200 volts and stained with ethidium bromide.

Data Analysis: Segregation of the parental PCR products among the progenies was scored visually. Using the software–carthagene (http://www.inra.fr/mia/T/CarthaGene/) scored data was analyzed. Genetic linkage groups were identified by LOD 3.0 and maximum map distance of 30 cM. Single marker analysis of Wilcoxon test in SAS 9.2 was performed to identify association between molecular marker and anthocyanin related trait.

RESULTS AND DISCUSSIONS: Sixty eight of 128 markers were identified as polymorphic in the AG population and the segregation analysis of 51 markers has been completed in the population. The markers with their linkage group are presented in Table. 1. Eight linkage groups were identified with 27 markers; rest of the markers (24) could not be assigned into linkage group in this preliminary study. Large number of unlinked markers may be explained here that we have selected few random markers mapped into different regions of linkage groups. However, based on the assignment of markers in common bean core map, we have identified at least five groups out of 11 linkage group of common bean (Fig.1). Anthocyanin is a quantitative trait and controlled by many genes. Our analysis identified a QTL for anthocyanin associated with three markers PVBR78, PVBR94, and GATS91, all of these markers are mapped onto linkage group 2 in core map.

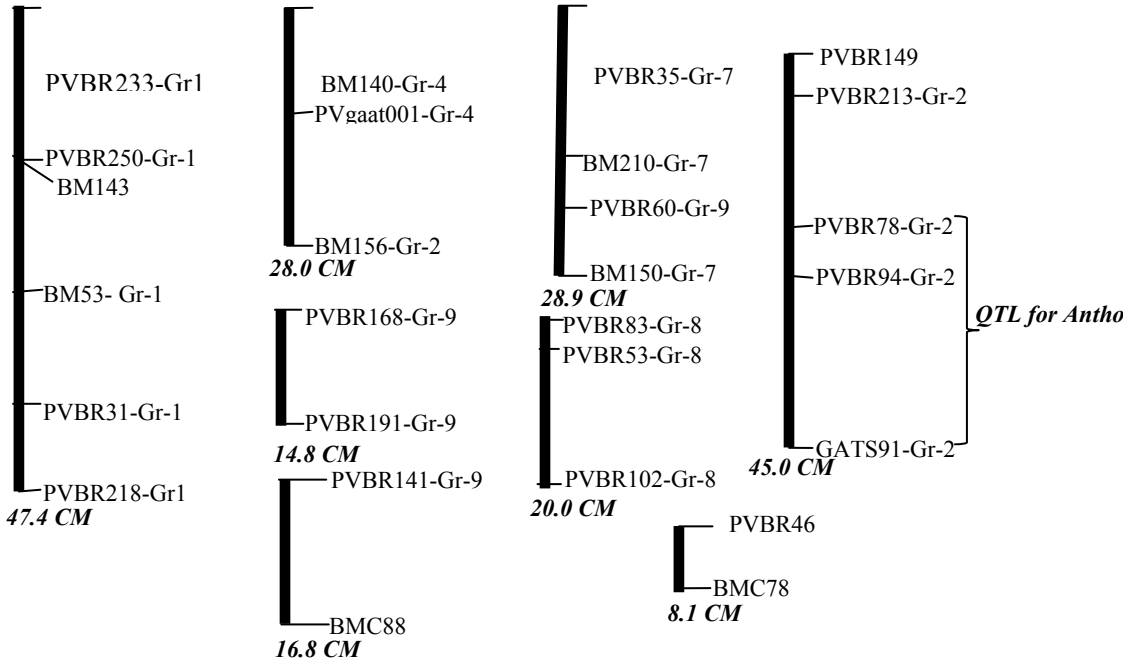

Fig.1. Linkage groups of common bean genome developed from AG population

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WATER SOAKING AND GRAIN COOKING OF COMMON BEAN CULTIVARS STORED FOR FOURTEEN MONTHS UNDER DIFFERENT CONDITIONS

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INTRODUCTION: The common bean is an important source of protein in the brazilians' diet. The grains of greatest national consumption are of the carioca type, but other types could be utilized, adding value to the product. For effective use of those types, however, it is essential to meet the consumers' requirements, which prefer grains with easy water absorption and reduced cooking time, characteristics which can be influenced by the storage prolonged. The objective of this work was evaluating the technological quality of the grains of cultivars of different commercial groups harvested in the 2010 fall-winter season and stored during 14 months under distinct conditions.

MATERIAL AND METHODS: The grains were produced in an experimental area at Uberaba, Minas Gerais State, Brazil, in the 2010 fall-winter season. At harvest, the statistical design was the completely randomized with five replicates and five cultivars:BRS Radiante (pinto), BRS Ouro Vermelho (red), BRS MG Talismã (carioca), BRS Supremo (black) and Bolinha (yellow). At 14 months, the randomized block design with 5 replicates and factorial scheme 5 x 2, involving the five cultivars and two storage conditions (room temperature-Amb and under refrigeration at 10 ^{o}C-Ref.), was assumed. The percentage of soaking before(PEANC) and after cooking (PEAPC), according to Garcia-Vela and Stanley (1989) and Plhak, Caldwell and Stanley (1989) and the average cooking time (TMC) by the Mattson Cooker in according to Proctor and Watts (1987) was determined.

The data were submitted to the analysis of variance. In the case of significance of the factors under study, the means were grouped by the Scott-Knott test at 5% of probability.

RESULTS AND DISCUSSION: In the analysis of variance of the harvest, PEANC, PEAPC and TMC presented significant F test for cultivars. As to the PEAPC, all the means were united together into a single group. As to the PEANC, however, cv. Bolinha presented lower value, which surely influenced its result in cooking, which went beyond 30 minutes. Good results were obtained by cv. Supremo both in the PEANC and PEAPC, as to the TMC shorter than 20 minutes.

After fourteen months' storage, the analysis of variance revealed that the TMC proved influenced by cultivars and by storage conditions, whereas PEANC and PAPC were affected by the cultivar x condition interaction. At 14 months of storage, differently from that observed at harvest, all the cultivars presented high TMC and do not differ from one another. In relation to refrigeration, the room condition increased by 78% the cooking time, reaching practically the double of the TMC obtained on the time of the harvest. Under refrigeration, the TMC at 14 months was greater than the initial one, but it did not go beyond 29 minutes (Table 2).

As compared with the initial situation, storage increased water soaking, a fact possibly owing to the overcome of impermeabilities or dormancies. Radiante and Ouro Vermelho cultivars stood out with higher PEANC in both the conditions. Under room temperature, the highest means for this characteristic were obtained by Bolinha, Supremo and Talismã cultivars. Under refrigeration, the superiority of Supremo cultivar kept, accompanied, nevertheless, by the

Radiante. Supremo cultivar stood out with higher PEAPC, while Ouro Vermelho was the one of lowest mean. The other cultivars presented intermediary and similar percentages among each other (Table 3).

Table 1. Average values of PEANC, PEAPC and TMC at harvest

Treatments Cultivar[1]	PEANC	PEAPC	TMC
	%		Minutes
B	89.33b	110.83a	32.21c
OV	97.73a	116.19a	26.61b
R	98.55a	123.20a	27.21b
S	97.42a	118.23a	19.61a
T	97.59a	120.21a	26.41b
Mean	96.12	117.73	26.41

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

Table 2 Average values of TMC at 14 months of storage.

Treatments Cultivar[1]	TMC
	Minutes
B	36.54a
OV	41.18a
R	39.56a
S	36.68a
T	44.11a
Condition	
Refrigerated	28.90a
Room	51.52b
Mean	40.21

Means followed by the same letter in the column belong to a same group according to the Scott-Knott test at 5% of probability..

Table 3 Average values of PEANC and PEAPC concerning cultivars and storage conditions at 14 months of storage.

Cult[-1]	PEANC		PEAPC	
	(%)			
	Amb.	Ref.	Amb.	Ref.
B	105.50aB	96.74bB	124.14aB	116.40bA
OV	100.37aC	98.27aB	111.05bC	118.50aA
R	103.90aB	104.52aA	120.56aB	120.91aA
S	108.52aA	103.90bA	129.95aA	120.95bA
T	104.28aB	99.17bB	119.84aB	120.58aA
Mean	104.51	100.52	121.11	119.47

Means followed by the same capital in the column and small letters in the row belong to the same group, according to the Scott-Knott testa t the level of 5% of probability

CONCLUSIONS: The storage for 14 months raises the common bean grain cooking time, an effect enhanced when there is no control of temperature and humidity of the storage place.

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COOKING QUALITY AND COLOR VARIABILITY IN A "FLOR DE MAYO" DRY BEAN POPULATION

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INTRODUCTION

In the Bean Program of the Campo Experimental Valle de México (CEVAMEX) Experimental Station, research is focused on developing new dry bean varieties which be resistant to pest, and have high yield, as well as nutritional and culinary quality, which contributes to improving the nutritional status of consumers. The new varieties are intended as well to be of preferred commercial type.

Flor de Mayo is a preferred market class for the central area of México (Castellanos *et al.*, 1997). During several years, a Flor de Mayo population made up of 130 lines, has been evaluated for disease resistance, short cooking time, protein content higher than 23.5 %, and outstanding lines were selected.

The objective of this study was assessing culinary quality and seed coat color of 16 Flor de Mayo recombinant inbred lines (RILs).

MATERIALS AND METHODS

During PV 2010, a group of 16 agronomically outstanding RILs was sown at Santa Lucía de Prías, Texcoco, Estado de México, also two commercial cultivars, Flor de Mayo M38, Flor de Durazno and a Bayo special line were sown as control. The experimental plot was one 4 m- long row with three replicates. All the plants of each plot were hand-threshed, and grain production was estimated.

Coat color of grains was measured using a CM-5 spectrophotometer (Konica Minolta, Inc., Osaka, Japan). Color reflectance was recorded in the CIE Lab color coordinate system, with D65 Illuminant and 10º observer.

The weight and volume of one hundred seeds wt. were measured, and cooking time measured in two samples of 25 grains, previously soaked in water for 18 hours according to a sensorial method (Guzmán *et al.,* 1995). Water absorption capacity, solids in broth were determined in replicated samples. Also broth color was measured using CM-5 spectrophotometer. Data were processed through an analysis of variance. Finally, the opinion of some bean sellers in the market about consumer's demand was requested in order to identify which of the RILs would have better acceptance in function of their color.

RESULTS AND DISCUSSION

RILs exhibited significant differences in grain color variables L*, a*, and b* ($P \leq 0.01$). Lightness (L*) varied in 24 units (table 1), associated to rose-pink mottled color up to almost black mottled colors.

According to consumers' preferences, mentioned by bean sellers, the genotypes with the preferred ones would be those in the range of L*= 43 up to 47; a*= 11 up to 13; b*= 10 up to 11.

The more pale-colored grains tended to absorb more water (r=0.39**), as well as the ones with yellow tones (b*), (r=0.40**). Results showed that bigger grains absorbed more water (r=0.58**) and tend to release more solids to cooking broth (r=0.37**). In the group of RILs, bigger grains exhibited paler color (r=0.32*) and were associated with thicker broths (r=0.49*).

The seed coat color L* values were not correlated with broth L* value. However variables a* and b* of the seed coat was correlated with L*, a* y b* values for broth color. When b* value in seed coat was low (all values were positive which correspond to yellow tones) broths were reddish (a*) (r= - 0.59**) which were related to darker broth color.

Even though some RILs were very similar in seed coat color, broth color was different among them.

There was as well a negative correlation between water absorption capacity and cooking time (r= -0.65**) The RILs with smaller grain size tend to show darker seed coat (-0.49**). Cooking time of the RILs varied from 55 to 108 minutes. Water absorption capacity was from 20 to 88 %, which indicates that some of them presented hard shell defect. The amount of solids in broth varied from 0.16 to 0.37 % (Table 1).

Five RILs surpass controls in yield. Among them four were of fast cooking, less than 64 minutes, while the control varied from 65 to 73 minutes.

Table 1. Color and cooking quality in Flor de Mayo dry bean RILs

	Average	Std dev	Min	Máx
Grain yield	1016.0	353.1	440.4	1571.0
Water absorption	59.7	25.6	20.0	88.0
100 seeds' weight	23.5	4.5	18.3	25.6
Cooking time	71.9	14.1	55	108.0
Solid in broth (%)	0.25	0.06	0.16	0.37
Seed coat color				
Lightness (L*)	45.2	5.6	36.7	60.7
a*	10.8	2.1	4.6	14.5
b*	11.5	5.4	5.5	32.5
broth color				
L*	0.6	5.7	70.7	94.6
a*	3.3	1.2	-0.6	5.0
b*	16.6	3.5	7.8	23.2

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EVALUATION OF THE LATE SEED-COAT DARKENING OF CARIOCA TYPE DRY BEANS

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INTRODUCTION

Most of the dry beans grown in Brazil are of the carioca grain type, that is, cream color and beige streaks. Among the characteristics associated with the carioca grain type, the lightest possible cream color is highly desirable. Recently, a carioca cultivar was identified which has a very light grain color and, in addition, it maintains this phenotype for some time; i.e., the grains remain light colored, which leads to greater commercial value (Silva et al., 2008). The present study was performed for the purpose of estimating genetic and phenotypic parameters of late seed-coat darkening trait of grains, with a view toward directing breeders in regard to selection of individuals and/or progenies with the desired phenotype.

MATERIAL AND METHODS

Field experiments were conducted in Lavras and Patos de Minas, MG, Brazil. The population of the cross between the cultivar BRSMG-Madrepérola (carioca type grain with very light cream background and this color persists for a long period of time) and the RP-2 line (carioca type grain with cream background that darkens rapidly) was used. The progenies of the $F_{2:3}$ generation were assessed in the "dry" growing period (sowing in February 2011) in Lavras and the progenies $F_{2:4}$ in the "winter" growing period (sowing in July, 2011) in Lavras and Patos de Minas. The grain darkening score were assessed at 30, 60 and 90 days after harvest (DAH) for $F_{2:3}$ and at 30 and 60 DAH for $F_{2:4}$. For that purpose, samples of grains from the progenies, after harvest, were placed in transparent plastic bags in a dark area. These samples of the progenies were assessed by two evaluators for the grain darkening trait by means of a scale of scores ranging from 1 to 5, with 1 for light colored grains and 5 for dark colored grains.

Analysis of variance of the grain darkening scores was carried out initially per generation. For joint analysis were considered the analyses at 30 and 60 DAH, involving the $F_{2:3}$ and $F_{2:4}$ generations or the locations (environments) in the $F_{2:4}$ generation. Genetic and phenotypic parameters were estimated by the expressions presented by Ramalho et al. (2012).

RESULTS AND DISCUSSION

The progenies $F_{2:3}$ showed significant difference ($P \leq 0.00$) in relation to the grain darkening scores. Nevertheless, the interaction progenies x time periods was not significant, indicating that the behavior of the progenies coincided in the different time periods of assessment. It was observed that the estimates of heritability for selection at the mean of the progenies, increased with the age of assessment (Table 1). However, the increase was not very expressive and, in almost all cases, there was overlap in the confidence intervals. The values obtained were similar to those reported by Silva et al. (2008) and shows that that it is possible to successfully perform selection for the trait of late grain seed-coat darkening scores and that this selection may be performed earlier; that is, even at 30 DAH.

Table 1. Means of the grain darkening scores of beans and estimates of heritability (h^2) between the $F_{2:3}$ and $F_{2:4}$ progenies in different time periods of assessment, and of joint analysis of the two environments in different time periods of assessment. Lavras/ Patos de Minas, MG, 2011.

Generation/ Location / Time period	Mean	h^2
$F_{2:3}$/ Lavras / 30 DAH[1/]	3.01	72.39 (59.00-81.40)[2/]
$F_{2:3}$/ Lavras / 60 DAH	3.53	85.85 (78.99-90.47)
$F_{2:3}$/ Lavras / 90 DAH	3.72	87.03 (80.74-91.26)
$F_{2:4}$/ Lavras / 30 DAH	2.27	76.13 (64.56-83.92)
$F_{2:4}$/ Lavras / 60 DAH	2.50	85.38 (78.28-90.15)
$F_{2:4}$/ Patos / 30 DAH	3.71	85.36 (81.23-91.49)
$F_{2:4}$/ Patos/ 60 DAH	3.87	87.24 (81.05-91.40)
$F_{2:4}$ /Lavras and Patos/30 DAH	2.99	70.33 (55.91-80.04)
$F_{2:4}$ /Lavras and Patos/60 DAH	3.18	71.45 (57.56-80.79)

[1/] Days after harvest. [2/] In brackets, limits of the confidence interval of h^2.

In joint analysis involving the $F_{2:4}$ generation in Lavras and Patos de Minas, significant difference ($P \leq 0.000$) was also observed between progenies and the interaction progenies x time periods ($P \leq 0.178$) was also not significant. Nevertheless, the effect of locations, and all the interactions involving locations were significant ($P \leq 0.00$). Although the interactions progenies x locations were significant, the genetic correlation (r_G) between the means of the progenies in the two locations was $r_G=0.74$, allowing one to infer that the interaction was predominantly simple because there was no great alteration in classification of the progenies.

In joint analysis to verify the effect of growing periods/generations on the experiments conducted in Lavras, in the $F_{2:3}$ and $F_{2:4}$ generations, it was observed that the effect of progeny was significant ($P \leq 0.000$), and the interaction progenies x time period once more was not significant ($P \leq 0.082$). The effect of generations/growing period was significant, with the same occurring for the interactions progenies x generations and time periods x generations ($P \leq 0.000$). The lowest mean was obtained in the $F_{2:4}$ generation, with the experiment being performed in the fall/winter growing period and sowing performed in July.

Although the interaction progenies x generations was significant, it was observed that the genetic correlation between the two generations was $r_G=0.81$, which shows that the interaction is predominantly simple, thus not contributing to change in classification of progenies in the different generations.

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DIFFERENCES IN STABILITY OF COMMON BEAN GENOTYPES TO DARKENING AND HARDENING PROCESS DURING STORAGE

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INTRODUCTION: The rapid loss of technological quality of common beans (*Phaseolus vulgaris* L.) is a challenge for its commercial market, making it very unstable. During storage the beans tend to darken the seed coat and become more resistant to cook, especially if stored under adverse conditions of temperature and relative humidity, as occurs in the main producing regions of this grain (BRACKMANN et al., 2002). The search for common bean cultivars with technological characteristics of grain quality is of great importance in breeding programs and to the supply chain. Consumers associate the darkening of the seed coat to its aging and thus to the hardening process. However, there is evidence that not all the old grains are hard and/or dark. The objective of this study was to evaluate whether there is variation between cooking time and color of different genotypes during storage in order to identify the most stable cultivars in relation to these characteristics.

METHODS: Samples of five carioca bean genotypes were grown at Embrapa Rice and Beans. Afterharvest (September/2009) the grains weredriednaturally to 13% moisture, packed in polyethylene bags and storedfor 180days under ambient conditions.The samples wereevaluated for cookingtime(PROCTOR; WATTS, 1987)at 0,90th and180th days, and monthly for the colorof the seed coatthrough readinginthree-dimensional system(L, a*, b*), using a colorimeter (ColorQuest XE, Hunterlab).

RESULTS AND DISCUSSION: The beans of different genotypes showed variation in their seed coat color during the storage period (Table 1). There was a trend of decreasing luminosity (L) in all cultivars, indicating that all grains darkened with storage. The red (a*) and yellow (b*) values oscillated during the storage period, showing no tendency. We could also observe that the grains had different color between each other, both in the beginning and at the end of the storage period, indicating that the genotypes have variability in color when freshly harvested, probably due to genetic characteristics, and they are all prone to darken throughout the storage period. However, some genotypes have lower levels of darkening after harvest, which should be due to the presence of a gene responsible for production of proanthocyanidins (BASSET, 1996). Among the cultivars, BRSMG Madrepérola was the one that stood out, due to its higher luminosity and yellow values, lasting throughout the storage period (180 days), in contrast to BRS Pontal which was darker in the whole period of study. Unlike color, all varieties showed statistically similar cooking time at the initial time of storage and tendency to hardening (Table 2). Genotypes Pérola, CNFC10467 and BRSMG Madrepérola were more stable than the others up to 180 days, showing the lower cooking times. BRS Requinte and BRS Pontal proved to be fairly stable until 90 days, but after this period had their cooking time abruptly increased. Relating the darkening to the hardness, it was observed that the genotypes BRSMG Madrepérola and CNFC10467 were the least darkened and kept low cooking time. Pérola and BRS Requinte darkened in the same proportion, but the last was more difficult to cook. BRS Pontal is the one that darken and harden with the storage period.

Table 1 – Changes in seed coat color of different bean grain genotypes during storage time under ambient conditions.

Genotypes	Storage time (days)						
	0	30	60	90	120	150	180
	L (luminosity)						
Pérola	52.60^{ab}	50.42^{b}	50.89^{ab}	49.32^{ab}	46.17^{b}	45.73^{b}	44.69^{b}
CNFC10467	54.00^{a}	53.06^{a}	51.29^{ab}	50.49^{ab}	50.25^{a}	50.08^{a}	49.59^{a}
Madrepérola	53.93^{a}	52.67^{a}	52.06^{a}	51.36^{a}	50.78^{a}	50.50^{a}	49.08^{a}
Requinte	51.72^{b}	49.73^{b}	48.85^{bc}	43.32^{b}	46.10^{b}	46.00^{b}	45.01^{b}
Pontal	51.33^{b}	48.99^{b}	48.02^{c}	46.67^{ab}	45.60^{b}	44.42^{c}	40.76^{c}
	a*						
Pérola	8.13^{b}	9.20^{b}	8.89^{c}	10.16^{ab}	9.81^{b}	10.30^{ab}	10.86^{a}
CNFC10467	8.42^{b}	8.71^{c}	10.49^{a}	10.33^{a}	10.74^{a}	8.94^{c}	10.18^{b}
Madrepérola	8.97^{a}	9.86^{a}	9.35^{bc}	9.27^{b}	8.86^{c}	9.78^{b}	9.17^{c}
Requinte	7.01^{c}	8.67^{c}	9.57^{b}	9.40^{b}	10.26^{ab}	9.96^{b}	10.71^{ab}
Pontal	8.15^{b}	8.55^{c}	8.99^{bc}	9.76^{ab}	10.27^{ab}	10.88^{a}	9.36^{c}
	b*						
Pérola	14.65^{c}	14.65^{c}	14.54^{c}	14.79^{bc}	13.78^{b}	13.78^{c}	13.73^{b}
CNFC10467	15.52^{b}	15.52^{b}	16.46^{b}	16.20^{ab}	16.35^{a}	14.93^{b}	16.12^{a}
Madrepérola	17.95^{a}	17.95^{a}	18.05^{a}	17.12^{a}	17.13^{a}	17.49^{a}	16.67^{a}
Requinte	13.33^{d}	13.33^{d}	14.42^{c}	13.84^{cd}	13.95^{b}	13.62^{c}	13.70^{b}
Pontal	13.53^{d}	13.53^{d}	12.93^{d}	12.84^{d}	13.15^{b}	13.53^{c}	10.71^{c}

Results are the mean of three repetitions ± SD. Within columns, means with same superscript are not significantly different by Tukey test ($p>0.05$).

Table 2 – Mean cooking time (min) of different carioca bean genotypes during storage time under ambient conditions.

Genotype	Storage time		
	0 days	90 days	180 days
Pérola	25.93^{a} ±0.35	39.79^{a}±0.19	45.20^{c}±1.03
CNFC 10467	28.95^{a}± 3.96	40.35^{a}± 0.96	44.30^{c}±0.95
VC3	32.86^{a}±0.37	42.41^{a}±2.49	49.00^{cb}±2.36
BRS Requinte	29.63^{a}±1.41	34.03^{b}±0.56	52.47^{b}± 1.65
BRS Pontal	31.42^{a}± 0.43	39.73^{a}± 0.67	75.96^{a} ±2.51

Results are the mean of three repetitions ± SD. Within columns, means with same superscript are not significantly different by Tukey test ($p>0.05$).

CONCLUSION: There is a trend of darkening and hardening during storage, but these two events occur at different intensities in each genotype. BRSMG Madrepérola and CNFC10467 are the most stable to the darkening and hardening process, and thus are more recommended for marketing under the technological aspect.

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BIOSTIMULANTS ON THE SEED GERMINATION AND SEEDLINGS VIGOR OF SNAP BEAN CULTIVARS

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INTRODUCTION

The snap bean is a legume of the same botanical species as the common bean plant (*Phaseolus vulgaris*). For being a vegetable of great economic return, the use of bioregulators in its production presents a great potential. The present work aimed to evaluate the effects of the application of four commercial chemicals upon the germination and vigor of snap-bean seedlings.

MATERIAL AND METHODS

The experiments were conducted at the Federal University of Lavras, Minas Gerais State, Brazil. In laboratory, the statistical design was the completely randomized with eight replicates and in germination plot, randomized blocks with four replicates. In both the conditions, the factorial scheme 5 x 5 was adopted, involving five cultivars (Trepador Torino, Feijão Maravilha de Veneza Amarelo, Teresópolis Manteiga, Hx10093000 and Macarrão Favorito) with certified seeds treated with fungicide Captan and four commercial chemicals intended for seed treatment (Stimulate®, Acadian ®, Ever® and Profol NiCoMo®) at the doses recommended by the manufacturers, plus one control treatment with water. In the laboratory were evaluated normal plants (PN) and abnormal plants (PAN). But in soil, the emergence speed index (IVE), emergence percentage (E), root length (CRaiz), shoot length (CPA), weight shoot fresh matter (MFPA), weight of root fresh matter (MFRaiz), weight of shoot dry matter (MSPA) and weight of root dry matter (MSRaiz) were determined. The data were submitted to the analysis of variance and in the cases of significance the means were compared by the test of Scott-Knot at 5% of probability.

RESULTS AND DISCUSSION

In the soil assay, cultivars Manteiga and Hx10093000 proved more responsive to the application of biostimulants. As to the biostimulants, increased weight of root dry mater was reached through the use of biostimulant Acadian (Table 1), but there were no differences as for the other variables evaluated, a fact also reported by Lima et al (2009), working with Stimulate at the two concentrations and liquid gibberellins on *Artocarpus heterophyllus* Lam. seeds. In the laboratory evaluations, there was significance of the interaction biostimulants versus cultivars (Tables 2 and 3). Product Acadian® was the one which furnished highest values to the normal seedlings in the several cultivars (Table 2). The least occurrence of abnormality was found in cultivar Hx10093000 (Table 3) with similar means and absence of statistical differences when in the presence of biostimulating treatment.

Table 1: Average values of CPA and CRaiz in cm; MFPA, MFRaiz, MSPA and MSRaiz in grams and percentage of emergence (E) of snap bean concerning the cultivars and biostimulating products

Cultivars	CPA	CRaiz	MFPA	MFRaiz	MSPA	MSRaiz	IVE	E
Veneza	9.34d	9.39b	13.41a	5.82	1.80	1.66	5.51d	47.50d
Torino	11.22c	11.41a	11.17b	4.95	1.69	1.36	9.84c	71.90c
Favorito	14.71b	14.71a	11.78b	5.31	1.50	1.49	18.21b	84.90b
Manteiga	15.59a	11.25a	14.21a	4.81	1.55	1.68	21.43a	94.40a
Hx10093000	16.20a	13.11a	13.78a	4.30	1.47	1.16	22.88a	96.20a
Products								
Ever	13.44	10.55	12.98	4.49	1.63	1.19b	15.58	79.90
Stimulate	13.16	10.95	13.31	5.11	1.62	1.44b	15.07	78.40
Acadian	13.31	1.14	12.25	5.36	1.63	2.05a	16.83	78.20
Profol	13.78	11.13	12.77	4.85	1.51	1.40b	15.29	79.20
Test	13.37	11.19	13.03	5.34	1.62	1.31b	15.10	79.20

Within each factor, means followed by the same letter belong to a same group by the Scott-Knott test at the level of 5% of probability.

Table2. Average values of the normal seedlings as regards the first (five days) and the second (9 days) counts of the emergence test of the snap bean conducted concerning the cultivars and biostimulating products

Cultivars	**Products**				
	Acadian	**Ever**	**Profol**	**Stimulate**	**Testemunha**
Veneza	50cA	46cA	39cB	50cA	38cB
Torino	92aA	88bA	78bB	85bA	87bA
Favorito	86bA	84bA	85bA	81bA	82bA
Manteiga	96aA	97aA	97aA	81bB	87bB
Hx10093000	96aA	95aA	91aA	99aA	98aA

Means followed by the same capital letter in the rows and small letter in the columns do not differ by the Scott-Knott test at the level of 5% of probability.

Table 3. Average values as regards the abnormal plants concerning cultivars and biostimulating products

Cultivares	**Produtos**				
	Acadian	**Ever**	**Profol**	**Stimulate**	**Testemunha**
Veneza	50cA	53cA	61cB	50cA	61cB
Torino	8aA	11bA	21bB	14bA	12bA
Favorito	14bA	16bA	15bA	17bA	17bA
Manteiga	3aA	2aA	8aA	18bB	12aA
Hx10093000	3aA	5aA	3aA	1aA	1aA

Means followed by the same capital letter in the rows and small letter in the columns do not differ by the Scott-Knott test at the level of 5% of probability.

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BIOSTIMULANT DOSES OF THE STIMULATE® ON THE SEED GERMINATION AND SEEDLING VIGOR OF THREE SNAP BEANS CULTIVARS

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INTRODUCTION: Often, products for incorporation as seed additives are released on market and some of them bring in their composition growth regulators, which can act upon the physiological processes of plants. In the literature, there are reports of positive results of the use of regulators on the common bean plant, nevertheless, in spite of being the same botanical species, little is known about those responses in snap-bean cultivars. Thus, the objective of this work was evaluating the effects of five doses of the biostimulant *Stimulate*® upon the seed germination and seedling vigor of three snap-bean cultivars.

MATERIAL AND METHODS: Two experiments (laboratory and soil) were conducted at the Federal University of Lavras, Minas Gerais State, Brazil. The statistical design was completely randomized with 8 replications of 25 seeds in the laboratory and 4 replicates in soil. In both the cases, the factorial scheme 3 x 5 was employed, involving three snap bean cultivars (Trepador Torino, Teresópolis Manteiga and Ht 10093000) and five doses of the biostimulant *Stimulate*® (0; 6.25; 12.50; 18.75 and 25 mL kg-[1] of seeds), which presents in their composition 0.009% of kinetin, 0.005% of gibberellic acid and 0.005% of indolbutiric acid. In laboratory was conducted the germitest paper germination test, determining the percentages of normal seedlings in first (PC) and last count (PN) and the percentage of abnormal seedlings (PA). In soil, the emergency velocity index (IVE) and the percentage of emergence (EP), length of root (CR) and shoot (CPA) were evaluated, in addition to the fresh and dry weights (PFR, PSR and PFPA, PSPA, respectively). The data were submitted to the analysis of variance and when there was a significant effect of cultivar, the means were grouped by the Scott-Knott test at 5% of probability. When the effect of doses was significant, the regression analysis was used.

RESULTS AND DISCUSSION: Only the cultivar factor (cv.) affected the results in laboratory, where cv. Ht presented the greatest PN while cv. Torino had the smallest PN (Table 1). According to Marcos Filho et al. (1987), samples which germinated faster are considered more vigorous; it is taken for granted, therefore, that hybrid Ht possesses seeds of greatest emergence potential, taking into account the fact that in first count, they germinated over 96% of the seeds (Table 1). Also in the soil, there was a significant effect of cultivar over all the characteristics, except CR (Table 2). The interaction was not significant in any situation. PFPA proved influenced by the doses of Stimulate (Figure 1), reaching highest weight with the dose of 13.79 mL kg^{-1} of seeds. Possibly, such a result was a consequence from effects on the turgescence in the seedlings with variation in the dose of the product, since PSPA was not affected.

In Table 2, it is found that, in general, cv. Teresópolis stood out in most of the evaluations, except CPA and IVE, where it was outyielded by hybrid Ht. The opposite occurred with cv. Torino, the means of which were lower in great part of the evaluations. The reduced IVE presented by cv. Torino is a strong indicative of seeds of poorer physiological quality, which is confirmed when EP is also evaluated (Table 2). The poor vigor of those seeds may have reduced the seedlings' emergence, with direct effects upon the biomass accumulation and growth. The reduction in the stand or the presence of less developed seedlings also account for by the lowest values of PFPA, PFR and PSPA and PSR, result from poorer root and shoot growth. Not all

those arguments, however, are applied to cv. Teresópolis, the EP of which overcame 95% and their values of PFR, PFPA, PSR and PSPA where the most elevated (Table 2).

Table 1 Average percentages of normal in first (PC) and last count (PN) and abnormal seedlings (PA) conercing three snap bean cultivars and five doses of the biostimulant Stimulate®

Treatments Cultivar	PC	PN	PA
	(%)		
Torino	84.40c	88.90c	11.10c
Ht	96.40a	97.80a	2.20a
Teresópolis Manteiga	89.60b	95.00b	5.00b
Dose (mL kg^{-1})			
0,00	87.67	93.50	6.50
6,25	89.83	94.33	5.67
12,50	90.17	92.50	7.50
18,75	90.33	94.17	5.83
25,00	92.67	95.00	5.00
Mean	90.13	93.90	6.10

Mean followed by the same letter belong to the same group according to the Scott-Knott test at the level of 5% of probability.

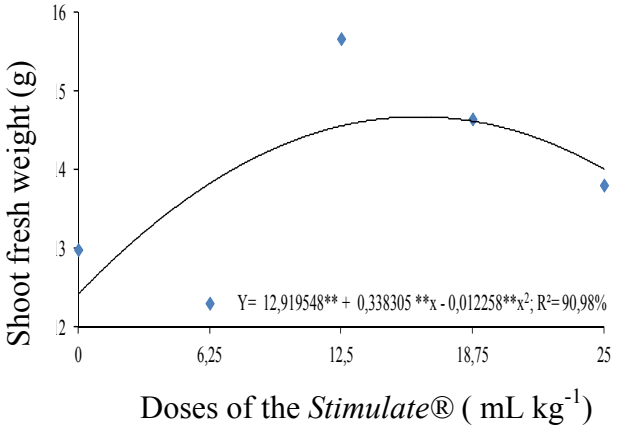

Figure 1 Fresh mass of the shoot (g) of three smnap bean cultivars owing to the doses of the biostimulant *Stimulate*®

Table 2 Mean values of length and fresh and dry weight of root and shoot of snap bean and germination percentage owing to the cultivars and doses of the biostimulant Stimulate®

Cultivar	CR	CPA	PFR	PFPA	PSR	PSPA	IVE	EP
	(cm)		(g)					(%)
Torino	13.31	14.38c	6.44b	13.12b	1.99b	1.57b	4.81c	81.67b
Ht	12.85	17.52a	6.10b	14.50a	1.75b	1.68b	6.82a	97.50a
Teresópolis Manteiga	12.53	15.54b	8.17a	15.20a	2.96ª	1.92ª	6.44b	95.40a
Dose								
0	13.05	15.13	6.00	12.98	2.28	1.63	5.90	90.61
6.25	13.26	15.53	7.89	12.30	2.29	1.80	6.04	91.83
12.50	12.24	16.40	7.50	15.66	2.15	1.81	6.03	91.67
18.75	12.33	16.25	6.05	14.64	1.86	1.77	6.05	91.17
25.00	13.59	15.60	7.07	13.80	2.57	1.62	6.10	92.33
Mean	12.90	15.78	6.90	14.27	2.23	1.72	6.02	91.52

Means followed by the same letters belong to a same group according to the Scott-Knott test at the level of 5% of probability

It should be noticed still that, although the seeds of the cultivars have been obtained from the same source, it was not possible to track back the conditions in which they were produced. As these conditions affect directly seed quality, reflecting in their germination and emergence potential, this fact should be taken into account in the interpretation of the results, that is to say, any interferences to the behavior of the cultivars, do not imply, necessarily, into differences among genotypes and can be related with the production conditions or storage of their seeds.

CONCLUSIONS: The doses of the biostimulant Stimulate® do not interfere in the physiological quality of snap bean seeds. The seeds of cultivar Teresópolis Manteiga stand out as to the physiological quality and seedling vigor.

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PHYSIOLOGIC AND SANITARY QUALITY OF BEAN SEEDS PRODUCED IN SINALOA, MEXICO

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In Mexico the use of certified seed free of diseases in dry bean is low and there is a great exchange and re-use of grain as seed among farmers. This has partly contributed to a great diversity observed in various pathogenic diseases that attack the rainfed bean crop and that can be dispersed as contaminants or within the seed. Seed health is an essential feature of dry bean production, since clean seed can reduce the potential damage by pathogens (4). The objective was to determine the physiologic and sanitary quality of seeds produced during the Fall-Winter cycle at different locations of Sinaloa. This information will indicate management strategies to minimize seed infection in seed production fields.

MATERIALS AND METHODS

Dry bean seeds produced in experimental plots in Los Mochis, and in commercial validation plots in Ahome and Guasave, Sinaloa during Fall-Winter 09-10 under irrigation were analyzed to determine its physiologic and sanitary quality. None special crop or seed management was provided and at harvest maturity seed samples were randomly taken and sent to the lab. The amount of surface pathogens on seeds (colony forming units per ml (cfu/ml) and internal pathogens (percentage of infected seeds) obtained on PDA, incubated at 25 ºC for 2 and 7 days was quantified. The standard germination and vigor tests were held in wet paper towels incubated at 25 ºC for four days to determine vigor and nine days for germination (2).

RESULTS

Different amounts of *Pseudomonas syringae* pv. *phaseolicola* (*Psp), Xanthomonas campestris* pv. *phaseoli* (*Xcp*) *and Fusarium* spp. were recovered from the tested seeds (Table 1). Seeds from plants grown in Ahome, in commercial validation plots, achieved high rates of *Psp* transmission, particularly in cultivars Azufrado Janasa and Azufrasin, that show100% of internal seed contamination. These results indicate the high susceptibility of these cultivars to the pathogen; therefore, care must be taken to control this bacterial disease in fields intended for seed production. The environmental conditions at Ahome and Guasave during the Fall-Winter season (cool nights), and the use of soil contaminated by dry bean debris, was favorable for the development of halo blight (*Psp*). 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).

A second pathogen recovered from the seed in large amounts was *Fusarium* spp. The risk of infection by *Fusarium* spp. to dry bean plants grown in monocrop or under a short crop rotation with cereals is high; therefore strategies to minimize seed infection should be carried out during the crop cycle to prevent damage from root rots and pathogenic bacteria. The seeds of all cultivars and locations show high physiologic quality (germination and vigor), but the seeds of Azufrado Janasa and Azufrasin produced in Guasave and Ahome, respectively, were infected with *Rhizoctonia solani* (data not shown), this fungus is responsible for the decay of seeds and seedlings, causing low percentage of emergency when infected seed is used.

The results obtained indicate that the locations included in this study are not apt for seed production of susceptibly cultivars, or that extreme care should be taken for seed production. The fall-winter season at the Pacific west coastal areas is used by most commercial seed companies for dry bean seed production, thus, is justifiable to apply extra care and chemicals as necessary to obtain disease-free seed.

Table 1. Pathogens colonies, diseases seeds, germination and vigor of bean varieties grown in different locations in Sinaloa, Mexico, cycle Fall-Winter, 2009-2010.

		Psp[+]		*Xcp*+	*Fusarium* sp.[+]			
Location	Cultivar	cfu/ml	Infected seeds %	Infected seeds %	cfu/ml	Infected seeds %	Germination %[&]	Vigor %[&]
Los Mochis	Azufrado Higuera	0	20	0	0	0	93.3	85
	Aluyori	0	35	0	0	0	93.0	100
	Azufrasin	1.2	66	15	0	0	90.0	85
	Azufrado Janasa	0	85	10	0	0	93.3	85
Guasave	A. Higuera	0	95	0	0	15	100.0	100
	Aluyori	0	15	0	0	0	100.0	100
	Azufrasin	0	55	0	0	50	93.3	100
	A. Janasa	0	100	0	0	60	86.7	100
Ahome	A. Higuera	0	95	0	1.2	10	90.0	85
	Aluyori	1.2	45	0	0	10	90.0	85
	Azufrasin	10	100	0	0	20	100.0	100
	A. Janasa	1.2	100	0	0	40	93.3	85

[+] PDA; cfu/ml: colony forming units /ml; *Psp: Pseudomonas syringae* pv. *phaseolicola, Xcp: Xanthomonas campestris* pv. *phaseoli;*[&]wet paper towels; average of four replications

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HISTOLOGICAL PATTERNS OF ABORTING EMBRYO SUSPENSOR IN PHASEOLUS VULGARIS EMBRYO DEFECTIVE LINE AND WILD-TYPE

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INTRODUCTION. A major objective of the research work conducted in our laboratory is to study the process of embryogenesis in *Phaseolus* genus. Embryonormal developmentdepends not only onthe perfect organizationof the embryo, but also that of the various structuressurroundingthe embryoi.e.endosperm, endotheliumandsuspensor. The suspensor is a terminally differentiated embryonic region that connects the embryo to surrounding tissues during early seed development and serves as conduitfornutrients and growth factors for the developing embryo (Rademacher and Weijers 2007) allowing its normal development. Malfunction of this conduit disturbs the normal development of the embryo and leads to its abortion. In this report, we compare the suspensor pattern in *Phaseolus vulgaris* embryo defective line and in wild-type during embryo development, using histological studies.

MATERIAL AND METHODS. The wild-type and EMS (Ethyl Methyl Sulfonate) embryo defective line of *P. vulgaris* genotype BAT93 were used as plant material. The EMS mutagenized plants were obtained as described previously by Silué *et al.* (2006). Plants were grown under following conditions: 27°C/23°C (day/night), 75% relative humidity and 12 hours photoperiod. For histological studies, four developing seeds each from wild-type and mutagenized plants deficient in embryo development were harvested, fixed in paraformaldehyde and embedded in resin. Embedded samples were sectioned with a microtome at 5-7 µm. The sections were stained with toluidine blue and examined by a Nikon microscope (Model Eclipse E800).

RESULTS AND DISCUSSIONS. Embryos from embryo defective line of BAT93 failed to grow normally, showing abnormalities mainly in suspensor and embryo proper from the early stages of development. These abnormalities increase during seed development and lead to the embryo abortion before maturity. Figure 1 shows the histological patterns of normal embryos (A, B and C) from wild-type plants compared to the abnormal ones (D, E, F) of the defective samples at 3, 7 and 10 DAA (days after pollination). Figure 2 represents the evolution of suspensor length in both wild-type and mutagenized samples during embryo development. During the last stages of seed development in wild-type sample, the suspensor's size starts to decline after 10 DAA until its complete disappearance. In contrast, in mutagenized samples, the suspensor continues to grow after 10 DAA (Fig. 2). These observations support the idea that continued growth of the suspensor in the wild-type sample is inhibited by the embryo proper at the last stages during normal development (Yeung and Meinke, 1993). In embryo defective plants, the embryo proper loses the capacity to inhibit the suspensor growth which continues to grow and contains longer cells in the basal part than in the wild-type sample at 10 DAA (Fig. 1C and 1F). In degenerated samples, the embryo development is affected in favor of the suspensor during embryo development. Similar results were obtained in *Arabidopsissuspensor* (*sus*) mutants (Schwartz *et al.*,1994). In these mutants, aberrant morphogenesis in the embryo proper consistently resulted in the formation of a large suspensor. Defects in the embryo proper

appeared at the globular stage of development while abnormalities in the suspensor were detected soon after at the heart stage. These abnormalities in the suspensor morphology could limit the nutrient transfer to the embryo and lead to its abortion.

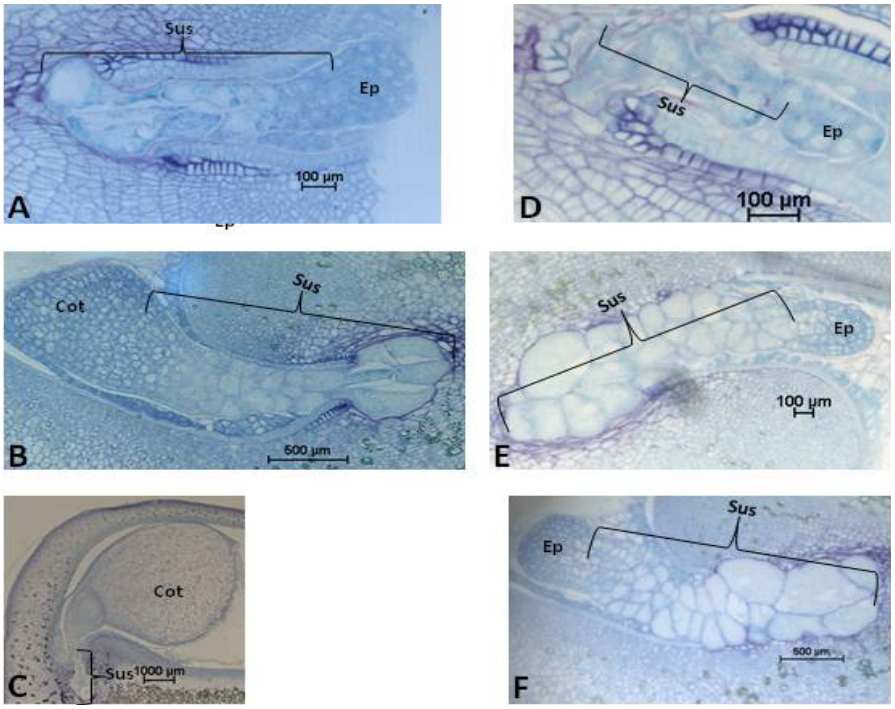

Figure 1. Longitudinal sections of *Phaseolus vulgaris* BAT93 developing embryos at 3 (A and D), 7 (B and E), 10 (C and F) days after anthesis (DAA). Sections A, B, C represent the wild-type samples while sections D, E, F represent the mutagenized samples. sus: suspensor, ep: embryo proper, c: cotyledon.

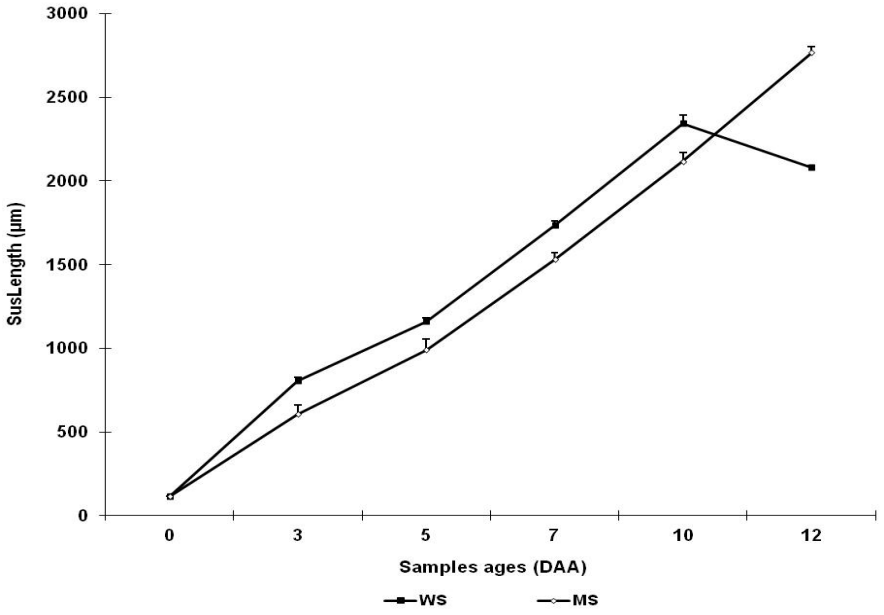

Figure 2. Suspensor length in wild-type and mutagenized samples during embryon development. DAA: days after anthesis, M: mutagenized sample, S: suspensor, W: wild-type. Four data were used for each sample age. Error bars represent the standard error of the mean (SEM).

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SILICON ACCUMULATION IN *PHASEOLUS VULGARIS* L. LEAVES OBSERVED BY SCANNING ELECTRON MICROSCOPY

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INTRODUCTION

Silicon (Si) can providethe bean (*Phaseolus vulgaris*L.) a greater resistance todiseasesandpests(Moraes etal., 2006)and improve resistance against salinity anddrought stress(Zuccarini, 2008). Plants canaccumulate Si in the form ofsilicabodies, and its distribution may varywithin the same species, as detected in leaves ofwheat(Andradeet al., 2012), or may be distributedin a dispersed manneras in the caseoflettuce(Andrade, 2012).

Theaimofthisstudywasto observe the regions of Si accumulation with the aid ofscanning electronmicroscopy (SEM) insnapbeanleaves.

MATERIALS AND METHODS

Thisstudywasconductedin a greenhouseat the StateUniversity ofLondrina,in pots filled withclay soil coming fromanOxisolof Londrina, ParanaState,Brazil. Analysis of thesubstrateshowedthe following chemical characteristics: pH ($CaCl_2$) = 4.1; K = 0.11$cmol_c/dm^3$, Ca = 2.6$cmol_c/dm^3$; Mg= 0.6 $cmol_c/dm^3$, Al =0,57 $cmol_c/dm^3$, H +Al=7.76$cmol_c/dm^3$, P = 3.1mg/dm^3; Organic Matter=1.87g/kg; C = 1.09.

Inthisexperiment,threesnapbeancultivars were used: UEL-1, Alessa andXera. The seeds were sown in 07/19/2011, oneplant per earthen vessel with a fiveliters capacity. The cultivars were fertilized before sowingwith40 g ofN-P-K(8-28-16) per pot, calcium silicateat doses ranging from0 and4$t.ha^{-1}$ for each cultivar. At 21 daysafter planting, top-dressing fertilization took place with2g ofurea and 2g ofKClper pot.

The oldest undamagedleaf was collected from eachplant for the SEM analysisin 09/12/2011. Theseleaves were cutin theircentral portionsand the sections wereimmersed in a fixative solutioncontaining 5%formaldehyde,90%ethanol and 5% acetic acid at room temperaturefor 24h. They were thendehydrated inan increasing series of ethanol (70%, 80%, 90%, 100%) and critical point dried usingCO_2(BalTecCPD030).The sampleswere placedin the "stubs" and carboncoated, using aSputterCoaterBal-TecSCD050.Todetect the presenceof silicon,the samples were analyzedby energydispersiveX-ray (EDS-Oxford) INCA software,coupled toSEMFEIQuanta200.

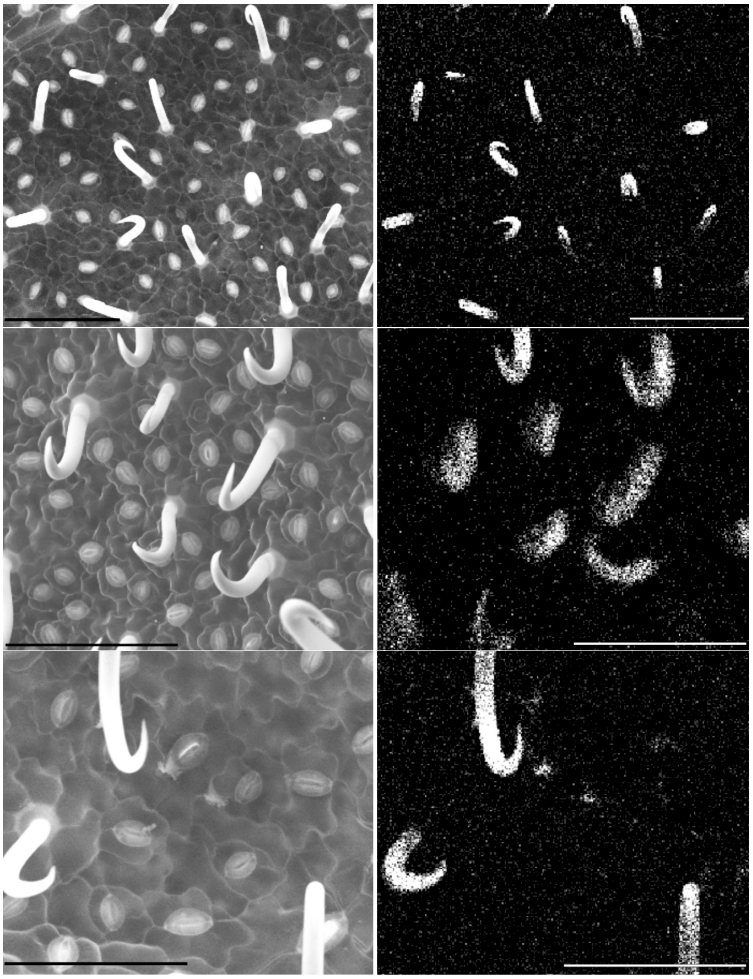
RESULTS AND DISCUSSION

AtSEM,the silicon accumulationin the leaf tissuewasdetected mainlyintrichomes(Figure 1), and there was noformation ofsilica bodies. The twodosesof calcium silicateused in the experimentshowed nodifferences inthe accumulation ofsilicon.Hayward andParry(1973)observed that thebarley awns accumulate large amounts of silica,having the highestconcentrations in thesclerenchymaandtrichomes. Moreover, Barber and Shone(1966) reported that cucumber plantsaccumulatedsiliconmainly on the basisoftrichomes. Theseresults can be explainedby the fact thatsiliconaccumulatesmainly inareas of heavyperspiration.

ACKNOWLEDGMENT

We ackowledge the CNPq, who provided support to Felipe Aranha de Andrade.

Figure 1 Scanningelectronmicrographsof the snap bean leaveslowerepidermisof the cultivars: UEL-1 (A);Alessa(B) andXera(C).The epidermisof each cultivar are on the leftand theirSi mappingsare on the right.



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MAIN PARAMETERS FOR DIRECT HARVESTING OF DRY BEAN (PHASEOLUS VULGARIS L.)

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Currently, the mechanized harvesting of common dry bean (*Phaseolus vulgaris* L.) is still a major problem of production (Georgiev, 1988). In the traditional technology for growing of bean, which uses a low level of mechanization and manual harvesting, the habit type is not important except in those cases when its morphology is in negative correlation with productivity. Habit type IIa is most suitable for direct harvesting (Fig.1). The main parameters of primary importance for direct harvesting are: (1) Habit type IIa with erect plant [cultivars: "Abritus" (Genchev et al., 1993), "Beslet" (Genchev et al., 2011)]; (2) Non-dehiscent pods but easy to harvest with a combine; (3) Suitable harvesting equipment.

Fig.1 Direct harvesting of dry bean cultivar 'Beslet'

The erect plant provides more sunlight for the crop which favors better photosynthesis and hence less problems caused by diseases and frequent rainfalls during harvesting. The non-dehiscent pods allow better maturation of plants. Often the plants in the bean crop are not loaded with enough pods and the stems remain green although the beans are mature and ready for thrashing (Fig. 2). Due to the elongated period of pod formation and maturation, it is necessary to wait till all pods of each plant are mature. There is variation of maturation also due to the different time of germination, flowering and full maturity of the plants. It is also necessary to wait in cases of long-lasting rainfalls during harvesting. The pods should also be easy to thrash with a combine harvester, i.e. they should not be of vegetable type.

The presence of weed plants and green stems in the bean crop during thrashing with combine (Fig. 2) becomes a source of moisture sufficient for sticking of soil particles on seeds thus deteriorating their market quality. This problem is eliminated if using plot micro-combine (with canvas conveyor belt and elementary conveyor units) (Fig. 1).

The combines for direct harvesting of dry bean should be with small header with a flexible cutterbar to better follow the relief of the soil surface. Another very important argument in support of small combine harvesters is that dry bean is a crop of the small area. In order to reduce to a minimum the mechanical damages on the seeds, it is necessary to perform direct harvesting during the twenty-four-hour period when seed are least breakable at full thrashing of

the pods. The degree of damage on the seeds is directly related to the speed of the seed coat absorption of humidity.

a **b**

Fig.2 Direct harvesting of dry bean cultivar 'Abritus' with combine (a) and "green stem" (b).

Significant cultivar diversity has been established towards seed coat absorption possibility. The speed of the seed coat absorption of humidity at 'Abritus' is 7h and 'Dobroudjanski 7' – 85h (Genchev1997). This seeds with maximum absorption values are practically unbreaked. The cultivars with high speed of absorption of seed coat humidity are with high breaking and suitable for canning and vice versa.

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COMMON BEAN KEEPERS AND CULTIVAR DIVERSITY AT TERRA INDÍGENA DO GUARITA INDIAN RESERVE IN BRASIL

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INTRODUCTION

Lately the role of genetic variability and species diversity has been stressed as a valuable support towards food safety (Bevilaqua and Antunes, 2008). The common bean (*Phaseolus vulgaris)* is a staple food for the Brazilian people, mainly for the low income classes. The species is reach in important nutrients like calcium, iron, proteins and vitamins as like those of the B complex. Indigenous populations constitute one of the most traditional human groups that make use of common beans as food, originally located in the South and Meso Americas. For these groups, common bean represents an important energy source for daily activities as fishing, hunting and agricultural practices.

Embrapa Temperate Climate, located in Pelotas, Rio Grande do Sul State, Brazil, has been conducting studies directed to characterize and preserve landrace germplasm of different crop species. One of the key elements in the landrace germplasm context is the so called "guardião", the seed keeper. "Guardiões" can be identified among the indigenous populations, and play a paramount role in the maintenance of biodiversity.

In order to identify indigenous Guardiões and characterize the crop and cultivar variability, Embrapa Temperate Climate in a joint venture with Tenente Portela municipality – Brazil, conducted a work at the Terra Indígena do Guarita Indian reserve. The present paper shows results obtained in relationship to the common bean.

MATERIALS AND METHODS

Field work was carried out in the January-March 2012 period at the Terra Indígena do Guarita indian reserve (TI), located in Northwestern Rio Grande do Sul State. The indigenous "Guardiões" were identified from a meeting comprehending the Tenente Portela Agriculture and the Environment Departments staff members, the Embrapa Temperate Climate common bean research staff and the indigenous representatives. Following this meeting, it was delivered a speech to the indigenous group on the goals of the research project which have been officially registered. A semi-structured quest was submitted to the Guardiões and the crop and cultivar inventories were built. Twelve Guardiões' families located at the Pedra Lisa, Linha Esperança, Três Soitas and Km 10 sectors of the TI were consulted. For sake of clarity, "Guardiões" are considered those farmers that keep their seeds for a long period of time, even for generations.

RESULTS AND DISCUSSION

Results show that all the twelve families consulted are common bean keepers. Ten cultivars were identified, namely, preto, carioca vermelho, carioca branco, amarelinho precoce, taquara, graúdo precoce, mouro, mouro graúdo, preto taquara and azulão. Another important factor detected, was

the existence of genetic erosion. It has been found that the cultivars carioquinha vermelho, carioquinha branco, enxofre, lentilha, carioca vermelho graúdo and chumbinho were lost. Among the causes for the losses drought is considered as the main one.

Besides common bean, maize and cassava are important crops for food. Cassava production however has been below the needs, also due to droughts. Peanuts (*Arachis hypogaea* L.) and sweet potato(*Ipomoea batatas* L. (Lam.)) are also crops used for food by the indigenous people at TI. The proportion of common bean and maize consumption among the indigenous people at TI are shown in Figure 1.

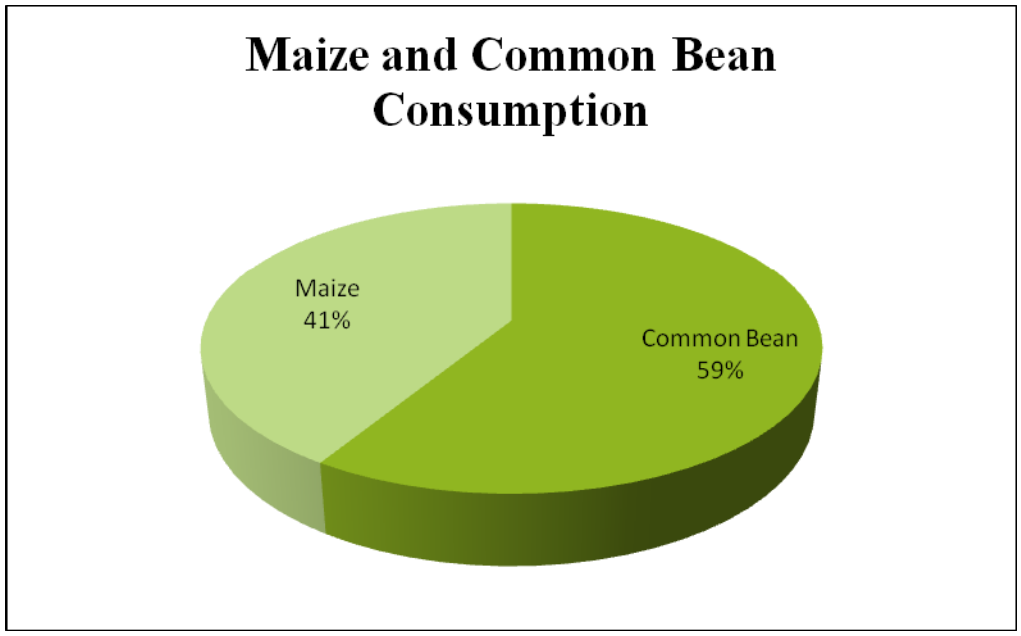

Figure 1: Common bean and maize consumption of indigenous families at Terra Indígena do Guarita Indian reserve

CONCLUSIONS

-All indigenous Guardiões are common bean keepers.
-Common bean constitutes the most important food source for the TI people.
-The detected existence of common bean cultivar genetic erosion, being drought as the main cause, justifies the need for collection and maintenance of the landrace germplasm.

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GENETIC DIVERSITY OF *PHASEOLUS COCCINEUS* L. GERMPLASM FROM VERACRUZ, MÉXICO

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The genus *Phaseolus* is world-wide distributed from tropical to temperate regions (1) reported 103 species into the genus and ayocote beans (*P. coccineus*) is the second most important species after common beans (*P. vulgaris*). Ayocote beans were originated in México and currently are mainly developed high and temperate regions through central and southern México. Ayocote beans are mainly used as human food and they are a species with high grain yields under rainfed conditions when are grown annual or bi-annually (3). This genus has enough genetic variation for the most of economic traits such as seed size, seed coat strength, protein contents and quality, among others which can be used for *Phaseolus* breeding trough recombination and selection (2). The study of genetic variability based on molecular markers could give us insights about the genetic structure of ayocote bean populations. The aim of this work was to analyze with SSR markers the genetic variability of 152 ayocote bean accessions from different regions of Mexico, mainly the state of Veracruz in order to obtain information that contributes to conservation and use in bean breeding.

The work included 164 accessions (Table 1): 152 from *P. coccineus* (137 cultivated and 15 wild populations). As controls we used seven *P. vulgaris* accessions from the state of Veracruz; three common bean cultivars (Pinto Villa, BAT 477 and Pinto UI-114); one accession of *P. glabellus* and one of *P. lunatus*. All accessions were kindly donated by the Genetic Resources Unit of INIFAP-Campo Experimental Valle de México located in the county of Texcoco, Estado de México. Most of ayocote accessions come from the states of Veracruz and Puebla (Table 1). For DNA isolation was needed three seeds per accession which were sown under greenhouse conditions in Reynosa, Tamaulipas, México during january 2011 and then 40 mg of leaf tissue was frozen in liquid nitrogen and macerated with mortar and pestle. DNA isolation was done using the Wizard Genomic Purification Kit (Promega). Quantity and quality of isolated DNA was evaluated in agarose gels 1%.

The gene flow between ayocotes from Veracruz is moderated due AMOVA showed the high genetic differentiation among populations and within accessions (Table 2). However, seed exchange among locations where ayocotes are cultivated through consumers and producers increase genetic diversity (4). Additionally, *P. coccineus* shows moderated open-pollination (14.7%) (3). Free pollination helps for high genetic variation. Results indicated the high genetic variability among and within accessions of ayocote beans from the state of Veracruz. Also, data suggest an important source of allele useful to be included in breeding programs of *P. coccineus* or *P. vulgaris*. Finally, we suggest that genetic diversity is a major challenge to botanist and

taxonomist as well as government institution of Mexico in order to maintain the natural populations both *in situ* and *ex situ*.

Table 1. Origin of *P. coccineus* accessions used in the genetic analysis with eleven SSR markers**.**

***P. coccineus* from Veracruz**	**No.**	***P. coccineus* from Puebla**	**No.**
Acultzingo	20	Chignahuapan	1
Altotonga	15	Mercado de Tlatlauquitepec	1
Jalapa	29	Ávila Castillo	1
Ciudad Mendoza	6	Mercado Serdán	3
Jalacingo	3	Mercado Zacapoaxtla	6
Perote	5	Nauzontla	3
Veracruz	29	Tlatlauquitepec	5
Wild *P. coccineus*			
Tlaxcala	1	P. Villa	1
Morelos	1	BAT 477	1
Veracruz	1	UI 114	1
Estado de México	3	*P. glabellus*	1
Distrito Federal	1	*P. lunatus*	1
Oaxaca	3	*P. vulgaris* (Veracruz)	**7**
Nayarit	1		
Michoacán	2		
Querétaro	1		
Hidalgo	1		

Table 2. AMOVA of 164 *Phaseolus* accessions analyzed with SSR markers.

Source of variation	**df**	**SS**	**MS**	**Explained variance (%)**	**P<F**
Among populations	18	336	19	7	0.074
Among accessions	145	1369	9	32	0.340
Within accessions	164	763	5	61	0.389
Total	327	2468		100	

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ASSESSMENT OF GENETIC DIVERSITY IN COMMON BEAN GERMPLASM CULTIVATED IN NORTHERN REGION FROM MEXICO USING AFLP MARKERS

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INTRODUCTION

Common beans (*Phaseolus vulgaris* L.) are a diverse food legume important to the diet of many people around the world. In Mexico,common beanis one ofthe most important crops. Common bean is produced in American continent in a wide range of climatic conditions ranging from the humid tropics in south of the continentto the Semiarid Highlands of Mexico and the High Plains from U.S. and Canada. Each region has different production practices. So that, breeding program objectives must be designed to address the needs of the farmers who will use the cultivars. Most public bean breeding programs are focused on dry bean improvement.Countries or regions within countries may differ in preferred seed type for dry edible beans. Mexico imports beans from other countries and is losing some of its native diversity due to seed introduction, especially of Pinto beans and shiny black beans. So the native beans planted have declined in the semiarid highlands states such as Chihuahua, Zacatecas and Durango. The aim of this study was to evaluate the genetic diversity of 12 common bean landraces collected in the highlands of Mexico.

MATERIALS AND METHODS

A total of 150 accessions of common bean were used, 40 accessions from Chihuahua (Pinto criollo 1, Ojo de cabra, Pinto criollo 2 and Flor de mayo media oreja "FMMO"); 40 accesiones from Durango (Canario 1, Canario 2, Negro 1 and Flor de junio 1) and 40 accesiones from Zacatecas (Bayo, Flor de junio 2, Negro 2 and Negro 3). Plant material included three out group accessions from the states Hidalgo, Guanajuato and Puebla (10 materials by group). Total genomic DNA was isolated of the young leaves for each accession following the standard Dellaporta method. Four AFLP was performed as described by Vos *et al*.,(1995). Every single PCR product was electrophoresed ondenaturing polyacrylamide gels and were visualized on a Li-Cor IR2 sequencing system (Li-Cor©, Lincoln, NE). Every AFLP primer combination was given a score (1 for presence or 0 for absence of bands in each accession) and a binary matrix was generated. The binary matrix obtained was used for assessing the discriminatory power of AFLP primer combinations by evaluating four parameters proposed by Laurentini and Karlovsky (2007): polymorphism information content (PIC), marker index (MI), resolving power (RP) and Diversity index (DI) and then a cluster analysis was calculated using the coefficient of Dice and UPGMA method.

RESULTS AND DISCUSSION

In total, all four primer combinations generated a total 406 fragments, of which 381 (93.8%) were polymorphic and 25 (6.2%) were fragments monomorphic. The percentage of polymorphic average was of 94 % per primer combination. Average PIC value per the primer combination was 0.34. The MI values ranged from 28.56 to 36.37 with an average of 31.81 per primer combination. The discriminatory potential of the primer combination ranged from 0.41 to 0.60

with an average of 0.51per primer combination. The index of genetic diversity ranged from 0.21 to 0.29 per combination with an average of 0.24 (Table 1).

Table1. Degree of polymorphism, information content and Marker attributes for AFLP primer combinations used in common beans grown in the semiarid highlands of Mexico.

Primer combinations	NMF[a]	NPF[b]	NTF[c]	POL[d]	PIC[e]	MI[f]	RP[g]	DI[h]
E-AGG/M-ACT	8	99	107	92.5	0.30	28.94	0.41	0.21
E-ACT/M-CTA	3	77	80	96.3	0.37	28.56	0.60	0.24
E-ACA/M-AGA	7	89	96	92.7	0.38	33.38	0.57	0.29
E-ACC/M-AGA	7	116	123	94.3	0.31	36.37	0.44	0.21
Total	25	381	406					
Average	6.3	95.3	101.5	94.0	0.34	31.81	0.51	0.24

[a] Number of monomorphic fragments; [b] Number of polymorphic fragments;[c] Total number of fragments generated; [d]polymorphism percentage; [e] Polymorphism information content; [f] Marker index; [g]Resolving power; [h]Diversity index.

The analysis cluster (Fig. 1) shows the formation of the two main groups of accessions with similarity coefficients that range from 0.0 to 1.6. Moreover, although there is a tendency for grouping by the type of seed color the spreading of some groups from different regions across the dendrogram suggests a limited genetic base. Cluster I is the most homogenous by color seeds and geographic origin (Durango). Cluster II is the most heterogeneous. This group includes accessions pinkish color and black type from Chihuahua and Zacatecas, also included the accessions from central Mexico (Hidalgo, Guanajuato and Puebla). Genetic differentiation between bean plots assessed by AFLPs was not clear, indicating that each parcel contains unique alleles result of handling each individual who has suffered bean morphotype.

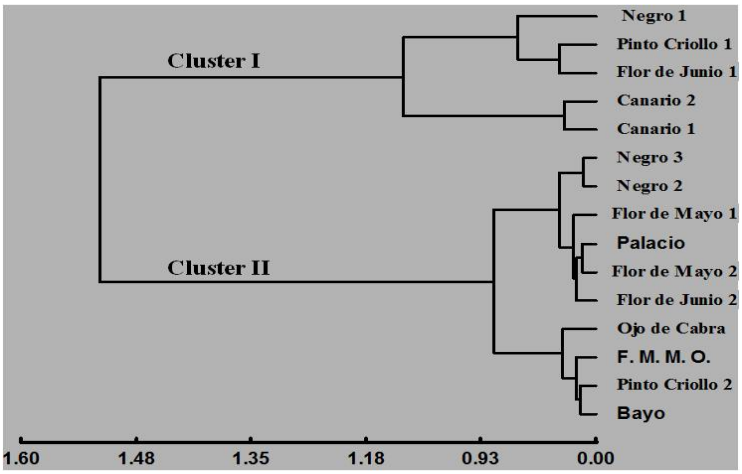

Figure. Dendrogram of 150 common beans accessions cultivated in northern region from Mexico based on genotypic data from 406 AFLP fragments obtained from four combinations.

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PHYLOGENETIC ANALYSIS OF *PHASEOLUS* SPP. FROM MÉXICO

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The genus *Phaseolus* is one of the more representative genetic resources of Mexico. From the more than 70 species known and distributed throughout the Americas, nearly half are endemic to the Mexican territory. This means that Mexico is the most biodiverse country regarding this genus (3). The legumes of major economic and agronomic importance in our country belong to the genus *Phaseolus*, particularly the common bean or *P. vulgaris*, which represents the main protein source of Mexican daily diet. Compared with *P. vulgaris*, little is known about the other four domesticated species and even less is known about the rest of the other wild species of the genus. To elucidate the phylogeny and endemism of *Phaseolus* spp. throughout our country more research is needed where the less known species are also included (2). The aim of this study was to collect germplasm from the greatest possible number of *Phaseolus* species from across different regions of Mexico and to analyze them at a molecular level to determine their phylogenetic relationships.

The selected germplasm (34 accessions) comprised 19 species, including two subspecies of *P. coccineus* (*P. coccineus griseus* and *P. coccineus striatus*) and were collected through-out México. Three species in this collection (*P. albiviolaceus, P. maculatifolius* and *P. rotundatus*) had not been studied before. All samples were analyzed using five (*trnT-trnL, trnL-trnF, rpl16, rpoC1-rpoC2, rps14-psaB*)non-coding regions of chloroplastic DNA amplified by PCR (1). The amplified fragments were sequenced and sequences were processed by bioinformatics processes to align the homologous characters and to create arrays of multiple alignments. Arrays were single and jointly analyzed by three phylogenetic methods (Maximum Parsimony, Maximum Likelihood and Bayesian posterior probabilities). For cluster analysis the cpDNA full sequence of *Vigna radiata* was obtained from GenBank and its homologous sequences were added to the multiple alignments of *Phaseolus* taxa as an outgroup.

Cluster analysis confirmed with strong bootstrap support that the genus *Phaseolus* is a monophyletic group which subdivides into two major lineages: one includes *P. pluriflorus, P. esperanzae, P. pedicellatus, P. microcarpus, P. glabellus, P. oligospermus, P. gladiolatus, P. zimapanensis* and *P. albiviolaceus*; and the other includes *P. filiformis, P. acutifolius, P. vulgaris, P. coccineus striatus, P. coccineus griseus, P. macvaughii, P. leptostachyus, P. lunatus, P. maculatus, P. maculatifolius* and *P. rotundatus* (Fig. 1). The topology of the distal subclades in all dendrograms obtained generally agrees with the topology of *Phaseolus* recognized to this date, which was obtained by ribosomal ITS and chloroplast *trnK* locus analysis (2). The exception was *P. albiviolaceus*, a species not studied before, which according to traditional morphological criteria belongs to the Pedicellatus group but that in this study appeared with the Tuerckheimii group. The other two species that were characterized for the first time in a

molecular phylogeny are *P. maculatifolius* and *P. rotundatus*, both of which were clustered within the Polystachios group.

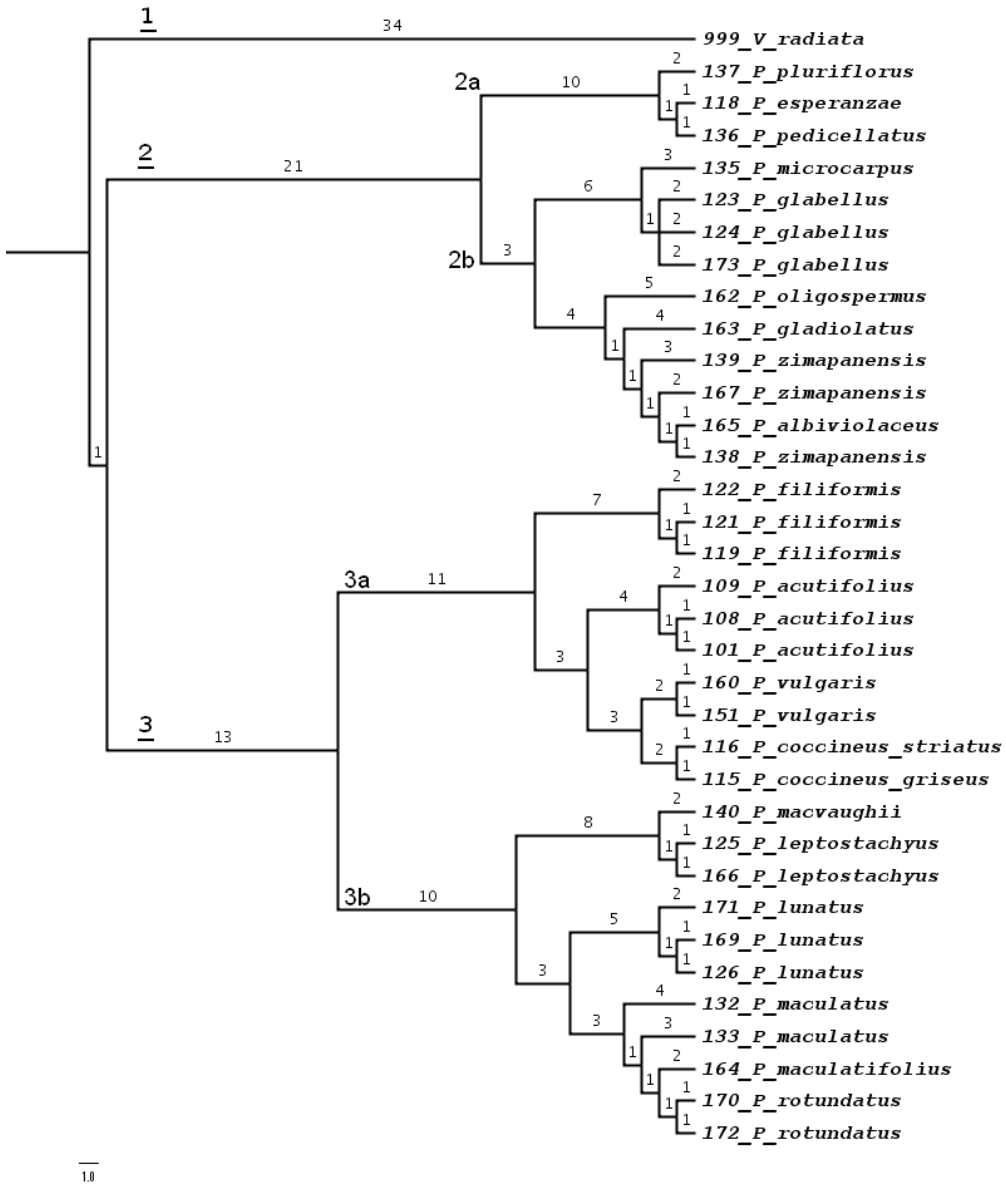

Figure 1. Consensus dendrogram of 19 *Phaseolus* species based on cluster analyses by three methods of phylogenetic construction.

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EMBRAPA COMMON BEAN BREEDING PROGRAM: A CURRENT OVERVIEW

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Common bean (*Phaseolus vulgaris* L.) is the most important legume for direct human consumption. It is grown and consumed worldwide in distinct areas and different seasons, mainly by subsistence level farmers with low-technology input. *P. vulgaris* is particularly important in the Americas, especially Latin America, as well as in Africa and Asia, once it is largely a subsistence crop used as a major source of dietary protein in these countries, as a complement to carbohydrate-rich sources such as rice, maize, and cassava. The common bean is also an important source of minerals, i.e., iron and zinc, and of certain vitamins. For this reason, it is considered as an economically, nutritionally, and socially important crop (Broughton *et al.* 2003). In Brazil, the current second main producer country (FAO 2012), *P. vulgaris* dry bean is a very popular and relevant crop, representing the major source of dietary protein. It is grown across all edafoclimatic areas of the country, with sowing dates happening almost every month. Its per capita consumption can be as high as 17 kg per year. The total growing area in 2010 was 2.1 million ha with a mean productivity of 1.285 kg/ha (FEIJÃO 2012). For this reason, provide Brazilian farmers with improved cultivars should be considered as mandatory and strategic to the country, both to increase farmers' income as to ensure food security.

Through the understanding, assessing, and exploration of allelic variability available at *P. vulgaris*, the Embrapa common bean breeding program drives its efforts on the development of high-yielding cultivars improved for tolerance to abiotic and biotic stresses, focusing the demand of the producing regions and consumer markets in the country and abroad. In this sense, 70% of its efforts are aimed at commercial grain type "carioca" (medium-sized cream-colored grains with brown stripes), the most consumed in the Brazilian market, 20% at black seeded type, and the remaining 10% at grain types "mulatinho", "roxo", "rosinha", "jalo", "rajado" (medium-sized cranberry-sugar bean) and "vermelhinho" (medium-sized red seeded beans), in addition to white seeded bean, cranberry-sugar bean, dark red kidney, light red kidney and calima, targeting the international market.

Currently, the core project of the Embrapa common bean breeding is being developed in partnership with other 42 research and academic organizations with extensive tradition and experience on agricultural research and development: 15 Embrapa research centers, 10 public state organizations for agricultural research and development, 13 academic organizations (universities and colleges), and four international organization on bean research. This partnership has been consolidated over many years, since the Embrapa bean breeding program began in 1978, with the strong collaboration in previous research projects. Hence, it has been possible to exchange staff and facilities to support all research goals, allowing the development of complementary actions for research, innovation and development, and maximizing the work efficiency.

The germplasm flow in the program pipeline is structured in actions of pre-breeding and breeding, which are organized into 10 subprograms or action plans (AP) that comprise 93

interrelated and interdependent activities of research, innovation and development. The management of the bean breeding project is in charge of Embrapa Rice and Beans (Santo Antônio de Goiás, GO) that deals with technical, administrative, and financial issues, being aided by a committee composed by the leaders of each project AP. The AP "Evaluation and Valuation of the Embrapa Bean Core Collection" conduces the phenotypic and molecular characterization of common bean accessions considered as representative of the genetic variability available at the Embrapa Bean Active Germplasm Bank. It also organizes a database with all information obtained from this characterization. There are five APs focusing on pre-breeding, i.e., identification and incorporation of desirable alleles in adapted genotypes to be used as donor parents of important traits such as disease resistance, biological nitrogen fixation, upright growth habit, tolerance to lodging, early maturity, drought tolerance, efficiency on nutrient absorption, tolerance to high temperature, and nutritional, culinary and commercial grain quality. These APs are entitled "Common Bean Breeding for Fungi Resistance", "Breeding for Resistance to Bacterial Diseases and Efficiency in Biological Nitrogen Fixation", "Breeding for Resistance to Viruses", "Breeding for Tolerance to Morphological, Physiological and Abiotic Stresses", and "Breeding for Grain Quality". The advanced lines generated from these efforts are combined to develop segregating populations aiming at the association and simultaneous selection of two or more important traits. This stage of the program is named as AP "Integrated Breeding", being realized separately for each commercial grain type and using conventional and participative breeding methods to develop elite lines. Some of these APs cited above are also assisted by molecular markers. The lines obtained are further evaluated during the APs "Initial Evaluation of Advanced Lines" and "Final Evaluation of Advanced Lines", on an evaluation network conduced by distinct institutions in the main growing areas of the country. The last trials are conducted in all Brazilian bean growing regions and seasons by a national bean assay network, named as Tests of Value for Growing and Use. If the superior agronomic performance of a bean line is confirmed in these tests, it could then be released as a new cultivar. When necessary, the final AP to be held is the extension of cultivar recommendations for those states and growing seasons that were not covered in the initial process of cultivar registration.

The data obtained during the execution of all cited APs are used to support basic and applied genetic studies aiming to optimize the processes of development and evaluation of elite lines. This has assured the indication of new cultivars with high agronomic performance. In addition, these studies are also being used as subsidy to develop human resources, i.e., training of graduate and undergraduate students. Additional information about the Embrapa common bean breeding program and its portfolio of cultivars are available upon request. As could be verified, there are many opportunities for national and international collaborative researches. The consolidation and ampliation of scientific partnerships have always been one of the priorities of the Embrapa common bean breeding program.

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HISTORY OF CARIOCA, THE MOST POPULAR LANDRACE CULTIVAR OF THE MODERN TIMES ON THE AMERICAN CONTINENTS

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The common bean landrace cultivar Carioca (bicolor seed: small striped greyish-cream seed) became most popular and widely disseminated within 25 years of its existence. Our objective is to document its authentic origin and provide a brief description.

ORIGIN AND EARLY HISTORY

Ing. Agr.Waldimir Coronado Antunes, Head, Coordinated Integrated Technical Extension Service (CATI), Ibirarema, São Paulo, Brazil, identified a few plants with small striped greyish-cream seeds in his field of 'chumbinho' (small solid greyish-cream seeds) in 1963. He collected plants with this atypical seed and multiplied them as curiosity. After having sufficient quantity of seeds he distributed to his neighbours, who reluctantly received seed, because it was uncommon. However, his neighbours were very impressed with its better resistance to diseases and higher yield in marginal as well in fertile soils than that of traditional cultivars. The new bean type was called 'Carioca' by one of the farm hands, because it had similar stripes and color as a hog called Carioca being raised on the farm. This is contrary to the common belief or speculation that the name Carioca came from the patterns of sidewalk on Copacabana beach, Rio de Janeiro, Brazil.

In 1966, Waldimir sent seed samples to Ing. Agr. Jacob Tossello, Director, Regional Agriculture Department (DIRA), Presidente Prudente, São Paulo. Jacob Tossello in turn sent seed samples to his brother Ing. Agr. André Tosello, at IAC (Instituto Agronômico Campinas, São Paulo, Brazil) who passed seed to Ing. Agr. Shiro Miyasaka, Chief Legume Scientist at IAC. Carioca was registered as I-38700 in the Plant Collection at IAC in August 1966 . Furthermore, Shiro asked Luiz D'Artagnan de Almeida to multiply Carioca seed and make a thorough multi-location evaluation. During the 3rd Meeting of Agricultural Technicians from 21 to 23 August 1968 Luiz D'Artagnan de Almeida, Hermógenes Freitas Leitão Filho and Shiro Miyasaka presented botanical, agronomical and culinary description of Carioca.

OFFICIAL CARIOCA RELEASE

The official release of Carioca was rejected in the meeting of Technical Commission for Grain Legumes in São Paulo on October 3, 1968 because of its bicolor seeds and fear of unacceptance by farmers. But, Shiro, Commission President, was determined to multiply Carioca seed due to its high yield and good agronomic performance as reported by D'Artagnan. The last push came from Ing. Agr. Alaor Menegário, an Extensionist at CATI who recommended seed production at CATI and asked D'Artagnan to officially release Carioca in 1969. Massive campaign for adoption by farmers and consumers was promoted through two brochures about Carioca.

In the mid 1970, D'Artagnan wrote in supplement for agriculture of the daily journal of the State of São Paulo for nationwide dissemination of Carioca. It also was published in Journal Bragantia of IAC in 1971 (D'Artagnan de Almeida et al., 1971) documenting Carioca's good agronomic

performance in 15 out of 22 locations in the State of São Paulo between 1967 and 1969. In 1977, Carioca was officially recommended as a new cultivar in the State of São Paulo.

CARIOCA CHARACTERISTICS

Carioca has an indeterminate prostrate semi-climbing growth habit Type III. Carioca has white flowers with chordate bracteoles. Carioca is relatively insensitive to long days or photoperiod. Carioca has small (18 – 22 g/100 seeds) cream-striped seeds. Carioca has moderate tolerance to acid soils, soil nutritional deficiencies, and water-stress. Carioca has the *I* gene resistance to all known strains of bean common mosaic virus. Carioca is resistant to some species of nematodes. Carioca is moderately tolerant to leafhoppers, angular leaf spot, anthracnose, and common bean rust.

WIDESPREAD CARIOCA DISSEMINATION AND USE

Between 1970 and 1975 Carioca established as the leading cultivar in the two main planting seasons in the state of São Paulo. Massive seed production jointly by IAC and CATI (especially by Alaor Menegário), testing of Carioca in numerous yield trials and adaptation nurseries nationwide followed by demonstration plots and on-farm testing facilitated rapid Carioca dissemination. Consequently, Carioca dominated common bean production in the state of São Paulo, and was accepted by consumers and traders as a new market class in 1976.

With irrigation in the third planting, Carioca is grown in all three planting seasons in Brazil. Carioca has conquered 48% of cultivated area in the States of Espírito Santo, Goiás, Minas Gerais, and Rio de Janeiro. Since 1980s Carioca and its derived cultivars are also grown in Mozambique, South Africa, Zambia, Argentina, and Bolivia. Carioca has been a donor parent in many breeding programs around the globe including at CIAT (Centro Internacional de Agricultura Tropical), Cali, Colombia.

DIVERSITY OF SOIL FUNGI IN TWO PLOTS USED FOR COMMON BEAN PRODUCTION IN DURANGO, MÉXICO.

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INTRODUCTION. Soil fungi play an important role as major decomposers in the soil ecosystem (Puangsombat *et al*., 2010).Saprophytic microbiota present in soils used to produce common bean (*Phaseolus vulgaris* L.) shows strong influence over root colonization by *Rhizobium* and the availability of macro and micro-nutrients, which are necessary for crop growth and development (Allegrucci, 2005). Monocropping system and seed introduction allows the increment in populations of pathogenic microorganisms that displace indigenous microflora. An increment observed for pathogen populations encourages the development of plant diseases and cause severe seed yield reduction. The objective of this study was to asses and compares the abundance and diversity of soil fungi observed at two common bean producing plots in Durango, México.

MATERIALS AND METHODS. During October of 2010, soil samples were taken in common bean monocropping plots at Durango and La Purísima in the municipality of Cuencamé, Dgo. Filamentous fungi were isolated from serial dilutions of 10 g soil samples, using phosphate buffer and then inoculated, by duplicate, using over-surface technique on Petri dishes containing potato dextrose agar (PDA) and Czapek Dox culture media. Different primary inoculation techniques were used such as: streaking, spread plate, direct inoculation onto liquid medium (Luria-Bertani) under constant movement, soil washing and direct inoculation on Petri dishes containing Czapek Dox and PDA growing media. Mixed cultures were obtained after 4-10 days of incubation at 27-30 °C. Data were registered for colony forming units per gram of soil analyzed (CFU/g) by serial decimal dilutions and streaking inoculation onto PDA plates. Axenic cultures were obtained in PDA medium and then identified by colony growth description and evaluating the microscopic morphology. Six independent experiments were performed for soil analysis in each location and data were recorded for species abundance and richness according to Margalef and Menhinick indices. Combined over locations analysis was also performed by using Dice, Jaccard, Kulczynski, Ochiai and Sorensen indices in order to evaluate relatedness among fungal communities.

RESULTS AND DISCUSSION. In Durango, nine species of filamentous fungi were isolated and identified. Most of the isolated fungal species are related to pathogenic symptoms observed in common bean plants. In La Purísima, Cuencamé, eight fungal species were isolated and dominant species were the saprophyte fungus *Penicillum* sp. and pathogenic fungi *Alternaria* sp. (Table 1). Highest CFU average number was observed in Durango (1,004 viable cells g^{-1} of soil sample) while in La Purísima 103 CFU g^{-1} was registered. Margalef and Menhinick indices showed that greater wealth and better diversity condition was observed in soil samples collected at La Purísima, compared to Durango, where only a few fungal species dominate the soil (Table

2). Highest seed yield was observed for Pinto Saltillo common bean cultivar in La Purísima (1,495 kg ha^{-1}), while in Durango an average of 717 kg ha^{-1} was registered under rainfed conditions.

Combined analysis showed very low or nil similitude between fungal communities isolated in soil samples collected in Durango and La Purísima (Table 3) with only one common species (*Alternaria* sp.). Differences in fungal communities could be associated to variations in soil texture and bean monocropping in Durango or crops rotation practiced occasionally at La Purísima plot. In Durango, fungal populations of pathogens related to common bean disease symptoms showed high prevalence.

Table 1. Fungal species identified at two locations of the State of Durango, México. 2010.

Durango	La Purísima
Colletotrichum sp.	*Chrysosporium* sp.
Rhizopus sp.	*Scopulariopsis* sp.
Cladosporium sp.	*Alternaria* sp.
Alternaria sp.	*Penicillium auleatum*
Beauveria sp.	*Penicillium implicatum*
Penicillium oxalicum	*Penicillium expansum*
Fusarium sp.	*Trichoderma* sp.
Epicoccum sp1.	*Phialophora* sp.
Epicoccum sp2.	

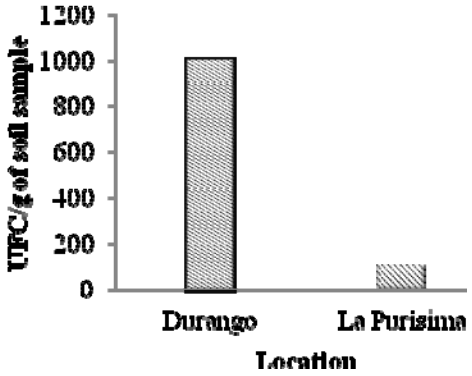

Figure 1. Abundance observed for fungal species at two locations of Durango ($\alpha \leq 0.05$).

Table 2. Richness indices observed in soil fungal evaluation at two locations of the State of Durango. 2010.

	Richness Menhinicl	Richness Margalef
Durango	0	1.16
La Purísima	0.79	1.51

Table 3. Relatedness indices among the fungal communities isolated at two locations of the State of Durango. 2010.

Index	Value	Similitude
Dice	0.1052	Very Low
Jaccard	0.0555	Nil
Kulczynski	0.0050	Nil
Ochiai	0.1176	Very Low
Sorensen	0.1054	Very Low

CONCLUSIONS. Diversity was observed among fungal communities obtained from soil samples collected at two locations where common bean was grown under rainfed conditions. Common bean pathogens dominance and low seed yield was observed at Durango, due to monocropping and recurrent use of introduced seed.

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COMPARING NITROGEN STABILITY INDEX AND PLANT BIOMASS IN AN 'EAGLE X PUEBLA 152' RIL POPULATION

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INTRODUCTION

Nitrate is the most common groundwater contaminant in Wisconsin (Kraft, 2000). Nearly 78,000 acres of snap beans (*Phaseolus vulgaris* L.) are produced in Wisconsin annually of which approximately 50% are located in the Central Sands region where the common soil type, Plainfield loamy sand, is susceptible to nitrate leaching. Recommendations for nitrogen fertilizer applications are 40 lbs/acre for a target yield of 1.5 to 6.5 tons (Laboski et. al., 2006). In commercial snap bean production, additional nitrogen fertilizer is commonly applied to counter abiotic and biotic stresses where rates can commonly exceed 110 lbs/acre.

The use of biological nitrogen fixation (BNF) has been known since the inception of agriculture (Graham, 1981). Modern reliance on synthetic nitrogen has been fueled by the favorable economic cost/benefit ratios, convenience, and timeliness of application. Development of genotypes with improved BNF can reduce the need for synthetic nitrogen applications (Graham, 1981). 'Puebla 152' a black seeded, Mesoamerican dry bean landrace from Mexico was identified as a genotype with high levels of BNF based on the acetylene reduction assay (McFerson, 1983) and later supported by ^{15}N-isotope analysis (Bliss, 1993).

MATERIALS AND METHODS

The objective of this research was to evaluate BNF based on the Nitrogen Stability Index (NSI) in a recombinant inbred line (RIL) population derived from a cross between 'Eagle', a commercial snap bean cultivar and Puebla 152. NSI is measured as :1 – the ratio of the difference in yield in a high N environment - yield in a low N environment divided by yield in a high N environment (Smith et al., 2011). Seventy-five RIL, the two parents and a non-nodulating check 'R99' were grown at the College of Agricultural & Life Sciences King Hall Greenhouses, Madison, WI in artificial soil solutions containing high (300 ppm) and low (100 ppm) nitrogen. Above ground biomass (dry weight) and nitrogen content were measured at flowering.

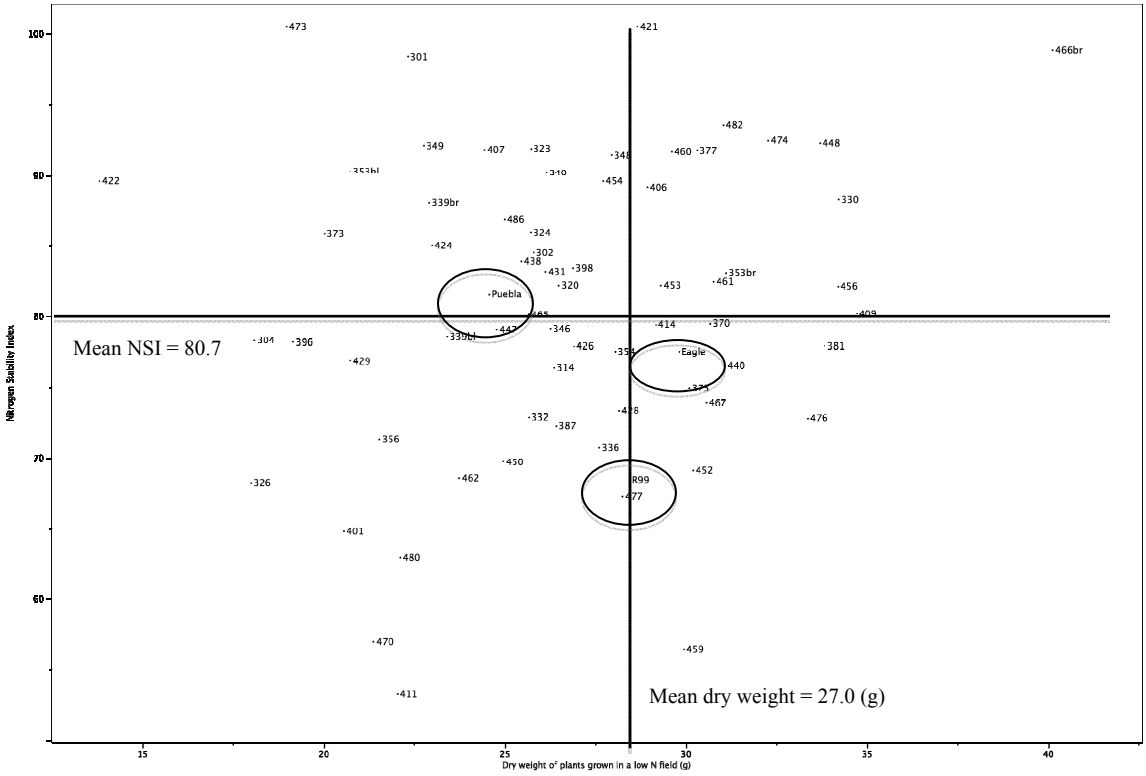
The RILs and checks were also field-evaluated under low N at the Saginaw Valley Research Farm in Richville, MI. Fertilization at planting was with Agro-culture Liquid Fertilizer (St. Johns, MI). This is a slow release fertilizer containing nitrogen, P_2O_5, K_20, and micronutrients. In the low N treatment 6.6 lbs N per acre was applied. A soil test at planting indicated the soil contained 6.5 ppm nitrate N and 3.5 ppm ammonium N and above ground biomass and nitrogen content was measured at mid pod fill.

RESULTS AND DISCUSSION

An ideal genotype would have the largest biomass (dry weight) when grown in a low N environment. As expected, the NSI rank of Puebla 152 was higher than Eagle (intermediate) and the non-nodulating check R99 was the lowest of the three (Fig. 1). Eagle and R99 have a higher mean dry weight compared to Puebla 152 when field-grown in a low N environment. Genotypes

expressing desirable NSI and the largest dry weight in a low N environment would be observed in the upper right-hand quadrant of the scatterplot (Fig 1). RILs were observed that appear to combine the BNF of the Puebla 152 parent with the larger plant biomass of Eagle. The results suggest that it may be possible to develop snap bean cultivars that can achieve high yields with reduced input of synthetic nitrogen.

Fig.1. Scatterplot of Nitrogen Stability Index based on plants grown at high (300ppm) and low (100ppm) nitrogen solutions in a greenhouse and dry weight of plants field-grown in low N environment.



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PRODUCTIVITY OF CULTIVARS AND NEW LINEAGES OF BLACK BEAN

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INTRODUCTION

The black bean is a part of traditional brazilian cuisine and it is cultivated mainly in the states of Rio Grande do Sul, Santa Catarina, Paraná, Rio de Janeiro, Minas Gerais and Espírito Santo (EMBRAPA, 2010). The crop productivity is still low in reason of different cropping systems and technological levels adopted by the producers. The study was conducted to evaluate the parameters of production and productivity of different black bean cultivars and lineages.

MATERIAL AND METHODS

The experiment was conducted in Dourados, Mato Grosso do Sul, Brazil (22 ° 16'S, 54 º 49'W) in 2010 year.The following cultivars and new black bean lineages were studied: Campeiro, Esplendor, Supremo, Uirapuru, CNFP-11973, CNFP-11976, CNFP-11978, CNFP-11979, CNFP-11983, CNFP-11984, CNFP-11985, CNFP-11991, CNFP-11994 and CNFP-19995. The experimental design was in randomized blocks with 14 treatments (cultivars and lineages) with three replications. The seeds were inoculated with Rhizobium tropici (CIAT 899 and PRF 81), as indicated by Pelegrin et al. (2009). The following variables were evaluated: number of pods per plant, number of grains per pod, weight of 100 grains and yield.

RESULTS AND DISCUSSION

The numbers of pods per plant and seeds per pod were not different between cultivars and the new lineages evaluated (Table 1). These results differ from those reported by Lemos et al. (2003), who observed differences. On the other hand, the mass of 100 grains ranged from 16.1 to 20.3 g and indicated differences between cultivars and between the new lineages, agreeing with Lemos et al. (2003). The new lineages CNFP-11973, CNPF-11978, CNFP-11991 and CNFP-11994 showed yields above 1900 kg ha^{-1} and differed from other lineages and cultivars. These results confirm previous observations of Brito et al. (2010), who worked with the same materials.

CONCLUSIONS

There are no differences regarding the number of pods per plant and grains per pod between commercial cultivars and the new lineages.

The new lineages CNFP-11978, CNFP-11991, CNFP-11973 and CNFP-11994 showed productivity above 1900 Kg ha^{-1} which differed significantly from the cultivars.

Table 1. Medium values for production parameters and productivity of different black bean cultivars and lineages. Dourados, MS, Brazil.

Cultivars and Lineages	Number of pods/plant	Number of grins/pods	Mass of 100 grains (g)	Yield (Mg ha^{-1})
Campeiro	9.50 A	4.1 A	20.3 A	1.580B
Esplendor	10.3 A	4.4 A	16.1 D	1.420 B
Supremo	8.90 A	5.1 A	17.3 C	1.176 B
Uirapuru	11.6 A	4.2 A	17.4 C	1.595 B
CNFP 11973	10.3 A	5.1 A	18.8 B	1.942 A
CNFP 11976	10.2 A	4.7 A	19.6 A	1.664 B
CNPF 11978	9.30 A	4.1 A	17.5 C	1.985 A
CNFP 11979	9.80 A	5.0 A	18.8 B	1.692 B
CNFP 11983	11.3 A	4.7 A	14.4 E	1.548 B
CNFP 11984	10.7 A	4.5 A	16.4 D	1.575 B
CNFP 11985	8.90 A	4.8 A	17.3 C	1.535 B
CNFP 11991	9.90 A	4.6 A	19.0 B	1.979 A
CNFP 11994	12.2 A	5.1 A	18.5 B	1.928 A
CNFP 11995	10.1 A	4.9 A	20.3 A	1.729 B
CV (%)	21.5	16.1	3.8	13.9

Means followed by same letter in column do not differ by Scott-Knott test at 5% probability.
VC = variation coefficient.

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CARIOCA BEAN: EVALUATION OF CULTIVARS AND NEW LINEAGES.

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INTRODUCTION

The common bean crop has a great socioeconomic importance in Brazil. Besides being the main daily protein source in brazilians diet, it is the source of income for many farmers. The national productivity of the bean crop is low, but with the adoption of simple technologies such as irrigation and improved seeds, it has been obtained high productivities.The study aimed to evaluate the performance and productivity of different cultivars and lineages of carioca common bean.

MATERIAL AND METHODS

The experiment was conducted in Dourados, Mato Grosso do Sul, Brazil (22 ° 16'S, 54 º 49'W). The experimental design was in randomized blocks with 17 treatments (carioca bean lines) and three replications. The strains tested were: CNFC 10429, CNFC 11944, CNFC 11945, CNFC 11946, CNFC 11948, CNFC 11951, CNFC 11952, CNFC 11953, CNFC 11954, CNFC 11956, CNFC 11959, CNFC 11962, CNFC 11966, Pérola, Juriti, Estilo and Cometa. The seeds were inoculated with Rhizobium tropici (CIAT 899 and PRF 81) as used by Pelegrin et al (2009). It was evaluated the following variables: number of pods per plant, number of grains per pod, weight of 100 grains and the crop yield.

RESULTS AND DISCUSSION

No significant differences were observed among the cultivars and the tested lineages for the number of pods per plant and grain number per pod, results that differed from those presented by Lemos et al. (2003). However, the results obtained for the 100-grain mass and the crop yield, which varied with cultivars and the lineages, are consistent with those obtained by Lemos et al. (2003). Considering only the productivity of crop was possible to group the evaluated cultivars and lineages in three categories: a) up to 1728 kg ha^{-1} (Cometa and Juriti); b) from 1934 to 2165 kg ha^{-1} (CNFC-11951, CNFC-11952, CNFC-11953, CNFC-11954, CNFC-11956, CNFC-11959 and CNFC-10429) and c) from 2280 to 2879 kg ha^{-1} (Estilo, Pérola, CNFC-11944, CNFC-11945, CNFC-11946, CNFC-11948, CNFC-11962 and CNFC-11966). Special attention to the new lineages CNFC-11944, CNFC-11945 and CNFC-11962 which had presented productivities above 2500 kg ha^{-1}.

CONCLUSIONS

There is no difference among carioca bean cultivars and the new lineages for number of pods per plant and number of grains per pod.

The productivity is different among lineages and cultivars, especially for lineages CNFC-11944, CNFC-11945 and CNFC-11962, which has presented productivities above 2.5 Mg ha^{-1}.

Table 1. Production parameters and productivity of cultivars and carioca bean lineages. Dourados, MS, Brazil.

Cultivars and Lineages	Number of pods per plant	Number of grains per pod	Mass of 100 grains (g)	Productivity (Mg ha^{-1})
Cometa	11.5 A	2.9 A	20.6 B	1.381 C
Estilo	10.3 A	5.4 A	21.9 B	2.417 A
Juriti	10.7 A	3.7 A	20.1 C	1.728 C
Pérola	9.5 A	4.0 A	23.5 A	2.649 A
CNFC 10429	12.3 A	4.1 A	21.2 B	1.997 B
CNFC 11944	12.8 A	4.2 A	21.4 B	2.635 A
CNFC 11945	12.6 A	4.6 A	22.0 B	2.879 A
CNFC 11946	11.1 A	4.4 A	22.3 B	2.356 A
CNFC 11948	12.8 A	4.5 A	23.6 A	2.383 A
CNFC 11951	13.3 A	4.6 A	22.0 B	2.165 B
CNFC 11952	8.10 A	5.3 A	21.1 B	2.030 B
CNFC 11953	13.9 A	5.4 A	19.4 C	2.089 B
CNFC 11954	12.2 A	3.9 A	22.1 B	2.135 B
CNFC 11956	11.1 A	4.6 A	18.9 D	2.090 B
CNFC 11959	11.6 A	3.6 A	18.7 D	1.934 B
CNFC 11962	10.6 A	5.4 A	17.8 D	2.520 A
CNFC 11966	9.70 A	4.4 A	18.4 D	2.280 A
VC (%)	20.5	18.5	3.4	11.8

Means followed by same letter in column do not differ by Scott-Knott test at 5% probability. VC= variation coefficient.

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INFLUENCE OF POPULATION DENSITY AND NITROGEN ON WATER USE EFFICIENCY, GRAIN YIELD AND YIELD COMPONENTS OF DRY BEAN

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INTRODUCTION

The influence of environmental changes and cultural practices to which the plant is exposed is uniform, not in the sequential development of different structures, among which are the major yield components (Gonzalez *et al*., 2008). The higher productivity of the intercropping on sole crop has been attributed to a better use of agricultural inputs (water, radiation and nutrients) (Willey, 1990). The aim of this study was to determine the effect of bean plant density and nitrogen fertilization on the water use efficiency, grain yield and its components in beans.

MATERIALS AND METHODS

This study was conducted in Colegio de Postgraduados, Montecillo, México. Sowing was carried out May 17, 2010, under rainfed condition, which corresponds to temperate climate, clay soils, pH of 7.8 and 3.8% organic matter. We used corn as living trellises and bean cultivar "Hav-14" of indeterminate climbing growth habit (Type IV). The experimental design was randomized block with four replications. Treatments were population density by one, two, three bean plants for a maize (D1) 6.6, (D2) 13.2 and (D3) 19.8 pl m^{-2} and levels of nitrogen of 0, 75 and 150 kg N ha^{-1} fractional in three applications, planting, first weeding, second weeding. A harvest was recorded yield (g m^{-2}) and its components. We estimated the water use efficiency (WUE, g m^{-2} mm^{-1}) where: WUE = grain yield / seasonal evapotranspiration (ETc). A response variables we performed a combined analysis of variance, and those with statistical significances, a comparison test of Tukey at 5% probability.

RESULTS AND DISCUSSION

The combined analysis of variance showed significant differences for yield and its components, except number of seeds per pod, a component that for some varieties of beans were found to be little plastic (Da Costa *et al*., 1983). The interaction density * fertilizer level that best expressed the behavior and yield components of beans associated with maize was three bean plants and fertilizer levels of 75 and 150 kg N ha^{-1}, mainly due to a greater number of pods per m^{-2} and a greater number of seeds m^{-2}, this agrees with that reported by Fageria and Santos (2008) who state that the increase in nitrogen levels increased the number of pods per plant, number of seeds per pod and weight seed, also, Escalante and Kohashi (1993) mentioned that the use of high population density and greater N fertilization favored a better response of the bean crop. On the other hand, the interaction of the factors studied, that less expressed behavior towards bean yield was greater population density and plant fertilization levels of 0 kg ha^{-1}. Thus the efficiency of water use by beans was different from the different combinations, and less efficient when lower population densities were studied and fertilizer of 0 kg N ha^{-1} probably due to reduced ground cover by the crop and increased evaporative demand (Escalante, 2001) and increase significantly with high population densities and high nitrogen levels (75 and 150 kg ha^{-1}).

Table 1. Population density and nitrogen fertilization on yield and its components in dry bean

Treatment		Dry bean					
		NP	NSP	NS	W100S	GY	$WUE_{dry\ bean}$
			(m^{-2})		(g)	(g m^{-2})	(g m^{-2} mm^{-1})
	0	59 g	6 a	321 e	27.5 c	25.6 g	0.10 g
D1	75	68 g	5 a	359 e	27.3 c	45.8 f	0.17 f
	150	104 f	5 a	545 e	33.5 b	92.3 e	0.35 e
	0	190 e	6 a	1045 d	34.5 b	159.5 d	0.61 d
D2	75	207 d	6 a	1137 c	46.8 a	214.4 c	0.82 c
	150	193 e	6 a	1061 d	31.5 b	172.5 d	0.66 d
	0	260 c	6 a	1430 b	32.5 b	222.3 c	0.86 c
D3	75	307 b	6 a	1686 a	31.5 b	260.6 b	1.00 b
	150	341 a	6 a	1873 a	32.5 b	377.8 a	1.45 a
Prob F		***	ns	***	***	***	***

1Means in the same column with same letter are statistically equal (Tukey, 0.05).
, * Significance P<0.05 and 0.001, respectively.
NP= Number of pods; NSP= Number of seeds per pod; NS= Number of seeds, W100S= Weight of 100 seeds; GY= Grain yield, EUA dry bean= Water use efficiency.

CONCLUSIONS

The highest yield of beans was found with the combination population density of three plants and a nitrogen fertilization level of 150 kg ha^{-1}.

Yield components that best expressed the effect of the factors studied were the number of pods and number of seeds per m^{-2}.

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SPLIT OF NITROGEN FERTILIZATION, AGRONOMIC EFFICIENCY OF NITROGEN, RADIATION USE EFFICIENCY AND YIELD OF BEAN

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INTRODUCTION

The application of nitrogen (N) under rainfed conditions in beans (*Phaseolus vulgaris* L.) increases the number of flowers, pods and consequently grain yield (Escalante *et al*., 2006). However, in addition to environmental costs, losses of N by improper handling of it may reduce the efficiency of N and crop productivity. The split application of N to the crop can increase efficiency in the use of N and their production. These studies are scarce in bean. In addition, due to variability in habit and growth cycle of the genotypes would expect different responses in the grain yield. The aim of this study was to determine the effect of split application of N on the agronomic efficiency of N, radiation use efficiency, biomass, yield and its components in bean cultivar "Cacahuate72" of determinate growth habit type I.

MATERIALS AND METHODS

The study was conducted under field conditions during the rainy season in Montecillo Mex., of temperate climate and 2,240 m of altitude. The soil is clay loam with 0.50 ppm of NO_3 (50 mg / kg soil) and pH 7.0. The "Cacahuate 72" bean of determinate growth habit type I, pink flower and elongatedgraincreamwith red stripes was sown on June 16, 2008, with a pattern of 20cm * 40 cm which generated 6.25 plants m^{-2} .Treatments consisted of applying 100 kg N ha^{-1}: T1) all before planting; T2) N application into two parts, 50 kg ha^{-1} before planting plus 50 kg ha^{-1} at 40 days after planting (six days before the R6 stage); T3) application of 100 kg of N divided into three parts: 33 kg before sowing, 33 to 40 days and 33 to 60 days (R8 stage); and T4) without N application (control). The experimental design was randomized blocks with four replications. Following thecriteria presented inthebook of Escalante and Kohashi (1993), were evaluatedthe phenology, biomass, grain yield (10% moisture), grain number, grain size (average weight of grain), pod number (PG) and grainsper pod (GP), number of empty pods (EP), total pod number (TP = PG +EP), pod grain index [(PGI = (PG / TP) * 100], harvest index [HI = (dry matter in grain / total dry matter or biomass) * 100]. Also calculated the agronomic efficiencyof nitrogen (EAN) by the equation; EAN = (RN-Rn) / F, where RN and Rn is the bean yield with and without application of N and F is the amount of fertilizer nitrogen, respectively. Was recorded weekly average temperature (° C) maximum (Tmax) and minimum (Tmin,), the amount of photosynthetically active radiation (PAR), precipitation (mm) and evaporation (mm). The efficiency of the PAR (RUE) was calculated for grain yield (RUE= grain yield / PAR (g MJ^{-1} m^{-2}).

RESULTS AND DISCUSSION

Emergence (V1) occurred at 8 days after sowing (das), the beginning of flowering (R6) was at 50 das, the pod filling beginning (R8) at 66 das and the physiological maturity 94 das. During the growth cycle, the Tmax and Tmin ranged from 20 ° C and 28 ° C and 7 ° C and 12 ° C, respectively. Rainfall and evaporation during the growing season was 326 mm and 408 mm respectively. The PAR seasonal was 826 MJ m^{-2}. The EAN, RUE, biomass, harvest index and higher yield (18.4 g g N^{-1} m^{-2}, 333 mg MJ^{-1} m^{-2}, 887 gm^{-2}, 32 and 275 gm^{-2}, respectively) was found with T2, which generated a larger number of grains, number of pods, pod grain index with higher grain and more grains per pod, followed by T3. This treatment possibly not achieved greater response because the N supplied in the phenological stage R8, could not be supplied to the pods and seeds (Escalante and Rodriguez, 2010), dueto decreasedactivityof the root system. The lower RUE, yield and biomass (110 mg MJ^{-1} m^{-2}, 91 gm^{-2} and 300 gm^{-2}, respectively) corresponded to the crop without application of N (T4). These results indicate that by proper management of nitrogen fertilization can be achieved more efficient use of production inputs and therefore a higher bean yields and reducing environmental pollution by N.

CONCLUSIONS

The split application of nitrogen increases the nitrogen agronomic efficiency, radiation use efficiency, grain yield and biomass. With the application before sowing and the flowering stage close to achieved a greater number of pods, pod grain index, grain number, grain yield and biomass of "Cacahuate" bean of determinate growth habit type I.

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FIELD PERFORMANCE OF FIVE NEW PINTO CULTIVARS TESTED IN SEVERAL ENVIRONMENTS OF THE MEXICAN HIGHLANDS

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INTRODUCTION. From 2005-2011, near to707,407 hectares on average of common bean (*Phaseolus vulgaris* L.) were harvested in the States of Zacatecas, Durango and Chihuahua, México. The annual value for total production was approximately 432,862 MT and the average seed yield was 0.61 t ha^{-1} (SAGARPA, 2012). In recent years (2006-2011), pinto seeds became the most popular commercial class of beans in the Mexican Highlands due to the higher yield and better market acceptance observed for Pinto Saltillo common bean cultivar. In 2010 five new pinto cultivars, derived from the cross 'Pinto Mestizo x Pinto Saltillo', were selected in INIFAP (Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias) using early maturity and large seeds as the main selection criteria and keeping the slow-darkening coat trait observed in Pinto Saltillo. Compared to P. Saltillo, the five new pinto cultivars (P. Bravo, P. Centauro, P. Centenario, P. Coloso, and P. Libertad) showed either similitude for coat slow-darkening, larger seeds, earlier maturity and darker broth color after cooking (Rosales *et al*., 2011). The objective of this study was to analyze the field performance, evaluating yield and seed size, of five new pinto bred cultivars grown in several environments of the Mexican Highlands.

MATERIALS AND METHODS. Six pinto cultivars were planted at several environments in the States of Durango, Chihuahua, Zacatecas and Aguascalientes. Cultivars included were Pinto Bravo (n= 12), Pinto Centauro (n= 9), Pinto Centenario (n= 11), Pinto Coloso (n= 13), Pinto Libertad (n= 11), and Pinto Saltillo as commercial check. Variable number of environments (n) and different row number and longitude were planted in each plot according to seed availability. Data were recorded for days to maturity, seed yield and 100 seeds weight. During harvest, five samples were taken in each cultivar, for grain yield and seed size determinations. Analysis of variance combined over locations and mean comparison test were performed for cultivars and commercial check, using SAS ver. 9.2 Proc GLM and Means $LSD_{0.05}$ commands.

RESULTS AND DISCUSSION. Compared to Pinto Saltillo, enhanced earliness of crop maturity was observed in most of the new cultivars varying from four days (d) in Pinto Bravo (89 *vs* 93 d), Pinto Centauro (90 *vs* 94 d), Pinto Coloso (92 *vs* 96 d), to three days for Pinto Centenario (89 *vs* 92 d) and Pinto Libertad (91 *vs* 94 d). Earliness observed in new pinto cultivars could be used to reduce risks caused by terminal drought and frosts registered at the end of the growing cycle in the Mexican Highlands. Analysis of variance showed similar yield between Pinto Saltillo and Pinto Centenario (1,242 *vs* 1,201 kg ha^{-1}) and Pinto Centauro (1,120 *vs* 1,213 kg ha^{-1}). In contrast highly significant ($p<0.01$) increase was observed for seed yield in Pinto Saltillo compared to Pinto Coloso (1,216 *vs* 1,396 kg ha^{-1}), Pinto Bravo (1,119 *vs* 1,214 kg ha^{-1}) and Pinto Libertad (983 *vs* 1,218 kg ha^{-1}). All the new cultivars showed larger seeds compared to Pinto Saltillo: Pinto Coloso (37 *vs* 30 g/100 seeds), Pinto Libertad (38 *vs* 31 g/100 seeds), Pinto Centenario (35 *vs* 30 g/100 seeds), Pinto Bravo (35 *vs* 30 g/100 seeds) and Pinto

Centauro (34 *vs* 31 g/100 seeds). Larger seeds will permit to improve market acceptance of common beans produced in Durango, Chihuahua and Zacatecas.

In most of the environments, Pinto Saltillo showed the highest and most stable seed yield (β_i= 1; S^2d=0) or better response in high yielding environments, consistently (β_i> 1; S^2d=0) (Eberhart and Rusell, 1966) (Figure 1a). Pinto Bravo showed similar yield stability compared to Pinto Saltillo and in contrast Pinto Libertad (Figure 1b), and the other new cultivars, registered better response under poor environments consistently (β_i< 1; S^2d=0).

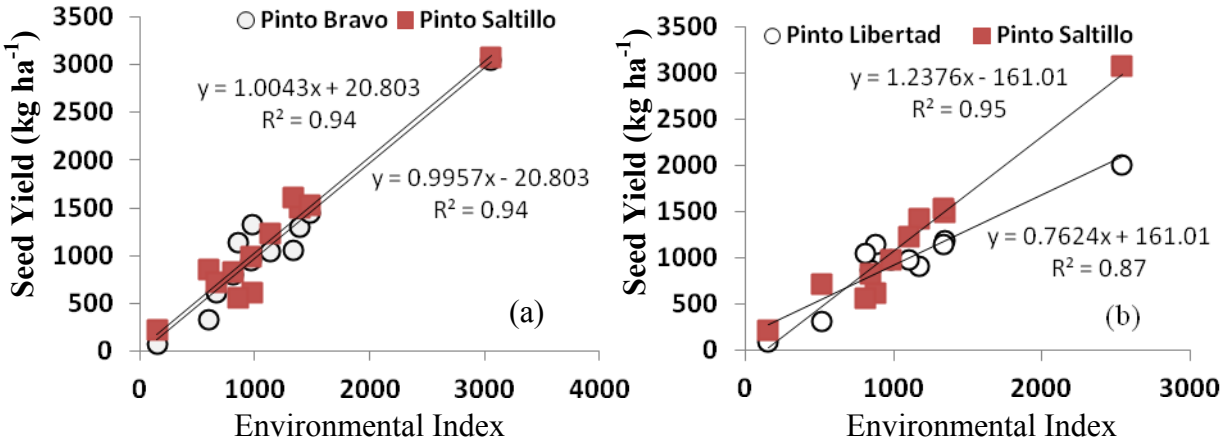

Figure 1. Seed yield observed and estimated from the regression (stability) equations obtained for Pinto Bravo (a) and Pinto Libertad (b) compared to the commercial check Pinto Saltillo.

CONCLUSIONS. Compared to Pinto Saltillo, early maturity, larger seeds and lower yield was obtained in most of the new pinto cultivars developed in Durango. Two of the new bred cultivars (Pinto Centenario and Pinto Centauro) could be used to obtain higher yields (similar to those observed for Pinto Saltillo) in the Mexican Highlands. Pinto Bravo showed similar yield stability to Pinto Saltillo while Pinto Coloso, Pinto Libertad, Pinto Centenario and Pinto Centauro showed better response under poor environments consistently.

ACKNOWLEDGMENTS

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AGRONOMIC EFFICIENCY OF NITROGEN AND YIELD WITH SUPPLEMENTAL IRRIGATION AND NITROGEN IN RAINFED BEANS

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INTRODUCTION

In Mexico, 85% of the area planted of beans (*Phaseolus vulgaris* L.) is under rainfed conditions with low grain yield. Generally, under temperate climate, rainfall occurs from May to September, with a dry period in August to coincide with the reproductive stage beans, even when planted short-cycle cultivars that are affected by this drought. The yield of rainfed can be increased with supplemental irrigation (as in Type III cultivars grain black and clear , Acosta *etal*., 2009) and nitrogen supply (Escalante and Rodriguez, 2010). This increase would be higher with supplementary irrigation, particularly in the reproductive stage. The answer may be variable and possibly would be determined by habit and growth cycle of bean. The aim of this study was to determine the effect of supplemental irrigation and nitrogen application on pod production, pod grain index, grain yield, biomass and agronomic efficiency of nitrogen on bean growth habit type I in warm climate.

MATERIALS AND METHODS

The study was conducted under field conditions during the rainy season in Montecillo Mex., of temperate climate and 2250m altitude. The soil is clay loam with 50 mg NO_3/ kg soil and pH of 7.0. The "Cacahuate 72" bean of determinate growth habit type I, pink flower and elongatedgraincreamwith red stripes was sown on June 16, 2008, with a pattern of 20 cm * 40 cm which generated 6.25 plants m^{-2}. Treatments were: T1) under rainfed and 100 kg of nitrogen (N) before sowing, T2) under rainfed without N; T3) under rainfed with supplementary irrigation of 5 cm at 52 days after sowing (das) (6 days after the R6 stage or early flowering), and at 70 das (4 days after R8 stage or early pod filling) and 100 kg of nitrogen (N) before planting ; T4) under rainfed with no supplemental irrigation without N. Moreover, the experiment was provided 100 kg of phosphorus (P_2O_5) ha^{-1}. The experimental design was randomized blocks with split plot arrangement and four replications. Following the criteria outlined in the book of Escalante and Kohashi (1993) recorded the phenology and evaluated the biomass, yield of grain (10% moisture), the number of normal grains, the grain size (average weight grain), the number of pods (GP) and number of grains per pod, harvest index (HI = (dry matter in grain / total dry matter or biomass) * 100), the number of empty pods (EP), the total pods (TP = GP + EP) and the pods grain index (GPI = (GP / TP) * 100). In addition we calculated the agronomic efficiency of nitrogen (AEN) by the equation: AEN = (RN-Rn) / F, where RN and Rn is the bean yield with and without application of nitrogen and F is the amount of fertilizer nitrogen (N), respectively. Weekly were recorded the average temperature (° C) (Tmax) and minimum (Tmin,), the amount of photosynthetically active radiation (PAR), precipitation (mm) and evaporation (mm).

RESULTS AND DISCUSSION

Emergence (V1) occurred at 8 das, beginning of flowering (R6) to 50dds, the beginning of pod filling (R8) at 66 das and the physiological maturity at 90 das in rainfed and 98 das with supplementary irrigation. The RFA was 792 MJ m^{-2} and 873 MJ m^{-2}, Tmax and Tmin ranged from 20 ° C and 28 ° C and 7 ° C-12 ° C, respectively. During the growing season rainfall was 326 mm and evaporation 408 mm indicating the magnitude of water deficit (WD) in the crop. The most severe DW was observed in R6 and R8 (3 mm rain per day, and evaporation of 9 mm per day, approximately). The highest yield (355 gm^{-2}) corresponded to the bean with supplementary irrigation and N, and was associated with greater biomass (792 gm^{-2}), higher HI (45% as a result of higher demand by the number of GP (200 m^{-2}), Escalante and Rodriguez, 2010), and especially larger number of grains (827 m^{-2}), and a higher GPI (76%), as the N stimulates the production of flowers and consequently the number of pods (Escalante and Rodriguez, 1999) and irrigation reduced falling pods. Such yield and biomass was followed by treatment of irrigation without N (177 gm^{-2} and 410 gm^{-2}, respectively), rainfed with N (143 gm^{-2} and 346 gm^{-2}, respectively).T1 and T2 were statistically equal. The lower yield (101 gm^{-2}) and biomass (255 gm^{-2}) corresponded to without irrigation and without N. The AEN was more than four times higher with supplementary irrigation (18 kg N ha^{-1}) in relation to rainfed only (4 kg N ha^{-1}), indicating that the amount of water supplied is critical for a greater response to N . In addition, the production of irrigation and rainfed was associated with incident PAR during the crop's growthcycle.

CONCLUSIONS

Supplementary irrigation in beans of rainfed increases the number of pods, pod grain index, grain number and hence the yield and biomass. In rainfed and rainfed with supplemental irrigation nitrogen increases the production of biomass, grain pod, pod grain index and grain yield. With supplemental irrigation achieving greater yield response to nitrogen and agronomic efficiency of nitrogen.

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GRAIN YIELD OF DRY BEAN VAR. PINTO SALTILLO UNDER SUSTAINABLE RAINFED PRODUCTION SYSTEM

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INTRODUCTION: In Mexico the new dry bean cultivars have contributed significantly to increase the national grain yield average, although those areas have limited and erratic rainfall, where most of 85% of the dry bean crop is grown (Acosta, 2008). In spite, of observed yield increases and particularly under arid and semi-arid environment, the varieties by themselves cannot guarantee to obtain good yields, it is necessary to complement their sowing with suitable agronomic practices that allow maximum advantage of rainfall and a greater sunlight interception by using narrow furrows to increase foliar area and thus increasing the efficiency in light interception per surface unit. However, to make possible the effective utilization of agronomic practices such as: minimum tillage, *in situ* rainwater harvesting practices (Aqueel, furrow dikes), sowing methods, plant densities and use of compact genotypes of early maturation in a sustainable rainfed production system for dry beans it is needed to evaluate them in integrated system at the semi-arid zones of the north center of the country. This is important mainly in relation to the water harvesting practices, sowing methods and management of surface soil cover, which generally increase the amount of available water to a crop. The purpose of this work was to validate a sustainable rainfed production system for dry beans, which can decrease runoff, improve soil water storage, plant water use efficiency and increase grain yield.

MATERIAL AND METHODS: In 2010, a study was conducted with dry bean grown under rainfed condition in Sandovales, Aguascalientes, Mexico. This site is located at 2100 m.a.s.l. Historical average rainfall during the growing season is 328.2 mm, annual mean temperature is 16.3 °C, and free frozen growing season is about 110 days. Main soil characteristics are: 0.45 m depth, 0.9% of organic matter content, sandy loam texture, 1.0% slope, and pH 6.8. The aim of this work was to validate an integral rainfed production strategy for dry beans including several improved cultural practices applied during the growing season: a) Variety: Pinto Saltillo; b) Sowing methods: conventionally one row per furrow, double row per furrow, triple and six row on 1.52 m width beds. Each plot had eighteen rows 50 m long and spacing at 0.76, 0.40 y 0.25 m with a plant distance of 14 cm between them. Plant population was 90,000, 180,000, 132,000 and 260,000 plants per hectare, respectively for each sowing method. Sowing was on July 31.Seed bed of all the plots were plowed using minimum tillage practice: vertical tillage (root cutter or Multiarado) and two water harvesting practices: 1) "*in situ*" Aqueel (the design of the Aqueel reservoirs ensures that far more of the rainfall is retained in the field for the benefit of the crop and vast reduction in run-off) and 2) furrow dikes were 0.15 to 0.20 m high and were installed in each furrow at a spacing of 2.0 m with a three row commercial diker. The N fertilization was applied to all plots at 25 days after emergency, using 40 $kg.ha^{-1}$ of N, as urea. At harvest, nine sampling points were randomly selected in each sowing method to take a 5 plants sample to determine number of pods per plant, grains per pod and weight of hundred grains. Grain yield was determined by the total grain weight of the nine 2 x 2 m plots.

RESULTS AND DISCUSSION: Total rainfall from sowing to harvest was 122.6 mm, but erratic and untimely distributed during the growing season. Nevertheless, results showed that improved technical modifications increased grain yields of evaluated variety Pinto Saltillo, mainly attributed to a better distribution of moisture in the soil profile. In addition, soil erosion was prevented due reduction of water runoff by using the Aqueel, sowing at beds with six plant rows, surface soil cover and furrows dikes. The dry bean variety Pinto Saltillo showed a different response in all the evaluated characteristics and its effect varied with the sowing methods.

Table 1. Average values of stem diameter, pods per plant, grains per pod, weight of 100 seeds and grain yield of dry bean Pinto Saltillo under four sowing methods and plant population. Aguascalientes, Mexico. 2010.

Sowing Methods	Plants. m^{-2}	Stem diameter (mm)	Pods. $plant^{-1}$	Grains. pod^{-1}	Weight of 100 seeds (g)	Grain yield ($kg\ ha^{-1}$)
Single row	9.0	3.54 c	7 c	3 b	25.6 b	383.1 c[¥]
Double row	18.0	3.94 b	11 b	4 a	28.0 a	520.6 b
1.52 m bed and triple plant rows	13.2	4.40 a	13 a	4 a	30.6 a	557.8 b
1.52 m bed and six plant rows	26.0	3.48 c	7 c	4 a	27.2 b	845.0 a
DMS 0.05		0.260	1.722	0.768	3.154	118.2
CV %		7.06	18.21	20.41	11.81	21.35

[¥]Means followed by the same letter within columns are not statistically different. ($LSD_{0.05}$).

Sowing method of 1.52 m width beds and six plant rows showed the highest grain yield, while the conventional single row 0.76 m sowing method had the lowest grain yield. This grain yield increase represent more than double as compared to the traditional sowing method used by farmers in the region of study and is attributed to a higher plant population, greater surface soil cover and water harvesting practices.

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PHOTOSYNTHESIS, TRANSPIRATION AND YIELD OF RAINFED BEANS WITH SUPPLEMENTAL IRRIGATION AND NITROGEN

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INTRODUCTION

The growth and yield of beans is the manifestation of the interaction genotype * environment through physiological processes. When the production of a crop is limited by water and nutrients and other supplies, it is expected that the effect of this limitation is observed in the first instance in these processes. Nuñez *et al*. (1998) indicate that bean photosynthesis, stomatal conductance and yield under water stress are reduced. The magnitude of this response depends on the genotype (Acosta, 2009) In bean cultivar Cacahuate72, Sanchez *et al*. (2000) report a rate of photosynthesis of 10-12 μmol CO_2 m^{-2} s^{-1} and stomatal conductance of 200 and 180 mmol m^{-2} s^{-1} at the stage of growth and pod filling, respectively. Moreover, N is crucial in photosynthesis (Sinclair and Horie, 1989) and bean yield (Escalante and Rodriguez, 2010). In addition, under water deficit conditions, plants with N may have osmotic adjustment allowing it to maintain cell turgor and thus less affected physiological processes (Smirnoff and Stewart, 1985). The aim of this study was to determine in beans of rainfed cv. "Cacahuate 72" of determinate growth habit type I, the effect of supplemental irrigation and nitrogen on the rate of photosynthesis, stomatal conductance, transpiration, water potential and its relationship to yield.

MATERIALS AND METHODS

The study was conducted under field conditions during the rainy season in Montecillo Mex., of temperate climate and 2250 m of altitude. The soil is clay loam with 50 mg NO_3 / kg soil and pH of 7.0. The "Cacahuate 72" of determinate growth habit type I, pink flower and elongatedgraincreamwith red stripeswas sown on June 16, with a pattern of 20cm * 40cm which generated 6.25 plants m^{-2} .Treatments consisted of two levels of moisture (ML) and nitrogen (N) that generated the following combinations: T1) only rainfed (T) and 100 kg N preplant (N +), T2) under rainferd without N (N0), T3) rainfed with supplementary irrigation (RS) of 5 cm at 52 days after sowing (das) (6 days after the R6 stage or early flowering), and at 70 das (4 days after R8 stage or early pod filling) and 100 kg N preplant (N +), T4) under rainfed with supplementary irrigation (RS) without N (N0). Moreover, the experiment was provided 100 kg of phosphorus (P_2O_5) ha^{-1}. The experimental design was randomized blocks with split plot arrangement and four replications. Following the criteria outlined in the book of Escalante and Kohashi (1993) was recorded the phenology and evaluated the biomass, grain yield (10% moisture). Measurements of photosynthesis (PS) and stomatal conductance (EC) were performed between 1100 to 1200 hours, in three leaflet fully exposed to solar radiation of five plants per experimental unit with a portable gas analysis in the infrared (IRGA , LCA-2 ADC, England) to 72 (early pod filling) and 80 days after sowing (das) (last pod filling). The reference was 300

ppm of CO_2.El water potential (Ψw) was measured at 72 das at 1300 hours with the pump pressure of Scholander. Transpiration (TRP, μg cm^{-2} s^{-1}) was measured at 72 das between 1100 and 1200 hours, with a diffusion porometer model LI-1600 (Licor Instruments Co. USA)

RESULTS AND DISCUSSION

The rate of PS and EC of bean showed significant changes were observed by ML, N and ML*N interaction in the stages that were evaluated. At 72 and 80 das, the PS and EC were highest in the treatment RSN + with 16 μmol CO_2 m^{-2} s^{-1} and 13 μmol CO_2 m^{-2} s^{-1} and 315 mmol m^{-2} s^{-1} and 327 mmol m^{-2} s^{-1}, respectively. Followed by treatment RSN0, TN + and the lowest values corresponded to TN0 with 11 μmol CO_2 m^{-2} s^{-1} and 7 μmol CO_2 m^{-2} s^{-1}, 154 mmol m^{-2} s^{-1} and 144 mmol m^{-2} s^{-1}, respectively . These PS and EC are slightly higher than those reported for the same variety but under irrigation by Sanchez *et al*. (2000). The Ψw was lower (-13 MPa) in TN0. With N the Ψw was higher. As for the bean TRP, RS and N showed the highest values being 15 μg cm^{-2} s^{-1} to RSN + and the lowest 11 μg cm^{-2} s^{-1} for TN0. This response was related to water availability and the possible osmotic adjustment due to N (Smirnoff and Stewart, 1985). The yield (355 gm^{-2}) and biomass (792 gm^{-2}) corresponded to higher RSN + and the lowest (101 gm^{-2} and 255 gm^{-2}, respectively TN0). Biomass and yield showed a strong relation with PS (r = 0.83 a 0.86 *), EC (r = 0.85 * to 0.93 **) and TRP (0.79 to 0.80 *) in the stages that were evaluated.

CONCLUSION

With supplementary irrigation during the reproductive stage and nitrogen supply can be achieved increases in the rate of photosynthesis, transpiration, biomass and grain yield in rainfed beans.

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USE OF PENERGETIC PRODUCTS P AND K IN THE SNAP BEAN PRODUCTION

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INTRODUCTION

The snap beans (Phaseolus vulgaris L.) is an important vegetable which is among the highest volume of marketing in Brazil (CONAB, 2011). Brazilian consumer's preference is for cultivars that produce cylindrical pods with low levels of fiber (HAMASAKI et al., 1998).

Beans pods cultivation is very demanding on soil fertility, but determined growth cultivars with short cycle are less demanding in fertilization (SIMS et al., 1977).

Penergetic International AG Company produces Penergetic products, P and K, from bentonite clays subjected to application of electric and magnetic fields. These products are suitable to increase the photosynthetic efficiency of plants (Penergetic P) or improve the performance of organic matter decomposing organisms of the soil (Penergetic K). These products are recommended for beans cultivation and have been tested to evaluate the response of snap bean, cv. UEL-2.

MATERIALS AND METHODS

The experiment was carried out in a greenhouse at UEL, Londrina, PR, Brazil, using pots with a capacity of 4.0 kg of soil. The following treatments were tested: T1= control, T2=chemical fertilization (250 $kgha^{-1}$ of 04-14-08), T3= 25% of chemical fertilization + Penergetic P and K, T4= 50% of chemical fertilization + Penergetic P and K, T5= 100% of chemical fertilization + Penergetic P and K, T6= 100% of Penergetic P and K recommended dose, T7= 75% Penergetic P and K, T8= 50% of Penergetic P and K recommended dose, and T9=25% of Penergetic P and K recommended dose. The experimental design was totally randomized, with four replications. During the experimental phase, the soil moisture was maintained in 80% of the field capacity by daily replacement of water loses by evaporation and plant transpiration. After 53 days of sowing were evaluated the following variables: numbers and dry mass of commercial pods (> 10 cm in length) and number and dry mass of small pods (<10 cm in length).

RESULTS AND DISCUSSION

The highest number and dry mass of commercial pods production was obtained in treatment 5 (Table 1), which did not differ of the chemical fertilization treatment and partial chemical fertilization treatments (25, 50 and 75) associated with Penergetic products P and K application. These results agree with those presented by MS Foundation (2005) that obtained highest productions of soybean culture with Penergetic products P and K use associated with chemical fertilization. As there was no difference for dry mass production of commercial pods between treatment 5 (100% of chemical fertilization + Penergetic P and K) and treatment 3 (25% CF + Penergetic P and K), these results indicate thatapplication of Penergetic productsP

andKassociated with theapplication of 25% of the recommendedchemical fertilizercan results in productivity similarto thoseobtained withtotal chemicalfertilizerapplication.

On the other hand, the exclusive application of the Penergetic products P and K always resulted in values for number and dry mass production of commercial pods that didn't differed from control, indicating that the isolated application of the Penergetic products P and K should not be recommended for snap beans cultivation.

Table 1. Average values for number and dry mass production (DM) of commercial and small pods of snap bean, cv UEL-2. Londrina, Brazil. 2011.

Treatments	Commercial pods		Small pods	
	Number	DM	Number	DM
1- Control	3.25 b	1.38 c	6.50 ab	0.55 a
2- Chemical Fertilization (CF)	7.50 ab	3.68 bc	11.00 ab	0.43 a
3- 25% CF + *Penergetic P e K*	9.33 ab	4.53 ab	5.66 ab	0.13 a
4- 50% CF + *Penergetic P e K*	9.25 ab	3.98 bc	9.25 ab	0.90 a
5- 100% CF + *Penergetic P e K*	12.25 a	7.03 a	11.75 a	0.48 a
6- *Penergetic* P e K 100%	3.50 b	1.20 c	4.75 b	0.10 a
7- *Penergetic* P e K 75%	4.25 b	1.73 bc	5.50 ab	0.23 a
8- *Penergetic* P e K 50%	4.25 b	1.55 c	5.25 ab	0.33 a
9- Penergetic P e K 25%	4.00 b	1.68 bc	5.25 ab	0.40 a

* Average followed by the same letter in the columns do not differ, by Tukey test at 5 %.

CONCLUSIONS

- The highest values for number and dry mass of commercial pods of snap bean, cv UEL-2, were obtained with Penergetic products P and K application, associated with chemical fertilization.

- The isolated application of Penergetic products P and K, is not recommended for the cultivation of snap bean, cv.UEL-2.

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EVALUATION OF RUNNER BEAN GERMPLASM IN BELARUS

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Runner (scarlet) bean (*Phaseolus coccineus* L.) is a popular ornamental and food legume in many countries, but it's spreading is very limited in Belarus now. There arenot any varieties of this species included in the National List of varieties of Belarus and more, the varieties of scarlet bean never tested in system of State varieties trials of Belarus. Some types of landraces with colored and white seeds but every time with indeterminate climbing growth type some time can be find out in small farms at the middle and south of Belarus. The red and bicolor-flowered samples usually used as ornamental plant. And the samples with white seeds only begin to be a food crop for self consumption in Belarus.

Runner beanhavedeserved our attention since 1998 due to the possibility of being a germplasm source to transfer to common bean the resistance to diseases and tolerance to some types of abiotic stresses. But now, after many years of germplasm investigations and following using of *P. coccineus* in crossings with *P. vulgaris*, we have the separate interest to runner bean as to novel crop for Belarus. We have started the collection of germplasm samples from missions to different parts of Belarus, and as the result 76 samples were included to our collection. Fivelocal Byelorussian samples were included into Genebank of the IPK, Gatersleben (http://gbis.ipk-gatersleben.de/gbis_i/): PHAS 8424 - 8428. Additionally we have received the samples from different genebanks: USDA (63 samples), IPK Gatersleben (156 samples), CIAT (18 samples), also from Slovak and Spain genetic resources centers. Some commercial varieties from Poland, Germany and UK also tested during our field trials for selection of appropriate samples to include to germplasm collection. As a result, our germplasm collection now consist of 294 accessions (varieties, local samples, breeding lines) which are able to produce full ripening seeds in enough quantity to reproduce and support sample in the field conditions.

The field experiments are carrying out in the south region of Belarus close to Kobrin city. We usually use the next scheme to design the plot: 2 repetitions, 3,2 square meters per 1 sample in repetition (2 rows 2m, 0,8m row-spacing). The distance between plants in row is 0,1m. The normal time to sow runner bean in Belarus is 15-25 May. The wooden poles are putted near plantsafter appearing of the first trifoliate for climbing.

Thus, all samples of germplasm collection are examining annually to determine the morphological and agronomical traits for estimation of perspectives of cultivation of this *Phaseolus* species in Belarus and for breeding of new varieties. Additionally some new samples testing annually to determine it's suitability to be included into collection.

The most part of our samples originated from the temperate zone: Belarus (76 samples), UK (22), Holland (20), Germany (31), Hungary (8), Slovak (15), Austria (88), Poland (13), other countries (Italy, Spain, USA, South Korea, China etc.) (21). Not one in the tested samples originated, for example, from Guatemala, Mexico, Columbia and other so southern regions is able to produce seeds in Belarus in outdoor conditions. Usually such samples begun flowering at September when the most part of germplasm finishing flowering and have many pods in different stages of ripening.

The evaluation of germplasm accessions was focused on morphological and agronomical traits. The morphological traits evaluated under UPOV TG 9/5 (http://upov.int/en/publications/tg-rom/tg009/tg_9_5.pdf). The agronomical and other traits evaluated under "Handbook on evaluation ofPhaseolusGermplasm" by C. De La Cuadra, A. M. De Ron and R. Schachl, also under IBPGR, 1983 (Phaseolus coccineus descriptors.

AGPG:IBPGR/82/74, Intern. Board Plant Genetic Resources Secretariat. Typescrit. Rome, Italy). 20 typical plants of each sample are evaluated to receive the data for morphological and statistical analysis of quantitative traits.

Under the classification of *P. coccineus* L. 160 samples of our collectionare *P. coccineus var. coccineus*, 85 samples are*P. coccineus var. albiflorus*, 46 samplesare*P. coccineus var. bicolor*, 2 samplesare*P. coccineus var. rubronanus*, and 1 sample is*P. coccineus var. albinanus*. All the local samples from Belarus are*var. coccineus* (45 samplesor 60 %), or are*var. albiflorus* (31 samplesor 40 %).

Studying of samples descriptions shows that approximately all variants of characters under UPOV TG 9/5 are presented in our collection, except "Height of plans: short and tall", "Beginning of climbing: early", "Leaflet size: small", "Flowering time: early". Thus, our germplasm collection is very polymorphic and it is especially important if to take into account that All-Russian Institute of Plants (VIR, S.-Petersburg, Russia) do not research and accumulate the germplasm of *P. coccineus* L.

Only 6 samples from 294 are fully physiological ripen at all years of tests, i.e. were without leaves, and with dry roots, and with all dry pods during plant maturity. All of them originated from Belarus: BSUPCC156, BSUPCC34, BSUPCC226, BSUPCC17, BSUPCC122 and BSUPCC5. The vegetation period of these samples vary from 110 to 125 days. The other samples have more or less number of fully ripening pods with dry seeds, but always with green leaves and pods, and sometime with flowers till the end of vegetation time which is limited by climatic conditions of Belarus. Such samples are harvesting after first frosts (usually end of September – October) with removing of leaves for the next afterripening in sheaf and evaluation.

The variation of some agronomical traits in germplasm collection is demonstrated in Table (Xav. – average value, min – minimal value, max – maximal value, V – coefficient of variation). This data is a result of not less than 3-year trials. Asterisk "*" means the theoretical case when all formed pods could be able to produce normal dry seeds.

Character	Value			V, %
	Xav.	min	max	
General number of pods per 1 plant, pcs.	28.1	7.6	45.7	94.5
Number of dry pods per 1 plant, pcs.	15.3	1.8	22.2	65.9
Number of dry seeds per 1 plant, pcs.	60.1	2.4	181.3	63.1
Potential number of dry seeds per 1 plant, pcs.*	220.3	41.3	298.9	74.5
Number of seeds per 1 pod, pcs.	5.9	2.4	8.2	46.9
1000 dry seeds weight, g	1115	834	1320	40.5
Weight of dry seeds per 1 plant, g	68.3	2.6	194.5	89.2
Potential dry seeds productivity of 1 plant, g*	212.6	45.5	320.9	72.4

As a result we have selected the number of early-matured, with large seeds, high yielding samples (additionally to named previously): BSUPCC354, BSUPCC1, BSUPCC68, BSUPCC55, BSUPCC41with appropriate characters to be produced in Belarus in small farms also for local market, and which could be selected for variety breeding and common bean improvement.

We are open for provision of the seeds of collection samples. Also the full information about germplasm accessions is available on request.

DRY BEAN COMPETITIVENESS WITH WEEDS AS AFFECTED BY BEAN GENOTYPE AND NITROGEN FERTILIZER APPLICATION RATE: AN EXAMPLE FOR RED ROOT PIGWEED

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INTRODUCTION

The natural genetic diversity that exists in some legumes for growth habit characteristics represents an excellent opportunity for farmers to use varieties that have a higher weed suppression ability (Wortmann, 1993). However, the nitrogen supply (N) has a big impact on canopy architecture, vegetative and reproductive growth and finally competitiveness of both crops and weeds (Westermann et al., 1981). The overall goal of this study was to determine the interaction among bean genotypes, weed competition and nitrogen management and the ultimate impact on dry bean yield in mixed dry bean cropping systems with weeds.

MATERIAL AND METHODS

Two experiments were conducted at the experimental station of Tarbiat Modares University, Iran, in 2009 and 2010. The soil type of the plot was a sandy clay loam soil (4% silt, 23% clay, 73% sand) with 1.03% organic matter and pH 7.73. The experimental design was a randomized complete block with factorial arrangement of treatments with three replications. The treatments included two dry bean genotypes, i.e., erect and semi-erect, four rates of N application, i.e. 0, 25, 50 and 100% of bean N requirement, and three levels of weed pressure, i.e., 0, 2 and 14 redroot pigweed plants m^{-1} per row. Nitrogen was applied at rates of 0, 50, 100 and 200 kg N ha^{-1} and 0, 35, 70 and 140 kg ha^{-1} for the semi-erect and erect bean genotypes, respectively. All plots contained four rows of bean spaced 50 cm apart. Dry bean genotypes were planted on 11 July, 2009 and 26 June, 2010 and thinned to the recommended plant density (40 plants m^{-2}). Redroot pigweed seeds were sown in a 1-cm-deep trench located 5 cm from each dry bean row. Soil volumetric water content was monitored daily using time-domain reflectometry (TDR). The soil was irrigated to field capacity with a sprinkler system when 40% of the available soil water (USDA-NRCS, 1997) was depleted in the top 0.6 m of the root zone in the weed-free treatments. At final harvest, the plot was hand-harvested for grain yield using a 3-m length of row from the two middle rows (3 m^2). The effects of treatments on seed yield were tested using ANOVA with the GLM procedure in SAS. Means were separated using the LSD test at the 95% level of probability. PROC RSREG was used to perform model fitting, canonical analysis of the surface shape, and ridge estimates for the region of optimum response.

RESULTS

For the weed-free condition, seed yield increased by 12% for the semi-erect genotype and 18 % for erect genotype across all N rates compared with no N application (Table 1). Under low weed pressure, the seed yield increased by 9.3% for the semi-erect genotype and 13.7% for the erect genotype across all N rates compared with no N application. Under high weed pressure the seed yield for both genotypes decreased with an increase in the N rate. The presence of redroot pigweed reduced the seed yield for the two bean genotypes, but the severity of this reduction was depended on weed pressure and N levels. Across all the N rates, the seed yield was reduced by

9.8 and 13.3% in the low weed pressure and 33.3 and 45.2% in the high weed pressure treatments for the semi-erect and erect genotypes, respectively. Ridge analyses of the surface response estimated that the ridge for the optimum response was 120 kg N ha^{-1} at a density of 0.13 weed plants m^{-1} for the semi-erect genotype and 84 kg N ha^{-1} at a density of 0.14 weed plants m^{-1} for the erect genotype (Figure 1). The estimated seed yield using a surface response model from actual N rate and weed density showed that the highest N addition for the weed-free condition could increase seed yield for both bean genotypes in the presence of redroot pigweed up to 4 plants per m^{-1} row. Under a high weed pressure, a reduced N dose could increase seed yield.

Table1. Seed yield and yield loss as a function of nitrogen (N) rate and redroot pigweed density (averages for 2009 and 2010).

Genotype	N %)	Yield (kg ha^{-1})			Yield loss (%)		
		Redroot density (Plant m^{-1})			Redroot density (Plant m^{-1})		
		0	2	14	14	2	4
Gholi	0	3303bA	3041cA	2988aA	1241aA	7.86aA	9.34cA
	25	3480.5bA	3159bcAB	2914aB	1195aB	9.23aA	16.08cA
	50	3717aA	3304abB	2595aC	1056bC	11.04aB	30.07bA
	100	3861aA	3508aB	1776.9bC	759.5cC	9.14aB	53.85aA
D81083	0	2108.5cA	1891bAB	1710aB	447.3abC	10.58aA	18.98dA
	25	2485bcA	1995abAB	1698aB	478.8aC	13.20aB	26.59cA
	50	2505abA	2138abB	1306bC	387.5bcC	14.63aB	47.95bA
	100	2625aA	2307aB	1023cC	318.0cC	12.10aB	61.00aA

Means followed by different letters are statistically different at P<0.05 0.05 by LSD test. Small letters and capital letters signify differences among N rate and redroot pigweed density treatments, respectively.

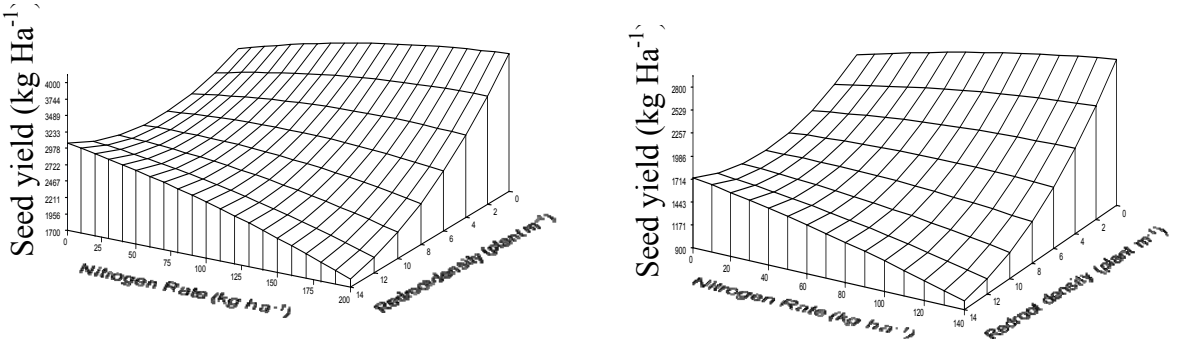

Fig. 1. Surface response model of bean seed yield as influenced by applied N fertilizer (N) and erect genotypes. Surface response regression for semi-erect genotypes: 3247.510152+5.7 0.677301WN + 7.953865W^2 (R^2 =0.89). Surface response regression for erect genoty 124.298864W – 0.011232N^2 – 0.660897WN + 6.920575W^2 (R^2 =0.80).

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EFFECT OF MAIZE SHADING ON BEAN BIOMASS, HARVEST INDEX, YIELD AND COMPONENTS

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INTRODUCTION

Bean yield is a function of agroclimatological and physiological factors such as leaf area, its duration, and consequently, the radiation intercepted by the canopy crops. Bean in association or competition that receives a greater proportion of incident radiation will be more efficient in the use of photosynthetically active radiation (PAR) (Wall and Kanemasu, 1990), and this will reflect upon a greater grain yield. On the other hand, bean yield, when is associated with maize, is reduce, mainly due to competition for radiation, when water and nutrients are not a limiting factor (Francis *et al*., 1978). Reduction is the result of the variation of yield in different stratum of the canopy due to climatic variation inside it. The aim of this study was to determine the effect of shading in each leaf stratum of maize on bean biomass, yield, and its components.

MATERIALS AND METHODS

Sowing was done on May 17th, 2010, under rainy conditions, in Montecillo, Mexico, temperate climate, with clay soil, pH 7.8, and 3.8% organic matter. A native blue maize variety chalqueño was used as support for the bean cultivar "Hav-14" with an undetermined climbing growth habit (Type IV). Treatments consisted on partial defoliation per stratum (30 cm from ground level) in pre-anthesis of the maize; they were: T1) defoliation lower stratum, T2) defoliation medium stratum, T3) defoliation upper stratum, T4) full defoliation, T5) control (with no defoliation). Incident PAR was registered at noon with a lineal quantum sensor. The experimental design was under random blocks with four repetitions. At harvest were evaluated: biomass, harvest index (HI), yield, and yield components. The proposed variables were subject to a variance analysis, and those with a statistical significance to the Tukey test at 5%.

RESULTS AND DISCUSSION

The variance analysis showed significant differences by biomass, HI, grain yield, and its components in bean, with the exception of the number of seeds per pod (NSP) (Table 1). Incident PAR measured at noon on the canopy was 1682 $\mu E\ m^{-2}\ s^{-1}$. During pre-anthesis, the elimination of shading caused by the upper stratum of the maize reduces PAR by 26%, which caused an increase in bean yield of up to 35%, which was related with a greater number of seeds, pods, and weight per hundred seeds. The elimination of all corn leafs caused a decrease in bean yield of 36%, showing in a lower number of pods per m^{-2} and a lower number of seeds per m^{-2}. This could be because the environmental conditions generated when eliminating the leaves in the canopy did not favor bean pod production, perhaps due to a photo-inhibition caused by the high radiation intensities (Sosa *et al*., 2000), which could have caused an inefficiency in the production of photosyntates translocated to the organs of interest (leaves, grain). Likewise, the

elimination of the leaves in the middle stratum of the maize caused a lower reduction (7%) in bean yield. Contrastingly, the elimination of the leaves in the lower stratum of the maize reduced yield by 31%. Gardiner and Craker (1981) mention that a greater radiation at different levels of the bean canopy, mainly in the early stages of development, traduces into a greater accumulation of dry matter and an increase in bean grain yield. The changes in yield are generally related with the number of pods at harvest (Escalante and Kohashi, 1986). The magnitude of this change is affected by environmental conditions (Escalante *et al*., (1980).

Table 1. Grain yield and components of bean Hav-14, in function of the degrees of defoliation of the maize.

Treatments	NP	NSP	NS	W100S	GY	Biomass	HI
		(m^{-2})		(g)	(g m^{-2})		(%)
Lower	129 d	5 b	642 d	33 b	138 d[1] (-31%)	429 c	32 d
Medium	178 b	6 a	1063 b	33 b	186 c (-7%)	382 d	48 a
Upper	244 a	6 a	1462 a	35 a	270 a (+35%)	627 a	43 b
Full	110 e	5 b	546 e	31 c	128 e (-36%)	283 e	45 b
No elimination	140 c	6 a	836 c	30 c	201 b	488 b	41 c
Prob F	***	ns	***	**	***	***	**

[1]Means in the same column with same letter are statistically equal (Tukey, 0.05).
, * Significance P<0.05 and 0.001, respectively.
GY= Grain yield; NP= Number of pods; NSP= Number of seeds per pod; W100S= Weight of 100 seeds; NS= Number of seeds.
% indicates increase or decrease

CONCLUSIONS

In the maize-bean association, the elimination of different leaf stratum before flowering causes changes in bean biomass, harvest index, yield, and its components.
Shading from the upper stratum of the maize canopy is the most limiting with regard to number of pods, number of grains, and consequently, bean yield and biomass production.

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'FLOR DE JUNIO DALIA' A NEW DRY BEAN CULTIVAR FOR CENTRAL MEXICO

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The Flor de Junio bean type, Jalisco race, is mainly grown and consumed in the central-west region of Mexico. Up until now, there is only one bred cultivar of this commercial seed type, 'Flor de Junio Marcela' (FJM) (Castellanos *et al*., 2003a) suited for irrigated conditions or rainfed areas with low disease pressure for rust. Since the seed type of FJM (medium size, oval shaped of cream color with pink stripes) takes a large share of the market, it is an irreplaceable parental genotype in our breeding program. This fact has hampered the development of new cultivars within the Flor de Junio type. In this report we describe the development of a new cultivar of this commercial class.

Line FJB 08004 (FJ04), derived from the simple cross of Flor de Junio Silvia (FJS), a sister line of FJM (Castellanos *et al*., 2003a), and Flor de Mayo Anita (Castellanos *et al*., 2003b), was tested along with 13 Flor de Junio lines and FJM and FJS as checks, in different environments in central Mexico during 2008 and 2009. Yield data were submitted to an AMMI analysis and although the yield level of FJ04 was similar to the checks, its G x E interaction was low whereas the checks show high interaction (Figure 1). The genotypes and environments in quadrant II are of high yield and those near the zero line are of low G x E interaction. In the case of the opaque black bean seeded cultivars grown in the humid tropics of Mexico, its yield stability depends upon multiple disease resistance and adaptation to acid soils (López *et al.,* 2003). Likewise, disease resistance is essential for bean genotypes to show stability, a feature equivalent to homeostasis in the curse of time (Bruzi *et al.*, 2007).

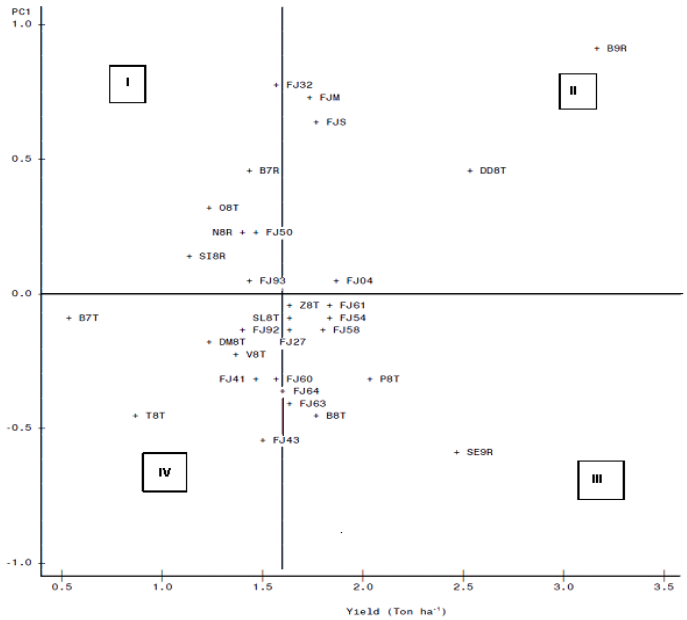

Figure 1. First Principal Component and seed yield of the genotypes and per environment graph.

During the winter-spring season of 2011 a plot of FJ04 was sown under irrigation at the Bajio Experimental Station at INIFAP, Celaya, Gto. In that plot, 340 individual plants of FJ04 were marked for sampling and young foliar tissue was taken for DNA extraction. On each DNA sample, the markers ROC 11 and SW13 (associated to the resistance to BCMV and BCMNV, respectively) were used for specific gene-segment amplifications following standard procedures (Johnson et al., 1997; Mukeshimana *et al*., 2005). Two hundred and six plants displayed the expected amplified segments derived from the use of both markers and the remaining plants gave rise to either one. All individual plants were harvested and sown a row per plant during the spring summer season under rainfed conditions to check for phenotypic traits such as days to flower and maturity, disease response, crop cycle and after-harvest and threshing data was recorded for the seed traits (100-seed weight, seed shape, shininess and color). At random, 150 rows coming from plants with both markers were sampled and the markers run again to verify its presence and all of the sampled rows showed both markers.

After carefully recording all mentioned traits in the field, the seed of 150 phenotypically similar progenies carrying both markers, was combined to recreate line FJ04. Sets of ten plants each of FJ04 carrying both markers were inoculated, under greenhouse conditions, with a strain of BCMV and BCMNV respectively, showing resistant phenotypes. This line has been chosen to be registered as new cultivar for central Mexico with the name of 'Flor de Junio Dalia'. For research purposes small amounts of seed are available from the corresponding author.

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PRIMAVERA
BEAN VARIETY, RAINFED GROWTH HABIT II FOR HIGH VALLEYS OF THE CENTRAL PLATEAU OF MEXICO

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Black bean, particularly the opaque type, is present in the southern zone of the country and is the one of most consumed in the capital, the Distrito Federal (Castellanos *et al.,*1997). The State of Mexico, together with the Distrito Federal, has the largest urban concentrations of the country, demanding 250 thousand tons of bean, part of which is covered with 85 000 tons, produced in the High Valleys of the Central Plateau. This region comprises the states of Tlaxcala, D.F., and part of Mexico, Puebla, Hidalgo, and Querétaro. Eighty-four percent are sown under rainfed conditions during the summer cycle, and the average yield is 670 kg ha^{-1}, whereas under irrigation it is 1220 kg ha^{-1} (SIAP, 2008).

Cross breeding was carried out at Campo Experimental Valle de Mexico (CEVAMEX) under greenhouse conditions in 1996; the progeny was advanced by mass selection until 2001, later on individual selection was done, and from 2003 to 2006, it was evaluated in an uniform yield assay at regional level; subsequently it was assessed in screening plots.

This variety is the product of the following cross breeding Negro 8025 / LINEA TLP-19-146-M-M-M-M-1-M, and its Plant Variety Protection register is FRI-075-041011.

Primavera is the first improved bean variety, opaque black, type II growth habit, adapted to the High Valleys of the Central Plateau. This region covers an altitude from 1800 to 2300 m above sea level. The variety has the following characteristics: vigorous emergence, it takes 50 days to flowering and flowers are purple-colored. The plant's vegetal cover is of good height (60 cm); with type II growth habit, adapted to environments with 250 to 300 mm of precipitation, and deep soils. The variety is resistant to anthracnose (*Colletotrichum lindemuthianum* (Sacc & Magn.) Briosi & Cav. and rust (*Uromyces appendiculatus* var. *appendiculatus* (Per.) Unger*.);* tolerant to halo blight (*Pseudomonas syringae* pv. *phaseolicola* (Burkholder) and root rots. It reaches physiological maturity at 102 days; its pods are small (10 cm-long), prismatic shaped, with 5 to 6 seeds, which are opaque black, small sized and heavy. The grain is of fast cooking (< 85 minutes), they have high protein content (26%) and good commercial acceptance.

Primavera variety is widely adapted, its highest yield potential, however, is expressed at sites of high altitude, from 2000 to 2300 m above sea level, deep soils (sandy-clay crumb) and with precipitation about 250 mm during its biological cycle.

At comparing Primavera to the varieties of the same commercial type, sown as control recommended for the same region, it had higher mean yield (> 2 tons ha^{-1}). Table 1 shows yields, year, and test sites of the three varieties.

Table 1: Yield in kg ha^{-1} of Primavera and the control varieties, assessed in the State of Mexico, during 2002 to 2009.

Site and year	Primavera	Negro 8025	Negro Jamapa
Santa Lucia, Estado de México, 2002	1,735	-	1,282
Santa Lucia, Estado de México, 2004	3,037	2,783	
Tepetlaxtoc, Estado de México, 2005	1,423	1,256	
Metepec, Estado de México, 2005	3,538	3,524	
Santa Lucia, Estado de México, 2005	1,230	1,453	
CEVAMEX, Estado de México, 2008	2,344	1,115	
Tlamincas, Estado de México, 2009	919	792	
Average	2,032.3	1,820.5	1,282
Difference compared to Negro Primavera	XX	-211.8 (10.4%)	-750.3 (36.9%)
Máximum	3,538	3,524	1,282
Mínimun	919	792	1,282

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RELEASE OF PARTIAL WHITE MOLD RESISTANT PINTO USPT-WM-12

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The Agricultural Research Service, Michigan State University AgBioResearch, and the University of Nebraska Agricultural Experiment Station, announce the release of USPT-WM-12 pinto bean (*Phaseolus vulgaris* L.) germplasm line with partial resistance to white mold caused by the fungal pathogen *Sclerotinia sclerotiorum* Lib. deBary. Scientists participating in the development of this germplasm were Phil Miklas (USDA-ARS, Prosser, WA), James Kelly (Michigan State University), James Steadman and Serena McCoy (University of Nebraska). White mold is rated the number one economic disease problem of dry bean production in the U.S., and is a major problem in pinto beans, which are highly susceptible to the disease. The disease is endemic in all production regions of the U.S., and is most problematic under moist conditions resulting from rains or excess irrigation during flowering and mid-pod fill stages from late July through August. The partial resistance in USPT-WM-12 is conferred in part by a QTL (WM2.2) that derives from ICA Bunsi navy bean (synonymous with Ex Rico 23 in Canada).

USPT-WM-12, previously tested as PS02-037-2 or 37-2, derives from the cross G99750/USPT-WM-1. Great northern breeding line G99750 with partial field resistance to white mold was derived from a cross between BelDakMI-RMR-11 (Stavely et al., 1997) and Matterhorn (Kelly et al., 1999) made in 1996. The cross was an attempt to introduce additional genes conferring resistance to rust (*Ur-6* and Ur-11) and *Bean common mosaic necrosis virus* (BCMNV) (*bc-3*) into the great northern market class in combination with wider adaptation and greater yield potential. BelDakMI-RMR-11 is a pinto bean line bred for resistance to rust and BCMNV in an upright architecture. Matterhorn is a great northern cultivar with upright architecture, wide adaptation and high yield potential, but is very susceptible to white mold. G99750 has late maturity and possesses the stay-green stem trait whereby the plant stems and branches remain green at harvest maturity. Both late maturity and stay green traits are associated with partial field resistance to white mold.

The other parent, pinto bean germplasm line USPT-WM-1 with partial field resistance to white mold, was released by USDA-ARS and collaborators in 2005. Similarly, USPT-WM-1 is later maturing and exhibits the stay-green stem trait. USPT-WM-1 derives from the cross 'Aztec'/ND88-106-04. Aztec is a semi-upright pinto bean cultivar from Michigan State University that is susceptible to white mold and BCMNV. ND88-106-04 is an upright navy bean breeding line from North Dakota State University with resistance to white mold derived from ICA Bunsi.

The cross G99750/USPT-WM-1 was conducted in the fall of 2002. The F1 seed was planted in the spring in the greenhouse and the selfed F2 seed from several F1 plants was harvested in bulk and spaced planted in the white mold field trial during the summer of 2003. This trial and all subsequent white mold trials used to select these materials were located at Paterson, Washington. Five single F2 plants exhibiting less white mold disease were selected and harvested individually. Subsequently the five F2:3 progeny were planted in separate rows in the white mold trial conducted in 2004. Three progeny rows with less white mold disease and pinto seed type were harvested. The plants with less disease within a row were selected and bulked. The three F3:4 bulked-selected families (lines) were not planted again until 2006, when they

were tested for white mold reaction with 27 other early generation F4 lines arranged in a randomized complete block design with three replications. A plot consisted of three rows of each line with 0.56 m row spacing and 3.04 m row length. Two lines PS02-037-2 and PS02-037-4 with less white mold infection and higher yields were advanced. In 2007, these two F3:5 lines, plus 28 other breeding lines and 10 checks, were planted in a replicated white mold trial similar to the previous year except that plots were four rows wide. Only one sib PS02-037-2 was selected based on low disease score and high yield potential for further testing in the national Bean White Mold Nursery (BWMN).

PS02-37-2 performed very well in the BWMN. Each year the BWMN tests nine advanced lines with putative partial resistance to white mold submitted by breeders and three checks. The checks include G122 that is partially resistant in both the field and greenhouse straw test, ICA Bunsi partially resistant in the field and susceptible in the greenhouse, and Beryl susceptible in both field and greenhouse environments. The BWMN is tested in five to nine different locations (CO, ID, MI, ND, NE, NY, OR, WA, WI) across the U.S. in any given year. The disease severity score in the field is rated on a 1 to 9 scale where 1 = no symptoms and 9 = all plants severely diseased and dying or dead. For the straw test, disease severity is rated with a similar 1 to 9 score, but a rating of 1 = no disease advancement at the cut stem site of inoculation and 9 = progression of infection past the third node from site of infection and severe wilting and plant death. The average score for USPT-WM-12 across the 2008, 2010, and 2011 BWMN trials in the field and greenhouse was 3.4 and 5.1, respectively, compared to 2.8 and 4.9 for G122, 3.8 and 7.3 for Bunsi, and 6.5 and 7.6 for Beryl. The level of field resistance is greater than ICA Bunsi and similar to USPT-WM-1. The level of resistance exhibited by USPT-WM-12 in the straw test was unexpected and amongst the best observed of the many lines from the Durango race (pinto, great northern, pink, small red) tested thus far in the BWMN.

Another indicator of partial resistance in USPT-WM-12 is its superior yield under white mold disease pressure. Of 64 lines tested in Entrican, MI, USPT-WM-12 was the highest yielding entry in 2010 and 2011 averaging 4160 kg ha. In comparison, the high yielding pinto bean cultivar Lariat yielded 3080 kg ha, 26% less than USPT-WM-12. The yield advantage for USPT-WM-12 in the absence of white mold in Michigan and Washington trials in 2011 was less when compared to Lariat, averaging 5% less yield, 3890 vs 4090 kg ha, respectively. Compared to other commercial pinto bean cultivars in the same non diseased trials, USPT-WM-12 yielded 3.5% less than La Paz, 3.5% more than Santa Fe, and the same as Stampede. This comparable yield with other pinto bean cultivars indicates USPT-WM-12 suffers minimal yield penalty for possessing partial resistance to white mold in the absence of the disease.

Agronomic traits for USPT-WM-12 are all commercially desirable. Maturity (98 days) is one to two days earlier than Lariat (100 days), La Paz (100 days) and Stampede (99 days), and a two days later than Santa Fe (96 days). Seed size for USPT-WM-12 (39.3 grams), as determined by weight of 100 seeds, was comparable to Lariat (40.3 g) and Stampede (39.4 g), slightly larger than La Paz (37.5 g), and slightly smaller than Santa Fe (43.3 g). USPT-WM-12 has upright Type II short-vine indeterminate growth habit similar to La Paz and Stampede and exhibits less lodging than Lariat or Santa Fe. The stay-green stem trait which can contribute to partial resistance to white mold but detract from harvest-ability is less prevalent in USPT-WM-12 than in the parent USPT-WM-1. This germplasm release possesses the *I* gene for resistance to BCMV and determination of reaction to bean rust is pending. The cream background color and light brown mottled patterns characteristic of the pinto market class is too dark for commercial acceptance in USPT-WM-12, and is the major reason why it is being released as a germplasm line instead of a cultivar.

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2011 FINANCIAL STATEMENT
BEAN IMPROVEMENT COOPERATIVE

BALANCE AS OF January 1, 2011			**$ 4,206.51**
INCOME			
2011 Dues	$	3,717.00	
Extra CDs	$	20.00	
Extra Books	$	90.00	
Extra Articles for 2011 Report	$	25.00	
2012 Dues	$	60.00	
Reimbursement for deposit for 2011 BIC Meeting	$	3,750.00	
2011 BIC meeting registrations	$	595.00	
2011 BIC meeting excess	$	739.07	
Back Issues	$	10.00	
Bank Interest	$	167.73	
TOTAL INCOME	$	9,173.80	
EXPENSE			
Labor Charges	$	983.61	
Graduate student awards - 2011 BIC meeting	$	200.00	
Postage, Copy Charges and Office Supplies	$	1,336.76	
Printing and shipment – Volume 54	$	1,941.27	
Google Checkout and PayPal Fees	$	100.94	
TOTAL EXPENSE	$	4,562.58	
BALANCE AS OF December 31, 2011			**$ 8,817.73**