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: Multi-line planting of dry beans in a furrow-irrigated field near Grant, Nebraska. Photo courtesty of H.F. Schwartz.

THE 53rd ANNUAL REPORT OF THE BEAN IMPROVEMENT COOPERATIVE

The Bean Improvement Cooperative celebrated the Twentieth Biennial Meeting in Fort Collins, Colorado. The highly successful meeting had approximately 125 registered participants and featured 44 oral presentations and 45 poster presentations. The BIC was able to sponsor travel to the meeting for six students from Bulgaria, Brazil, Spain, Kenya, Puerto Rico, Cornell and North Dakota State who contributed either an oral or poster presentation. The quality of both the oral and poster presentations was excellent. The meeting began with two Frazier-Zaumeyer Distinguished Lectures, entitled: '*Health Benefits Associated with Consumption of Dry Beans'* and '*Strategic Approaches to Tapping the Human Health Potential of Common Bean (Phaseolus vulgaris L.)'*. The stimulating lectures were presented by Dr. Maurice Bennink, Professor at Michigan State University and Dr. Henry Thompson, Professor at Colorado State University.

The meeting received generous support from: ADM Edible Bean Specialties Inc.; Bush Brothers & Company; Harris Moran Seed Company; Seminis Vegetable Seeds, Inc.; Syngenta Seeds, Inc.; BASF Corporation; Northern Feed & Bean; Seneca Foods Corporation; Trinidad Benham Corporation; Central Bean Company; Crites Seed Inc.; Jack's Bean Company; Kelley Bean Company; Michigan Bean Commission; and Servi-Tec. On behalf of the BIC, I wish to acknowledge the substantial role of the organizing committee, Howard Schwartz and Mark Brick, and would like to thank them, the sponsors and the participants for making the meeting a success.

At the Awards Banquet, the Frazier-Zaumeyer Lecturers were recognized, the Meritorious Service Award was presented to Dr. Mark Brick, and two student awards were presented for the best oral and poster presentations at the BIC meeting.

> The outstanding student oral presentation was entitled: '*Development and Screening of BIBAC Libraries from Two Sources of CBB Resistance in P. vulgaris'* presented by Gregory Perry, University of Guelph, Guelph, Ontario, Canada – Peter Pauls, advisor [p.38-39].
>
> The outstanding poster presentation was entitled: '*Phenotypic and genotypic evaluation of common bacterial blight resistance in a resistant inter-cross population of Phaseolus vulgaris'* presented by Kelli Durham, University of Guelph, Guelph and Harrow, Ontario, Canada – Peter Pauls and Ali Navabi, co-advisors [p92-93].

On behalf of the BIC, I would like to recognize Jim Kelly for his 12 years of dedicated service as BIC President from 1998-2009. Dr. Kelly championed the BIC into the Information Technology Age by developing a highly informative website, digitizing the BIC Annual Reports by the National Agricultural Library, and enabling internet-based payment of membership dues. Jim was instrumental in establishing the Frazier-Zaumeyer Lectureship, and continued the pattern of past presidents by raising the bar of excellence for the BIC overall during his tenure.

The next BIC meeting is planned in San Juan, Puerto Rico in October, 2011. The local organizing committee consists of Tim Porch, Jim Beaver and Mildred Zapata. Details for the 2011 BIC meeting will be posted on the BIC Web page www.css.msu.edu/bic.

Dr. Phillip N. Miklas, BIC President

BIC COMMITTEE MEMBERSHIP - 1957 to 2010

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

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
2010 Hosfield, Schwartz, Singh

Genetics Committee

2007: Tim Porch (Chair), James Beaver, Matthew Blair, Paul Gepts, Phil McClean, Phil Miklas, Carlos Urrea, Molly Welsh (ex officio).
2008: Tim Porch (Chair), Kirstin Bett, Matthew Blair, Paul Gepts, Phil McClean, Phil Miklas, Carlos Urrea, Molly Welsh (ex officio).
2010: Tim Porch (Chair), Kirstin Bett, Matthew Blair, Paul Gepts, Phil McClean, Phil Miklas, Carlos Urrea, Molly Welsh (ex officio).

GENETICS COMMITTEE MINUTES

2009 BIC Meeting, Colorado

Meeting location: Hilton Garden Inn, Ft. Collins, Colorado
Date: Oct. 28, 2009
Start Time: 11:15 AM

Attendance:

Kirstin Bett	U. of Saskatchewan, Saskatoon	Member
Paul Gepts	U. of California, Davis	Member
Phillip Miklas	USDA/ARS, WA	Member
Tim Porch	USDA/ARS, PR	Chairperso
Carlos Urrea	University of Nebraska	Member
Molly Welsh	USDA/ARS, WA	(ex officio)
Talo Pastor-Corrales	USDA/ARS, Beltsville	
Jim Beaver	U. of Puerto Rico	
Jim Steadman	U. of Nebraska	
M. Celeste Goncalves-Vidgal	UEM-Maringa, Brazil	
Pedro Soares Vidigal	UEM-Maringa, Brazil	
Jim Myers	Oregon State U.	
Giles Waines	U.C. Riverside	
Kirsten Bett	U. of Saskatchawan	
John Wamatu	Brotherton Seed Company	
Steve Noffsinger	Seneca Foods Corp.	
Dan Walquist	Syngenta Seeds	
Parthiba Balasubramanian	AAFC-Morden, MB	
Dedrie Fourie	ARC-Grain Crops Research Institute, South Africa	
Alyson Thornton	Harris Moran Seed Co.	
Bob Gehin	Harris Moran Seed Co.	
John Thomas	U. of Nebraska	
Mark Brick	Colorado State U.	
John Hart	Cornell U.	
Phillip Griffiths	Cornell U.	
James Kelly	Michigan State U.	

Old Business

1. **Approval of the Genetics Committee meeting minutes from the W1150 Meeting in Isabela, Puerto Rico on Feb 21st 2009**

Decision: Motion to approve Minutes by P. Miklas and seconded by P. Gepts, and then approved by those attending.

2. **Recently accepted gene symbols were presented.**

For general information of the committee, the gene symbols accepted during the February 2009 meeting and by email since that date were presented. No decision was made.

New Business

1. **Update of the Fusarium wilt gene symbols**

There are two gene symbols for Fusarium wilt, *Fop-1* and *Fop-2*. Jim Kelly indicated that they appear to have been named backwards. 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. In terms of origin, *Fop-1* is an Andean gene with resistance to Mesoamerican race 2, and *Fop-2* is a Mesoamerican gene, but we don't know whether race 1 is Andean or Mesoamerican. In addition, other races include race 3 from Colombia, race 4 from Colorado, and race 5 from Greece.

Decision: No decision was made about switching the race designation for race 2 and race 1. This decision will be delayed until the next meeting when Mark Brick and Howard Schwartz are present.

2. **Addition of the *Phg-1* gene symbol to the List of Genes**

A manuscript (Goncalves-Vidigal et al.) was presented by Maria Celeste Goncalves-Vidgal for review by the Genetics Committee that mapped *Phg-1* and showed linkage to *Co-1*^{4}. *Phg-1* has not been included on the List of Genes, nor on the common bean map. In the manuscript, *Phg-1* is linked to SCAR $SH13_{520}$ from AND 277 in crosses to 'Ruda' and 'Ouro Negro,' which were evaluated in F2 (Ruda) and F2:F3 families (ON) through simultaneous inoculation with races 63-23 of *P. griseola* and race 73 of *C. lindemuthianum*. Ruda segregated 3R:1S for race 73 indicating dominant resistance through *Co-1*^{4}. Ouro Negro segregated 1:2:1 for both 2047 and 63-23, indicating single dominant genes. In AND 277, *Phg-1* was linked to *Co-1*^{4} and to SH13, with no recombinants, and *Co-1*^{4} mapped to B01.

Discussion: *Phg-1* and *Phg-2* are on separate linkage groups, but allelism tests have not been completed with other resistance genes. SH13 has not been mapped independently due to lack of polymorphism in the BAT x Jalo population. However, the soybean map has a homologous sequence at the bottom of B11. Juan Jose Ferreira's group in Spain has a polymorphic population and has agreed to map SH13. It is possible that a gene other than *Co-1*^{4} is linked to *Phg-1*, and that this gene reacts to the same races. In this case, *Phg-1* could be located on a different LG.

Decision: The addition of the *Phg-1* gene symbol and the mapping of this gene will be tabled until further evidence for the map location of SH13 can be generated. The mapping location can be generated quickly as Dr. Ferreira's group is actively mapping the SH13 marker. The discussion will continue through email and Tim Porch, Jim Kelly and Talo Pastor-Corrales will take leadership in reviewing and organizing the *Phg* genes and gene symbols.

3. **New gene symbol, *Rk*^{r}, for garnet brown seed coat color** (Phil Miklas)

The authors propose the gene symbol *Rk*^{r} for a dominant gene for garnet brown seed coat color in 'Dorado' (Bassett and Miklas manuscript). The proposed *Rk*^{r} gene symbol needs to be reviewed for addition to the List of Genes. There was not sufficient time to address this manuscript. The manuscript will be reviewed by email instead.

Meeting Adjourned: 12:15 PM (Motion to adjourn by P. Gepts)
Minutes completed by: Jim Myers and Tim Porch

2009 BIC AWARD RECIPIENTS

THE BEAN IMPROVEMENT COOPERATIVE

Proudly Presents the

Frazier - Zaumeyer Distinguished Lectureship

to

MAURICE R. BENNINK

Professor

Food Science and Human Nutrition at Michigan State University

&

HENRY J. THOMPSON

Director

Cancer Prevention Laboratory at Colorado State University

Meritorious Service Award

to

MARK A. BRICK

Colorado State University
Fort Collins, CO

MAURICE R. BENNINK

Maurice R. Bennink, Professor of Food Science and Human Nutrition at Michigan State University, has played a seminal role in promoting the awareness of health benefits of dry beans. He received his B.S. degree from Michigan State University and M.S. and PhD. Degrees from Colorado State University and the University of Illinois, respectively. Dr. Bennink has conducted research on dry bean for over 25 years. Much of his research on the effects of high fiber diets, especially soluble bean fiber, on the reduction of serum lipid levels in rats has spawned numerous research investigations in the U.S. and internationally. Since 1984, Dr. Bennink has been a Co-Principle Investigator of Bean/Cowpea CRSP projects in Guatemala, Costa Rica, and Africa. He was part of a team in collaboration with the CRSP host country of Guatemala, and the USDA-ARS Dry Bean Genetics group at Michigan State University, that elucidated that bean starch and fiber were the major contributors to flatulence and accompanying gastro-intestinal disorders prevalent in most people after consuming beans. This work countered the thinking of the time that indigestible oligosaccharides (complex sugars) of the raffinose family were the main contributors to flatulence in humans. The discovery of the starch-fiber link to flatulence helped resolve the carbohydrate "X-Factor" contributor to flatulence. He also collaborated on a project that demonstrated bean starch to be extremely resistant to thermal breakdown.

Since 2000, a major part of Dr. Bennink's research has been on the relationship of bean diets and the improvement of human health and well-being. His research group demonstrated in 2001 that eating beans reduced colon cancer by more than 50% in a rodent model of human colon cancer. This work captured the attention of all sectors of the bean community, the United States Agency for International Development, and the United States Department of Agriculture. Further clinical research indicated that beans had the potential to prevent and/or reduce other chronic diseases, namely Type II diabetes and cancers of the prostrate, colon, and breast. He has proposed a mechanism by which beans could inhibit cancer through regulation of blood glucose and insulin levels. It is well documented that eating beans produces low serum glucose and insulin concentrations compared to most other sources of dietary carbohydrates. The enthusiasm generated was the spearhead used to secure funding from the United States Agency for International Development to form the "Beans for Health Alliance". The Beans for Health Alliance in turn has initiated research into the health benefits of beans by many other scientists.

Dr. Bennink's literature reviews are often used by other scientists to substantiate their argument that production agriculture can play an important role in public health and that dry beans are an important commodity that requires funding at the state and federal levels. As an invited speaker, he promotes the health benefits of beans to a wide audience - various scientific disciplines, federal agencies, international programs, and general public.

Dr. Bennink's current international work in Africa demonstrated that beans can form the basis for an inexpensive food supplement to prevent malnutrition in small children and ameliorate the effects of hunger deprivation in malnourished children. Perhaps, the most important contribution Dr. Bennink has made to human nutrition and public health is his research demonstrating that a bean based diet helps improve the immune systems of children infected with the Human Immune Deficiency Virus.

HENRY J. THOMPSON

Henry J. Thompson is a Professor in the College of Agricultural Sciences, and Director of the Cancer Prevention Laboratory at Colorado State University (CSU) in Fort Collins. He is a member of the American Association for Cancer Research and the American Society for Nutrition. Dr. Thompson earned his PhD from Rutgers University in nutritional sciences with an emphasis in biochemistry. Thompson received postdoctoral training in the Department of Molecular Medicine at the Mayo Clinic in Rochester, MN where he investigated the underlying causes of diabetes. From 1977 to 1979, he assumed the role of a senior research nutritionist at IIT Research Institute in Chicago, IL and was trained in experimental carcinogenesis, learning methods for the chemical induction of cancer in five organ sites thus launching his career in cancer research. From 1980 to 1989, Dr. Thompson served on the faculty of the University of New Hampshire and directed the Human Nutrition Center at that institution. Beginning in 1990, Thompson moved his laboratory to Denver, Colorado where he was the Head of the Center for Nutrition in the Prevention of Disease at AMC Cancer Research Center.

In January 2003, Henry joined the faculty of CSU and established the Cancer Prevention Laboratory (CPL) in the Department of Horticulture and Landscape Architecture. His decision to do this was based in part on the results of three dietary intervention studies that he conducted in women at high risk for breast cancer. The studies were designed to discover the effects of plant food rich diets on biomarkers for cancer risk. Because of modest effects observed in women eating as much as 16 serving of vegetables and fruits per day, Thompson initiated discussions with plant breeders and producers of staple food crops which led to the formulation of the hypothesis that the most health beneficial cultivars of staple plant foods are currently not known, in part, because plant breeders and biomedical scientists have not had the opportunity to interact to determine "human health-related plant characteristics". These discussions ultimately led to Thompson's lab moving to CSU where his current research investigates the human health benefits of staple food crops, namely dry beans, potato, wheat and rice.

Dr. Thompson played a leadership role in defining the field of Biomedical Agriculture and in establishing the "Crops for Health" program at Colorado State University. He presented the Betty Klepper Endowed Lectureship at the 2007 Crop Science Society of America meeting in New Orleans, LA, titled "Biomedical Agriculture: A New Approach to Improving the Human Health Attributes of Staple Food Crops". This presentation initiated a cascade of events, including the development of the provisional division C-9, "Biomedical, Health-Beneficial, and Nutritionally Enhanced Plants". This division will hold its first symposium and scientific paper sessions at the 2009 CSSA meetings. He has a long standing interest in the associations between diet and breast cancer, and maintains an active program of clinical and laboratory research that addresses this topic.

Dr. Thompson has published more than 140 journal articles and book chapters. In summary, Dr. Thompson has contributed extensively to interest in the health benefits of staple food crops and is now leading an effort to establish a transdisciplinary program that will foster contemporary approaches to crop improvement for biomedically important traits.

MARK A. BRICK

Dr. Mark A. Brick was born October 6, 1947 in Green Bay Wisconsin. He grew up on a dairy farm in eastern Wisconsin and operated the family farm for two years after High School. He served in the U.S. Navy from 1966 to 1968, then entered the University of Wisconsin-River Falls in 1969 where he received the BS degree in Crop Science. He received the M.S. from the University of Arizona in 1975, and Ph.D. from the University of Minnesota in 1980. He worked as a Research Station Manager for Cal West Seeds in Wisconsin for two years and taught at University of California-Fresno for one year after completing the Ph.D. From 1981 to 1986, Mark served as Manager of the Colorado Seed Growers Association, the official seed certification agency in Colorado. During this time he was appointed to serve as the U.S. representative to the1996 Pan American Seed Seminar in Cali, Colombia, served as liaison to improve relations between AOSCA and Latin American seed certifying agencies, and developed the first variety descriptors for common bean for the Plant Variety Protection Office. In 1986, he became leader of the Dry Bean Breeding Project at Colorado State University, and currently is a Professor of Crop Science in the Department of Soil and Crop Sciences at CSU.

Mark has been a member of the Bean Improvement Cooperative since 1985, and received the BIC Distinguished Achievement Award in 2001. He has been involved in teaching, research and outreach at the university level for 29 years. His teaching responsibilities have spanned thirteen different courses at four universities, as well as short courses taught internationally. He advised graduate students from Argentina, Burma, China, Ethiopia, Macedonia, Mexico, Saudi Arabia and Turkey. In 2001, he received the highest award given by the College of Agriculture at Colorado State University, the NACTA/
Shepardson Teaching Award for innovative classroom teaching. Mark has served as major professor for 28 M.S. and 4 Ph.D. students. He became a fellow in the American Society of Agronomy in 2003. He has collaborated with scientists, farmers and industry clientele in the U.S. and internationally.

Mark is involved with community service and outreach. In collaboration with Dr. Howard Schwartz, and faculty at the University of Wyoming and University of Nebraska, they formed the Tri-State Bean and Beet Workers Group. This group developed a regional dry bean bulletin that has over 20,000 copies in circulation. Also in collaboration with Dr. Howard Schwartz and the Colorado bean industry, Mark helped form the Colorado Dry Bean Administrative Committee (CDBAC), the organization that manages and distributes check-off money from bean production. He currently serves on the Board of Directors to the Certified Seed Growers and is a member of the Colorado Foundation Seed Program. To help the local community, he initiated a program to donate three to six tons of surplus beans from his breeding project to feed needy families through the Larimer County Food Distribution Center and has participated in community service work in Bolivia and El Salvador. Mark is also an honorably discharged veteran of the US Navy and received the Viet Nam Campaign and Viet Nam Service medal for his service during 1968.

As the leader of the Colorado State University Dry Bean Breeding Project, he is responsible for development of dry edible bean cultivars for irrigated and non-irrigated production in the High Plains. His professional areas of expertise are plant breeding for yield, disease resistance, and crop improvement to enhance human health. Since 1986, this project has released seven cultivars and ten germplasm lines. The cultivars have widespread adaptation in the region, and have provided both producers and certified seed growers with cultivars that have high yield, disease resistance, and excellent seed quality. He published research on the use of carbon isotope discrimination, the application of selection indices and traits associated with drought stress, and resistance mechanism and screening protocols for resistance to *Fusarium* wilt and white mold. More recently he has collaborated with Dr. Henry Thompson, the director of the Cancer Prevention laboratory at CSU. Together, they have demonstrated that dry beans in the diet can reduce the incidence and severity of cancer in a dosage dependent manner using a preclinical animal model system. Mark has authored or coauthored more that 60 refereed publications.

IN MEMORY OF PETER H. GRAHAM

Peter H. Graham died suddenly on May 9, 2009. He was a world expert on soil microbiology as related to symbiotic nitrogen fixation in common bean and other legumes.

A native of Perth, Australia, Peter earned his B.Sc. Agric. (Hon.) and Ph. D. degrees from the University of Western Australia. From 1963 to 1971 he served as a Lecturer, Senior Lecturer, and Acting Head, in the Dept. of Microbiology at the University of Sydney where he taught courses in agricultural microbiology, soil microbiology, and bacterial systematics. From 1971 to 1982 he worked as a soil microbiologist at the International Center for Tropical Agriculture (CIAT) in Cali, Colombia.

Peter's studies at CIAT centered on the practical nature of the symbiotic relationship of legumes and rhizobia - his ultimate goal was to help farmers increase their seed yields without increasing their production costs. His early efforts focused on the development of effective *Rhizobium* inoculants for farm use, including an examination of carriers as well as extensive strain testing. His research also encompassed the plant side of the bacteria-plant symbiotic relationship. He conducted numerous studies on common bean, including an examination of carbohydrate partitioning in cultivars which differed in growth habit and agronomic factors, breeding for increased N_2 fixation potential and the ways that these physiologic and morphologic characteristics affect N_2 fixation. Peter's papers on bean inoculation and variation in strains and cultivars leading to differences in their ability to fix N_2 have been cited frequently. Of particular importance was his work identifying Puebla 152 as a high yielding, high N2 fixing cultivar, and his characterization of *Rhizobium* strain CIAT 899 (UMR 1899) as a superior common bean inoculant for crops grown in acid-soil regions.

While at CIAT, Peter taught post-graduate short courses and supervised post-graduate research trainees in soil microbiology. Peter's fluency in Spanish allowed him to have a strong connection and high impact with students he trained throughout Central and South America through theoretical and practical courses taught in Colombia, Brazil, Argentina, Uruguay, Venezuela and Mexico. Peter trained many of today's prominent soil microbiologist researchers in Latin America during his tenure at CIAT.

Peter moved to the Department of Soil, Water and Climate at the University of Minnesota, in 1982, where he was an active faculty member up to the time of his death. At Minnesota Peter continued his work on *Rhizobium* taxonomy and on the *Rhizobium – Phaseolus* symbiotic relationship. He expanded his activities to include improvement of nitrogen fixation in soybean and in prairie legumes used for revegetation. In 2000, the Peter H. Graham Inoculant Laboratory in Quito, Ecuador was named in his honor for the research he coordinated during his 12 years working on the Bean/Cowpea CRSP Project. He served as long term Editor-in-Chief for Field Crops Research Journal.

Peter was a friend and mentor to many. He gave of his time freely and provided sage advice based on his knowledge and broad experience. Peter was noted for his strong support and mentorship of students and junior colleagues. He taught an undergraduate course and a graduate course in soil biology. He advised 12 Ph.D. students, 13 M.S. students, and many undergraduate students throughout his career.

IN MEMORY OF MATT SILBERNAGEL

Mathias Joseph Silbernagel, retired USDA-ARS Research Plant Pathologist, died in Grandview, WA, on April 14, 2009 at home after an 18 year battle with prostrate cancer. Dr. Silbernagel spent his entire 33-year career from 1962 to 1995 working for the Bean Project at the USDA-ARS Vegetable and Forage Crop Research Station & WSU Irrigated Agriculture Research and Extension Center in Prosser, WA.

The son and grandson of farmers, Dr. Silbernagel was born on the family wheat farm outside Hague, ND, on May 13, 1933. His parents lost the farm due to the 'Great Depression' and moved their family to Yakima, WA, when Matt was 10. He graduated from Marquette high school in Yakima. He served two years in the Marines, and shortly thereafter in 1955 married high school sweetheart Gladys Marie Herring. They moved to Seattle, WA, where he earned a B.S. degree in Botany from the University of Washington in 1957. He achieved a Ph.D. in Plant Pathology from Washington State University in 1961.

Matt is recognized most for his research on snap bean production, snap bean germplasm development, and characterization of bean virus diseases prominent in the Pacific Northwest namely *Bean common mosaic virus* (BCMV) and *Beet curly top virus* (BCTV). In conjunction with colleagues he identified the necrotic variant BCMNV and its origins and spread from East Africa to Europe and the U.S. Matt released snap bean cultivars 'Apollo', 'Gold Crop', 'Blue Mountain', 'Greenlight', and 'VR-Romano', and numerous germplasm lines during his career including FR266, 8BP3, 5BP7, CTR Sprite, and USWA-64. In addition to resistance to the aforementioned virus problems these germplasms represented novel sources of resistance to Fusarium root rot, bean rust, and other diseases and stresses such as heat, soil compaction and drought. He was a leader in development of threshers that enhanced snap bean seed quality. He received international invitations to serve as a consultant on snap bean breeding and production.

Dr. Silbernagel assumed full responsibility for dry bean germplasm development after the retirement of Dr. Burke in 1984. Important dry bean cultivars and germplasm contributions included pinto beans 'Othello', 'Burke', 'Quincy', USWA-20, and 92US-1006. Matt received the Meritorious Service Award from BIC in 1987 in recognition of his contributions to bean research worldwide. During his overseas travels, Dr. Silbernagel ingratiated himself to researchers at many plant research institutions in Africa and Eastern Europe. He was exceedingly adept at persuading foreign scientists to work on bean problems. Moreover, Matt had an exceptional knack for bringing researchers together to work on bean problems of mutual interest. These engagements led to numerous fruitful collaborations, including Matt's mentorship of many students and young scientists in his role as Principal Investigator for the US-AID, Bean-Cowpea CRSP project in Tanzania that spanned 15 years. With colleagues at Sokoine University of Agriculture in Morogoro Tanzania, he initiated the breeding program that led to the release of 'SUA 90' and 'Rojo' dry beans and established the germplasm base for more recent releases from that program.

Matt enjoyed many hobbies including hunting, fishing, rock-hounding, and camping. He is survived by his wife, five children, nine grandchildren, two great grandchildren, brother, and sister.

IN MEMORY OF DAVID MAURICE WEBSTER

Family, friends and colleagues were shocked and saddened when they learned that David M. Webster had been killed while riding his bicycle correctly on June 25, 2009. He was devoted to his family, a community leader and an award-winning, highly-renowned pathologist and plant breeder.

David was born July 10, 1951, in Ft. Bragg, N.C. Growing up with his parents Maurice W. Webster and Margaret M. Webster, he lived in many different places in the U.S. and in Japan. He graduated with a B. A. in Chemistry from Kalamazoo College in Kalamazoo, MI in 1973 and then enrolled in the University of Wisconsin-Madison where he earned a M.S. degree (1975) and the Ph.D. degree in plant pathology (1978).

Dr. Webster began his career with Asgrow Seed Company on June 26, 1978, thus having completed 31 years with the company later known as Seminis and now part of Monsanto. He was hired to develop new varieties of peas and beans and what a career it was! Worldwide, Seminis sells $40,000,000 yearly in pea seed, which represents 35% of commercial pea sales. So when you buy a can of peas or a bag of frozen peas, there is about a 1 in 3 chance that you are eating peas developed by David. Sales of foundation seed of dry beans are also impressive and increasing. His accomplishments with individual variety releases were equally impressive. At last count, David had 96 active PVP certificates worldwide with another 27 applications pending examination.

David applied both science and art to plant breeding. His education gave him the scientific background needed to succeed, and he was also an artist whose palette was the pea and bean plants that he worked with. He created novel plants and brought to commercialization gene combinations that would not exist without his efforts. His ultimate commercial success was achieved when he applied for a pea patent in March, 2005, that involved a unique combination of genes that does not exist in nature. One of the special moments in his career as breeder was the recognition Meritorious Service & Achievement Award at the BIC meeting in 2005.

The quote from the plaque he received as part of the Seminis Special Recognition Career Award in 2004 is an apt tribute; "*World renowned pea breeder with a history of extraordinary product sales in North and South America, Europe and Australia/New Zealand. David's continuous dedication to the development of widely adapted pea and bean varieties has allowed Seminis to set and maintain the benchmark in these crops. David has demonstrated great skill in developing widely adapted varieties with leading agronomic performance and important disease resistance combinations. David's curiosity and drive to improve has led to a highly distinguished record for variety sales*."

David had an active life outside of work and built up a wide network of relationships, a group of friends and close associates that included staff and crew at the research station in Twin Falls. It also included other researchers worldwide, people in sales, production and marketing within the company and extended to include people at universities and the USDA as well as competitors at other companies, with whom David interacted and shared new varieties.

David brought enthusiasm to all of his pursuits. He had extraordinary energy and great respect for health and physical conditioning. He worked long hours but enjoyed being home to tend garden, tutor their children, renovate home and farm, and read history and biographies. He enjoyed simple pleasures of sharing companionship and telling jokes that he could hardly get through without cracking himself up.

David is survived by his wife Charlene, daughter Rachel M. Webster, stepchildren, Leah (Phil) Knight and Austin Hollingshead; mother, Margaret Webster; sister, Elizabeth; nieces, Sarah (David) Glass and Lauren (Jeremy) Cox; nephew, Tim VanDenBerg (Tiffany) and many extended family and friends.

HEALTH BENEFITS ASSOCIATED WITH CONSUMPTION OF DRY BEANS

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Dietary and lifestyle habits that promote excess glucose (hyperglycemia) and excess insulin (hyperinsulinemia) in the blood and excess body fat facilitate development of several chronic diseases including Type 2 diabetes, cardiovascular diseases, and cancer at several sites in the body. Hyperglycemia, hyperinsulinemia, and excess body fat are responsible for a milieu of changes – hormones, growth factors, inflammatory products, oxidative stress, etc – that contribute to development of chronic diseases.

The extent to which different foods or meals raise blood glucose depends on the glycemic index of the consumed foods and the quantity of carbohydrate consumed. A key study (1) used meta-analysis and meta-regression in the analysis of 45 publications to determine the outcome of substituting low glycemic foods for high glycemic foods. This study determined that overall control of blood glucose is strongly related to the glycemic index and glycemic load of the diet and the amount of unavailable carbohydrate (fiber) consumed. It was suggested that optimum control of blood glucose is achieved when the diet has a glycemic index < 45, a glycemic load < 100g per day and a fiber intake of ≥ 25g per day.

There has been a steady increase in the percentage of overweight and obese individuals in most industrialized countries (2-5) and even in urban areas of under developed countries. On a worldwide basis, more than one billion adults are overweight and more than 300 million are obese (3, 4). In the U.S. more than 60% of the adult population is overweight or obese (5). Obesity and overweight account for approximately 300,000 deaths per year in North America (6, 7) and the cost associated with excess body fat is estimated to be greater than 117 billion dollars per year (8). Most of the costs associated with excess body fat are related to Type II diabetes, heart disease, and high blood pressure (9). Twenty-three studies (1) examined changes in body weight that occurred when subjects changed from a high to a low glycemic index diet. The most significant factors related to successful weight loss and maintenance were a reduced glycemic load along with a concurrent reduction in total caloric intake.

Beans are the perfect food to improve and/or promote glycemic control. Beans have a low glycemic index, varying from 27-42% relative to glucose and 40-59% that of white bread (10). Beans are also high fiber - total unavailable carbohydrate is 27 - 29%. Substituting beans for foods prepared from white flour (on an equal dry weight basis) will reduce the glycemic index of the diet by about two-thirds and glycemic load by about 80%. Furthermore, consuming beans will significantly increase your intake of dietary fiber and that is particularly important for controlling blood glucose concentrations. Clearly, if bean consumption could be increased and if there was a concomitant decrease in body weight, the incidence of Type II diabetes, heart disease, and high blood pressure would be decreased and *the public health benefit would be enormous! Since increasing bean consumption would not increase the cost of the diet, it is hard to imagine a more cost effective intervention!*

Data from several human intervention trials indicate that consumption of canned and cooked beans reduce serum cholesterol. Generally small (6 - 10%) but statistically significant reductions in total and LDL cholesterol occur when beans are added to the diet. In carefully controlled clinical studies where the macronutrient intake was matched and the fiber content in the bean fed group was at least twice that of the control diet, significant reductions in both total and LDL cholesterol occur.

Changes in HDL cholesterol and triglyceride concentrations are inconsistent. The small reductions in blood cholesterol that occur due to consuming beans are not likely to attract much interest by the medical profession. However, the study by Kabagambe *et al.* (11) suggests that eating beans provides protection from CVD beyond what can be explained by a small depression in blood cholesterol. They reported that 1 serving per day of beans was associated with a 38% lower risk of myocardial infarction. However, more than one serving per day did not elicit a further decrease in risk for myocardial infarction. It is quite likely that the wide variety of phytochemicals in beans along with lower blood glucose concentrations provide the protection observed by Kabagambe *et al*. (11).

We have shown in several experiments that feeding beans (black, navy, pinto) reduces chemically induced colon cancer in rodents. Feeding beans typically reduces colon cancer by 50 - 75% which is as efficacious as any pharmaceutical intervention. Eating beans protects against development of colon cancer by minimizing translocation of bacteria and bacterial products across the colonic mucosa into the submucosa and deeper tissues thereby minimizing inflammation and promoting terminal differentiation of cells in the colon mucosa. Epidemiologic studies support the concept that eating beans helps to reduce colon cancer incidence. In addition, inflammatory diseases of the colon increase the odds of developing colon cancer. Thus, the human and animal studies are consistent and provide mutually supportive evidence.

The most recent work that I have been involved with involves malnourished children in Africa. In Tanzania, malnourished children were fed an inexpensive bean based, mineral and vitamin fortified supplement. All children that did not have secondary illnesses such as malaria or HIV infection recovered from malnutrition within four to six weeks. This study demonstrated that local foods combined in correct proportions and with vitamins and minerals were capable of rehabilitating malnourished children and were able to prevent malnutrition. Expensive, imported foods were not required. Two studies were conducted with children and adolescents infected with HIV. The children in Botswana had been receiving anti-retroviral drugs for a minimum of a year prior to initiation of the study. A bean-sorghum supplement promoted growth and improved the immune system (increased CD4%) better than a supplement lacking beans. Fewer children in the group receiving beans failed to respond to drug treatment compared to the group receiving the supplement without beans. Tanzania has fewer resources and HIV infected individuals are not placed on anti-retroviral drugs until their immune system is severely compromised. One hundred and seven children not receiving drugs were fed a bean-based supplement for six months. Most children showed a dramatic improvement in growth and their immune system improved such that none of them required drugs based on Tanzanian criteria. These studies show the importance of eating beans when infected with the HIV.

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STRATEGIC APPROACHES TO TAPPING THE HUMAN HEALTH POTENTIAL OF COMMON BEAN (*PHASEOLUS VULGARIS L.*)

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In many parts of the world, common bean is an underutilized staple food crop with remarkable yet unappreciated potential to reduce the risk for chronic diseases such as obesity, diabetes-type II, cardiovascular disease and cancer. Methods are discussed that are being developed to facilitate the identification of QTL that account for human health beneficial properties of common bean. Included in this discussion are strategies to evaluate common bean for traits related to obesity and type-2 diabetes, weight loss, and plasma cholesterol reduction. The use of metabolic profiling and the longevity extension of *Caenorhabditis elegans* for screening a RIL population of common bean is described. The identification of a candidate mechanism for cancer inhibitory activity of common bean is also presented.

RESULTS AND DISCUSSION

Obesity and Type-2 Diabetes. The prevalence of overweight and obesity has increased at epidemic rates over the last twenty-five years (Reviewed in (1)). While the impact of excessive weight for height is significant in itself, over weight and obesity are predisposing to increased risk for type-2 diabetes, cardiovascular disease, and cancer. Studies were initiated to develop a model for testing the effects of dry bean consumption on body weight regulation and on insulin resistance and chronic inflammation, which are components of type-2 diabetes and the metabolic syndrome. For this purpose, a diet-induced obesity model that uses C57black-6 mice was adapted to crop testing. Cooked and canned dry bean was drained, freeze-dried, and milled into a homogenous powder. The powder was incorporated in to the reference, high fat diet formulation that induces obesity. Sixteen week old, obese male mice were randomized to diet groups containing 30% (w/w) dry bean cultivars from either the Andean or the Middle American Center of Domestication. Effects on body weight were compared to those of mice that were either continued on the high fat obesogenic diet or that were switched to a low fat diet that is known to induce weight lose. Bean-fed mice experienced weight loss comparable to mice consuming the low fat diet despite the fact that they consumed the high fat obesogenic diet. Blood and tissue are being evaluated to determine effects on metabolic parameters associated with obesity, type-2 diabetes, and the metabolic syndrome.

Cardiovascular Disease. Cardiovascular disease is the leading cause of death in the United States and blood lipid profiles, particularly the circulating level of cholesterol (total, LDL-cholesterol, and HDL-cholesterol), are known to have prognostic significance. Accordingly, a rodent model has been established that will permit screening of common bean cultivars for cholesterol lowering activity. To this end, a one week whole-animal feeding assay has been established in which cooked and canned common bean, which has been freeze-dried and milled, is incorporated into a purified diet. Dietary concentrations can be varied between 5 and 60% (w/w). This assay can be conducted using either the rat or the mouse. The use of the obese mouse model described in the preceding section has particular value because the obese mice have elevated circulating plasma lipids such that there is greater sensitivity to detect changes in cholesterol metabolism induced by common bean consumption. However, a disadvantage is the cost of using the obese mouse model. The assay assesses both circulating lipids and hepatic levels of a panel of enzymes that regulate lipid

metabolism. Using this model in a non-obese rat, a dose response study of common red bean was conducted and indicated the steps in metabolism that common bean is likely to impact in exerting beneficial effects on cholesterol metabolism; however, the non obese model had limited sensitivity to detect changes in circulating levels of cholesterol. Experiments are in progress to validate the use of this screening tool and to determine if common bean varies in cholesterol lowering capabilities based on a bean cultivar's center of domestication.

RIL Population. Based on the results of our work on cancer inhibition by common bean (2), a RIL population has been created as an initial step in the process of identifying traits that account for protection against cancer. In order to screen this RIL population, we have established a model for longevity extension using the nematode *C. elegans (3).* Longevity extension in this model invertebrate organism has been shown to predict health benefits in mammalian species. We have also adopted a 4-phase chemical extraction technique and the use of a high throughput LC-MS analysis platform to establish cultivar-specific metabolomic fingerprints that identify bean cultivars that extend *C. elegans* longevity. The goal is to pinpoint the genetic loci within common bean that regulate health benefits.

Mechanisms of Cancer Inhibition. Published work from our laboratory indicates that common bean inhibits cancer cell proliferation and induces apoptotic cell death (4). In order to identify the signaling pathways that are modulated by common bean consumption and that account for these beneficial effects, tumor tissue was probed for candidate mechanisms using proteomic techniques. A strong candidate pathway was implicated, i.e. the pathway of which the mammalian target of rapamycin (mTOR) is a component. This finding is considered particularly significant since the mTOR pathway is misregulated in the majority of human cancers.

Conclusion. Investigators working to identify the human health benefits of common bean are strongly encouraged to perform experiments designed to identify the traits of common bean that are responsible for human health benefits rather than to focus on a single chemical with the hope that it will be the magic bullet that accounts for disease prevention. The goal of identifying health beneficial traits is most likely to be achieved via the application of the same 21st century tools used in crop improvement for agronomic traits.

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BEAN CONSUMPTION AS A MARKER OF CULTURAL IDENTITY

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Beans have been an integral part of human diets long before the wide-spread practice of agriculture. Each geographic area or culture has characteristic legumes that are part of the local cuisine. Some examples of these are fava beans in the Middle East, soybeans in Asia, cowpeas in Africa, and pinto beans in Latin America. Continued consumption of these foods is often a strong marker of ethnic identity or cultural affiliation by group members (1,2).

With the shift to a more Westernized diet due to changes within a country, the use of legumes and other cultural food traditions often declines. Immigration to a different country such as the United States frequently alters the culturally specific diet pattern as well. Despite a decrease in daily consumption, items may still be featured as ethnic-specific dishes during holidays, or family gatherings.

Although some traditional diets are deficient in micronutrients or calories, most are nutritionally adequate and it is disturbances in the availability of food that causes classic malnutrition disorders. Unfortunately, with the shift away from traditional diets to Westernized diets chronic disease risks increase as overall diet quality goes down. Preservation of beans in diets may negate risks of cardiovascular disease, diabetes, and obesity development, and contribute to reducing hunger and malnutrition.

Despite these observations of dietary pattern changes with immigration and the Nutrition Transition, or shift to a Westernized diet, there is a global research gap in understanding the motivations behind these changes in traditional diets. Macro level consumption statistics document that bean consumption declines, but the reasons why are more complex. The changes in diet are not based solely on economics. Cultural factors either promote or discourage legume consumption. Some food patterns change due to availability, lifestyle factors such as working out of the home, and sociocultural preferences or desires to fit in with the majority culture or 'modernization' (1) One Kenyan immigrant stated, "Once they have enough money to buy other foods, people run away from eating beans."

Much of the research in this area implies that the native or traditional diet is healthier before acculturation takes place assuming that undernutrition is not an issue. Without examination, this may not be true. To preserve positive traditional foods, we need to know which foods are more likely to change for a particular population, place, and time. Although we know that peripheral foods change quicker than core staples like beans or rice, the exact pattern of dietary change is going to be situation specific and vary by level of acculturation as well as the culture.

Since the health benefits of beans are many, it is logical from a global health perspective to retain beans in the diet of people around the world. In this sense, it becomes critical to identify these situation-specific barriers and motivators to bean consumption. Qualitative applied research is essential to drive the next stages of research in diverse settings. Many agricultural programs and

public health service agencies rely on survey methodology to collect quantitative data quickly. One-on-one qualitative interviews and interactions with consumers, farmers, primary food preparers, and store owners is often a missing piece of understanding.

Some efforts to collect qualitative applied research have been done in the area of farmer acceptance of new crops, consumer acceptance of modified bean varieties, and other agricultural concerns (3). Public health surveys, anthropological inquiries, and economic sector research have different objectives than agricultural research and vs. versa. Since qualitative field research can have high time and funding costs, as well as requiring sound investment and cooperation from the country/region of investigation, it is best to maximize the depth of qualitative research conducted for the benefit of multiple health perspectives.

In addition to conducting qualitative field investigations on legume consumption in developing countries, immigrants in the United States or other country setting can serve as models for change. Immigrants can provide insights about dietary change patterns overall and specifically for assessing the promoters and barriers to retaining legumes in the diet and the role of legumes in cultural identity. For example, before conducting field research in rural Guatemala on the acceptability of iron-enhanced black bean varieties with farmers and villagers, these items can be evaluated by Guatemalan immigrants. Cowpea varieties could be tested with Rwandan or Ugandan immigrants in urban US cities. Although not exact, immigrant studies can inform researchers by a preview of consumer acceptability in the region of interest.

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BEANS REDUCE GLYCEMIC RESPONSE AS PART OF A RICE MEAL

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INTRODUCTION

Legume or dry bean consumption may be beneficial in the prevention and treatment of diabetes and diabetes-related diseases, including coronary heart disease (CHD) and metabolic syndrome[1]. Legumes contain a considerable amount of resistant starch (RS), and a higher ratio of slowly digestible (SDS) to readily digestible (RD) starch, compared to other carbohydrate foods[4]. RS and SDS are associated with reduced glycemic responses, and lower postprandial glucose levels compared to high GI carbohydrates[5] which can benefit insulin-resistant individuals and people with diabetes[2, 3]. The low glycemic response of beans alone has been documented, but little research has been conducted on the glycemic response to traditional food combinations such as black beans and rice, or chickpeas and rice. The results of a series of studies we conducted that examined the effects of bean consumption in combination with a high glycemic index food on glycemic response will be discussed.

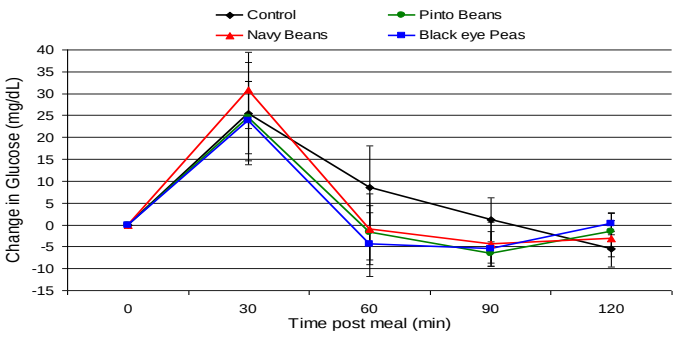
MATERIALS AND METHODS

Study #1[6]*:* This study examined the effects of pinto bean, blackeye pea and navy bean consumed in 2 amounts, low-dose (~1/2 cup) and high-dose (~1 cup), on the glycemic response to a high glycemic index (GI) treatment (calculated GI = 96) in insulin sensitive adult men and women. In a randomized, crossover, placebo-controlled design, 12 participants consumed each of the low-dose treatments and 11 participants consumed each of the high-dose treatments in conjunction with a high glycemic index meal on different mornings, at least 7 days apart. Blood samples collected at time 0 (fasting), and 30, 60, 90, 120 minutes post-treatment were analyzed for glucose and insulin.

Study #2: This study examined the effects of consumption of 50 grams of available carbohydrate from 3 test meals: plain white rice (control), black beans with rice, and chickpeas with rice in insulin sensitive adult women. Food portioning was by gram weight and the carbohydrate content of the meals was equal. The weighed meal portions were ~ ¾ cup of rice for the control, and ~ ½ cup of beans with ½ cup of rice for the black bean and chickpea treatments. Black beans and chickpeas have similar carbohydrate content per gram weight despite being different species. Using a randomized, crossover, placebo-controlled design, 9 participants consumed each of the 3 test meals on different mornings, at least 7 days apart. Blood samples collected at time 0 (fasting), and at 30, 60, 90, 120 minutes post-meal were analyzed for glucose.

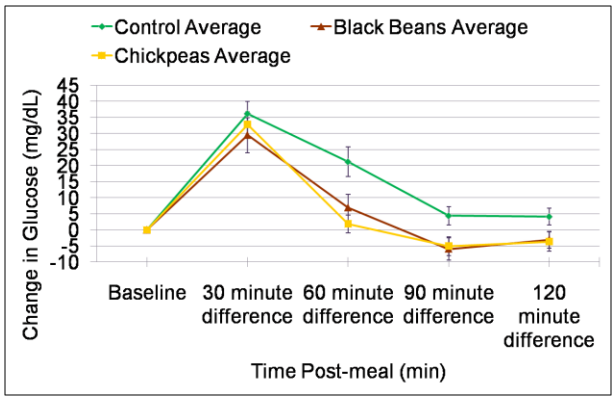
RESULTS AND DISCUSSION

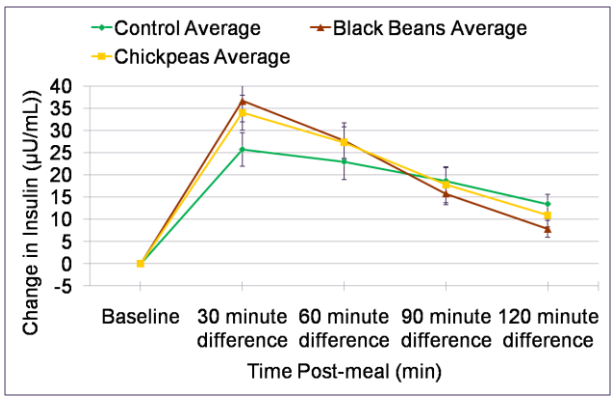
Study #1: Data were analyzed in 2 groups: low-dose (n=12) and high-dose (n=11) treatments. The low-dose treatments were also compared to the high-dose treatments (n=11). There were no significant time-by-treatment interactions for any of the legume treatments at either the low-dose or high-dose amounts. No



significant difference in insulin response, whole body insulin sensitivity or Homeostasis Model Assessment for either low-dose, high-dose, or low- vs. high-dose treatments was found.

Study #2: The glucose response based on the incremental area under the curve to the rice/bean meals showed a significant difference by treatment (ANOVA, p=0.017). Results of a paired t-test indicated that the glucose response curve was significantly different at two of the four time points for the black bean meal as well as the chickpea meal in comparison to rice alone, 60 minutes post-prandial (p=0.041), and 90 minutes post-prandial (p=0.002).





CONCLUSIONS

Study #1: When provided in the form of a spread, pinto bean, navy bean or blackeye pea intake did not reduce glycemic response to high glycemic index foods. Making the beans into a spread destroyed the cell walls which may have compromised the GI and GL of the beans.

Study #2: Black bean and chickpea intake, when consumed as a whole bean as part of a meal, can reduce the glycemic response to high glycemic index foods, such as white rice.

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CALCIUM NUTRITION AND BIOAVAILABILITY OF SNAP BEANS: STUDIES IN PLANT AND HUMAN NUTRITION

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Calcium (Ca) is an essential human nutrient that is important in bone growth and metabolism. Snap beans are a potential source of dietary Ca, as well as a source for all other essential minerals and various health-beneficial phytochemicals. The bioavailability of Ca from this food has not been previously determined, nor has there been much research into the absorption of other compounds from this food crop. There have, however, been efforts to characterize mineral composition in snap bean germplasm, and to understand the factors that contribute to mineral accretion (and specifically Ca accretion) into developing snap bean pods.

Previous research (Grusak et al., 1996b; Quintana et al., 1996; Grusak and Pomper, 1999) has shown that Ca concentration in snap beans varies by genotype, as well as by pod size. Calcium is transported to developing pods via the xylem pathway, although the presence of non-functional stomates on snap bean pods (Grusak and Pomper, 1999) indicates that non-stomatal transpirational flow predominates, and that genotypic differences in the Ca concentration of xylem fluid can have a significant impact on Ca delivery to the pods. Furthermore, because atmospheric humidity will impact non-stomatal diffusional water loss (Pomper and Grusak, 2004), the microenvironment around developing pods is also a critical factor in determining water flow and Ca delivery to pods.

The USDA Food Nutrient Database (http://www.nal.usda.gov/fnic/foodcomp/search/) indicates an average Ca concentration of 37 mg/100 g fresh weight (or 37 mg per a 1 cup serving) for raw snap beans (**NDB No:** 11052; sample size = 153). This value (equivalent to 3.8 mg Ca/g DW) falls at the low end of a range of Ca concentrations (3.5 to 6.6 mg Ca/g DW) identified in a field study with 64 genotypes (Quintana et al., 1996). This suggests that breeding for higher levels of Ca in this crop is feasible.

From the standpoint of human dietary Ca requirements, the Ca concentration in snap bean is not the only issue of importance. Other factors include the life stage of the individual (recommended dietary intakes [RDIs] vary by age, gender, and pregnancy/lactation status), as well as the percent absorption of Ca from this food (i.e., its Ca bioavailability). The RDI for Ca ranges from 210-270 mg/d for infants to 1300 mg/d for teens and pregnant or lactating women. How well snap beans can contribute to these requirements had not been previously determined. Thus, we were interested in assessing the Ca bioavailability of this food, which can be defined as the proportion of ingested Ca that is digested, absorbed, and ultimately utilized by an individual. This factor is critical to determining intake recommendations. For instance, although the bioavailability of Ca from plant foods can range from ~3% to 35%, an average value of ~30% is assumed for many foods. This explains why the DRI for teens was set at 1300 mg/day; their total daily Ca requirement has been determined to be ~400 mg/d and thus a daily intake of foods containing a total of 1300 mg Ca (at ~30% absorption) would potentially yield a net functional acquisition of 400 mg.

To assess snap bean Ca absorption directly in humans, we needed a way to track Ca coming specifically from snap beans, when these are consumed in a complete diet (i.e., with other foods also containing Ca, and Ca already circulating throughout one's body). We grew plants hydroponically and labeled them intrinsically with a low-abundance, non-radioactive stable isotope of Ca (^{42}Ca) (Grusak et al., 1996a). Several low-abundance isotopes of Ca are available, which allows one to use triple labeling to compare absorption from different foods, as well as to get a good measure of the total body circulating pool of Ca. The fact that these isotopic forms are naturally in low abundance means that they will not be present in high concentration in other foods, or body pools. In this study, thirteen teenage subjects (7 girls, 6 boys) were recruited for a two-week stay in our Metabolic Research Unit (MRU) and were fed ^{42}Ca-labeled snap beans along with ^{48}Ca-enriched milk; an intravenous dose of ^{46}Ca was also administered. Blood, urine and fecal samples were collected during the two-week study. All subjects were maintained on Ca adequate diets during the two weeks of the study and during a one-week run-in prior to entering the MRU. The bioavailability of Ca averaged 28 $\pm$ 3% from snap beans, which was comparable to that of milk Ca (27 $\pm$ 2%). No differences were seen in the percent absorptions between boys or girls. These results indicate that snap beans are a potentially good source of Ca; their comparability with milk suggests that they do not contain significant levels of any anti-nutrients that would inhibit Ca absorption. Nonetheless, the low content of Ca in a single serving (~37 mg per cup), being much lower than milk (288 mg per 8 oz. serving), demonstrates that a teenager would have to consume 5 cups of snap beans to attain the Ca intake of one 8 oz. glass of milk, or ~35 cups of snap beans daily to meet their RDI for Ca! If this level of intake could be achieved, it would certainly assist sales in the snap bean industry, but of course it is clear that these levels of intake are unrealistic. However, with snap bean now demonstrated to be a good source of bioavailable Ca, breeding efforts to increase pod Ca concentrations and further enhance the nutritional value of this food are definitely warranted.

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QUALITATIVE METABOLOMICS OF CANCER PREVENTION IN *PHASEOLUS VULGARIS L.*

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Epidemiological results indicate that when dry beans are eaten as a staple food, there is an inverse relationship between dry bean consumption and cancer risk. This has been shown to be true for several primary cancer sites including breast (Adebamowo et al. 2005; Murtaugh et al. 2008), prostate (Kolonel et al. 2000), and colon (Correa 1981). The Thompson lab has previously reported that *Phaseolus vulgaris L.* (dry bean) inhibited experimentally-induced breast cancer by as much as 70% in female Sprague Dawley rats, an effect which is COD and market class dependent (Thompson et al. 2008). In another pre-clinical study, Bobe *et al. (2008)* found that both the soluble and insoluble fractions of dry bean inhibit colon cancer growth. Differences in cancer prevention activity are most likely due to variations in the small molecule (metabolite) profile of each market class.

METHODS

Navy bean and white kidney bean market classes were selected for this study to represent the Middle American and Andean COD, respectively. These two genetically distinct market classes were also selected because of previous pre-clinical results from our laboratory indicating white kidney beans had the highest level of cancer prevention activity whereas navy beans offered the lowest level of cancer prevention activity. Plasma, mammary gland tissue, and mammary carcinomas were collected previously (Thompson et al. 2008) and analyzed here using LC-MS following small molecule extraction using a modified Bligh and Dyer technique as published by Sana *et al.* (2008) To account for differential solubility of various compounds and extract the largest number of compounds possible from the dry bean powders, extractions were carried out at pH 2 and 9. The acidic (pH 2) and basic (pH 9) samples were combined in equal volumes in LC vials for analysis.

LC-MS was used to collect metabolomics data. Chromatographic separation was carried out using a flow rate of 0.6 mL/min with a 2% to 98% linear gradient of water/methanol over 13 min followed by a solvent hold until 19 min. 0.2% acetic acid was used as a mobile phase modifier. Data was collected in both positive and negative modes for m/z range of 50-1000. Mass Profiler Professional was used to analyze and compare diet groups in this study.

RESULTS AND DISCUSSION

The relationship between the metabolite profile of dry bean and that found in plasma, mammary gland, and tumor tissue was investigated to gain insight about components likely to be exhibiting biological effects in the animal and to determine the effect of dry bean consumption on mammalian metabotypes associated with the carcinogenic process. Metabolite fingerprinting of the dry beans showed that white kidney bean (high cancer inhibitory activity) and navy bean (low cancer inhibitory activity) are clearly distinguishable using principal components analysis (PCA) demonstrating the genetic dependence of dry bean metabolite profiles. Little overlap is seen when comparing the bean secondary metabolites to the animal metabolite fingerprint due to phase 1 and 2

metabolism in the animal. However, changes in the animal small molecule profile as a result of indirect effects of dry bean consumption are observed when comparing diet treatment groups. Differences in plasma, mammary gland tissue and tumor tissue were observed to be diet dependent with the bean diet groups clearly distinguishable from each other and the control group using PCA. The work presented here begins to establish a metabolite profile of cancer prevention for dry bean by comparing two market classes. These data provide a foundation for identifying the pathways affected by metabolic reprogramming characteristic of breast cancer development and tumor progression.

ACKNOWLEDGEMENT

Thank you to members of the Thompson laboratory for carrying out the animal studies and to Agilent Technologies for the use of the LC-MS and laboratory facilities. This research was supported in part by a grant from the American Institute for Cancer Research (grant #08A032).

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EFFECT OF SOIL COMPACTION AND IRRIGATION MANAGEMENT IN DRY BEAN PRODCUTION

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INTRODUCTION

Limited data suggests that certain dry bean varieties or germplasm 'tolerate' soil water stress better than other varieties. In addition, soil compaction limits water use efficiency because it limits root performance and water infiltration. Soil compaction not only restricts root function, but also promotes root diseases, increases herbicide injury, and causes yield reduction in dry beans (Smith and Pearson 2004). Delaying the initiation of irrigation by one and two weeks reduced yield by 5 and 15%, respectively (Yonts, 2006). Water stress induced at the end of the growing season also delayed maturity and suppressed yield, but the impact was less severe because the plants had more extensive root systems later in the season (Yonts, 2006). We evaluated these factors in combination to develop the optimal combination of variety, water stress, and levels of compaction.

MATERIALS AND METHODS

Plots were established at Scottsbluff, NE 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, moderately compacted (driving a tandem axel truck weighing 21,000 lbs), 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. Four irrigation treatments were assigned to subplots, including full irrigation (100%), two limited irrigation schemes (75%, 50%), and no supplemental irrigation (0%) after flowering. Nine varieties, Marquis, Matterhorn, 99-131, Emerson, Orion, Tara, Beryl-R, 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.

RESULTS AND DISCUSSION

The experiment was planted on June 13, 2008. Total rainfall was 8.52 in. from June 13 to September 31. Total water, (irrigation + precipitation) was 19.4, 17.3, 15.2, and 11.1 inches for full irrigated (100%), limited irrigated (75%), limited irrigated (50%), and no supplemental irrigation (0%), respectively.

Water stress significantly affected yield. On average yield was reduced by 35% when no supplemental irrigation was used after flowering (Table 1).

Soil compaction affected yield, days to flowering, days to maturity, and 100-seed weight significantly. There were differences among genotypes for all parameters evaluated in this study. The interaction of soil compaction by variety affected yield and days to maturity. The interaction of irrigation by variety affected yield, days to flowering and maturity.

Yield was significantly reduced by 71% and 84% when soil was moderately and heavily compacted, respectively (Table 2). Under non-compaction, Roza (2957 kg ha^{-1}) had the highest yield followed

by Marquis (2903 kg ha^{-1}). In soils moderately and heavily compacted, UI-537(1224 and 594 kg ha^{-1}, respectively) had the highest yield.

UI-537 (1552 kg ha^{-1}) had the highest yield and significantly yielded more than Matterhorn (1135 kg ha^{-1}), Orion (1172 kg ha^{-1}), Tara (1223 kg ha^{-1}), Beryl-R (1270 kg ha^{-1}), Emerson (1307 kg ha^{-1}), Gemini (1352 kg ha^{-1}) and Marquis (1404 kg ha^{-1}). Matterhorn had the lowest yield and significantly yielded less than Roza (1270 kg ha^{-1}), Marquis, Gemini, and Emerson.

Days to flowering were delayed by 2 and 3 days when soils were moderately and heavily compacted, respectively (Table 2). Days to maturity were delayed by 11 and 9 days in soils moderately and heavily compacted, respectively (Table 2). Gemini flowered and matured earlier (48 and 103 d) and Emerson had the largest seed size (33.7 g 100-seed weight). On average, seed size was reduced by 14.2 % when soils were heavily and moderately compacted (Table 2).

Table 1. Effect of irrigation scheduling on yield (kg ha^{-1}) at Scottsbluff during 2008.

Irrigation scheduling	Yield
	----------kg ha^{-1}----------
100%	1438a†
75%	1390a
50%	1503a
0%	932 b

† Within columns, data followed by the same letter are not significantly different in the Duncan test at p=0.05

Table 2. Effect of soil compaction on yield (kg ha^{-1}), days to flowering and maturity, and 100-seed weight (g) at Scottsbluff during 2008.

Soil Compaction	Yield	Days to Flowering	Days to Maturity	100-seed weight
	--kg ha^{-1}--	--------days--------	-------days--------	----------g--------
Non-compacted	2722a†	50 b	99 b	32.3a
Moderately	795 b	52a	110a	28.7 b
Heavily	428 c	53a	109a	26.7 b

† Within columns, data followed by the same letter are not significantly different in the Duncan test at p=0.05

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The authors appreciate the financial support of the Anna Elliot Fund and the Nebraska Dry Bean Commission. Technical support of John Thomas, Dave Reichert, and Dick Gatch is highly appreciated.

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FARMER CHOICES AND ENVIRONMENTAL ADAPTATION OF *PHASEOLUS* BEAN SPECIES IN OAXACA, MEXICO

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INTRODUCTION

In this era of increasing concern over human population growth, climate change, and increased resource scarcity it is becoming ever more important to conserve crop genetic resources. For *in situ* conservation, it is crucial to understand the ways that farmers perceive and manage diversity. The smallholder farmers of highland Oaxaca regularly interplant a great diversity of bean landraces of three different species: common bean (*P. vulgaris*), runner bean (*P.*), and year bean [*P. dumosus* (= *P. polyanthus*)]. In this study we tested the hypothesis that the presence of these three bean species is a risk-mitigating strategy on the part of the farmers by conducting interviews and making germplasm collections from farmers' fields in the village of Santa Maria Jaltianguis in the Sierra Juárez of Oaxaca.

MATERIALS AND METHODS

Collections of *Phaseolus* landraces were made in ten fields in Santa Maria Jaltianguis during December, 2008. A total of seven farmers participated in the study with either one or two distinctly managed fields represented in the collections. All participating farmers were interviewed about their *Phaseolus* production and consumption habits and asked to provide local names for their seeds at the time of collection. The amount of genetic diversity present in the 287 samples collected from the farmers' fields was then assessed with molecular marker analysis. The diversity present was evaluated at ten nuclear microsatellite loci well distributed over the entire *Phaseolus* genome (Kwak et al., 2009). The population structure of these accessions was then analyzed using the STRUCTURE program and principal coordinate analysis performed in GenAlEx 6 (Peakall and Smouse, 2006; Pritchard et al., 2000). The optimal number of distinct population subgroups within the collection was determined based on the ΔK statistical test (Evanno et al., 2005).

RESULTS AND DISCUSSION

Three genetically distinct populations were identified within the germplasm collections from Santa Maria Jaltianguis (fig. 1). Based on pod and seed characteristics (Singh et al. 1991), the first two populations were identified as *P. vulgaris* eco-geographic races Mesoamerica and Jalisco and the third population was comprised of samples of *P. coccineus* and *P. dumosus*. The principal coordinate analysis corroborates the population assignments inferred by STRUCTURE (fig. 2). The first axis, which explains 39% of the variation, separates the *P. vulgaris* samples from *P. coccineus* and *P. dumosus*. The second axis, which explains 24% of the variation, differentiates between *P. vulgaris* accessions of eco-geographic races Mesoamerica and Jalisco.

Each of the fields represented in the collections is comprised primarily of a single *Phaseolus* species or eco-geographic race. Race Mesoamerica beans, which are grown in monoculture and are intended for sale in the market, are managed in isolation from the Race Jalisco beans, which are grown in

polyculture with maize and squash for home consumption. The three farmers with multiple fields represented in the collections (Farmers 1, 3, and 6) maximize available diversity with two different strategies. Farmers 1 and 3 maintain two fields comprised of different eco-geographic races, whereas Farmer 6 maintains two differentiated fields of the same eco-geographic race. These results indicate that farmers are able to perceive real genetic distinctions between their seeds and adapt their management strategies accordingly. The field of Farmer 7 was fallowed at the time of collection but showed volunteers of *P. coccineus* (a perennial species) and *P. dumosus* (a semi-perennial species or long-lived annual species).

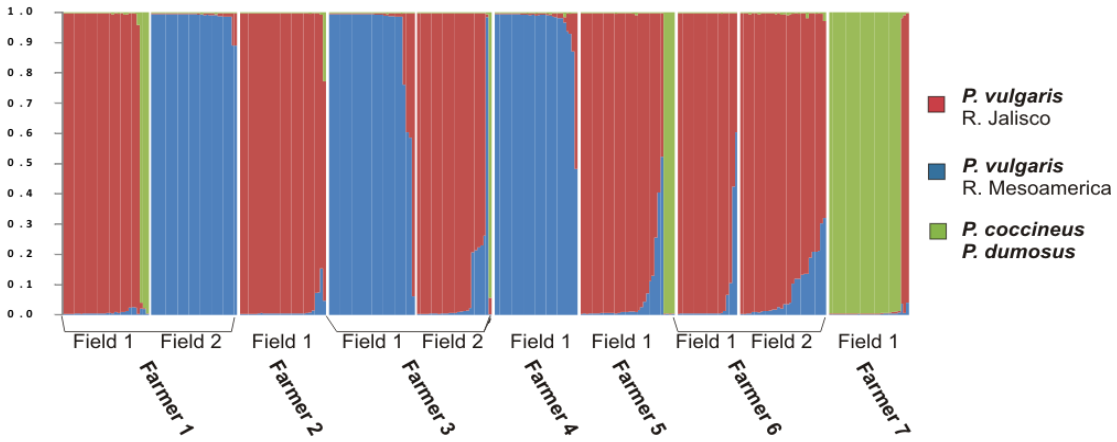

Figure 1. STRUCTURE analysis (Pritchard et al., 2000) of the Oaxacan *Phaseolus* collections. The optimal clustering number (K=3) was calculated using the *ad hoc* statistic ΔK (Evanno et al., 2005).

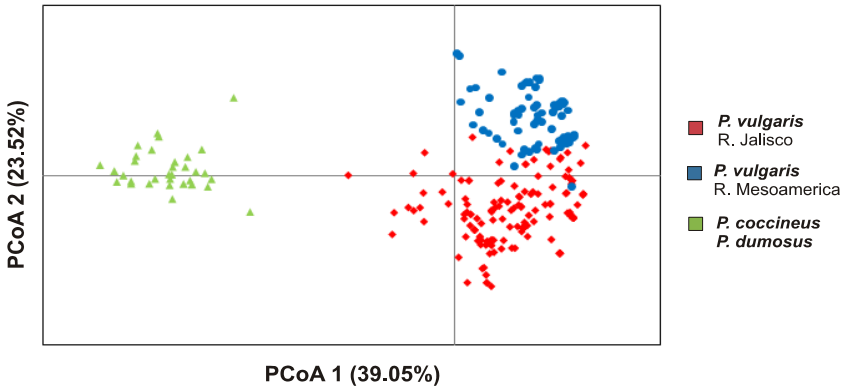

Figure 2. Principal coordinate analysis of microsatellite diversity among the 287 accessions.

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DRY BEAN YIELD LOSS OF COMMERCIAL CULTIVARS UNDER CONDITIONS OF DIRECT HARVESTING

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INTRODUCTION

North Dakota is the leading producer of dry bean in United States (USDA-ERS, 2009). It is necessary to find ways to obtain optimal yields at the lowest input costs possible. Direct harvest reduces equipment investment, harvest time and operational costs, but it is often associated with reduced seed yield and quality (Costa and Pasqualetto, 1999; Aidar et al., 1990; Gregoire, 2007). The objective of this study was to evaluate seed yield and seed loss, of the most commonly grown cultivars in North Dakota and Minnesota under conditions of conventional and direct harvest.

MATERIALS AND METHODS

This study was conducted at four environments (Carrington and Prosper, ND, in 2008, and Carrington and Hatton, ND, in 2009). Nine dry bean cultivars were used for this study. It included the three most important market classes grown in the region (pinto, navy, and black). Within each market class, three cultivars were evaluated. The experimental design was a RCBD in a split-plot arrangement with three replicates. The whole plots were the two harvest methods (conventional and direct harvest). The subplots were the three market classes with three cultivars nested within each market class. Only comparisons of cultivars within the same market class were considered to be meaningful. The cultivars were planted in a four-row 76 cm apart and 10.6 m long plot, at the recommended seeding rates (222,500 plants ha^{-1} for black and navy, and 173,000 plants ha^{-1} for pinto). The two center rows were harvested discarding 2.3 m from each end of the row, so the effective harvested area was 9 m^2 (6 m x 1.52 m). The same combine was used for the conventional method, where plants in the plots were first cut with a rod Pickett® cutter/weeder, windrowed, and about two hours later the entire plot material was fed into the combine. Harvest loss was estimated by counting the seeds on the ground from two samples in each plot. Analysis of variance was performed within environments and then across the four environments (combined).

RESULTS AND DISCUSSION

Harvest method and cultivar were consistently the most important factors in determining seed yield and yield loss in this study, which proves that to optimize field results, farmers have to take in consideration not only the harvesting equipment to be used, but also choose the cultivar most suited to that particular piece of equipment. Significant differences in seed yield and yield loss among harvest methods occurred across all environments. Under conventional harvesting, there was no difference in yield loss of cultivars within market class for the three market classes tested (Table 1). However, under direct harvest, the cultivars Lariat (pinto), T-39 and Eclipse (black), and Vista (navy) showed the lowest yield loss when compared to the other cultivars within the same market class. There was no statistical difference among the navy cultivars for seed yield and yield potential (Table 2). Furthermore, the cultivars Lariat, T-39, and Eclipse showed the best yield potential and lowest yield loss among all the cultivars tested.

There was variability in the yield loss of the harvest methods across environments. Under conventional harvest, the mean yield loss of the best-five plots was 20 kg ha^{-1} (0.9%), and the five worst was 384 kg ha^{-1} (14.9%). Under direct harvest, the loss of the best-five plots averaged 119 kg ha^{-1} (9.6%), and the five worst 818 kg ha^{-1} (45.0%) (data not shown).

This research emphasizes the importance of equipment set up and operator care on the yield loss under direct harvest. This research also emphasized the important role of genotype and environmental conditions at harvest time in determining the seed yield under direct harvest. Growers should take in consideration the cultivar to be planted, the environmental conditions, and the equipment set up and operator care when deciding on which harvest method to use.

For the conditions in which this study was conducted, Lariat had the greatest seed yield and the lowest yield loss under direct harvest (highest seed yield and lowest yield loss).

Table 1. Yield loss of cultivars within market class under two harvest methods averaged across four North Dakota environments, in 2008 and 2009.

Market Class	Cultivar	Harvest method	
		Conventional	Direct
		Yield loss	
		---------- % ----------	
Pinto	Lariat	5.9 a	16.3 b
	Stampede	4.1 a	28.3 a
	Maverick	7.7 a	29.5 a
Black	Eclipse	2.9 a	20.8 b
	T-39	3.0 a	20.8 b
	Jaguar	3.4 a	25.6 a
Navy	Avalanche	4.8 a	32.4 a
	Vista	3.7 a	19.7 c
	Mayflower	4.6 a	26.7 b

Table 2. Mean yield, yield loss, and yield potential of nine dry bean cultivars averaged across two harvest methods and four North Dakota environments, in 2008 and 2009.

Market Class	Cultivar	Seed yield	Yield loss	Yield potential
		kg ha^{-1}	%	kg ha^{-1}
Pinto	Lariat	2,232 a	11.1 b	2,499 a
	Stampede	1,939 b	16.2 a	2,269 b
	Maverick	1,713 c	18.6 a	2,070 b
Black	Eclipse	1,880 a	11.8 a	2,091 a
	T-39	1,815 a	11.9 a	2,028 a
	Jaguar	1,527 b	14.5 a	1,738 b
Navy	Avalanche	1,783 a	18.6 b	2,120 a
	Vista	1,798 a	11.7 a	1,985 a
	Mayflower	1,737 a	15.6 b	2,001 a

*Only letters within the same column of means of cultivars within the same market class should be compared. If letter behind number is similar, the numbers are not significantly different at $p<0.05$.

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PHENOTYPIC CHARACTERIZATION OF CONDENSED TANNIN ACCUMULATION IN FIVE DRY BEAN GENOTYPES

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INTRODUCTION

Condensed tannins (CT; syn. proanthocyanidins) are important groups of polyphenolic compounds found in the seed coats of many dry beans. CT can be harmful and/or beneficial to human health and the environment depending on the concentration. Manipulating the production, accumulation and form of CT in the seed coat of dry beans may be beneficial to bean producers, consumers and breeders. The main objective of this experiment was to evaluate the difference in the pattern of CT accumulation in the seed coats of five different genotypes of beans.

MATERIALS AND METHODS

Two separate experiments were conducted. Genotypes from each of the experiments were evaluated for their accumulation of seed coat CT during seed development. In one experiment, three genotypes, DOR364, RIL89 and RIL58, which had moderate, high and low CT, respectively, at maturity were compared. In the second experiment a similar comparison was made between two pinto bean genotypes: CDC Pintium and 1533-15. Preliminary trials suggested that CDC Pintium has relatively higher CT concentrations compared to 1533-15. Plants of each of the five genotypes were grown under a 12-hour photoperiod and developing seeds were harvested every other day starting from 6 days after flowering (DAF) up until 40 DAF. The experiments were organized in a randomized complete block design and replicated three times.

The seed coats from each of the 20-time points/genotype/replication were separated from their cotyledons, weighed, freeze dried, weighed again, ground and three technical replications from each of the 20-time points/genotype/ replication were assessed for CT concentration. A modified BuOH-HCl assay (Lees et al., 1993) was used for the CT assessment. A control (ground, freeze dried mature DOR364 seed coat tissue) was also assessed using the same BuOH-HCl assay described above. The DOR364 control tissue was used to generate a standard curve. The absorbance values of each sample were then used to calculate DOR364 equivalents using the linear regression equation of the standard curve. A qualitative assessment of CT accumulation was also conducted for all five genotypes.

RESULTS AND DISCUSSION

The results demonstrated that CT begins to accumulate very early on in the seed coat of dry beans (Figure 1). It was also determined that CT accumulates quite rapidly from 6 – 14 DAF for all five genotypes. Following this rapid increase in CT one of two observations were recorded: (1) A slower increase or stabilization of CT in the seed coat was apparent from 14-40 DAF for those genotypes that were found to have relatively high and moderate levels of CT at maturity, namely RIL89, DOR364 and CDC Pintium. (2) By contrast, genotypes that were found to have low concentrations of CT at maturity, RIL58 and 1533-15, showed declines in CT concentration from 14-40 DAF. Finally, genotypes that had relatively high CT concentrations at maturity tended to have

high concentrations of CT throughout seed coat development when compared to moderate and low CT lines.

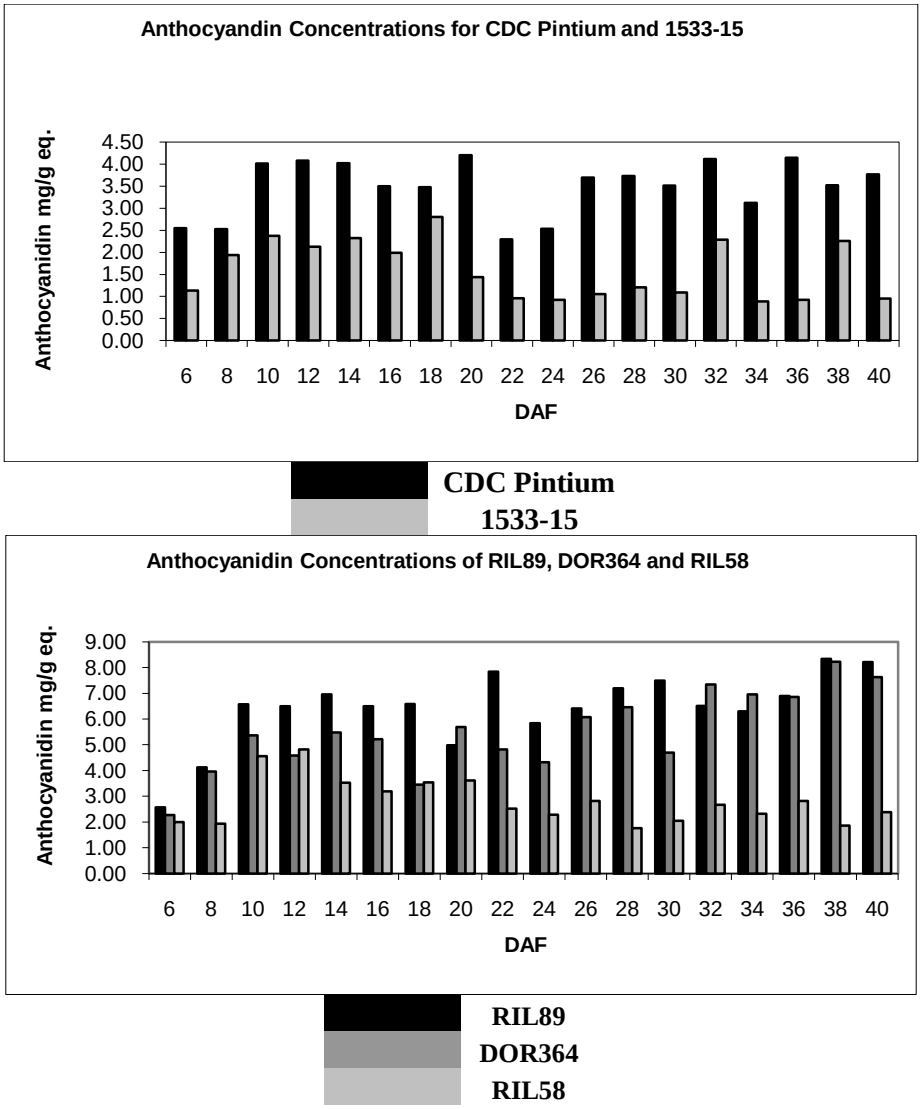

Figure 1. Average condensed tannin accumulation throughout seedcoat development in DOR364, RIL89, RIL58, CDC Pintium and 1533-15. All data averaged over three field (biological) replications and nine laboratory (technical) replications

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PHENYLPROPANOID PATHWAY GENE EXPRESSION PATTERNS ASSOCIATED WITH NON-DARKENING IN CRANBERRY BEANS

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INTRODUCTION

Several coloured bean classes, including cranberry beans, darken during storage. Because consumers associate darkened beans with poor quality they are priced lower. Recently a few pinto bean cultivars, that are "slow-darkening", have been developed (Junk-Knievel *et al.* 2008). Although the biochemical mechanism behind the post-darkening phenomena is not well understood, Beninger *et al.* (2005) correlated decreases in proanthocyanidin and flavonoid levels with the slow-darkening in pinto bean 1533-15, relative to conventional darkening pinto germplasm. The current study was initiated to identify cranberry bean germplasm that is slow to darken and to identify the patterns of phenylpropanoid pathway gene activity that lead to the slow/non-darkening phenotype.

MATERIALS AND METHODS

Seeds from approximately 700 cranberry-type lines, from the USDA National Germplasm system (Washington, USA) and the University of Guelph bean breeding collection were assessed for their tendency to darken after treatment with UV (Junk-Knievel *et al.* 2007). Crosses were made between a pale nondarkening cranberry-like bean (Wit-rood boontje) and the cranberry varieties Hooter, Etna or Capri. The F_1s and F_2s were allowed to self and the F_3 seed was tested with the UV-protocol to identify darkening and nondarkening F_3 seeds. The plants from the seeds were selfed to establish several non-darkening and nondarkening cranberry-like siblines. Proanthocyanidins were extracted from ground seed coats in 70:30 acetone: water and analysed for proanthocyanidin levels using a colourimetric protocol (Sun et al. 1998). The expression of genes leading to the synthesis of proanthocyanidins was determined in cDNAs produced from RNA samples from immature seeds harvested from siliques 1-3 mm in length. Approximately 60 to 70 seeds were pooled for each sample from three separate plants of each plant type (Wit-rood, Etna, non-darkening F_4 progeny from Wit-rood by Etna, darkening F_4 progeny from Wit-rood x Etna, 1533-15 and CDC Pintium). Real-time PCR analysis was performed on a BioRad iCycler with the Multicolor Real-Time PCR Detection System.

RESULTS

A pale cranberry-like bean (Wit-rood boontje) was identified that darkened significantly less than conventional cranberry beans. From crosses made between Wit-rood and the cranberry varieties Hooter, Etna or Capri and Wit-rood, F_2 seeds with red stripes on a white background were selected and plants established from them were selfed. A screen of the resulting F_3 seeds identified several non-darkening cranberry-like lines as well as lines that darkened. Significantly lower levels of proanthocyanidins were measured in Wit-rood and nondarkening F_3 seed obtained from Etna x Wit-rood than in Etna, or darkening F_3 seeds (Fig1).

The non-darkening Wit-rood had very reduced expression levels of flavone -3-hydroxylase (F3H), dihydroflavonol-4-reductase (DFR), anthocyanidin synthase (ANS), flavanol synthase (FLS), leucoanthocyanidin reductase (LAR), anthocyanidin reductase (ANR) and vacuolar transporter (VT). No differences were found for flavanol synthase (FLS), anthocyanin 5-0-glucosyltransferase (UF5GT), homeodomain protein (HD) or anthocyanin 5-acyltransferase (Fig 2). In most cases the

severely reduced levels of enzyme expression that were noted for Wit-rood also occurred in the non-darkening progeny of both the Hooter by Wit-rood cross and the Etna by Wit-rood cross, but not to the darkening progeny of either cross. The results suggest that the nondarkening trait in Wit-rood and its nondarkening progeny is related to reduced proanthocyanidin synthesis and indicate that the nondarkening trait can be incorporated into cranberry bean varieties. It should be noted that the observed differences in gene expression found between the darkening and non-darkening cranberry-type bean samples were not the same as the observed differences found between the darkening and slow-darkening pinto bean samples. This suggests separate explanations for the reduced darkening trait found in the 1533-15 pinto vs. the Wit-rood cranberry.

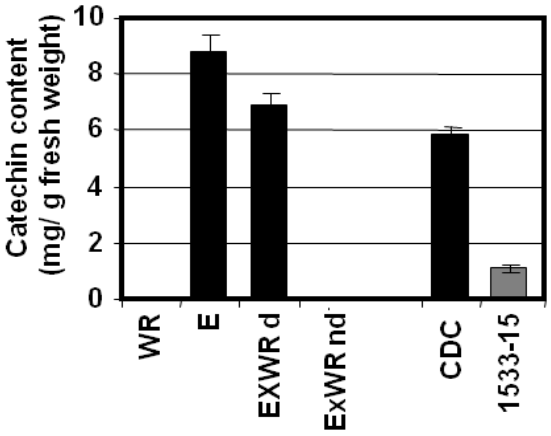

Fig 1. Catechin content as determined by the Vanillin assay. WR, non-darkening parent Wit-rood; E x WR d, darkening progeny of Etna x Wit-rood cross; E x WR nd, non-darkening progeny of Etna x Wit-rood cross; CDC, CDC Pintium a darkening pinto; 1533-15, a slow-darkening pinto.

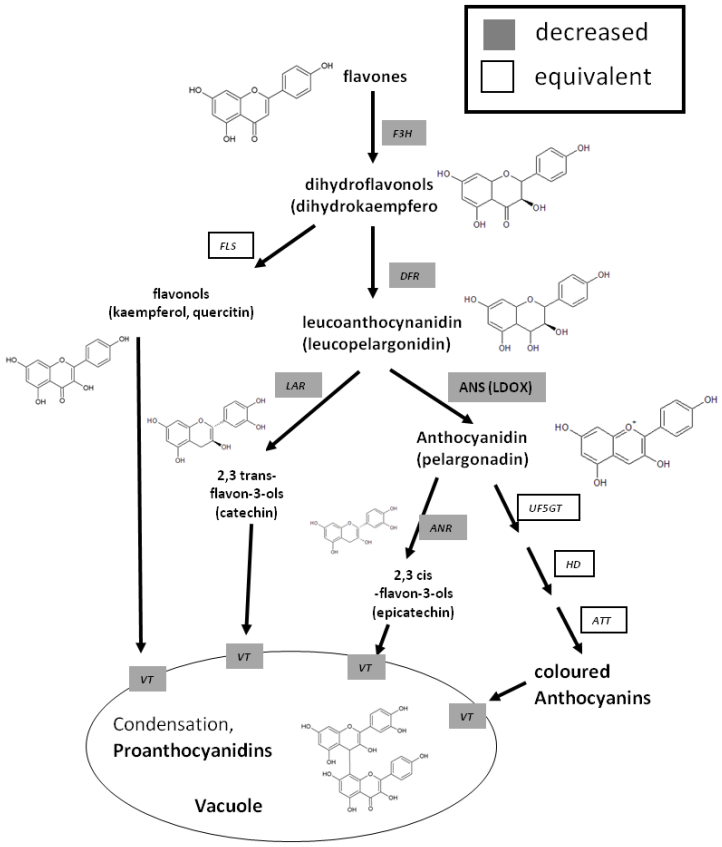

Fig 2. Gene expression patterns (as analysed by real time PCR) in Witrood and non-darkening progeny of Witrood by conventional cranberry bean variety (Hooter, Capri or Etna) crosses, as compared to expression in darkening cranberry bean genotypes. AAT (anthocyanin 5-acyl-transferase), ANR (anthocyanidin reductase), DFR (dihydroflavanol reductase), F3H (flavonoid 3'-hydroxylase), FLS (flavanol synthase), HD (homeodomain protein), LAR (leucoanthocyanidin reductase), UF5GT (anthocyanin 5-0-glucosyltransferase), VT (vacuolar transporter).

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LEGUME IPMPIPE — A NEW OPTION FOR GENERATING, SUMMARIZING AND DISSEMINATING REAL-TIME PEST DATA TO STAKEHOLDERS

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Legume ipmPIPE (Pest Information Platform for Extension and Education) enhances the role of extension specialists in IPM by providing near real-time access to observations, model output, pest management information, and diagnostic images at http://legume.ipmpipe.org. Communication tools also allow specialists to customize information for dissemination to crop consultants and growers. The diversity of pathogens, pests and hosts are uniquely suited to demonstrate the value of the ipmPIPE as a "one-stop shop" for legumes. Educators and stakeholders can easily obtain information on pathogens and pests identified in a specific area or general region. Progress to date was presented to and input solicited from the BIC meeting participants; which included 78 responses to the following 10-question survey which will help guide future efforts of the Legume ipmPIPE team. Additional information on the Legume ipmPIPE is summarized in the BIC Poster Proceedings by M. Langham et al.

Summary of Responses presented as {percentage} of 78 respondents; some questions allowed multiple responses:

1. What is your interest in legumes?
   A. Grower, processor, broker or marketer {10.3 %}
   B. Research, extension, education, student {85.9 %}
   C. Agribusiness, marketing {11.5 %}
   D. Crop consultant, insurance adjustor, other {1.3 %}

2. Select your legume production region(s) of interest:
   A. CA, CO, ID, MT, NM, OR, WA, and/or WY {32.0 %}
   B. Other state(s) in U.S. {38.5 %}
   C. Canada {25.6 %}
   D. Other - Mexico, Europe, Africa {25.6 %}

3. Select your legume crop(s) of interest:
   A. Common beans (dry, snap, and/or fresh market) {98.7 %}
   B. Cool-season legumes (lentil, chickpea, field pea) {16.7 %}
   C. Warm-season legumes (lima bean, cowpea) {9.0 %}
   D. Other (adzuki, mung) {2.6 %}

4. What is your experience with the Legume ipmPIPE
   A. I was not aware of it until today {46.2 %}
   B. Aware of it, but have not used it yet {38.5 %}
   C. Used it 1-3 times {5.1 %}

D. Used it more than 3 times {9.0 %}

5. What is the value of the Legume ipmPIPE to you or the industry?
   A. Too early to tell {37.2 %}
   B. No value {0.0 %}
   C. Limited value {15.4 %}
   D. High value {41.0 %}

6. What is the future need for the Legume ipmPIPE?
   A. Useful but not essential {11.5 %}
   B. Non-essential and should be discontinued {0.0 %}
   C. Valuable and should be continued if possible {60.3 %}
   D. Very essential and must be continued {19.2 %}

7. Legume ipmPIPE should focus on the following (non-soybean) legume groups:
   A. Common, cool-, and warm-season {65.4 %}
   B. A in addition to others such as adzuki, mung {7.7 %}
   C. Common and cool-season only {11.5 %}
   D. Common and warm-season only {3.8 %}

8. Legume ipmPIPE should focus on the following disease/pest groups:
   A. Rusts, bacteria, other fungi (white mold), viruses, foliar insects{62.8 %}
   B. A + soil-borne problems, abiotics {24.4 %}
   C. Fewer disease and pest groups {2.6 %}
   D. Fewer examples within each disease and pest group {3.8 %}

9. Legume ipmPIPE should focus on the following outreach efforts:
   A. Public Web Site with current resources, add listserv {55.1 %}
   B. Add more pest management commentary and files {23.1 %}
   C. Add production and marketing commentary and files {11.5 %}
   D. Add other formats, e.g., print, newsletters, field days, educational meetings {28.2 %}

10. Legume ipmPIPE should focus on the following outreach resources:
    A. Additional Diagnostic Profile Cards {25.6 %}
    B. Add weekly summary of weather, forecasts {24.4 %}
    C. More frequent commentary {15.4 %}
    D. Add disease forecast models and IPM {53.8 %}

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YIELD POTENTIAL FROM INTERSPECIFIC CROSSES OF COMMON AND RUNNER BEAN

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INTRODUCTION

Aluminum (Al) toxicity is estimated to affect 40% of common bean (*Phaseolus vulgaris* L.) production in the tropics. Al inhibits root elongation and can exacerbate the effects of drought by limiting access to soil moisture. Our objective in the work reported here was to combine resistance to drought with resistance to aluminum, to obtain common bean genotypes with multiple abiotic stress resistance.

Runner bean (*Phaseolus coccineus)* evolved in moist highland environments on volcanic soils with the potential of low pH and high aluminum saturation. This suggests that runner bean could have been exposed to Al toxicity during its evolution and could be a source of Al resistance to improve common bean. However, runner bean is very aggressive with great biomass and low harvest index, implying problems to recover yield capacity in crosses with common bean.

MATERIALS AND METHODS

A core collection consisting 153 accessions of *P. coccineus* and *P. polyanthus* was evaluated in unreplicated rows for vegetative vigor in a field in Santander de Quilichao, Colombia, with severe Al toxicity (> 70% Al saturation). The most vigorous accessions were subsequently tested in Al toxic media in the greenhouse in both hydroponic culture and in soil tubes. An accession that was superior in the three tests, G35346-3Q, was crossed to drought resistant common bean SER 16 that expresses excellent remobilization of photosynthates to grain under drought –an important drought resistance mechanism (Beebe et al., 2008). The F_1 was backcrossed to SER 16, and recombinant inbred lines (RIL) were developed. Simultaneously, pedigree selection was practiced on the segregating populations for 4 generations in the Al toxic field site, and in one cycle under drought. In 2008 a lattice trial of 100 selected lines and checks was tested under intermittent drought. In 2009, 33 elite RIL and selected lines plus 3 checks were yield tested in conditions of Al toxicity, terminal drought, and irrigation in trials planted in lattice design with from 3 to 5 replications.

RESULTS

Among the 36 lines in yield trials, many lines expressed greater biomass than SER 16, indicating introgression from runner bean. Data from the trial under Al stress appear in Fig. 1. SER 16 was among the lowest in biomass, but was intermediate in yield due to excellent remobilization. Some lines combined both good biomass accumulation under Al toxicity with good remobilization, resulting in better yield.

Under intermittent drought in 2008, some lines yielded more than SER 16, and as much as 1 MT more than drought resistant check BAT 477 (Tab.1). However, under terminal drought and in the irrigated treatment in 2009, no line significantly out-yielded SER 16. Nonetheless, many lines equaled SER 16 in yield under drought and irrigation, as well as presenting good yield in the Al treatment. Thus, some lines presented multiple abiotic stress resistance.

DISCUSSION

Biomass accumulation combined with remobilization of photosynthate to grain (or harvest index) is the basic formula for yield improvement. In this sense the cross of SER 16 and G35346 should have the potential for improving yield potential. Greater yield compared to SER 16 was

observed in some lines under Al toxicity, and under intermittent drought in 2008. However, in a combined analysis of terminal drought and irrigated conditions in 2009, no line yielded significantly more than SER 16. It appears that in these environments remobilization in the derived lines was inadequate to take advantage of the biomass derived from runner bean. In that season the lines may have expressed some sensitivity to high temperatures that was inherited from runner bean. In any case, augmenting biomass accumulation may induce more vegetative development in the crop, with a concomitant reduction in sink strength and remobilization. Maintaining good remobilization while increasing biomass is a particular challenge.

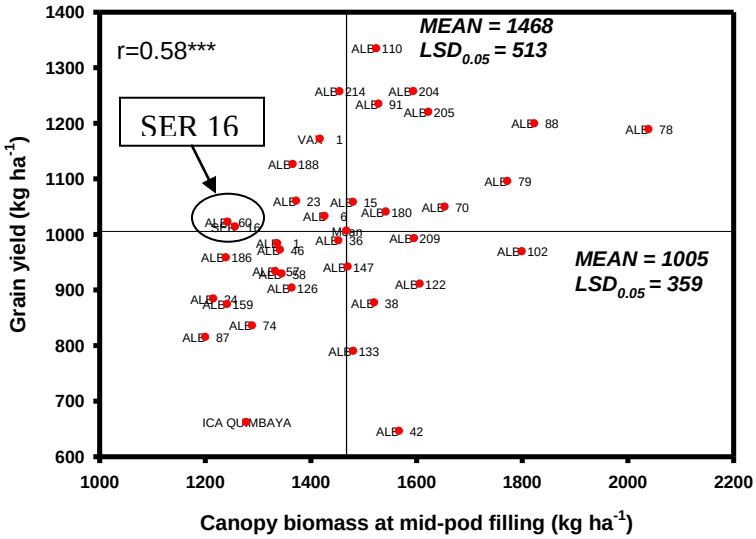

Figure 1: Yield (kg ha^{-1}) and biomass accumulation of 36 bean lines under aluminum toxic field condition

Table 1: Yield (kg ha^{-1}), days to maturity and yield per day of selected interspecific lines under intermittent drought. CIAT, Palmira, Colombia, 2008.

	Kg ha^{-1}	Maturity	Yield d^{-1}
Interspecific lines			
ALB 205	3199	68	47
ALB 167	3174	69	46
ALB 213	3029	67	45
Drought checks			
SER 16	2520	63	40
BAT 477	2165	68	32
LSD (0.05)	568	2.4	8.1

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CLONING THE MAJOR CBB RESISTANCE QTL OF COMMON BEAN THROUGH MAP-BASED CLONING AND GENE PROFILING APPROACHES - CURRENT STATUS AND FUTURE PROSPECTS

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Common bacterial blight (CBB), incited by *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Dye (*Xap*), is one of the most destructive seed-borne diseases of common bean throughout the world. Sources of genetic resistance to CBB have been identified in common bean and its related species, tepary bean (*P. acutifolius*) and runner bean (*P. coccineus*), but they are all inherited as quantitative trait loci (QTL), vary in their levels of genetic effects and their expression is influenced by environmental conditions. Two major CBB resistance QTL, BC420 and SU91, derived from tepary bean have been mapped to Chromosome 6 and 8 respectively. The two QTL together can provide durably higher level of resistance to CBB. In order to develop QTL-specific molecular markers for marker-assisted selection (MAS) and to understand the molecular mechanisms for CBB resistance, attempts have been made to clone the BC420 and SU91 QTL through map-based cloning and transcript profiling approaches to identify candidate genes that underlie the CBB resistance found in the bean line, HR45.

MATERIALS AND METHODS

Map-based cloning: The BC420 and SU91 QTL were mapped by Liu et al. (2008) and Pedraza et al. (1997). Both QTL were present in HR45 that derived its resistance from tepary bean. The Leaf nuclei procedure as described by Zhang et al.1995 was used to isolate high molecular weight DNA of HR45 for construction of a bacterial artificial chromosome (BAC) library using the pIndigoBAC-5 vector (Epicentre Biotechnologies, Madison, USA). The BAC library was screened with tightly linked molecular markers to identify positive clones in the QTL genomic regions. Positive BAC clones were analyzed by DNA fingerprinting to develop a physical map and a minimum tiling path (MTP). BAC clones in the MTP were selected for sequencing with 454 DNA sequencer at Genome Quebec. Candidate genes were identified and annotated after sequence analysis.

Transcript profiling: cDNA-amplified fragment length polymorphism (AFLP) analysis of the mRNA, which was isolated from HR 45 leaves at different time point post inoculation with the pathogen, were conducted according to the manual from Invitrogen, USA to identify differentially expressed transcripts (DET). DETs were cloned, sequenced, BLAST searched, *in silico* mapped and submitted to NCBI database. Expression of DETs were analysed by qRT-PCR. Cluster analysis of DETs was conducted in the TIGR Microarray Data Analysis System (www.tm4.org).

RESULTS AND DISCUSSION

Currently, one BAC clone containing the BC420 marker was sequenced, whereas another BAC harboring the SU91 marker has been selected from the BAC library. The sequenced BAC was assembled into a 64kb single contig for functional annotation. Since the BAC was selected by marker BC420, the entire sequence of BC420 was fully recovered from this BAC sequence. Sixteen

novel genes were *ab initio* predicted by FGENESH using Medicago gene model, including 6 from sense chain and 10 from anti-sense chain. Although no homology to any previously identified common bean genes was found, six of the putative genes were supported by common bean ESTs and three of them were supported by runner bean ESTs. The expression of 6 putative genes with supported bean ESTs was assessed and verified by RT-PCR. For each putative gene, one or two primer pairs were designed and tested in the contrasting NILs (Near Isogenic Lines) (Vandemark et al. 2008). Fifty-seven percent (8 of 14) of the primer pairs were polymorphic. Seven of them are dominant markers present in the NILs harboring the BC420-QTL, but one is a co-dominant marker. Based on the simple repetitive elements found in the BAC sequences, seven SSR markers were designed and tested in the contrasting NILs. Three of them turned out to be polymorphic, including two dominant and one co-dominant markers. Overall, eleven new markers have been identified in association with CBB resistance in HR45.

In parallel, cDNA-amplified fragment length polymorphism (AFLP) technique was used to identify the genes that are differentially expressed in the leaves of HR45 sampled at different time-periods after inoculation. Selective amplifications with 34 primer combinations allowed the visualization of 2,448 transcript-derived fragments (TDFs) in infected leaves; 10.6% of them were differentially expressed. Seventy-seven differentially expressed TDFs (DE-TDFs) were cloned and sequenced. 50.6% (39 of 77) of the DE-TDFs representing modulated bean transcripts were not previously reported in any EST database then. The expression patterns of 10 representative DE-TDFs were further confirmed by real-time RT-PCR. BLAST analysis suggested that 40% (31 of 77) of the DE-TDFs were homologous to the genes related to metabolism, photosynthesis, and cellular transport, whereas 28% (22 of 77) of the DE-TDFs showed homology to the genes involved in defence response, response to stimulus, enzyme regulation, and transcription regulation. Thus, the 22 pathogenesis-related DE-TDFs were selected as functional candidate genes (FCGs) in association with CBB resistance. Meanwhile, six of the FCGs were *in silico* mapped to the distal region of the chromosome 6 (the genomic region of the previously identified CBB resistance QTL in HR45) and were chosen as positional candidate genes (PCGs) for comparative mapping. Comparing the CGs found from map-based cloning to the CGs derived from cDNA-AFLP, none of them is overlapped. This indicates that gene expression studies may characterize the downstream transcriptional cascade of the QTL. The PCGs could be the genes for CBB resistance, whereas the FCGs genes that map to other locations may be involved in the molecular responses related to the QTL. Future works of this project will include: 1) sequence analysis of the SU91 BAC clone; 2) genetic mapping of the CGs identified from cDNA-AFLP approach; and 3) functional analysis of the CGs through Virus induced gene silencing (VIGS).

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DEVELOPMENT OF PHASEOLUSGENES, A GENOME DATABASE FOR MARKER DISCOVERY AND CANDIDATE GENE IDENTIFICATION IN COMMON

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Recent years have seen tremendous, technology-driven advances in DNA sequencing. This trend is continuing and further advances can be expected that will lead to unprecedented amounts of information on the organization of genomes and the DNA basis of traits. For example, whereas only a few years ago, the development of a single whole-genome sequence was seen as an achievement (e.g., human: Lander et al. 2001; Arabidopsis: Kaul et al. 2000), the availability of multiple whole-genome sequences in a species will soon become the norm (e.g., rice: *japonica*: International Rice Sequencing Project 2005; *indica*: Yu et al. 2005; McNally et al. 2006). Furthermore, DNA by its very nature as the biochemical vehicle of heredity, is also the common language of biology across ranges of seemingly disparate organisms. Hence, comparisons across these organisms become possible and can lead to a better understanding of the function and evolution of genomes and organisms.

In legumes, rapid progress has been made in developing genomic tools in three species selected as key species for legume genomics: soybean (for its economic importance) and *Medicago truncatula* and *Lotus japonicus* (for certain characteristics that make them convenient experimental models) (Gepts et al. 2005). Common bean genetics can benefit from genomics advances in two main ways: 1) through the development of its own genomic resources (e.g., physical map, Expressed Sequence Tags (ESTs), Bacterial Artificial Chromosome (BAC) libraries; reviewed in McClean et al. 2008; Gepts et al. 2008); and 2) through the utilization of genomic information available in other species, both within and outside the legume family.

The integration of these different types of genomic information can be achieved in a database (e.g., TAIR: The Arabidopsis Information Resource: http://www.arabidopsis.org/; Solanaceae: Sol Genomics Network or SolGenes: http://solgenomics.net/; Poaceae: Gramene: http://www.gramene.org/). The purpose of the current work, funded by the Kirkhouse Trust (U.K.) and the Bean Coordinated Agricultural Project (BeanCAP; USDA-NIFA), is to develop a genome database (PhaseolusGenes: http://phaseolusgenes.bioinformatics.ucdavis.edu/) focused specifically on common bean. The motivation of such a database came originally from the African Bean Consortium project funded by the Kirkhouse Trust (ABC-KT), whose overall goal is to introduce a marker-assisted selection capability among East African bean breeding program. The ABC-KT project focuses solely on five bean diseases prevalent in East Africa: BCMV/BCMNV, anthracnose, angular leafspot, common bacterial blight, and Pythium root rot. The project uses existing markers developed by bean breeders in North America, South America, and Europe (Miklas et al. 2006) but also seeks to develop alternative markers in case the current ones are not functional (e.g., lack of polymorphism). Hence, this project has also pursued a sequencing of the BAT93 genome (PvBAT93-MF) with an approximate 1x coverage) targeted at hypo-methylated genome regions, which most likely contain expressed genes. BeanCAP funding greatly expands the scope of the PhaseolusGenes database to other targets of marker selection and to other phenotypic traits (including the BeanCAP focus on nutritional traits and multistate nurseries in the U.S.).

PhaseolusGenes is being developed because: a) the current genetic and marker data are dispersed across journals, reports, and other databases like GenBank; b) there is an influx of bean genomic data, such as ~ 89,000 BAC-end sequences corresponding to a nascent physical map (Schlueter et al. 2008), ~ 84,000 ESTs (Ramírez et al. 2005; Melotto et al. 2005; Thibivilliers et al. 2009; K. Bett, pers. comm.),~ 2,400 nucleotide entries in NCBI, ; c) the whole-genome sequences of other legume species and associated data such as mutant phenotypes associated with specific genes (soybean: Schmutz et al. 2010; Medicago: http://www.medicago.org/genome/downloads/Mt3/; and Arabidopsis) facilitate the identification of candidate genes and, through synteny, closely linked sequences that can become new markers.

The core of the database consists of three co-equal parts: a) a marker database; b) a GBrowse representation; and c) a CMap representation. The three parts are hyperlinked to facilitate data gathering and comparison. The marker database includes markers or sequences that have or can be mapped, including SCARs for disease and insect resistance, sequence-tagged sites (converted RFLP markers, Leg markers for cross-legume comparisons, and g markers based on conserved expressed sequences) and SSRs markers (PV, BM, BMc, BMd, PVBR, SSR-IAC, and FJ) for a total of ~1400 markers. Additional markers will be added to this table as they become available. Each of these table entries will be hyperlinked to the GBrowse and CMap representations. The GBrowse representation (representing genes at a scale up to several Mbp) is currently anchored onto the soybean whole-genome sequence (obtained from http://www.phytozome.net/soybean; Schmutz et al. 2010). Additional tracks in the current representation include assemblies of the *P.* vulgaris and *P. coccineus* ESTs of JCVI, the g markers developed by P. McClean, and the PvBAT93-MF sequences BLASTed against the soybean WGS. The CMap representation (representing the genome at a scale from cMorgans to entire chromosomes) is built currently on the g markers developed by P. McClean. Development of a full CMap representation will require collating all the segregation data in the BAT93 x Jalo EEP558 populations. However, already at this stage, the database has reached one of its goals, i.e. provide additional sequences that can serve as raw material for additional PCR-based, linked markers.

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UTILIZING SYNTENY BETWEEN *PHASEOLUS VULGARIS* AND *GLYCINE MAX* AS A MEANS OF REFINING GENOME FUNCTION

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INTRODUCTION

Synteny can be described as the preserved co-localization of genes on chromosomes between related species. It has been shown that synteny drops off as evolutionary relatedness drops off (Mudge et al. 2005). Large blocks of macrosynteny and microsynteny have been shown to exist among the legumes (Choi et al. 2004; Hougaard et al. 2008). Since Glycine and Phaseolus are both members of the Phaseoleae, it is likely that high degrees of synteny exist between species within and between these genera. Utilizing existing synteny between *Phaseolus vulgaris* and *Glycine max* can facilitate answering such questions as how the legume genome has evolved and what functional and structural components comprise the genome. Synteny can also help develop novel markers and expedite the search for candidate genes underlying useful agronomic traits.

Previous work in *Phaseolus vulgaris* has identified a candidate gene, *PvTFL1y*, for determinate growth habit (Kwak et al. 2008). It was found that *PvTFL1y* mutations cosegregate with the phenotypic determinate growth habit locus *fin* on linkage group 1. The most prevalent mutation (70%) found was a 4.1kb retrotransposon insertion in the 4^{th} exon of the *PvTFL1y* open reading frame. We aim to confirm *PvTFL1y* as a locus contributing to the regulation of determinate growth habit using quantitative PCR (qPCR) and Agrobacterium-mediated transformation. We also intend to employ synteny around the *PvTFL1y* locus to find candidate genes for determinacy in *Glycine max*.

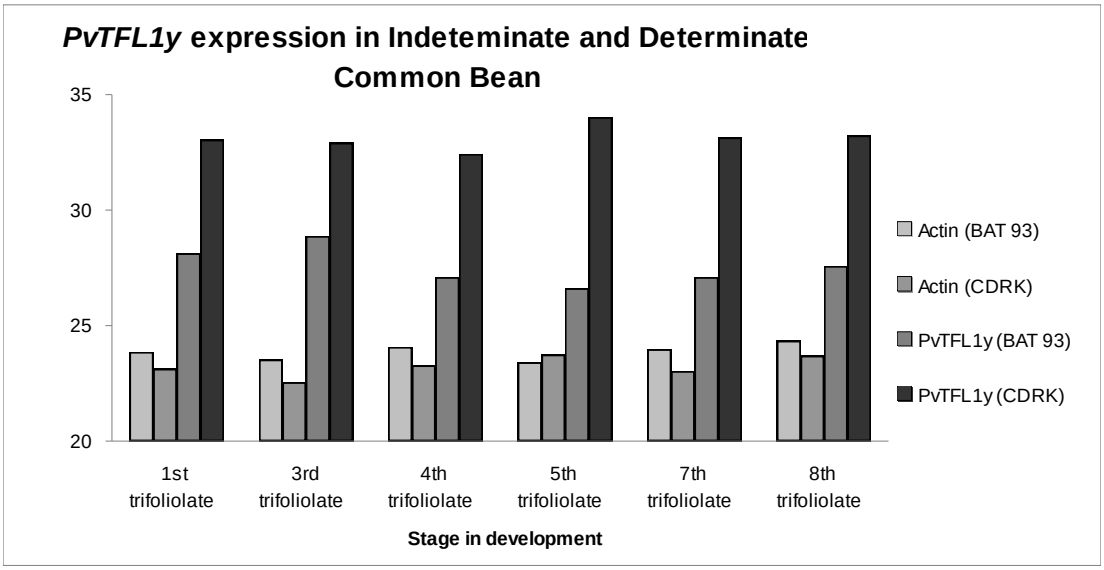
MATERIALS AND METHODS

qPCR -Plant tissue was collected from the shoot apical meristem of the indeterminate variety BAT93 and the determinate variety CDRK (retrotransposon haplotype) at the onset of the 1^{st}, 3^{rd}, 4^{th}, 5^{th},7^{th} , and 8^{th} trifoliolate nodes. Next, RNA was isolated using a Total RNA Isolation kit (Cartagen Seattle, WA). The RNA was then made into cDNA to be used for qPCR using SuperScript First-Strand Synthesis Supermix (Invitrogen Carlsbad, CA). Ct values were set using a threshold of 0.2. Fold change was estimated using the standardized ΔΔCt value (adjusted for standard deviations). Actin was used as a control during qPCR, since it has continuous expression throughout development and has been used in previous qPCR experiments (Reid et al. 2006). All reactions were run in triplicate.

Synteny studies – The *PvTFL1y* sequence from BAT93 (GenBank: EF643249.1) was used in a BLAST search of the masked soybean whole genome sequence at Phytozome.net. Regions with an E value less than $1.0e^{-50}$ were then used to find possible candidates. *Glycine max*- anchored markers from Cmap (comparative-legumes.org) were than used to map the physical positions of the candidate genes to the genetic map. Any candidate gene with an anchored marker found to be closely linked to a *Glycine max* determinacy locus was selected as a strong candidate and will be used in transformation studies.

RESULTS AND DISCUSSION

We have found *PvTFL1y* transcripts from determinate type CDRK to have a 13 to 98-fold decrease in abundance as compared to indeterminate BAT93 plants (Figure 1). Expression in BAT93 was found to peak at the onset of flowering. Contrary to this finding, the housekeeping gene actin was found in equal abundance in BAT93 and CDRK at all developmental time points. These findings suggest that the determinate variety CDRK has a decrease in *PvTFL1y* mRNA transcript abundance and is likely to have lower, if any, protein levels.



P. vulgaris candidate *PvTFL1y* was found to have two homologs in *G. max* on chromosome 19 (*GmTFL1yA*) and 03 (*GmTFL1yB*). We expected to see two *G. max* homologs for every one *P. vulgaris* candidate gene due to the whole genome duplication in *G. max* that has taken place after the evolutionary split of these two groups (Schlueter et al. 2004; Shoemaker et al. 2006). By mapping the *GmTFL1yA* homolog on the genetic linkage map, we found that it was tightly linked to the phenotypic locus *dt1*. Currently, wild-type *PvTFL1y* and *GmTFL1yA* are being transformed into *tfl1-1* Arabidopsis mutants to verify function. If the genes are able to restore indeterminate growth habit in the transformed offspring it is likely these genes play a role in the regulation of determinate growth habit.

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EVOLUTION OF THE EUROPEAN BEAN FROM THEIR AMERICAN ANCESTORS

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INTRODUCTION

Microsatellite markers have been enough proved to be ideal markers to distinguish the fine-scale relationships within bean gene pools and between lines or populations in many species (Kwan and Gepts 2009, Diaz and Blair et al. 2006, 2007). They may help to reveal the history of bean introduction in the Iberian Peninsula and its widespread to other countries of the Mediterranean area. This research could help to give support, with experimental data derived from microsatellite polymorphisms to the existence of a new European genetic pool in common bean. Increasing the knowledge about the variability of the Mediterranean bean genotypes is essential in order to select the most suitable for breeding, both for hybridization and selection of lines, from populations. As well as this, it is quite important to gain a better understanding about what part of the genome of bean varieties from the New World are still present in nowadays Mediterranean bean varieties. With this aim, a set of microsatellite markers was analysed in a large and representative set of common bean populations of both American and European origins. Investigation of genetic similarity between populations from both continents made it possible to identify various types of American common bean introduced into Europe at different times or in different places and which have given rise to distinctive intermediate or recombinant types (Santalla et al. 2002).

MATERIAL AND METHODS

The set of analysed populations consisted of a total of 532 of white seeded *Phaseolus vulgaris* accessions collected from different countries of the Mediterranean area, genotypes from America, and the gene pool check controls: ICA Pijao, Calima and California Dark Red Kidney. We have genotyped 62 polymorphic microsatellite markers by standard procedures. The analysis of population structure was accomplished by using STRUCTURE software. The genetic diversity analysis of both the studied loci and groups defined by STRUCTURE software was performed with PowerMarker V3.2. Genetic relationships among entire accessions were analysed by Principal Component Analysis (PCA) using the DARwin 5.0.158 program.

RESULS AND DISCUSSION

After applying STRUCTURE software to the genotypes of the total set of accessions, Mesoamerican and Andean accessions were defined based on their membership coefficient (MC). Population structure of Mesoamerican and Andean set of accessions was analysed independently running structure at K from 1 to 12 and the optimal values of K were estimated by following the procedure of Evanno and collaborators (2005). K=3 was estimated as an optimal clustering number for each one of Mesoamerican and Andean set of accession. Clusters performed by STRUCTURE in both sets were named as follows: Andean -Nueva Granada/Turkey (which also included the cDRK control), Nueva Granada (NG) accessions (not Turkey NG), Peru accessions from the whole Mediterranean area-; Mesoamerican –Pure Mesoamerican from South-Western Europe, Pure Mesoamerican from

Eastern Europe and Middle Orient countries and a final group of Intermediate Mesoamerican accessions. PCA of microsatellite diversity showed the genetic relationships among bean Mesoamerican and Andean populations (figure 1) and displayed similar results to those found with STRUCTURE software. As diversity concerns, the lowest values were found for the Nueva Granada/Turkish group (0.37) and the highest differentiation was shown between Eastern and Western Mesoamerican accessions and Turkish ones(0.28). The highest flow (Nm) was found between Mesoamerican accessions from both East and West geographic Mediterranean areas (7.03). The study also highlights the large percentage of accessions that are carrying alleles from both original American pools and the broad genetic diversity shown by European common bean populations.

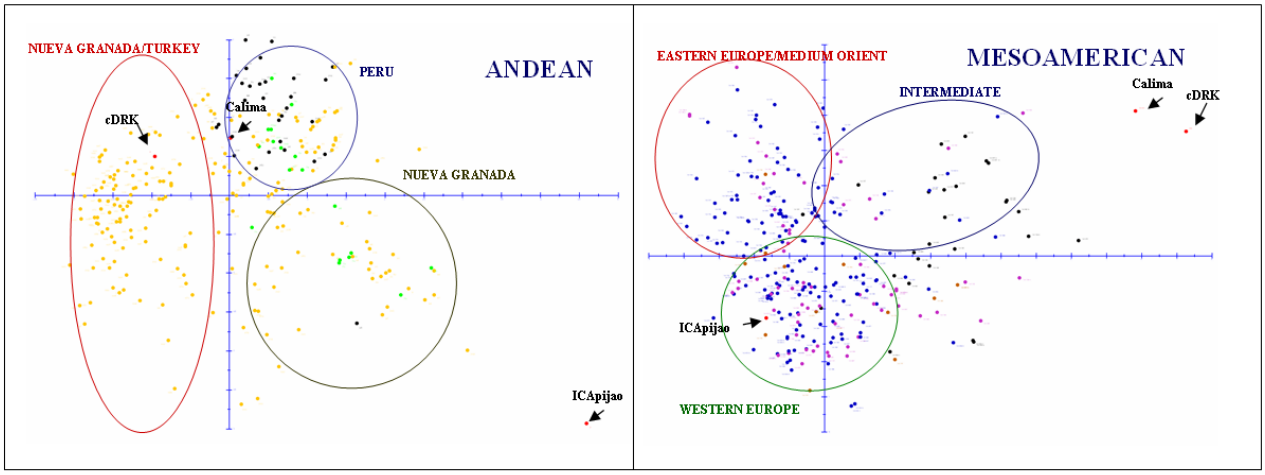

Figure 1: Genetic relationships among Andean and Mesoamerican accessions of white seeded common bean from the Mediterranean area.

ACKNOWLEDGEMENTS

Research was supported by projects AGL2008-02091/AGR, INCITE07PXI403088ES and RF2006-00001 from the Spanish Government and the Xunta de Galicia, respectively. The authors are grateful to the Germplasm Banks of: The Agronomy Faculty of Buenos Aires ("N. I. Vavilov" Plant Genetic Resources Laboratory), the International Center of Tropical Agriculture (Cali, Colombia), the Western Regional Plant Introduction Station (Pullman, Washington, USA) and IBPGR from Bulgary. M. De La Fuente is grateful to the Xunta de Galicia for awarding her a fellowship grant, which allowed her to carry out this study, and to BIC for the Foreign Award to assist to the 2009.

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DEVELOPMENT AND SCREENING OF BIBAC LIBRARIES FROM TWO SOURCES OF CBB RESISTANCE IN *P. VULGARIS*

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INTRODUCTION

Common bacterial blight (CBB) is endemic to all regions of the world where dry beans (*Phaseolus vulgaris*) are cultivated, and represent a significant barrier to crop production. The disease is caused by the bacterium, *Xanthomonas axonopodis* pv. Phaseoli, and results in reduced seed yield and the contamination of future seed (Broughton *et al.*, 2003).

To aid in the identification of lines possessing CBB-resistance genes, a number of molecular markers have been identified for various lines, with OAC-Rex being the first commercial variety with significant CBB resistance (Tar'an *et al*. 2001). Although these markers are useful tools for breeding CBB-resistant lines, the actual genes involved in resistance are not yet known. The objectives of this work were to: develop a BAC library for OAC-Rex, characterize the region surrounding the major CBB-resistance QTL, and develop a screening technique using the model plant *Arabidopsis thaliana*.

MATERIALS AND METHODS

DNA Isolation and Library Construction

High molecular weight (HMW) DNA from the fully expanded leaves of four week-old OAC-Rex was extracted and encapsulated according to an established protocol (Zhang *et al.*, 1995), and the fragments between 100-400kb were ligated into the BiBAC2 vector according to the protocol of Hamilton *et al*. (1996).

Library Screening

The OAC-Rex library was spotted onto nylon membranes in a 5-by-5 matrix using a Biomek 2000 automated workstation (Beckman) with a 96-pin high-density replication tool. The membranes were prepared according to the protocol of Olsen *et al*., (1993). Hybridization with the DIG-labeled pvCTT001-derived probe was performed according to the manufacturers' instructions (Roche). Clones that were identified by probe hybridization were characterized using a gel-based restriction fingerprinting method (Chang *et al.* 2001). End sequencing of the identified clones was conducted using standard T7 and SP6 primers flanking the insertion site. Selected clones were sequenced by 454-sequencing and assembled using CLC Genomics Workbench software.

Determining X. axonopodis Compatibility with A. thaliana

Cultures *X. axonopodis* pv. Phaseoli were grown overnight at 28°C to an OD_{600} of 0.6. Inocula with optical densities of 0.06 and 0.006 were created by diluting the overnight culture with 10mM $MgCl_2$. Mature rosette leaves of *A. thaliana* (Columbia ecotype) were infiltrated with approximately 0.2ml of solution and covered to maintain the humidity >80%. Infected leaf samples were collected at 0, 48, 96, 144 and 192 hours post infection. Bacterial counts were obtained by grinding the leaves in 10mM $MgCl_2$ and plating the samples on XCP media (McGuire *et al*., 1986).

RESULTS AND DISCUSSION

Library Construction and Analysis

The OAC-Rex library consisted of 31,776 clones and had an average insert size of 150 Kb, providing a library depth of 5.6. Initial screens of the OAC-Rex library with the pv-ctt001 marker-derived probe identified 8 positive clones. These results were confirmed by PCR using primers for the pv-ctt001 marker (data not shown).

After digestion with *Hind*III and *Bam*HI restriction endonucleases the samples were separated by electrophoresis and the bands from each clone were analyzed using FPC software (Sanger) and aligned into a contig (Figure 1). The size of the contig was expanded by sequentially probing the library with probes designed from end sequences of the identified clones (Figure 1) to a final size of approximately 700Kb.

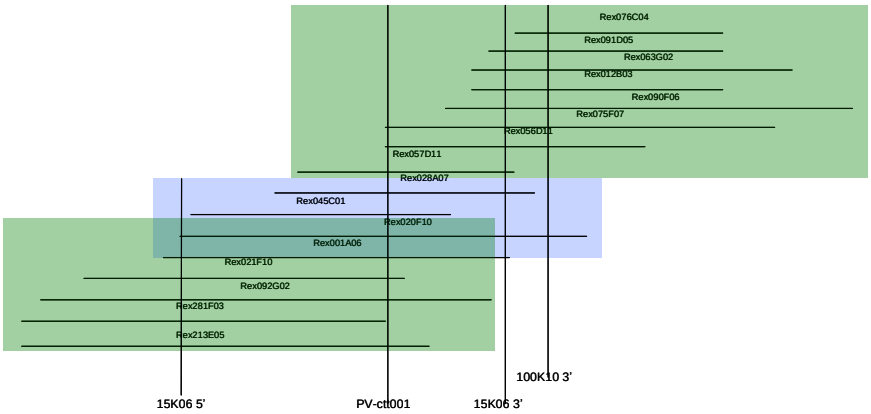

Figure 1: Contig construction of BICAC clones from the OAC-Rex library centered around a marker (PV-ct001) for CBB resistance. The library was probed with the PV-ctt001 SSR marker and the end sequences were used to construct the 15K065', 15K063' and 100K103' probes that were used to identify additional clones for the contig. The locations of the probes in the contig are indicated by vertical lines.

High-throughput sequencing, using the Roche 454 platform, identified 1,309 individual contigs. Sequence comparison using BLAST analysis identified several regions homologous with *Glycine max* chromosome 19 between 50Kb and 4Gb, with several NBS-LRR and protein kinase genes identified in both species (Schmutz et al., 2010). Additional sequence analysis has indicated that the overall order of the contigs is conserved between P. vulgaris and *G. max* however, there does appear to be some rearrangement as fragments from the terminal end of the contig appear on the opposite end of *G. max* chromosome 19.

Growth of X. axonopodis in A. thaliana

After infection, the bacterial counts for the leaves inoculated with 0.06 and 0.006 OD cultures rapidly climbed between 0 and 144 h.p.i, (Figure 2) with the 0.06OD and 0.006OD treatments reaching maximum densities of 2.79×10^{8}CFU•g^{-1} at 96 h.p.i and 1.86×10^{8} CFU•g^{-1} after 144 h.p.i, respectively. The infected leaves showed marked chlorosis and necrosis around the site of inoculation, as well as the margins of the leaves. After 192 h.p.i. secondary infections were seen on the surrounding leaves, and the bacteria could be isolated from the surrounding leaves as well as stems.

These results showed that *X. a.* pv. *phaseoli* is capable of infecting *A. thaliana*. By transforming *A. thaliana* with BiBAC2 clones carrying bean DNA we will be able to screen for bean genes for CBB-resistance.

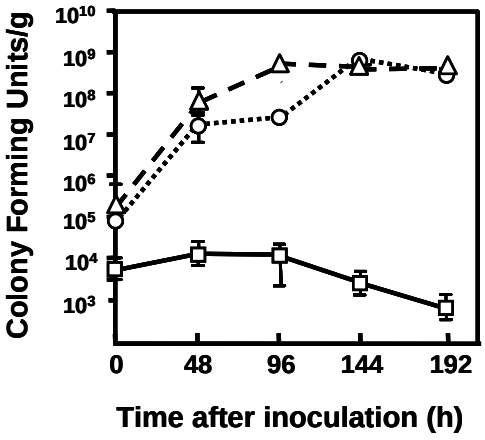

Figure 2: Bacterial multiplication in *Arabidopsis* leaves infiltrated with 0.2 ml of 0.06(∧), 0.006 (○) overnight *X. a.* pv. *phaseoli* culture, or mock inoculated with 10mM $MgCl_2$(□).

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MARKER-ASSISTED PYRAMIDING OF RESISTANCE TO COMMON BACTERIAL BLIGHT AND ANTHRACNOSE IN NAVY BEAN

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ABSTRACT

Navy bean cv. OAC Rex is moderately resistant to common bacterial blight (CBB) and the resistance is partially associated with the QTL linked to the molecular marker SU91. Morden003 is susceptible to CBB, but has resistance to anthracnose races 73 and 105 as indicated by the presence of the associated molecular marker SAS13. To pyramid resistance to CBB and anthracnose, crosses were made between Morden003 and OAC Rex, and the progenies were backcrossed four times to Morden003. Artificial inoculation screening identified progeny lines with improved resistance to CBB and resistance to anthracnose race 73, or 105. Genotyping with associated molecular markers confirmed lines with resistance to CBB or anthracnose or to both diseases.

INTRODUCTION

Common bacterial blight (CBB; *Xanthomonas axonopodis pv. phaseoli*) and anthracnose (*Colletotrichum lindemuthianum*) are two of the most important foliar diseases in dry bean production in Manitoba. Infection of CBB or anthracnose causes considerable yield losses and seed quality reduction. Use of resistant cultivars is considered as the most efficient approach to control these diseases for dry bean commercial production. OAC Rex is a navy bean cultivar with moderate resistance to CBB (resistance derived from PI 440795) (1). Morden003 is a navy bean with resistance to anthracnose races 73 and 105 and adaptation to Manitoba (2). Backcross was made to transfer CBB resistance from OAC Rex in to Morden003. The BC_4F_5 progeny lines were evaluated.

MATERIALS AND METHODS

For CBB evaluation, the BC_4F_5 progeny lines were grown in a field CBB nursery at Morden. Each line was planted in a single 5-m row with 60 cm row spacing. Plants were inoculated prior to flowering and evaluated in a month for CBB severity (1-5) and disease index (percent leaf tissue infected). Anthracnose screening was conducted in controlled growth chambers. Each line was screened in three replications with four seeds per replication. Seedlings were inoculated 10 days after emergence with anthracnose races 73 or 105 and rated in 10 days for disease severity. The experiment was repeated once. The BC_4F_5 lines were also genotyped with molecular markers associated with CBB (SU91) (3) and anthracnose (SAS13) (4), following standard PCR protocols.

RESULTS AND DISCUSSION

Among the 112 BC_4F_5 lines evaluated, 17 showed moderate or high resistance (similar to resistant check OAC Rex), 48 lines were intermediate between resistant and susceptible checks, and 47 lines were as susceptible as Morden003. For anthracnose resistance, 40 lines were pure lines with resistance to both races 73 and 105, 55 segregated for resistance and 19 lines were susceptible.

Separated by CBB severity, 48 lines possessed moderate resistance to CBB along with resistance to anthracnose races 73 and 105. In genotyping with molecular markers associated with disease resistance to CBB and anthracnose, five lines were identified to possess both molecular markers of SU91 and SAS13. Two lines had only SU91 marker, and 82 lines had only SAS13 marker, and 23 lines were not associated with any molecular markers. Five of the lines were identified to have resistance to both CBB and anthracnose, and also possess the associated molecular markers. The plants selected with improved resistance to anthracnose and CBB are being further evaluated in replicated field trials for disease resistance and agronomic performance.

ACKNOWLEDGEMENTS

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

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INTRODUCTION

Anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib. fungus, is the major disease of common bean, which upon favorable conditions is able to result in high reduction not only in yield, but also in grain quality. In Brazil, more than 54 physiological races of *C. lindemuthianum* were identified in several productive common bean regions, considering that 64, 65, 73, 81, 87 and 89 races are the most frequent. The highest variability of *C. lindemuthianum* was detected in Paraná State, with 40 identified races, followed by Goiás with 17, Santa Catarina with 16, and Rio Grande do Sul with 14. Several strategies have been adopted for anthracnose control. However, the use of resistant cultivars is considered the most efficient control method due to the low cost and reduced environmental damage as well. Until now, ten resistant genes of Mesoamerican origin and three of Andean origin were identified. Among them, Andean genes (*Co-12 and Co-13)* are those with greatest importance since they are present in common bean landraces in Paraná. Previous studies, carried out at Nupagri-UEM, had shown that Pitanga genotype, a landrace collected in small farms in Paraná State, is resistant to 23, 64, 65, 73 and 2047 races of *C. lindemuthianum.* The present work had as objective to characterize the genetic resistance of Pitanga cultivar to *C. lindemuthianum*.

MATERIALS AND METHODS

Seeds from Pitanga, AB 136, SEL 1308, G 2333, Michelite, Michigan Dark Red Kidney (MDRK), Cornell 49-242, BAT 93, Mexico 222, TU, Ouro Negro, Jalo Vermelho, Jalo Listras Pretas (JLP) and PI 207262 cultivars were sown in plastic vases containing substrate and kept at greenhouse, in order to obtain posterior F_1 and F_2 seeds. The inheritance test was conducted in F_2 population from the cross Pitanga x AB 136, inoculated with 2047 race. Allelism test was also carried out with F_2 populations from the crosses (R x R) Pitanga x SEL 1308 and Pitanga x G 2333, both inoculated with 2047 race. On the other hand, F_2 populations from the crosses Pitanga and the Michelite, MDRK, Cornell 49-242, Jalo Vermelho and PI 207262 cultivars were inoculated with race 64. Meanwhile, the F_2 populations from the crosses between Pitanga and Mexico 222, BAT 93 and Ouro Negro cultivars were inoculated with race 23. Race 65 was used in F_2 populations from the crosses between Pitanga x Tu and Pitanga x JLP cultivars. Inoculum was prepared according to the methodology proposed by Cárdenas et al. (1964). After the emergence of the first trifoliate leaf, plants were inoculated with a spore suspension prepared with 23, 64, 65 and 2047 races of *C. lindemuthianum,* adjusted to a concentration of 1.2×10^6 mL^{-1}. Visual evaluation was done ten days after inoculation, using a scale from 1 to 9 (Pastor-Corrales, 1991). Plants scoring from 1 to 3 were considered resistant, whereas the others (4 to 9) were susceptible. Genetic analyses of F_2 population were done by using Chi-Square test (χ^2).

RESULTS AND DISCUSSION

The observed segregation ratio of 3R:1S, in F_2 population from the cross Pitanga (R) x AB 136 (S) cultivars inoculated with race 2047, indicates the action of a dominant resistant gene present in Pitanga cultivar. Allelism tests demonstrated that the gene present in Pitanga cultivar is independent from *Co-1* (MDRK); *Co-2* (Cornell 49-242), *Co-3* (Mexico 222), *Co-4*^{3}+*Co-3*^{3} (PI 207262), *Co-3*^{3} (BAT 93), *Co-4*^{2} (SEL 1308; G 2333), *Co-5* (TU), *Co-6* (AB 136), *Co-10* (Ouro Negro), *Co-11* (Michelite), *Co-12* (Jalo Vermelho), *Co-13* (JLP) genes, and from the gene in Corinthiano (Table 1). Considering that G 2333 cultivar possesses only *Co-4*^{2} allele (Silvério et al., 2002) for resistance to race 2047, it demonstrates that the resistant gene present in Pitanga cultivar is independent from *Co-4*^{2} (SEL 1308 and G 2333) genes and it may be used in order to obtain cultivars with ample resistance spectrum to *C. lindemuthianum*. Therefore, we propose that the anthracnose resistance gene in Pitanga conditioning resistance to races 23, 64, 65 and 2047 be designated as *Co-14*.

Table 1. Allelism tests in F_2 populations from R x R crosses inoculated with races 23, 64, 65 and 2047 of *Colletotrichum lindemuthianum*

Crosses	Race	Resistance Gene	Observed Ratio		Expected Ratio	χ^2	P-Value
			R[a]	S[b]	R:S		
Pitanga x Ouro Negro	23	*Co-10*	92	6	15:1	0.03	0.96
Pitanga x BAT 93	23	*Co-3*^{3}	85	6	15:1	0.02	0.89
Pitanga x México 222	23	*Co-3*	98	7	15:1	0.03	0.86
Pitanga x Michelite	64	*Co-11*	103	5	15:1	0.48	0.49
Pitanga x MDRK	64	*Co-1*	105	6	15:1	0.13	0.71
Pitanga x PI207262	64	*Co-4*^{3}*; Co-3*^{3}	102	2	63:1	0.09	0.76
Pitanga x Jalo Vermelho	64	*Co-12*	96	5	15:1	0.29	0.60
Pitanga x Cornell 49-242	64	*Co-2*	88	6	15:1	0.03	0.98
Pitanga x TU	65	*Co-5*	90	6	15:1	0.00	1.0
Pitanga x AB 136	65	*Co-6*	95	7	15:1	0.06	0.80
Pitanga x Jalo L. Pretas	65	*Co-13*	53	3	15:1	0.07	0.78
Pitanga x G 2333	2047	*Co-4*^{2}	94	6	15:1	0.01	0.92
Pitanga x Corinthiano	2047	*?*	60	4	15:1	0.00	1.00
Pitanga x SEL 1308	2047	*Co-4*^{2}	95	6	15:1	0.03	0.86

R[a] = Resistant; S[b] = Susceptible

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SUCCESSES AND CHALLENGES OF THE BEAN BREEDING PROGRAM OF THE ARC-GRAIN CROPS INSTITUTE, SOUTH AFRICA

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Agricultural Research Council – Grain Crops Institute, Potchefstroom, South Africa

The bean breeding programme of the ARC- Grain Crops Institute concentrates on two main seed types, namely red speckled sugar (RSS) and small white canning (SW) beans, with dark red kidney (DRK), large white, painted lady, carioca & *Phaseolus coccineus* filling niche markets. The programme consists of three branches, namely the main breeding programme and the bacterial and fungal resistance breeding programmes. The main breeding programme creates new variability, sets standards for adaptation and increased yield, quality and field resistance to diseases. The breeder makes crosses to improve yield and quality. Greenhouse increases are followed by field selection, then evaluation in yield trials. This is reliable when done over multiple sites and seasons. The breeder also undertakes field evaluation of disease resistance. However, except for BCMNV (which is now successfully controlled) this is unreliable due to uneven infection and the presence of races in some diseases. The main breeding programme also supplies the well adapted cultivars for backcrossing, the most important of which are the red speckled sugar cultivars Kranskop, Jenny, OPS-RS1, -RS 2, -RS 4, -RS 5, and -RS 6 and the small white cultivars Teebus, Helderberg and OPS-KW 1. The breeder evaluates lines from both resistance breeding programs against the yield and quality standards and releases any promising line as a cultivar. The breeder is also responsible for the national cultivar trials and increases of breeder's seed.

The resistance breeding programs undertaken by the bacteriologist and pathologist include race studies, the importation and identification of new sources of resistance, and backcrossing, using well adapted local cultivars as recurrent parents. Resistant individuals are identified by means of artificial inoculation, and more advanced F-generations are evaluated in the field for adaptation and yield. Multiple resistance genes are now being stacked in advanced backcrosses. For common bacterial blight (CBB), a number of QTLs are available. Good resistance is available against halo bacterial blight (HBB) in the form of the single recessive gene from Edmund, which is race non-specific. Some work is now also being undertaken on bacterial brown spot (BBS). Work on fungal diseases includes rust and angular leaf spot (ALS) with some attention to anthracnose, root rot, aschochyta and powdery mildew. For rust, the most important genes have been *Ur-3, -5, -11, -13* and others. ALS resistance from CAL 143, G 5686 and several other sources has been utilised. Good progress has been made (summarized in Table 1) and a total of 27 cultivars have been released, 25 since 1988,

New molecular markers have been developed for HBB and rust in collaboration with Dr PN Miklas (*Pse-1* and *Pse-2*) and Dr CMS Mienie (*Ur-13*), the latter with a view to retaining this useful hypostatic Middle-American gene in the RSS cultivars. Many existing markers, especially for CBB, *Ur-3*, *Ur-5*, *Ur-11*, anthracnose and ALS, have been tested, with varying success, for use in our local material.

There have been three main challenges to these programs. Firstly, acceptable canning quality has been difficult to attain. The variable abiotic conditions (esp. soil, which is inclined to be acidic) experienced in South Africa play an important role. Canning factories have also changed standards over the years, for instance OPS-KW 1 was accepted, but later rejected, partly due to extended power cuts which effect the soaking period. Teebus, which has exceptionable and consistent canning quality in SA, is the only cultivar acceptable. Teebus-RR 1, a backcross 3, has now been accepted by the canning factories. However, it seems that the CBB resistance from XAN 159 used

in developing Teebus-RCR 2 (RR BC3/CBBR BC4) might have a negative affect on the canning quality of this cultivar. Secondly, funding is becoming an increasing problem and we have recently had serious cuts, especially for the Biotechnology program. Thirdly, manpower is now also a serious problem, and no replacements are in sight for three key posts (out of a staff of 9)

Table 1. Characteristics of the most important cultivars released by ARC-Grain Crops Institute

Cultivar	Gene(s)	% Yield Increase	BCMV	Rust	ALS	HB	CBB
Large seeded (red speckled sugar)							
Bonus (SA) (standard)	Unknown	0	S	S	S	S	S
Kranskop	*Ur-13*	5-8	R	I	S	S	S
Kranskop-HR 1	*Ur-13*;HR from Edmund	14-26	R	I	I-S	R	S
OPS-RS 1	*Ur-13*	12	R	I	S	S	S
Werna*	*Ur-13?; CR from VAX 4*	27*	R	R-I	R	S	R
OPS-RS 2	*Ur-13*	0-5	R	I	S	S	S
OPS-RS 4	*Ur-13*	22-27	R	I	I	S	S
OPS-RS 5	*Ur-13*	7	S	I	S	S	S
OPS-RS 6	*Ur-13?*	18	R	I	S	S	S
Jenny	*Ur-13*	11-16	R	I	S	S	S
Sederberg	*Ur-11,Ur-13*	14-24	R	R	R	S	S
Tygerberg*	*Ur-11, Ur-13*	33*	R	R	R	S	S
Small seeded (small white canning and carioca)							
Teebus (standard)	Unknown	0	R	S	R-I	I	S
Kamberg**	*Ur-3+***	<25**	R	R	R	I	S
Helderberg**	*Ur-3+*	<29**	R	R	R	I	S
OPS-KW 1	*?Ur-3+***	11-17	R	R	R	I	S
Teebus-RR 1	*?Ur-3+***	19-30	R	R	R-I	I	S
Teebus-RCR 2	*Ur-5+*; CR from XAN 159; BC420 & SU91 markers	20	R	R	R-I	I	R
CAR-2008	Unknown	21*	R	R	R	I	I

* One year's data ** Previous data *** The rust resistance additional to *Ur-3* varies Improvement on standard

CONCLUSIONS

Although success has been satisfactory, this is highly dependant on the dedication and concerted effort of team members, where everybody must buy into the same objectives. Expertise, as well as sufficient funding, must also be available for a long enough period,

The products of this work are available to other breeders and comprise well adapted cultivars with high yield potential and, hopefully, fairly stable resistance genes. Some cultivars, especially those that are Teebus-related, are day-length sensitive and therefore not adapted to higher latitudes, whereas the large seeded cultivars are inclined to be susceptible to *Beet curly top virus* in the United States (PN Miklas, personal communication). Some of our cultivars and released breeding lines are also planted with success in other African countries. Genetic markers are available for use by pathologists and breeders.

POTYVIRAL VPG-INTERACTING PROTEINS AND *BEAN COMMON MOSAIC VIRUS* RESISTANCE IN *PHASEOLUS VULGARIS* L.

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ABSTRACT: A study was made to find candidates for the *bc*-genes conferring resistance to systemic movement of potyviruses *Bean common mosaic virus* (BCMV) and *Bean common mosaic necrosis virus* (BCMNV) in *Phaseolus vulgaris*. Homologues of *Arabidopsis thaliana PVIP1* and *PVIP2* (potyviral VPg-interacting proteins) genes important in systemic movement of a wide range of potyviruses were cloned from different genotypes of *P. vulgaris*. Two alleles of *PVIP2* gene, annotated as *PvPVIP2*^{1} and *PvPVIP2*^{2}, differentiated the majority of *BC-1*^{1} from *bc-1*^{1} cultivars according to their deduced amino acid levels. A CAPS marker was developed for *PvPVIP2* alleles but no positive correlation was found between the mutated *PvPVIP2*^{2} allele and *bc-1*^{1} resistance, and instead plants homozygous for *PvPVIP2*^{2} appeared to support high level of viral multiplication

INTRODUCTION: Recessive resistance to BCMV and BCMNV strains in *P. vulgaris* is mediated by three strain specific genes *bc-1, bc-2* and *bc-3*, all of which need a strain non-specific gene *bc-u* to confer resistance (Drijfhout 1978; Kelly, 1997). A single allele of *bc-3* gene is known to confer immunity to all known strains of BCMV and BCMNV with NL-3K of BCMNV as the only reported exception (Miklas *et al.*, 2000; Larsen *et al.*, 2005). In contrast, both *bc-1* and *bc-2* possess *bc-1*^{1}, *bc-1*^{2} and *bc-2*^{1}, *bc-2*^{2} alleles, respectively, and confer strain specific resistance against systemic movement of these viruses (Drijfhout, 1978; Silbernagel *et al.*, 2001). None of these genes have been cloned at the molecular level but molecular markers linked to *bc-1*^{2} and *bc-3* have been developed and used to map these genes to linkage groups b03 and b06 in the *P. vulgaris* genome (reviewed in Kelly *et al.,* 2003). Due to the importance of PVIP1 and PVIP2 proteins in potyviral systemic movement (Dunoyer *et al.,* 2004) they were likely candidates for the BCMV resistance genes. In the present study, we addressed this hypothesis by cloning both genes from different host groups of *P. vulgaris* carrying different combinations of *bc*-genes; developing a marker for one of the *PvPVIP* genes and placing it on *P. vulgaris* genetic map.

MATERIALS AND METHODS: Seeds of *P. vulgaris* cultivars with different combinations of *bc*-genes (table 1) were obtained from CIAT (Colombia). cDNA of *PvPVIP1* and *PvPVIP2* genes were amplified from all cultivars applying degenerated primer pairs that were designed on the basis of homologues of these genes from *G. max, A. thaliana, P. sativum* and *M. truncatula*. A CAPS-*Hpa*II marker discriminating *PvPVIP2* alleles was developed and segregation of the marker with *bc-1*^{1} resistance was studied using BCMV-NL1 strain and an F_2 population derived from DW and Immuna. Genetic mapping of the *PvPVIP2* gene was conducted in a RIL population derived from DOR364 x G19833.

RESULTS AND DISCUSSION: Both genes *PvPVIP1* and *PvPVIP2* were cloned and sequenced at least two times for each cultivar. For the *PvPVIP1* locus, two alleles *PvPVIP1*^{1} and *PvPVIP1*^{2} were found within the genotypes tested. The alleles differed at a single codon, but the *PvPVIP1*

polymorphy had no relation to a specific *bc*-genotype. For *PvPVIP2* two alleles, annotated as *PvPVIP2*^{1} and *PvPVIP2*^{2}, differentiated the majority of *BC-1*^{1} from *bc-1*^{1} cultivars. cDNAs corresponding to these alleles differed at 18 nucleotide positions affecting 5 amino acids. . However, in the F_2 population segregating for *bc-1*^{1} and *BC-1*^{1} there was no positive correlation between the presence of *PvPVIP2*^{2} allele and *bc-1*^{1} resistance. In contrast, plants homozygous for *PvPVIP2*^{2} appeared to support high level of viral multiplication. Therefore the *PVIP* genes were ruled out as candidates for the known BCMV/BCMNV resistance genes. It is, however, interesting that the presence of *PvPVIP2*^{1} allele was associated with lower viral multiplication. This could suggest *PvPVIP2*^{1} or a linked gene as a quantitative resistance factor that protects bean against BCMV. *PvPVIP2* was mapped to linkage group b08 by application of the CAPS marker on a RILs population derived from DOR364 x G19833.

Table 1. *P. vulgaris* cultivars with the proposed srain-specific *bc*-genes against BCMV and BCMNV and *PvPVIP2* allele determined for each cultivar.

Allele	Cultivar
PvPVIP2^{1}	DW, CRM, Widusa, BTS, [RGB, Amanda (*bc-1*^{2})], Sanilac (*bc-2*), IVT7214 (*bc-2, bc-3*)
PvPVIP2^{2}	The Prince, SGR, [Immuna, Topcrop, ITG (*bc-1*)]

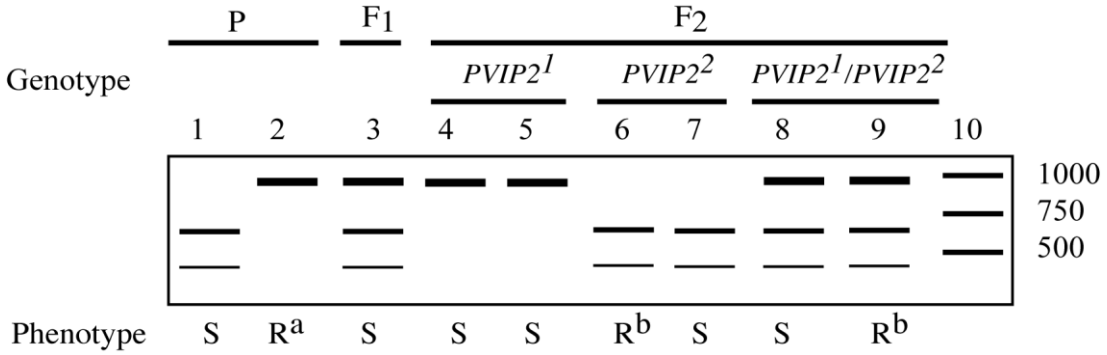

Figure 1. Segregation analysis of BCMV-NL1 susceptibility and *Hpa*II-CAPS marker differentiating *PvPVIP2*^{1} (Susceptible: S) and *PvPVIP2*^{2} (Resistant: R) alleles. An F_2 population (lanes 4-18) derived from DW [(*Bc-u, Bc-1*^{1})(S, *PvPVIP2*^{1}, lane 1)] X Immuna [(*bc-u, bc-1*^{1})(R, *PvPVIP2*^{2}, lane 2)] together with parental genotypes (lanes 1-2) and F_1 hybrids (lane 3) were inoculated with NL1 strain. BCMV susceptibility was analyzed by symptomatology, ELISA and back inoculation on susceptible cultivar DW. F_2 plants with OD_{405}> 2.5 times of mock-inoculated plants and showing viral symptoms were rated as susceptible (S). Asymptomatic plants having OD_{405}< 2.5 times of control plants were rated as resistant (R). $^{a/b}$ BCMV was recovered from inoculated leaves/ un-inoculated leaves by back-inoculation on susceptible cultivar DW. All plants were genotyped for *PvPVIP2* alleles using *PvPVIP2 Hpa*II-CAPS marker and were grouped into homozygous *PvPVIP2*^{1}, homozygous *PvPVIP2*^{2} and heterozygous plants. DNA marker is shown in lane 10.

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A POSSIBLE ROLE FOR *BC-U* IN *BC-U, BC-3* GENE COMBINATION IN RESISTANCE TO *BEAN COMMON MOSAIC VIRUS* IN *PHASEOLUS VULGARIS* L.

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ABSTRACT: Recessive resistance against *Bean common mosaic virus* (BCMV) and *Bean common mosaic necrosis virus* (BCMNV) in *P. vulgaris* is mediated by three loci, *bc-1*, *bc-2* and *bc-3*, and a fourth locus, *bc-u*, has been suggested as a complementary strain-unspecific gene for resistance conferred by the *bc*-numbered genes. To elucidate a possible function to the *bc-u*, we present genetic evidences on the basis of *Bc-u, bc-3* and *bc-u, bc-3* gene combinations that suggest fundamental role for the *bc-u* in preventing the *bc-3* gene from being overcome by BCMV.

Introduction: Resistance to the potyviruses BCMV and BCMNV in *P. vulgaris* is affected by three strain-specific loci, *bc-1*, *bc-2* and *bc-3* and a single strain-unspecific *bc-u* genes (Drijfhout, 1978). Resistance controlled by alleles at these loci is inherited as recessive characters. In addition to the recessive *bc*-genes, the dominant *I* gene in *P. vulgaris* confers resistance to BCMV and other potyviruses through a hypersensitive response (Kelly *et al.*, 1997; Collmer *et al.*, 2000). Despite that some functions have been suggested to the strain-specific genes, no clear function(s) has been suggested so far to the *bc-u* locus except that it is necessary for the strain-specific genes to be completely expressed (Drijfhout 1978; Kelly *et al.*, 1995; Kelly 1997). In the present study we address a possible role for *bc-u* in *bc-u/bc-3* combination.

MATERIALS AND METHODS: Seeds of *P. vulgaris* genotypes SGR (*i, bc-u*) and USCR8 (*i, bc-3*) and BCMV strains RU1 and NL1 were obtained from Dr. Richard Larsen (USDA-ARS, Prosser, Washington, USA). Seeds of cultivar Dubbele Witte (DW; *i*) were obtained from CIAT (Colombia). An F_2 population segregating for *bc-u* and *bc-3* was generated by crossing genotypes SGR and USCR8. All three cultivars and F_2 individuals were inoculated with RU1 and NL1 strains in individual experiments and the susceptibility to virus was checked by symptomatology, ELISA and RT-PCR. SGR and DW carry an allele of *eIF4E* designated $eIF4E^1$ and USCR8 carries an allele designated $eIF4E^2$ that co-segregates with *bc-3* resistance (Naderpour *et al.*, 2008). All F_2 plants were genotyped for *eIF4E* allele. Six primer pairs amplifying the whole RU1 genome and a single primer pair amplifying NL1-VPg were designed on the basis of RU1 (AY863025) and NL1 (AY112735) sequences in public databases. Viral cDNAs corresponding to the primer pairs were amplified using high fidelity reverse transcriptase (Roche, Mannheim, Germany) and the amplified PCR fragments were sequenced at MWG-Biotech, Germany.

RESULTS AND DISCUSSION: Inoculation of RU1 or NL1 strains on susceptible cultivars DW, SGR produced typical symptoms of BCMV (mosaic, malformation) about ten days after inoculation. F_2 individuals carrying $eIF4E^1$ in either homozygous or heterozygous condition showed the same response as DW and SGR. Inoculation of RU1 or NL1 strains on USCR8 resulted in very mild mosaic symptoms on some plants, and these symptoms appeared about four weeks post inoculation (WPI). F_2 individuals homozygous for $eIF4E^2$ showed the same response as USCR8. RU1 was isolated from symptomatic USCR8 and DW 6 WPI. Sequence analysis of the complete sequence

revealed two codon differences (a/b in DW and c/d in USCR8) in the region encoding the viral VPg. Sequencing of the VPg coding region of RU1 obtained from five DW, five SGR and 15 USCR8 plants showed that only virus from USCR8 displayed mutations to codons c and d (figure 1A). In the case of NL1 strain, only the VPg domain was amplified from DW, SGR, USCR8 plants and individuals of an F_2 population derived from a cross between SGR and USCR8 that segregates for *eIF4E*^{1}/*eIF4E*^{2}. Sequencing of the PCR products revealed one codon difference (e or f) at a single position. Sequencing of the VPg coding region of NL1 obtained from five DW, five SGR and 10 USCR8 plants showed that only virus from USCR8 displayed the mutated codon f (figure 1B). In the segregating population, virus from all individuals carrying *eIF4E*^{1} contained the e codon variant. A mixture of e and f was found in one heterozygote. In plants homozygous for *eIF4E*^{2}, the f codon variant was predominant and only in one plant it was not possible to detect the f codon variant. We have previously shown that resistance mediated by *bc-3* in genotype USCR8 co-segregates with *eIF4E*^{2} (Naderpour *et al.*, 2008). Several publications have reported that breaking resistance mediated by *eIF4E* or *eIFiso4E* is associated with mutations in the region encoding the central part of VPg (Robaglia and Caranta, 2006, Bruun-Rasmussen *et al.*, 2007). In the F_2 population derived from SGR (*bc-u)* x USCR8 (*bc-3)*, we found that 8 of 126 of the F_2 plants were completely resistant. These were all homozygous for *eIF4E*^{2} and the segregation ratio is close to 1/16 as expected when two recessive genes are segregating (data not shown). If *bc-3* is indeed *eIF4E*^{2}, the possible role for *bc-u* could be protecting *bc-3* from being overcome by BCMV strains.

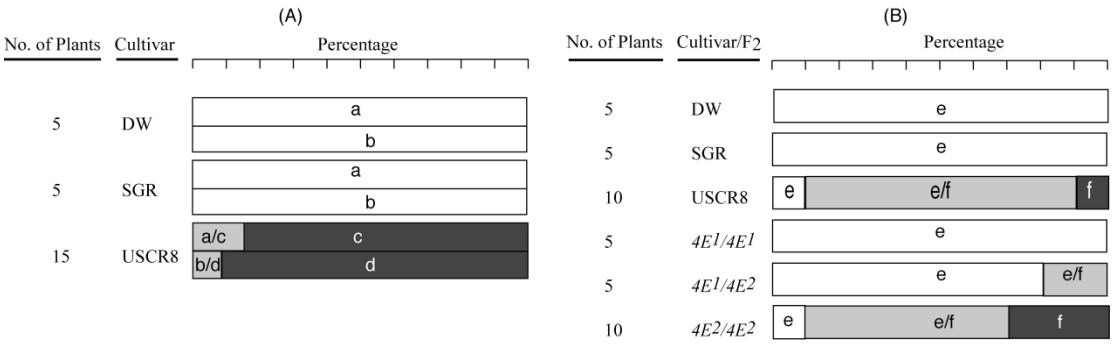

Figure 1. VPg types found in BCMV isolated from *P. vulgaris* DW, SGR and USCR8 and a F2 population segregating for *eIF4E*^{1}/*eIF4E*^{2}. (A) VPg types of BCMV RU1. Letters a and c refer to the amino acid codon at the first mutated position and letters b and d to the amino acid codon at the second mutated position. Bars represent the percentage plants carrying VPg type a/b (white), c/d (black) and mixed (grey). (B) VPg types of BCMV NL1. Letters e and f refer to the amino acid codon at the mutated position. Bars represent the percentage plants carrying VPg type e (white), f (black) and mixed (grey).

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GENETIC DIVERSITY IN CANADIAN CONTEMPORARY COMMON BEAN: A PEDIGREE ANALYSIS

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INTRODUCTION

Genetic improvement of common bean (*Phaseolus vulgaris* L.) in Canada has a history of more than 120 years. Early breeding efforts, which started soon after the establishment of the Central Experimental Farm in Ottawa in 1886, included testing of dry and garden bean introductions. Over the years, the genetic diversity in the natural gene pools of *P. vulgaris*, introduction from other national and international bean breeding programs, along with variation derived through hybridization and recombination, as well as occasional inter-specific hybridizations have been the main sources of genetic variation employed by the Canadian bean breeders. The coefficient of parentage, also referred to as coefficient of co-ancestry or kinship, is one of the tools used to study genetic diversity among genotypes. First used by Malecot (1948), the coefficient of parentage (f_{ij}) is computed based on the pedigree data and estimates the probability that, at a single locus, a random allele from the i^{th} individual and a random allele from the j^{th} individual are identical by descent (Bernardo 2002). The objectives of this research were to develop a pedigree database for the Canadian common bean and to assess the genetic diversity among common bean varieties released in Canada since 1930, using pedigree information.

MATERIALS AND METHODS

The pedigrees of dry bean varieties of different Canadian market classes (navy, black, great northern, pinto, pink, small red, dark red kidney, light red kidney, and cranberry) were collected. The pedigrees were traced back, as far as possible. The coefficient of parentage was estimated for all possible pair-wise combinations of varieties using the software 'KIN' (Tinker and Mather 1993). Cluster analysis (PROC CLUSTER in SAS) was employed to study the genetic diversity of Canadian contemporary common bean varieties. Dendrograms were generated separately for varieties of Mesoamerican, Durango and Nueva Granada races. Furthermore, A RAPD fingerprinting dataset (S Mack, T Michaels, and KP Pauls; unpublished), which included a sub-set of 28 bean genotypes with 150 RAPD fragments (400-3000 bp) were used to compare the results of pedigree- with RAPD-based diversity analyses.

RESULTS AND DISCUSSION

The mean and median values of dissimilarity indices were highest for the varieties of Durango-origin and lowest for varieties of Nueva Granada-origin (Fig 1.). However, the range of dissimilarity indices was wider for the varieties of Mesoamerican origin compared to varieties of Durango and Nueva Granada origin with 75% of the values higher than 0.87 and 0.75 for varieties of Durango and Mesoamerican origin, respectively, compared to 0.50 and higher for the varieties of Nueva Granada origin (Fig. 1). This indicates narrow genetic diversity within varieties of Nueva Granada origin

(kidney and cranberry market classes) compared to the other two races. Pedigree-based cluster analysis of the varieties of race mesoamerica (navy and black), in the first fusion level, resulted in two main clusters; represented by varieties Seafarer and Ex Rico 23 with highest average f_{ij}. These two diversity groups, in the second fusion level, formed four sub-groups, represented by navy varieties OAC Rico, AC Compass, OAC Seaforth and Fleetwood. Two major diversity groups were identified in Canadian beans of race Durango (pinto, great northern, small red, and pink), represented by varieties Agrinto and UI 111, while varieties of race Nueva Granada- (dark and light red kidney) were classified into two groups represented by varieties Montcalm and OAC Lyrick. The pedigree- and RAPD-based dendrograms were somewhat similar suggesting that pedigree information will continue to be useful to inexpensively identify diverse parents in the bean breeding programs.

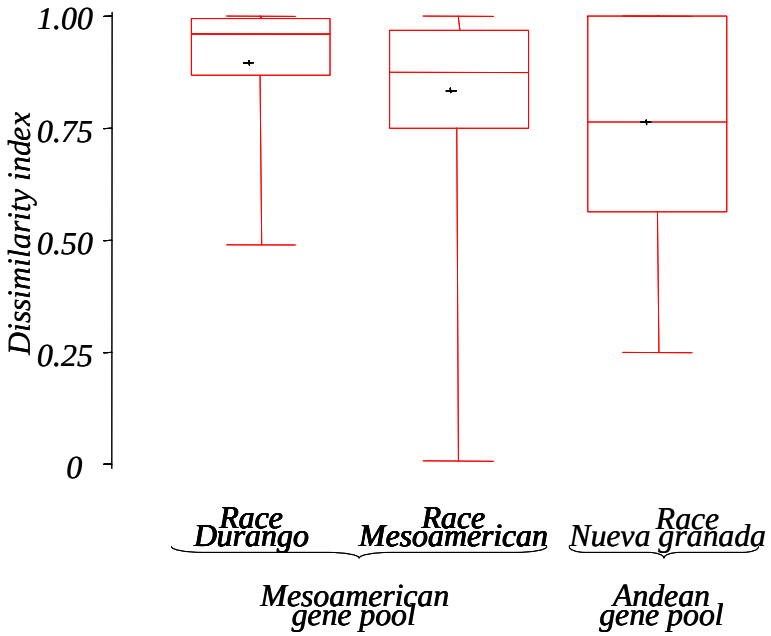

Figure 1. Frequency distribution of dissimilarity index values between pair-wise combinations of varieties of different evolutionary race- and gene pool-origins. Boxes represent the inter-quartile range. The whiskers represent the range. The solid line across the box indicates the median, while + indicates the average.

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MAPPING RESISTANCE TO PEANUT MOTTLE VIRUS IN COMMON BEAN

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Peanut mottle virus (PeMoV) causes severe symptoms of systemic vein necrosis in susceptible snap beans, resembling those caused by *Bean common mosaic necrosis virus* (BCMNV). PeMoV is a member of the genus *Potyvirus* and is transmitted by several aphid species in a non-persistent manner. During 2008 and 2009, PeMoV was identified in snap bean fields in Frio County, TX. Snap beans were observed to have high incidences of PeMoV with infection rates ranging from 5 to 25 % in some fields, resulting in significant yield losses. Vein necrosis was observed in leaves and stems, and pods showed extensive orange-brown necrotic lesions. The snap beans were located in the vicinity of peanut fields that likely served as the virus reservoir source. When plants were evaluated by ELISA, the virus did not react with the group-specific potyvirus monoclonal antibody but was positively identified as PeMoV using RT-PCR.

An incompletely dominant gene *Pmv* which confers resistance to PeMoV was described by Provvidenti and Chirco (1987). However, *Pmv* has not been assigned to any linkage group. In lieu of the recent PeMoV outbreak, our objective was to identify available sources of PeMoV resistance, and to characterize and locate the gene(s) on the *Phaseolus* core map.

MATERIALS AND METHODS

A preliminary screening of germplasm materials for reaction to PeMoV revealed that BelNeb-RR-1 great northern and G 122 landrace possessed resistance. A RIL population consisting of 75 F_9-derived lines from the cross between BelNeb-RR-1 (PeMoV-resistant) and A55 (PeMoV-susceptible), henceforth referenced as the BA population, was obtained from a previous study (Ariyarathne et al., 1999). A second RIL population was also evaluated consisting of $F_{5:7}$ RILs derived by Johnson (1997) from a cross between the landrace cultivar G122 (resistant) and A55 (AG population). Each RIL population and their respective parents were mechanically inoculated at the primary leaf stage with the PeMoV isolate from Texas. All plants in each population were rated in multiple experiments as resistant or susceptible at 21 dpi based on symptom response. In order to rule out false negatives, a random sampling of plants exhibiting a resistance response within each population was collected and evaluated for the virus by RT-PCR using primers designed specifically to detect PeMoV.

RESULTS AND DISCUSSION

All plants in each population exhibited necrotic local lesions and vein necrosis on primary inoculated leaves. At 10 to 14 days post-inoculation, the virus in all susceptible plants caused systemic necrosis (top necrosis) in secondary trifoliate leaves and stems resulting in eventual death of the plant. Resistant plants in the BA and AG populations exhibited a hypersensitive response on inoculated leaves with no systemic movement of the virus. However, one line (recombinant) in the AG population showed an intermediate reaction of systemic mosaic and no systemic necrosis. PeMoV was not detected by RT-PCR in lines absent of systemic symptoms (resistant). Within the BA RIL population, 34 R and 41 S fit the 1:1 segregation ratio expected for a single resistance gene. Within the AG RIL population, 26 R and 34 S (with one recombinant) also fit the 1:1 segregation ratio.

Resistance to PeMoV derived from BelNeb-RR-1 was mapped to linkage group 3 on the *Phaseolus* core map (Fig. 1). Similarly, resistance in the AG population was determined also to be located on LG 3. Integration of these groups on the core map suggested the gene was located in the vicinity of the *bc-1*^{2} gene conferring resistance to *Bean common mosaic virus* (BCMV) and BCMNV. To examine this relationship further, SCAR marker SBD5.1330 linked with *bc-1*^{2} (Miklas and Larsen, 2000) was mapped in the BA population. Results showed that resistance to PeMoV derived from BelNeb RR-1 is directly linked (0 recombinants) to SBD5.1330 as determined by co-segregation with the marker (Figure 1). BelNeb-RR-1 contains *bc-1*^{2} and *bc-u* that confer resistance to BCMV/BCMNV in Pathogroups I, II, III and V (e.g., US1, US7, NL8, and US2, respectively). SBD5.1330 was also determined to be present in G122, and when mapped in the AG population, resistance to PeMoV derived from G122 was found to be tightly linked (1 recombinant) to the marker. Interestingly, G122 does not express *bc-1*^{2} or any other known genes for resistance to BCMV or BCMNV. In an extended host range study that included seven different varieties containing *bc-1*^{2} (Ivory, Red Kloud, Redlands Greenleaf B, UI-129, UI-31, UI-59, US 1140), all were resistant to PeMoV. However, other lines with different gene backgrounds such as Hystyle (*I*), Jubila (*bc-1*), UI-34 (*bc-u*, *bc-2*), Othello (*bc-u*, *bc-2*^{2}) were also resistant to the virus. Hence, we conclude that resistance to PeMoV does not appear to be dependent on *bc-1*^{2} and that the relationship of the previously identified *Pmv* gene to the *bc-1*^{2} locus is unknown.

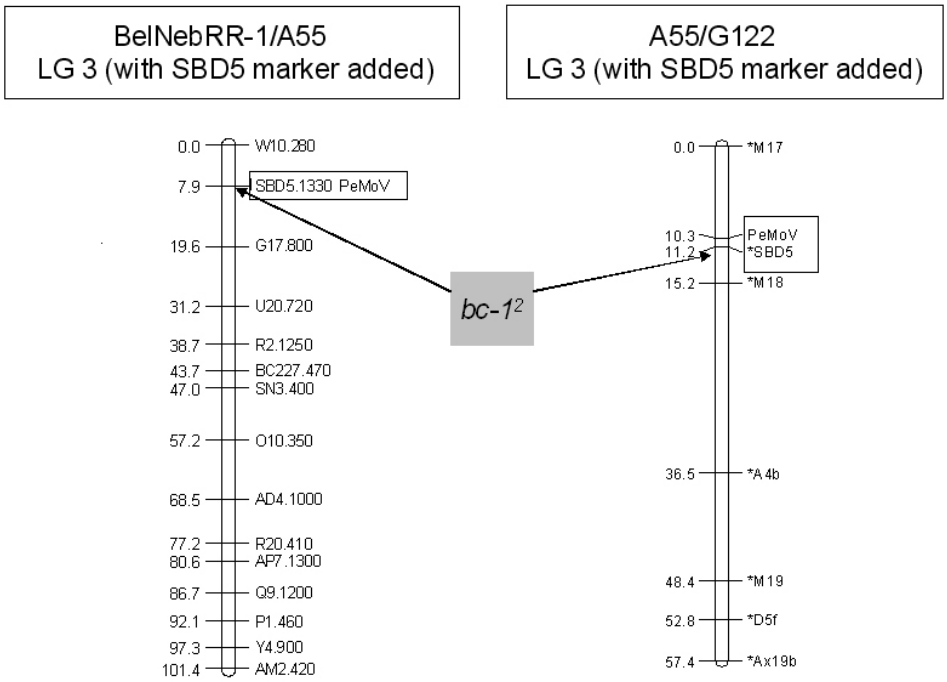

Figure 1. Map showing integration of resistance to *Peanut mottle virus* in the BelNeb-RR-1/A55 and A55/G122 RIL populations on linkage group 3. Resistance is linked with the SBD5.1330 SCAR marker which is tightly linked to the *bc-1*^{2} gene conferring resistance *Bean common mosaic virus* and *Bean common mosaic necrosis virus*.

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VIRUS SURVEILLANCE IN BEANS USING TISSUE BLOT IMMUNOASSAY: THREE YEARS EXPERIENCE OF THE LEGUME IPM-PIPE

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The Integrated Pest Management Pest Information Platform for Extension and Education (ipm-PIPE) began with the soybean rust (SBR) monitoring/communication system and was expanded to include soybean aphid (SA) in 2006 and to legume pests and diseases in 2007 and later. One of the initial objectives of the Legume ipmPIPE was to monitor for selected viruses, as increased prevalence of bean and soybean viruses had been reported with the introduction of the soybean aphid. A second objective was to increase preparedness of NPDN labs to conduct virus assays for the ipm-PIPE. A working group was formed to review viruses known to occur in common bean and assess virus diagnostic methods suitable for economical, high throughput surveillance programs. Viruses selected for monitoring were: *Alfalfa mosaic alfamovirus* (AMV), *Bean common mosaic potyvirus* (BCMV), *Bean yellow mosaic potyvirus* (BYMV), *Cucumber mosaic cucumovirus* (CMV), *Bean pod mottle comovirus* (BPMV) and *Soybean mosaic potyvirus* (SMV). The tissue blot immunoassay (TBIA) was selected as a practical, high-throughput method that could be performed with minimal equipment and time. In this test, leaves are pressed directly onto a nitrocellulose membrane, which traps virus particles that can be detected by an enzyme-linked antibody method from dry and stored membranes. A complete experimental kit with a TBIA card for testing up to 50 samples, in duplicate to detect two viruses, was developed for the ipm-PIPE and distributed by Agdia, Inc. to NPDN laboratories participating states. A training session and an S.O.P. were available on-line for NPDN laboratories with instructions on processing and reading the immunoassays. Completed TBIA cards were collected from all states in 2007 and observed to assure quality and consistency, to verify the assays, and to direct research for improving and enhancing the assay.

Protocols for sentinel plot number and design, sampling, and monitoring were established by the Legume ipm-PIPE. Leaves were collected two times in 2007 and 2008, and one time in 2009. In 2007, leaves were taken from 9 transect samples, each of 5 consecutive plants, in bean or soybean sentinel plots. In 2008 and 2009, in addition to 5 transect samples of 5 plants, 20-25 random or symptomatic plants were selected to enhance the proportion of positive samples, and the scope of legume species sampled was broadened. States could select to receive TBIA kits to test for AMV/BCMV (mainly in western states) and/or CMV/BYMV (mainly in eastern states) in legumes. In 2007, soybean SBR plots in all states were monitored for BPMV/SMV. This kit was available only on demand in later years. Nylon membranes were also supplied to western states choosing to test for *Beet curly top virus* (BCTV), a DNA virus known to be prevalent in western states, but not amenable to immunoassays. The same leaves were pressed onto nylon membranes, which were processed by nucleic acid hybridization at a central location. Data from TBIA and BCTV tests were uploaded by NPDN labs to the website (http://legume.ipmpipe.org).

Kit-based immunoassays were developed for the six viruses and used for monitoring viruses in at least 158 common bean sentinel plots and mobile plots in 27 states (Table 1). The distribution of viruses was shown to vary across the USA. The priority viruses were detected in one or more of 12

states, and in one or more years. The greatest number of positive fields in the eastern region was found in MI, NY and WI, with CMV being the most prevalent. In the western region, BCMV was detected in 5 states and AMV in three. Nine states reported none of these 4 viruses, or failed to enter a report. In pea, AMV was detected in South Dakota (2008) and BCMV in WA in 2009. In lima bean (2007 only), AMV was found in DE and WA, BCMV in ID, and BYMV in DE. Reports of soybean positive for BPMV were from DE, IA, MI, SC and VA in 2007, and from SD and WV in 2009. SMV was reported only from MO and VA (2007). States in the western region in which BCTV positive beans were detected included AZ, NM, and WA in all years, and CO, MO, and OR in one or two years. Examination of the TBIA cards at the conclusion of the season showed inconsistencies in the quality of the processed membranes, suggesting that additional enhancement in reagents and instructions may be needed to validate the method. However, TBIA offered a novel method for extensive surveillance of legume viruses.

Table 1. Viruses detected in common bean by Tissue blot immunoassay reported by NPDN laboratories for the Legume ipm-PIPE.

	2007	2008	2009
Eastern Region[a]			
Delaware	BYMV, CMV		
Iowa		BYMV, CMV	
Kansas		BYMV, CMV	
Michigan	CMV	AMV, BYMV, BCMV	AMV, BYMV, BCMV
New York	BYMV, CMV	BYMV, CMV	CMV
South Dakota		AMV	
Wisconsin	CMV	BYMV	
Western Region[b]			
Colorado	AMV, BCMV	BCMV	BCMV
Idaho	BCMV		
Montana	AMV, BCMV		
New Mexico	BCMV	BCMV	
Washington	AMV	AMV	BCMV, BYMV

[a]No viruses detection reported by FL, IL, IN, TX, VA
[b]No virus detection reported by AZ, CA, OR, WY

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SAT gratefully acknowledges the help of Dr. Chet Sutula of Agdia, Inc. for the research and development for scale-up of TBIA and production of self-contained kits, and funding from USDA-CSREES, that made this assay possible. Agdia also conducted BCTV assays. We also acknowledge the input of virologists who selected the viruses to be included; the dedicated work of the state extension specialists, their field and laboratory workers, and their affiliated organizations for growing and sampling legume plots; the contributions of the NPDN diagnosticians and their labs and all those who processed TBIA and entered data; the regional IPM centers; and the Risk Management Agency for funding.

CHARACTERIZATION OF A NEW WHITEFLY-TRANSMITTED VIRUS FROM A WILD LEGUME IN PUERTO RICO THAT INFECTS BEAN, AND MOLECULAR SURVEY OF BEAN VIRUSES IN PUERTO RICO, DOMINICAN REPUBLIC, AND NORTHERN MEXICO

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Whitefly-transmitted geminiviruses, or *Begomoviruses*, have been recognized as emergent pathogens of vegetable and fiber crops in tropical and fringe temperate zones, worldwide. Begomoviruses are transmitted in a persistent manner by a suite of variants, or 'biotypes', of the whitefly vector, *Bemisia tabaci* (Genn.). 'Topcrop' and 'Red Kidney' bean (*P. vulgaris*) are highly susceptible to nearly all begomoviruses isolated from vegetable crops and studied systematically during the past decade in the Arizona (J.K. Brown) laboratory. These viruses are typically quite virulent in bean as well as other vegetable crop species, are widespread, and cause economic losses in most vegetable growing regions of the Americas (Brown, 1990; 1994; Brown et al., 1999; Idris and Brown, 1998). Begomoviruses from the Eastern Hemisphere also can infect bean, including *Tomato yellow leaf curl virus* from Israel, which was recently introduced in the US Sunbelt States, the Caribbean Basin (Bird et al., 2001), and Mexico (Idris et al., 2007; Isakeit et al., 2007).

In the Caribbean Basin and Central American countries, the whitefly-transmitted bean golden mosaic virus (BGMV) has historically been recognized as the primary bean-infecting begomovirus in the Caribbean region. This virus has been targeted as the primary viral pathogen of bean in the region for over 30 years. A distinct strain of BGMV emerged in Florida in 1993 as a major bean disease problem (Blair et al., 1995). In addition, a begomovirus species has been characterized from *M. lathyroides* in Florida (erroneously reported as BGMV) (Blair et al., 1995; Hiebert et al., 1991) and has now been shown to be distinct from all strains of BGMV and from MaMV from Puerto Rico (Idris et al., 1999; 2003).

More recently, several previously unidentified bean-infecting viruses were identified in bean and indigenous species in Puerto Rico. Specifically, *Macroptilium mosaic virus* (MaMV) from *Macroptilium lathyroides (*and not BGMV) was prevalent, as had been expected. Ironically the once prevalent BGMV and major focus of the bean breeding program for the tropical Americas (Faria et al., 1994; Molina and Beaver, 1998; Singh et al., 2000; Velez et al., 1998) has disappeared as an important pathogen, likely owing to a shift in viral population dynamics resulting from the displacement of the 'local' Sida race of *B. tabaci* by the invasive B biotype (Africa) of the whitefly vector, which has a distinct host range and is more fecund than the Sida race (Brown, 2007). Other begomoviruses of bean identified recently include MaMPRV in *Macroptilium lathyroides*; *Rhyncosia mosaic virus* from *Rhyncjosia minima*; and *Jatropha mosaic virus* from *Jatropha gossypifolia, Passiflora edulis* and *P. foetida*.

In 2006, *R. minima* plants exhibiting mild mosaic symptoms that are reminiscent of begomovirus infection were observed in PR during the summer of 1997. Total nucleic acids were extracted from symptomatic *R. minima* leaves using the C-TAB method (Doyle and Doyle, 1987). The DNA was subjected to rolling circle amplification to amplify circular DNA molecules and cloned into *Sac*I-digested pGEM7Zf+ in *E. coli* strain DH5α. Eight clones bearing a fragment of

about 2.6 kb were fully sequenced using primer-walking approach. Analysis of the obtained sequences showed that five clones were DNA-A and three were DNA-B of a begomovirus (Idris and Brown, in preparation).

The genome organization of both components is typical of other bipartite begomoviruses in that six and two open reading frames (ORF) of characteristic size, position and orientation were identified in DNA-A and DNA-B, respectively. Inspection of the common region (CR) revealed that these molecules are cognate components. Comparative analysis of the nucleotide sequence DNA-A indicated that these five molecules shared 98-99% nucleotide identity and therefore, according to the ICTV guidelines (Fauquet et al., 2003) there are considered isolates of a single begomovirus. On the other hand, three DNA-B molecules shared 99% nucleotide identity with each other. The nucleotide sequences of each component were blasted in GenBank and closes relatives were included in multiple alignment. The distance analyses for the DNA-A indicated that these isolates shared 80% nucleotide identity with their closest relatives, MaMPRV and *Rhynchosia golden mosaic virus* (RhGMV). This confirmed that the cloned begomovirus isolate meets the <89% nucleotide identity and based on the ICTV guidelines it represents a distinct virus that has not been reported before. The nucleotide sequence of the DNA-B of this new virus shared 64% and 62% nucleotide identity with RhGMV and *Cabbage leaf curl virus* (CaLCV), respectively (Idris and Brown, in preparation).

Virus surveys. Survey for begomoviruses infecting bean and uncultivated indigenous plant species were carried out in Dominican Republic, Sonora Mexico, Arizona, and Puerto Rico during 2006-2009. Results indicated that a possible new strain of *Bean golden yellow mosaic virus* was present in bean in Dominican Republic (courtesy Graciela Godoy). In Puerto Rico [weeds, bean]: *Sida golden yellow virus, Abutilon mosaic v, Tomato virus-Nicaragua; Okra yellow mosaic virus; Tobacco leaf rugose virus, Clitoria virus*; [88-92%]; *Rhynchosia mosaic virus* [97-98%]; and *Malva alcefolia virus-PR* [96%] were detected. In Sonora, Mexico [bean]: *Abutilon mosaic virus, Sida golden mosaic virus, Malvastrum yellow mosaic virus, Okra yellow mosaic virus* [87-92%] and *Tomato yellow leaf curl virus* [98-99%] were detected.

SELECTION FOR WHITE MOLD RESISTANCE IN COMMON BEAN

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INTRODUCTION

White mold [caused by *Sclerotinia sclerotiorum* (Lib.) de Bary] is a severe and widespread disease of dry and green common bean (*Phaseolus vulgaris* L.). Partial resistance to white mold is found in Andean (e.g., A 195, G 122, MO 162, PC 50, RedKloud, VA 19) and Middle American (e.g., AB 136, ICA Bunsi) dry and green (e.g., Black Valentine, CORN 501) common bean. But, higher levels of resistance occur in *P. coccineus* (e.g., G 35172, PI 433246, PI 439534) and other secondary gene pool species. Interspecific breeding lines that derive white mold resistance from *P. coccineus* and *P. costaricensis* have been reported. Also, plant architectural avoidance has been reported. Both physiological resistance and architectural avoidance are quantitatively inherited with low to moderate heritability and controlled by >30 quantitative trait loci (QTL) distributed across the genome. Also, a single recessive and dominance resistance gene control of white mold resistance has been reported. However, resistance of individual genotype, irrespective of its origin, is inadequate for combating white mold in North America. Little or no effort has been made for pyramiding and introgressing high levels of pyramided white mold resistance into cultivars. Furthermore, the traditional backcross and pedigree methods of breeding with or without use of molecular markers have been inadequate for introgressing high levels of white mold resistance in cultivars. The goal of this research is to systematically pyramid white mold resistance from *Phaseolus* species of the primary and secondary gene pools and introgress the highest levels of pyramided resistance into pinto bean, the largest market class in North America. The specific objectives are to (1) determine complementation or lack thereof among the white mold resistant large- and small-seeded dry and green bean and interspecific breeding lines derived from *P. coccineus* and *P. costaricensis*, and (2) simultaneously pyramid white mold resistance from across *Phaseolus* species and introgress the highest levels of pyramided resistance into pinto bean. We also will discuss progress made thus far in pyramiding and introgressing white mold resistance.

Verification of White Mold Resistance of Dry and Green Common Bean and Interspecific Breeding Lines Derived From *Phaseolus* Species of the Secondary Gene Pool.
White mold reaction of known contemporary resistant large-seeded Andean (A 195, G 122, MO 162, PC 50, VA 19) and small-seeded Middle American (AB 136, ICA Bunsi) dry and green (CORN 501) common bean and interspecific breeding lines (VCW 54, VCW 55, VRW 32, 92BG-7, I9365-25, 0785-127-1, 0785-127-2, 0785-220-1) was verified in two greenhouse environments in Idaho and Colorado. Five large-seeded dry bean genotypes (A 195, G 122, MO 162, PC 50, VA 19) and three small-seeded interspecific breeding lines (VCW 54, 92BG-7, 0785-220-1) derived from *P. coccineus* and one interspecific breeding line (VRW 32) derived from *P. costaricensis* with the highest white mold resistance across greenhouse environments were selected for the complementation study.

Complementation Study of White Mold Resistance.
Five large-seeded dry bean genotypes, namely A 195, G 122, MO 162, PC 50, VA 19 and three white mold resistant interspecific breeding lines derived from *P. coccineus*, namely VCW 54, 92BG-7, 0785-220-1 were crossed within the group. Also, G 122 was crossed with VCW 54. Approximately 50 seeds of each cross were produced. The parents and a part of seed from the F_1 of eight single-crosses were evaluated for their reaction to white mold to determine complementation or lack thereof (preliminary test) and produce the F_2 seed. All five large-seeded Andean dry beans and their four F_1 were resistant to white mold (score of 4 on a 1 to 9 scale, where 1= healthy with no white mold symptoms and 9= severely diseased or dead), indicating that they probably carried similar resistance genes/QTL. While the VCW 54/0785-220-1 F_1 also was resistant, in crosses with 92BG-7 both interspecific breeding lines exhibited a susceptible white mold reaction. Thus, very likely VCW 54 and 0785-220-1 had the same resistance genes/QTL, but both were different from 92BG-7. ICA Bunsi and Cornell 501 would need to be crossed with G 122 and VRW 32 would need to be crossed with VCW 54 and 92BG-7. Also, all parents, F_1, and F_2 will need to be evaluated in a replicated trial to verify and determine the complementation or lack thereof for white mold resistance.

Simultaneously Pyramiding and Introgressing White Mold Resistance into Pinto Bean.
Selected interspecific breeding lines were crossed with partially resistant germplasm from the two gene pools to develop two double-cross populations: Pop I = USPT-WM-1 / CORN 601 // USPT-CBB-1 / 92BG-7 and Pop II = 'Chase' / I9365-25 // ABL 15 / A 195. Eight hundred and forty-one F_1 plants from each double-cross population were subjected to gamete and recurrent selection. The gamete selection was practiced from F_1 to F_4 by selecting white mold resistant single plants followed by progeny testing in the subsequent generation. Two cycles of recurrent selection were practiced by intermating selected white mold resistant plants in each cycle. Thirteen selected families in each method and parents were evaluated at 16, 23, and 33 days post first inoculation in replicated trials in two greenhouse environments. There were higher frequencies of families with lower white mold scores in gamete selection than in recurrent selection. Average genetic gain due to gamete selection was 19.6% and recurrent selection was 7.9%. Mean white mold scores increased from 16 (4.3 for population I and 4.2 for population II) to 33 (5.6 for population I and 5.4 for population II) days post inoculation evaluations. Use of multiple-parent-crosses with parents of diverse evolutionary origins, delayed white mold evaluation, and application of gamete selection are suggested for improving physiological white mold resistance in common bean.

AGRONOMIC AND ECONOMIC ASSESSMENT OF INTENSIVE PEST MANAGEMENT OF EDIBLE DRY BEAN – PART 2. WHITE MOLD EXPERIMENT

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INTRODUCTION: Dry beans compete poorly with weeds and are susceptible to many diseases. As a result, pest management decisions determine a large portion of the yield potential. White mold (*Sclerotinia sclerotiorum* (Lib.) de Bary*)* is a destructive disease in dry beans, resulting in yield losses up to 100% (Tu 1989, and Steadman 1983). White mold epidemiology depends on multiple factors including plant density (Tu, 1997) and weed density (Burnside et al. 1998, Blackshaw et al., 2000).

Thiamethoxam seed treatment is marketed for its insecticidal properties. Additional benefits have been reported regarding plant health, where thiamethoxam has assisted plants to overcome environmental stresses, compared to untreated seed. Environments were selected to evaluate two herbicide programs and three foliar fungicides, for their ability to control weed and white mold disease pressure. In addition, the study investigated the potential of thiamethoxam to counteract these stresses. An economic analysis was done to determine the most profitable pest management strategy. An extensive literature search found no evidence of studies that investigate the interaction of these three factors, or an economic analysis of a similar pest management program.

OBJECTIVE: To investigate the agronomic and economic interactions of a complete pest management program for edible dry beans growers.

METHODS: Field experiments were conducted at the Ridgetown Campus of the University of Guelph, Ridgetown ON, the Huron Research Station, Exeter ON and the Honeywood Research Farm, Plattsville ON in 2007 and 2008, using a split plot RCBD design. Thiamethoxam seed treatment was applied to half the treatments at 50 g/100 kg seed, and the remainder used untreated seed. Two herbicide programs were examined, an economic program consisting of one PPI application of triflualin at 600 g/ha, and a premium program consisting of S-metolachlor at 1144 g/ha plus imazethepyr at 45 g/ha applied PPI. Three white mold foliar fungicides were evaluated, consisting of fluazinam at 500 g/ha, cyprodinil/ fludioxonil at 609 g/ha and boscalid at 539 g/ha. Fungicides were applied at 20-30 percent bloom, and reapplied 2 weeks later. The economic analysis was done by subtracting the pesticide and pesticide application costs from the crop value per hectare to determine the net return on investment.

RESULTS AND DISCUSSION: All data was subjected to analysis of variance, using the PROC MIXED procedure of SAS. Where there was no site by treatment interaction, the data was combined. It should be noted that a wide range in weed and white mold pressure was observed between environments.

Thiamethoxam increased plant vigour in 3 of 12 environments, even though the conditions at planting and emergence were considered ideal for dry bean development. This suggests some potential plant health benefits from the product. Thiamethoxam decreased plant emergence in 3 of

12 environments, but it was determined that this was due to human error. In addition, a decrease in plant vigour was noted in two of the three environments. The early effects of thiamethoxam did not impact the weed or white mold ratings taken later in the season. Thiamethoxam increased seed yield in 3 of 12 environments, and decreased seed yield in 3 environments, compared to untreated seed. The three environments that had a yield decrease did not correspond well to the environments where differences in emergence and vigor were measured.This evidence suggests that any early plant health benefits of thiamethoxam are relatively short lived.

The premium herbicide program reduced total weed ground cover in 12 of 12 environments, compared to the economic herbicide program. The premium program reduced weed ground cover by an average of 20% at 56 days after planting, and increased seed yields in the 8 environments with the highest weed pressure. The premium program reduced plant emergence in 3 of 12 environments, and no explanation is provided for this effect. Plant vigour was not affected by either herbicide program. The premium herbicide program gave increased economic returns, but this was observed only in environments with high weed pressure.

White mold was present in 10 of 12 environments. In 8 of these 10 environments the fungicides reduced white mold severity and increased seed yield, compared to the untreated check. In 4 high disease environments, fluazinam decreased disease pressure and increased economic returns, compared cyprodinil/ fludioxonil and boscalid. The remaining environments showed no economical benefit to fungicide application.

Six environments had moderate to heavey white mold and weed pressure. An interaction between factors was observed in 2 of these 6 environments. White mold severity increased by up to 20% in the economic herbicide program, where the total weed ground cover was higher.

CONCLUSIONS:

- Thiamethoxam had inconsistent effects on emergence, vigor, yield and economic returns.
- The economic herbicide program had higher weed ground cover and white mold severity, and reduced yield and economic returns in high weed pressure environments
- Foliar fungicides reduced white mold severity, increased yield and economic return in environments with moderate to severe white mold disease pressure.
- Fluazinam was superior to cyprodinil/ fludioxonil and boscalid in 50% of the environments with moderate to severe disease pressure.
- An interaction between weed and disease factors occurred in 2 environments. High weed pressure increased white mold severity by up to 20% and reduced yield by up to 20%.

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CHARACTERIZATION OF THE RUST RESISTANCE GENE PRESENT IN THE COMMON BEAN CULTIVAR 'OURO NEGRO', THE MAIN RUST RESISTANCE SOURCE USED IN BRAZIL

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Thirteen rust resistance (RR) genes (*Ur-1* to *Ur-13*) have been identified in the common bean which are named according to the nomenclature proposed by Kelly et al. (1996). In addition to these genes, other important unnamed genes have been identified. This is the case of *Ur-ON* gene which is present in the Mesoamerican cultivar 'Ouro Negro', the main RR source used in Brazil. This black seeded common bean line showed resistance to several isolates of *Uromyces appendiculatus* in Brazil and in the USA (Souza et al. 2008).

Previous works conducted by our research group showed that 'Ouro Negro' possesses single resistance genes to rust and anthracnose located 12.3 cM apart on chromosome 4 – linkage group B4 (Faleiro et al. 2000; Souza et al. 2008). Independence of the anthracnose resistance gene in 'Ouro Negro' has been demonstrated and it was designated *Co-10* (Alzate-Marin et al. 2003). Information is lacking on the independence of the RR gene in this cultivar, which has been temporary named *Ur-OuroNegro* or *Ur-ON*. The main goal of the present work was to characterize the Mesoamerican RR gene *Ur-ON*.

We compared the 'Ouro Negro' RR spectrum with those of other bean lines harboring known RR genes when inoculated with nine selected races of *U. appendiculatus*. In addition, all bean lines have been screened with molecular markers linked to *Ur-ON* aiming to identify additional evidence for the presence of alleles for this locus in the screened RR sources (Table 1). Finally, we tested the allelic relationships of *Ur-ON* with RR genes already characterized from lines resistant to at least one race of the pathogen. We also accomplished allelism tests between 'Ouro Negro' and 'CNC' and 'CSW 643', important RR sources in Brazil harboring unnamed RR genes (Table 2).

The results showed that the major dominant gene conditioning RR in 'Ouro Negro' is positioned at a locus distinct from those with which it was compared. We propose this gene – or complex gene locus – is unique and be designated *Ur-14*.

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Table 1. Differential reactions of common bean rust resistance (RR) sources to selected races of *Uromyces appendiculatus* and presence/absence of DNA markers linked to the 'Ouro Negro' RR gene (*Ur-ON*) in these lines.

Cultivar	Gene	*U. appendiculatus* race (isolate)[a]									DNA marker[b]		
		21-3	29-3	29-15	53-3	53-7	53-19	61-3	63-3	63-19	OPX11	SBA08	SF10
AxS 37	*Ur-2*^{2}	R	R	R	R	R	-	R	R	R	0	1	1
Aurora	*Ur-3*	S	S	S	S	S	S	S	S	S	0	0	0
Ecuador 299	*Ur-3*$^+$	R	R	S	R	R	-	R	R	S	0	0	0
Mexico 235	*Ur-3*$^+$	R	R	S	R	R	R	R	R	R	0	1	0
Early Gallatin	*Ur-4*	S	S	S	S	S	S	S	S	S	0	0	0
Brown Beauty	*Ur-4*	R	R	R	R	R	-	R	R	R	0	0	0
Mexico 309	*Ur-5*	R	R	S	R	S	R	R	R	R	0	1	1
Golden Gate Wax	*Ur-6*	S	S	S	S	S	S	S	S	S	0	0	1
Pinto Olathe	*Ur-6*$^+$	S	S	S	S	S	-	S	S	S	0	0	0
GN 1140	*Ur-7*	S	S	S	S	S	S	S	S	S	0	0	0
U.S. #3	*Ur-8*	-	S	S	S	S	-	S	S	S	0	0	1
PC-50	*Ur-9*	R	S	S	R	R	R	S	S	S	0	0	1
Resisto	*Ur-10*	S	S	S	S	S	S	S	S	S	0	0	0
PI181996	*Ur-11*	R	R	R	R	R	R	R	R	R	0	0	0
Redlands Pioneer	*Ur-13*	R	R	R	R	R	R	R	S	S	0	0	0
CNC	*Ur-?*	R	R	R	R	R	S	R	R	S	0	0	1
CSW 643	*Ur-?*	R	R	R	R	R	-	R	R	R	0	1	0
US Pinto 111[c]	-	S	S	S	S	S	S	S	S	S	0	0	0
Ouro Negro	*Ur-ON*	R	R	R	R	R	R	R	R	R	1	1	1

[a] Resistant (R) or susceptible (S) reaction; not available (-). [b] Presence (1) or absence (0) of DNA marker. [c] Susceptible control cultivar.

Table 2. Crosses and races of *Uromyces appendiculatus* used for characterization of the RR gene (*Ur-ON*) present in the common bean cultivar 'Ouro Negro' (ON)

Cross	Tested locus	Studied population	Race	No. of plants	Expected ratio (R:S)[a]	Observed ratio (R:S)[a]	χ^2	*P*(%)[b]
US Pinto 111 × ON[c]	*Ur-ON*	F_2	Multiple[d]	303	3:1	224:79	0.1859	66.63
US Pinto 111 × ON[c]	*Ur-ON*	$F_{2:3}$	Multiple[d]	303	1:2:1	66:155:82	1.8514	39.62
Ruda × ON[c]	*Ur-ON*	RILs	61-3	152	1:1	80:72	0.4210	51.64
Golden Gate Wax × ON	*Ur-ON*	F_2	29-3	217	3:1	165:52	0.1244	72.43
AxS 37 × ON	*Ur-2*^{2} × *Ur-ON*	F_2	63-3	125	57:7	114:11	0.5862	44.38
Ecuador 299 × ON	*Ur-3*$^+$ × *Ur-ON*	F_2	21-3	142	15:1	134:8	0.0920	76.16
Mexico 235 × ON	*Ur-3*$^+$ × *Ur-ON*	F_2	63-3	81	15:1	75:6	0.1851	66.69
Brown Beauty × ON	*Ur-4* × *Ur-ON*	F_2	63-3	128	15:1	119:9	0.1333	71.50
Mexico 309 × ON	*Ur-5* × *Ur-ON*	F_2	29-3	208	15:1	193:15	0.3282	56.67
PC-50 × ON	*Ur-9* × *Ur-ON*	F_2	21-3	297	57:7	263:34	0.0793	77.81
BelMiDak RR3 × ON	*Ur-11* × *Ur-ON*	F_2	29-3	64	15:1	60:4	0.0000	100.00
ON × PI181996	*Ur-11* × *Ur-ON*	F_2	29-3	49	15:1	46:3	0.0013	97.06
Redlands Pioneer × ON	*Ur-13* × *Ur-ON*	F_3	29-15	335	55:9	285:50	0.2063	64.96
CNC × ON	*Ur-?* × *Ur-ON*	F_2	63-19	163	63:1	160:3	0.0818	77.47
CSW 643 × ON	*Ur-?* × *Ur-ON*	F_2	63-19	177	15:1	166:11	0.0003	98.45

[a] Resistant (R) or susceptible (S) reaction. [b] Percent probability (*P*) of the Chi-square (χ^2) test; α=5%. [c] Inheritance studies previously conducted by our research group.

[d] Mixture of spores of races 29-3, 53-3, 61-3, and 63-19.

EVALUATION OF SNAP BEAN GENOTYPES COMBINING RUST RESISTANCE AND HEAT TOLERANCE TRAITS IN EAST AFRICA

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INTRODUCTION

The major biotic and abiotic constraints to snap bean production in East Africa include diseases such as common bean rust (caused by *Uromyces appendiculatus*) and high ambient temperature that causes heat stress (CIAT 2008; Kelly 2004; Wortmann et al. 1998). Cultivation of rust susceptible and heat sensitive cultivars exacerbate yield loss associated with the two constraints. Most snap bean cultivars grown in East Africa are very susceptible to rust (Hillocks et al., 2006; CIAT, 2008). Rust resistant snap beans that perform well under both cool and hot agro ecological conditions are needed to increase production in this region. The objective of the present study was to evaluate at East African field sites selected snap bean breeding lines with rust resistance and heat tolerance traits combined in the same genetic background and to identify lines to be utilized in the genetic improvement of cultivars presently grown in East Africa.

MATERIALS AND METHODS

Four snap bean breeding lines were developed, – three combining common bean rust genes *Ur4* and *Ur 11* with heat tolerance ((601BJ) L9, (BF601)L4, and (BF611)11) and one breeding line with the two rust resistant genes in a heat sensitive type as a control ((601BJ)L4) which were evaluated in 2009 alongside 12 commercial snap bean cultivars. The cultivars evaluated (Table 1) included types targeted for South Africa, East Africa, North Africa and the US in small and large sieve types. Entries were evaluated at six sites in East Africa. The sites in Kenya were: Homabay, Kibos, Maseno, Sabatia and Kitale while that in Tanzania was located at Arusha. The sites differed in soils, altitude and climate. Homabay was the hottest of the sites while Arusha was the coolest. Rainfall at the sites followed a similar pattern commencing in late March increasing in intensity in April and May and reducing in June. The 16 entries were grown in a randomized complete block design with four replications. Planting was done at the onset of the 2009 long rains. The entries were scored for the common bean rust at flowering and at pod filling stages. Pod yield data was also collected.

RESULTS AND DISCUSSION

High rust incidence and severity was observed at Arusha, Homabay and Kitale sites. Similar trends in rust incidence and severity on the 16 genotypes were observed across the three sites and genotypes significantly differed in terms of reaction to rust. Three of the four breeding lines: (BF601)L4, (601BJ)L4, and (601BJ)L9 which had been selected for the combination of *Ur-4* and *Ur-11* rust resistance genes, had no visible rust symptoms at all three sites, while (BF611)L11 segregated for one of the two rust genes. Of the commercial cultivars only PV698 and PV712 were consistently rust resistant at all the three sites, the remainder were either susceptible or only partially resistant. The breeding lines (601BJ)L4 and (601BJ)L9 were the highest yielding among genotypes tested while PV712 and Palati had the lowest yields across the three sites. Compared to the cultivars

presently grown in East Africa, the newly developed breeding lines had insignificant variation in yield between sites despite contrasting growth temperatures indicating that they were tolerant to higher temperatures at the lower altitude sites (Table 1).

Table 1. Yield of 16 snap bean genotypes at field sites in East Africa.

Genotype*	Yield, pods plant^{-1}			
	Arusha	**Homabay**	**Kitale**	**All sites**
(601BJ)L4	24.4 b-e	27.4 ab	29.3 a-c	27.0 ab
(601BJ)L9	26.0 bc	31.4 a	30.4 a-c	29.3 a
(BF601)L4	24.1 b-e	26.0 a-c	25.9 a-d	25.3 b-d
(BF611)L11	21.2 d-f	24.8 bc	23.3 cd	23.1 c-f
Amy	23.6 c-e	20.3 c-e	29.5 a-c	24.5 b-e
Barrier	21.4 c-f	25.0 bc	24.4 b-d	23.6 b-e
Brio	17.0 f	26.5 ab	31.7 ab	25.0 b-e
Bronco	20.7 ef	27.1 ab	32.1 ab	26.6 a-c
Hystyle	21.0 ef	28.5 ab	19.4 d	22.9 d-f
PV 712	25.7 b-d	11.3 f	22.6 cd	19.9 f
Juliet	28.5 ab	23.5 b-d	27.0 a-d	26.3 a-d
Masai	21.9 c-e	17.7 de	32.2 ab	23.9 b-e
Opus	22.7 c-e	28.8 ab	28.3 a-c	26.6 a-d
Palati	22.9 c-e	18.0 de	23.5 cd	21.5 ef
Teresa	24.5 b-e	23.0 b-d	32.9 a	26.8 ab
PV 698	32.4 a	16.9ef	29.5 a-c	26.2 a-d

**Within a site/column, means followed by the same letter are not significantly different*.

Concurrent selection of the breeding lines for rust resistance and heat tolerance conferred yield stability in their performance at the three rust infected sites that also differed in ambient temperatures. The commercial cultivars which are presently grown or are targeted for production in the East African region were highly sensitive to temperature variations at the sites even though some of them exhibited resistance to the local rust races. The responses of the breeding lines and the commercial cultivars indicate that genetic improvement for heat tolerance may contribute expanded production at lower altitudes. There was also significant rust pressure at the sites including those at lower altitudes highlighting the contribution that a combination of rust resistance and heat tolerance would make in snap bean cultivars targeted for the East African region. The breeding lines BF601)L4, (601BJ)L4, and (601BJ)L9 have potential utility in genetic improvement of snap bean cultivars for East Africa and other tropical regions.

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CURRENT STATUS OF *UROMYCES APPENDICULATUS* IN BULGARIA

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Common bean rust, caused by hypervariable fungal pathogen *Uromyces appendiculatus (*Pers.:Pers.) Unger limits common bean (*Phaseolus vulgaris* L.) production worldwide. Bean rust in Bulgaria was reported for the first time by Kovachevski in 1930. The disease occurs annually in the Rhodoppi Mauntains and has sporadic occurrence in plain regions. Until 2004, 10 physiological races had been determined in Bulgaria (Kiryakov and Genchev, 2003; Kiryakov, 2004). The objectives of this study were to identify: 1) the virulence variability of the pathogen in Bulgaria; 2) the reaction of bean cultivars and lines to the pathogen under field conditions; 3) the genetic control of resistance to bean rust in a Bulgarian cultivar 'Beslet'.

MATERIALS AND METHODS: The virulence variability of bean rust was studied under greenhouse during 2007-2009. Bean rust populations were collected from five locations in Rhodoppi mountain and four locations in North Bulgaria. One hundred and ten single-uredinium isolates were made from the collections and their virulence phenotype were determined by inoculation the standard set of 12 differential cultivars proposed by Steadman *et.al*.(2002). Resistance of 379 accessions (including *Ph. vulgaris, Ph. coccineus, Ph. lunatus, Ph. accutifolius*) was studied under field conditions against collected rust populations in artificial infection background during 2007-2008. The rust intensity at the first and the last assessment for each accession was used to calculate the area under disease progress curve (AUDPC) individually and according to the adjacent susceptible check (rAUDPC). To identify genetic control of rust resistance in cultivar Beslet five crosses were made: Beslet (Resistant)/Dobrudjanski ran (Susceptible); Dobrudjanski ran/Beslet; Early Gallatin (*Ur-4;* R)/Beslet; Redland Pioneer (*Ur-13*; R)/Beslet and CNC (*Ur-CNC*; R)/Beslet. F1, F2 plants and F3 families of these crosses were evaluated for reaction to race 20-1 (pathotype 74).

RESULTS AND DISCUSSON: Ninety pathotypes have been identified which have to be referred to nine physiological races: 20-0, 20-1, 20-2, 20-3, 20-19, 28-1, 52-3 (Beleva and Kiryakov, 2009) and 29-0 and 29-1 of bean rust pathogen. The pathotypes of race 20-0 was the most frequently observed followed by pathotypes of races 20-3 and 20-2 (Table 1). Thirty two pathotypes (races 20-0 and 29-0) overcame only specific resistance genes in the Andean gene pool and had to be referred to the Andean-specific pathotypes of *U. appendiculatus.* The rest of pathotypes had virulence phenotype typical for the group of Andean-Middle American isolates of the rust pathogen. The pathogen showed higher virulence variability in the mountain areas than in the plain areas of Bulgaria. Only two of the identified races were observed in both plain and mountain areas (20-2 and 20-3) and one pathotype of race 20-3 was identified in Bostina and North Bulgaria. Therefore we suppose that the pathogen overwintered and made sexual recombination annually in the Rhodoppi mountain and was spread by wind to the other regions of Bulgaria. Rhodoppi mauntain is probably the center of diversity of *U. appendiculatus* for the South Balkan peninsula in Europe.

Forty eight *P. vulgaris* accessions, five accessions *P. accutifolius* and one accession *P. lunatus* were immune to the bean rust pathogen under field conditions. Twenty-two common bean accessions showed high level of partial resistance. The rust intensity of these cultivars was up to 10 % and rAUDPC values were lower than 0.333. Eight of the accessions had high level of pubescence. In five of them the pustules on the upper leaves had smaller infection type than on the primary and first trifoliate leaf. Simultaneously, smaller pustules were also observed on accessions with low trichome density, which was an indication that the pubescence is not the only one mechanism of partial resistance.

Variety 'Beslet' (HR 45//Sataya425/Trudovetz) is resistant to bacterial blight, halo blight and antracnose. 'Beslet' has upright IIa plant habit type, navy seed type, 100 seed weight 20g, immune to bean rust under field condition and resistant to the 90 pathotypes under greenhouse (Table 2). F_1 plants of all crosses were resistant to race 20-1. The results from F2 generations of Beslet/Dobrudjanski ran and Dobrudjanski ran/Beslet fit a 3(R_): 1(rr) segregation ratio[χ^2= 0.3936; χ^2= 0.2865, respectively] and F3 families 1RR:2Rr:1rr - segregation ratio [χ^2= 1.2857, a χ^2= 1.9669, respectively] indicating that the resistance of variety "Beslet" to race 20-1 of the bean rust pathogen is determined by a single dominant gene. The segregation ratio of F2 generation of Early Gallatin/Beslet, Redland Pioneer/Beslet and CNC/Beslet was 15R_:1rr [χ^2 =0.284; χ^2 =0.3061 and χ^2 =1.0051, respectively] indicating that the gene or complex gene locus present in Beslet does not correspond to genes *Ur-4*, *Ur-13* and *Ur-CNC*. The resistance of 'Beslet' to the ninety pathotypes of the nine races identified in Bulgaria up to now supposed that the resistant race-specific gene in the cultivar is not identical to genes *Ur-3* (Aurora), *Ur-6* (Golden Gate Wax), *Ur-7* (Great Northern 1140), *Ur-9* (Pompadour Checa 50), *Ur-260* (PI 260418) and the resistance gene unidentified in cultivar Montcalm. The results from the allelism test with 'Early Gallatin' and 'CNC' confirmed this hypothesis. Additional crosses must be made to determine allelic relationship between this gene and genes *Ur-3+ (*Mexico 235), *Ur-5 (*Mexico 309), *Ur-11* (PI 181996) and other named genes *Ur-BAC 6* (BAC 6), *Ur-Dorado-53* (Dorado), *Ur-Dorado-108* (Dorado), *Ur-ON* (Ouro Negro). For the time being we named this gene *Ur-Beslet.*

Table 1. Distribution and virulence potential of 90 pathotypes and 9 races of *Uromyces appendiculatus* in Bulgaria

Location/Number of pathotypes						Number of pathotypes	Race	Differential set											
								Andean						Middle American					
North Bulgaria	Devin	Smilyan	Rakitovo	Bostina	Kostandovo			Early Gallatin	Redland Pioneer	Montcalm	PC 50	GGW	PI 260418	GN 1140	Aurora	Mexico 309	Mexico 235	CNC	PI 181996
-	7	10	7	3	2	25	20-0	-	-	+	-	+	-	-	-	-	-	-	-
3	7	7	5	2	-	22	20-3	-	-	+	-	+	-	+	+	-	-	-	-
4	2	2	7	-	-	15	20-2	-	-	+	-	+	-	-	+	-	-	-	-
-	2	-	2	3	-	7	29-0	+	-	+	+	+	-	-	-	-	-	-	-
-	4	-	2	2	-	7	20-1	-	-	+	-	+	-	+	-	-	-	-	-
-	-	2	4	1	-	7	29-1	+	-	+	+	+	-	+	-	-	-	-	-
-	3	-	-	-	-	3	20-19	-	-	+	-	+	-	+	+	-	-	+	-
-	1	-	-	-	-	1	28-1	-	-	+	+	+	-	+	-	-	-	-	-
-	-	3	-	-	-	3	52-3	-	-	+	-	+	+	+	+	-	-	-	-

Table 2. Reaction of the differential set (summary) and variety Beslet to 90 pathotypes of *Uromyces appendiculatus* identified in Bulgaria

Differential set	Andean						Middle American					
Ur-gene	*4*	*13*	*-*	*9*	*6*	*260*	*7*	*3*	*5*	*3+*	*CNC*	*11*
Differential set	S*	R	S	S	S	S	S	S	R	R	S	R
Beslet	R	R	R	R	R	R	R	R	R	R	R	-
Possible gene	No	No	No	No	No	No	No	No	?	?	No	?

***** S – Susceptible; R - Resistant

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REACTION OF COMMON BEAN CULTIVARS TO TWO NEW RACES OF RUST PATHOGEN FROM MICHIGAN AND NORTH DAKOTA

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INTRODUCTION

Two similar but not identical races of the bean rust pathogen (*Uromyces appendiculatus*) were discovered in Michigan (race 22-3) and North Dakota (race 20-3) in 2007 and 2008, respectively (Markell et al, 2009, Wright el al, 2009). The objective of this study was to evaluate the reaction of 70 U.S. dry bean cultivars in various U.S. market classes to the two new races from MI and ND and to other selected races of *U. appendiculatus.*

MATERIALS AND METHODS

Seed of dry bean cultivars was provided by bean scientists from U.S. state universities, industry, and government. Published methods were used for sowing seeds, inoculum preparation, and inoculation of beans. Twelve plants per cultivar were inoculated with each race. Checks were included in all inoculations. Cultivars with resistance to at least one of these races were subsequently inoculated with some or all races 41, 44, 47, 53, 67, 73 and 108 used in the identification of rust resistance genes *Ur-3*, *Ur-4*, *Ur-6*, and *Ur-11*.

RESULTS AND DISCUSSION

Fifty four of the 70 (77%) cultivars were susceptible to the new MI and ND races: Pintos: Baja, Durango, La Paz, Maverick, Santa Fe, Stampede, Lariat, Topaz, P239222, PT7-8, 06UI17, 06UI19, GTS2828, GTS2824, GTS903, GTS904, CO33875, CO34142, CO54150, CO55119, C055646, C055658, Croissant; Navy: Navigator, Ensign, Vista, Seabiskit, 5027557, N5039540, N252185, 1054N, T10601, T9905, ND012103, N05324; Blacks: Eclipse, T-39, B210237, B201240, B5019630; MI cultivars: Beluga, Chinook, Red Hawk, Capri, Matterhorn, Sedona, Merlot, B05055, B07863, Condor, Jaguar, Tacana, Zorro; and ND 307. The reaction of these cultivars to both races was identical to that of the rust differential cultivar Aurora (*Ur-3)* and Golden Gate Wax (Ur-6), and of cultivars such as CO 33875 that combine *Ur-3* and *Ur-6*. Several of these 54 susceptible cultivars are known to have *Ur-3* rust resistance gene, while other cultivars appear to lack rust resistance genes. Ten (14%) of the 70 cultivars were resistant to both races and six (9%) cultivars were resistant to the MI race but susceptible to the race from ND (Table 1). Most of the cultivars with resistance to both races were inoculated with two or more races (41, 44, 47, 49, 53, 67, 73, and 108) used in the identification of rust resistance genes *Ur-3*, *Ur-4*, *Ur-6*, and *Ur-11* (Table 1). The results from these inoculations suggest that some cultivars have one of the broadly *Ur-5* or *Ur-11* rust resistant genes. Among these are CO 24972 and CO 29258 (which are the progeny of BelDakMi-RR-3 with *Ur-6* and *Ur-11*), as well as Montrose and Rosa Nativa which are the progeny of BelNeb-RR-1 with *Ur-5*, *Ur-6* and U*r-7*. Other cultivars, such as Norstar, Buster, Olathe, pinto 813 and the rust differential cultivar GN 1140 (*Ur-7*, were resistant to the MI race but susceptible to the ND race.

These results suggest that perhaps at least some of these cultivars, such as Buster, which was derived from BelNeb-RR-1, may also have the *Ur-7* rust resistance gene.

One of the most interesting and useful results of this study was the discovery that the pinto cultivar Stampede, derived from BelDalMi-RMR-14 with *Ur-3*, *Ur-6*, and *Ur-11*, was comprised of at least two different populations. One population, called here Stampede, has only the *Ur-3* rust resistance gene. This population was susceptible to races from MI and ND. The population, called here Stampede-R, was resistant to the MI and ND races as well as to other races (see Reaction of Stampede –R in Table 1), suggesting that Stampede-R has the *Ur-3* rust resistance gene in combination with the *Ur-11* gene or *Ur-3* combined with *Ur-6* and *Ur-11*.

TABLE 1. Checks and other bean cultivars with resistance to new races of the rust pathogen from Michigan (MI) and North Dakota (ND) and to other selected races of the bean rust pathogen used in the identification of rust resistance genes.

Cultivars	Races of the Bean Rust Pathogen [a]Reaction Grade and Disease Category						
	MI	**ND**	**47**	**49**	**53**	**67**	**108**
[*]**Aurora (Ur-3)**	4,5 S	4,5 S	5,6 S	5,4 S	2,2+ R	4,5 S	2 R
E. Gallatin (Ur-4)	2,2^{+} R	2 R	5,4 R	2,2+ R	4,5 S	5 S	2 R
Mexico 309 (Ur-5)	f2,3 R	f2,3 R	3,f2 R	5,4 S	f2,3 R	5,6 S	4,5 S
Great N. 1140 (Ur-7)	3,f2 R	5,4 S	4,5 S	3,f2 R	4,5 S	3 R	3 R
PI 1818996 (Ur-11)	f2 R	f2 R	f2 R	f2 R	f2 R	f2 R	5,4 S
Redl. Pioneer (Ur-13)	4,5 S	3,f2 R	4,5 S	5,4 S	4,5 S	5 S	4,5 S
Stampede-R	3,f2 R	3,f2 R	3,f2 R	3,f2 R	2 R	3,f2 R	2 R
Montrose	f2 R	f2,3 S	f2 R	3,f2 R	f2,3 R	f2,3 R	f2,3 R
Pink Floyd	3 R	3 R	2 R	2 R	2 R	3,f2 R	3 R
P35161	f2,3 R	3,f2 R	5,4 S	4,5 R	2 R	3 R	2 R
CO 24972	2 R	2 R	f2 R	2 R	2 R	2 R	5,4 S
CO 29258	2 R	2 R	f2 R	2 R	2 R	2 R	5,4 S
NO5324	2 R	2 R	f2,2 R	f2,3 R	f2,2 R	2 R	5 S
B315039	3,f2 R	3 R	4,5 S	NE	NE	3 R	3 R
Rosa Nativa	f2,3 S	f2,3 S	NE	NE	NE	3,f2 R	f2,3 R
115 M	3 R	3 R	NE	NE	NE	5 R	5 R
P 813	3,f2 R	4.5 S	f2,3 R	f2,3 R	2 R	3 R	2 R
P 0868	3,f2 R	4,5 S	NE	NE	NE	NE	NE
Norstar	3 R	4,5,6 S	4,5 S	NE	NE	3 R	2 R
Buster	3,f2 R	4,5 S	2 R	NE	NE	3,f2 R	2 R
Olathe	3 R	4 S	2 R	3,2 R	5,6 S	f2.3 R	3,f2 R
Maria	3,f2 R	5,4,6 S	3,f2	3,f2	2 R	3 R	2 R

[a]**Reaction grade and Disease Category**: 2, 2^{+} = Necrotic spots (HR), no sporulation - Resistant; f2 = faint spots – R; 3 = Tiny sporulating pustules (diameter less than 0.3mm) – R; 4, 5, 6 = Large pustules – S.

[*]**Check cultivars:** Aurora, Early Gallatin, Great Northern 1140, PI 181996, and Redlands Pioneer.

THE CONNECTIONS BETWEEN SOIL HEALTH AND ROOT HEALTH

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INTRODUCTION

Intensive production of agronomic crops in New York State and the Northeast has contributed to a gradual overall deterioration in soil health and function and resulted in reduced crop productivity and perceived farm profitability. Signs of unhealthy soils include erosion, compaction, surface crusting, low organic matter content, poor nutrient cycling, and increased damage from diseases, parasitic-nematodes, weeds, and other pests. In addition, growers and extension educators have become aware and concerned with lower soil productivity that no longer can be compensated by increasing production inputs (fertilizers, pesticides, tillage intensity, etc). Thus, there is a great interest by growers and other land managers in assessing health status of their soils and in the implementation of sustainable soil management practices. As a result, numerous conferences and symposia have been organized to discuss soil health issues and to identify practical solutions. The latter also resulted in numerous publications on defining soil health, methods for assessing soil health status and identifications of major constraints, soil processes impacted, and possible management practices (Doran et al., 1994; Doran and Jones, 1996; Magdoff and van Es, 2000). The emerging concept of soil health deals with integrating and optimizing the soil physical, chemical and biological properties for improving soil functions and crop productivity in a sustainable and environmentally friendly manner. Soil health management practices consist of numerous modifications and combinations of reduced tillage systems, crop rotations, cover crops and/or soil amendments. Individually or in combinations, these practices significantly impact the soil physical, chemical and/or biological properties, thus soil health in general. Accordingly, soil health assessment and management is a holistic, log-term, and whole-farm process.

The Cornell Soil Health Program Work Team (PWT)

The Soil Health PWT at Cornell was established in 2003 to address soil health issues, develops a cost-effective assessment protocol, develops practical solutions to identified constraints, and provide the needed educational program for their implementation. The team consists of growers, extension educators, multi-disciplinary faculty and staff as well as other interested agricultural service providers. Our team has made significant progress in increasing awareness of soil health issues (Gugino et al., 2007), developing a cost-effective protocol for assessing soil health status (Idowu et al., 2008), facilitated on-farm soil health demonstrations by interested growers, promoting multi-disciplinary research and outreach, establishing a new 14-acre, long-term soil health site at the Gates farm of the NYSAES, and establishing a soil health website (http://soilhealth.cals.cornell.edu).

The Cornell Soil Health assessment Protocol/Test (CSHT)

Currently, soil health status can only be assessed indirectly by measuring a set of soil quality indicators. Initially, 39 potential soil properties were measured on large number of samples collected from long-term, replicated research sites; commercial production fields; and other sites throughout New York State. The developed CSHT consists of measuring four physical, four biological and 7 chemical indicators. Also, the textural composition of the soil sample is determined and provided in the report. The selection of the indicators was based on their sensitivity to management practices, precision of the method, relevance to important functional soil processes, ease and cost of sampling,

and cost of analysis (Moebius-Clune, et al., 2007). The results of the soil health analyses of each submitted sample are presented in a visually enhanced format in the CSHT report, which is color coded to aid practitioners in targeting their management practices to identified soil health constraints. Also, a set of short-term and long-term soil management practices are suggested as guidelines for consideration in improving identified constraints. Detailed information on soil health sampling, indicator assessment procedures, development of scoring functions, interpretation of the auto-generated report and general management recommendations can be found in the Cornell Soil health Training Manual, which can be downloaded from the website.

Connections between Soil Health and Root Health

All soil health management practices directly or indirectly impact populations of root pathogens, severity of root diseases, and often the quality and quantity of marketable yield (Abawi and Widmer, 2000; Widmer, et al., 2002). In addition, root pathogens are most damaging to beans and other agronomic crops in poor quality soils. Thus, root health assessment is a good indicator of overall soil health status, as roots are influenced greatly by soil physical, chemical and biological properties. In contrast, improving soil health will result in healthier roots and reduced damage caused by root pathogens. Roots growing in healthy soils are generally large, coarse, white, penetrate deeper into soil, have large number of fine fibrous rootlets, and exhibit limited or no symptoms of damage by root pathogens. Accordingly, the challenge is to implement soil health management practices that are also suppressive to root pathogens as well as other pests. The latter requires the diagnosis and frequent monitoring of root pathogens and other pests and to implement new or modify current practices on as needed basis.

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DRY EDIBLE BEAN PATHOGENS PREVALENT IN NORTH DAKOTA WITH SPECIAL EMPHASIS ON ROOT ROTS

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Diseases have been a primary concern in dry bean production areas in mid-western United States where more than fifty percent of the acreage is concentrated. Surveys conducted over the past two years have shown that white mold, bacterial blight, rust and root rots are currently the most prevalent diseases in the state (Goswami et al., 2009). Grower surveys conducted by NDSU researchers in 2008 also supported these findings. Among these diseases root rots have been a yield-limiting problem for growers in the Northarvest area for several years (Knodel et al., 2008). This disease is known to be caused by a complex of pathogens, including *Fusarium solani* f.sp. *phaseoli*, *Rhizoctonia solani* and *Pythium* species. In North Dakota and Minnesota, *Fusarium solani* was considered to be the most common causal agent of root rot followed by *Rhizoctonia solani.* However, comprehensive surveys of root pathogens affecting dry bean has not been conducted in the recent past. Therefore, the objectives of this study were to assess the prevalence of root rots in the dry bean growing counties of North Dakota and to identify the major pathogens associated with this disease in this region.

MATERIALS AND METHODS

Samples were collected from 39 fields in 2008 and 45 fields in 2009 located in the Grand Forks, Pembina, Steele, Trail, Walsh counties in ND. The roots were washed in running water and rated for disease severity using a modified 1-7 rating scale based on discoloration (adapted from Schneider and Kelly, 2000). Infected roots were plated with and without surface sterilization on Potato Dextrose agar (PDA) for 7-8 days. After 7-8 days, mixed cultures of fungal species were obtained. Fungal colonies were separated by sub-culturing and mono-sporic cultures were established. Species were identified by morphological characteristics and DNA sequencing. Morphological characteristics evaluated included fungal growth, color, texture, mycelium and spores. Selected isolates from each morphologically identical group were used for molecular identification. DNA was extracted from 7-8 days old fungal mycelium grown in potato dextrose broth using a DNeasy™ Plant Tissue mini kit (Qiagen, Valencia, CA). Polymerase Chain Reaction (PCR) was carried out to amplify a portion of the Translation elongation factor I alpha (TEF) gene region from potential isolates belonging to *Fusarium* species and the Internal Transcribed Spacer (ITS) region from other fungal species. Molecular identification was based on comparison of our sequences with those in publicly available databases such as GenBank and Fusarium-ID (Geiser *et al.,* 2004). Pathogenicity tests for all the *Fusarium* species isolated from dry beans was initially conducted by direct inoculation of germinating seeds of the root rot susceptible kidney bean variety "Montcalm" in petri-dishes. The method involved placing mycelial plugs from 7 day old fungal cultures grown on PDA on the hypocotyl of pre-germinated seeds, incubating them in sealed petri-dishes at room temperature under light and dark cycles of 12 hr each, removal of plugs after seven days and scoring presence or absence of lesions. The pathogen was reisolated from the host tissue to establish Koch's postulates. Additional, growth chamber trials were conducted to assess the ability of *Fusarium* species that had not been reported on dry beans to infect this crop. The sand cornmeal inoculation layer method was followed to evaluate pathogenicity of fungal isolates (Bilgi *et al.* 2008). Six pots

of each isolate were planted and kept in growth chamber under controlled conditions. The plants were evaluated after 18 days using the same scale mentioned above. Each experiment was repeated three times under similar conditions.

RESULTS AND DISCUSSION

Almost all the fields surveyed appeared to be affected by root rot though the disease severity varied and ranged from a few roots with small lesions to more than 95% roots being discolored (a rating of 2 to 5 on a 1-7 rating scale). The pathogens isolated primarily included *Fusarium* species followed by a small number of *Rhizoctonia solani* isolates. *Fusarium* species isolated included *F. oxysporum* (primarily known as a wilt pathogen that can potentially cause root rot) which accounted for more than half of the isolates. Among the species known to be associated with root rots *F. solani, F. graminearum, F. sporotrichiodes, F. acuminatum* and *F. redolens* were isolated with their prevalence being in the same order. All these species were found to be pathogenic on the kidney beans cultivar 'Montcalm'. In greenhouse trials some isolates of *F. graminearum* and *F. acuminatum* were found to cause more disease than an aggressive isolate of *F. solani*. Several of these species, including *F. graminearum, F. sporotrichoides* and *F. acuminatum* are toxigenic in nature and are known to affect cereals. Therefore, in addition to demonstrating a possible expansion in the range of *Fusarium* species associated with root rots of dry beans, these findings also suggest that *Fusarium* species from cereals may prove to be a potential threat to dry bean production under rotations.

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EVALUATION OF LIMA BEAN LANDRACES FROM PUERTO RICO

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The Lima bean (*Phaseolus lunatus* L.) is a heat and drought tolerant grain legume crop that is produced and consumed throughout the Caribbean. Most landrace varieties in Puerto Rico are indeterminate plants that produce pods during the dry season. Because Lima beans grow well in fence rows or on walls, the crop is well suited for urban agriculture. Lima bean landraces have been cultivated in the Caribbean for at least 500 years and have unique traits of economic importance. For example, the Lima bean accession L-136 from Puerto Rico was used as a source of root knot nematode resistance in the development of the cultivar 'Cariblanco N' (Helms et al., 2004). Montgomery (1965) noted that the highest HCN concentration in *P. lunatus* seed was found in a black-seeded line from Puerto Rico. A Lima bean landrace variety from Puerto Rico was used to study anti-A1 hemagglutinating activity (Schertz et al., 1960). Unfortunately, the USDA and CIAT bean germplasm collections contain very few accessions from the region. These collections currently have 2 accessions from Haiti, ≤ 3 accessions from Puerto Rico and no accessions from the Dominican Republic. Fifteen landrace varieties of Lima beans, collected from different locations in Puerto Rico, were planted at the Isabela Substation in October 2008, and in Honduras and Delaware in June 2009. Morphological and agronomic traits of the landrace varieties were evaluated during the growing season and compared with the 'Sieva' and 'Christmas' Lima varieties from Seed Savers' Exchange (Table 1). Although most of the varieties were collected within a few kilometers from the Isabela Substation, a wide range in seed types were observed among the landrace varieties. Seed size and altitude of cultivation of landraces from Puerto Rico are consistent with the values reported by Gutiérrez Salgado et al. (1995) for Lima beans of the Middle American gene pool. All of the varieties had an indeterminate growth habit whereas there were differences among varieties for leaf and pod shape. Days to flowering of the landrace varieties ranged in Puerto Rico from 46 to 100 days after planting (DAP). When planted in Honduras in June, four landraces (PL08-01, PL08-02, PL08-03 and PL-08-18) flowered < 60 days after planting, suggesting that these varieties could be planted in the tropics throughout the year. When planted in Delaware in June, the earliest landrace varieties (PL08-01, PL08-02 and PL08-03) flowered at 81 days after planting versus 35 for 'Sieva' and 47 days for 'Christmas'. The mean number of seed per pod in Puerto Rico ranged from 2.7 to 3.4. Mean seed yield per plant during the first 180 days of the growing season in Puerto Rico ranged from 149 g to 1475 g. PL-08-1 and PL-08-2 were the highest-yielding lines in Puerto Rico and Honduras. Only four of the landraces had seed HCN concentrations < 100 ppm which is the maximum concentration recommended for Lima bean varieties released in the U.S. Leaf concentrations of HCN ranged from 200 to 800 ppm. PL-08-14 was the only landrace variety that had early flowering in Puerto Rico (51 DAP) and Honduras (71 DAP) and a seed HCN concentration < 100 ppm. Seed of the landraces from Puerto Rico will be sent to the USDA germplasm collection and the most promising line may be considered for release in Puerto Rico as a variety.

Table 1. Morphological characteristics and HCN concentrations in the seed and leaves of fifteen Lima bean landrace varieties from Puerto Rico and two cultivars from the U.S.

Identity	Source	Seed type[1]	100 seed weight (g)	Leaf shape	Pod shape	Seed HCN conc. (ppm)	Leaf HCN conc. (ppm)	Days from planting to flowering at 3 test sites (planting month)		
								PR (Oct.)	Honduras (June)	Delaware (June)
PL-08-01	Isabela	2,0,0	33	ovate	slightly curved	500	400	51	57	81
PL-08-02	Isabela	2,3,12	36	ovate	slightly curved	400	300	56	57	81
PL-08-03	Isabela	7,0,0	37	ovate lanceolate	slightly curved	400	200	56	57	81
PL-08-05	Isabela	9,0,0	35	round	slightly curved	75	500	76	No flowers	101
PL-08-06	Isabela	10,0,0	30	ovate	slightly curved	60	200	100	No flowers	No flowers
PL-08-07	Isabela	5,3,4	39	lanceolate	straight	200	200	76	68	91
PL-08-08	Isabela	10,0,0	30	round	straight	75	400	69	85	No flowers
PL-08-09	Isabela	6,3,10	32	lineate	slightly curved	400	700	87	90	No flowers
PL-08-10	Isabela	5,3,7	33	ovate	slightly curved	400	400	69	90	No flowers
PL-08-11	Isabela	6,3,10	35	ovate	slightly curved	300	400	76	80	101
PL-08-12	Isabela	6,3,10	35	round	slightly curved	150	800	69	90	101
PL-08-13	Isabela	6,3,10	36	lineate	slightly curved	400	600	76	90	101
PL-08-14	Mayaguez	9,3,11	43	ovate	slightly curved	75	300	51	71	101
PL-08-15	Hatillo	9,0,0	48	lanceolate	slightly curved	200	600	76	90	101
PL-08-18	Aguada	8,4,10	35	ovate	slightly curved	200	700	46	45	101
Christmas	Seed Savers Exchange	5,5,12	110	ovate	slightly curved	10	700	56	50	47
Sieva	Seed Savers Exchange	2,0,0	41	round	slightly curved	50	300	56	94	35

[1]Background color, pattern color and seed coat pattern based on Lima bean descriptors (IBPGR, 1982).

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GENETIC DIVERSITY OF MESOAMERICAN AND ANDEAN WILD BEANS USING MICROSATELLITE MARKERS

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INTRODUCTION

Knowledge of the center(s) of bean domestication is important to identify potential area(s) of origin for agriculture several millennia ago and to better understand the genetic, physiological, and ecological characteristics of the domesticated bean gene pool. Common bean was domesticated at least twice, in the southern Andes (from southern Peru to northwestern Argentina) and in Mesoamerica (in west-central Mexico). The putative Mesoamerican domestication center of *Phaseolus vulgaris* is located in the Lerma-Santiago Basin of Mexico (Kwak *et al.* 2009). Our objective in this study is to analyze the genetic structure of wild Mexican and Andean bean populations and to study their relationship with landraces of the same regions. As a first step, we are characterizing genetic diversity among these accessions with a set of microsatellite markers distributed throughout the genome.

MATERIALS AND METHODS

A sample of 50 accessions (total no. of individuals n=236) of wild and domesticated Mesoamerican and Andean common beans from Argentina (20), Bolivia (4), Peru (4), and Mexico (22) were analyzed. Genetic relationships among accessions were studied using ten microsatellites markers distributed over the entire bean genome. The amplified fragments were multiplexed depending on their size variation and analyzed in an ABI 3730 (Applied Biosystems). Marker genotypes were determined using the GeneMarker program version 1.85. A STRUCTURE analysis (Pritchard *et al.* 2000) was conducted. As a preliminary step to this analysis, ten independent runs were performed using the admixture model, a length of burning period of 10000 and 100000 MCMC replicates after burning. A neighbor-joining tree was constructed using Powermarker (Liu and Muse 2005).

RESULTS AND DISCUSSION

The population subdivision showed significant Andean-Mesoamerican gene pool divergence (Figure 1, K=2 analysis). One hundred twenty-seven and 109 individuals fell into the Andean and Mesoamerican groups, respectively. For K=5, the groups were identified as Argentinean wild (K1), Argentinean domesticated (K2), Bolivia and Peru wild (K3), Mexican domesticated (K4) and Mexican wild (K5).

There were admixed accessions in the Argentinean wild beans involving Argentinean domesticated types suggesting gene flow between sympatric populations as seen with other markers (Galván *et al*., 2010). Some genotypes similar to Bolivia and Peru wild types were observed.
Hybridization between Mexican wild and domesticated types was also seen. Wild beans from Mexico were divided in three groups. Most of the recently reported wild bean populations (Zizumbo

et al. 2009) showed different genotypes than the wild beans from the putative domestication sites in Jalisco, suggesting that more explorations should be done in the region.

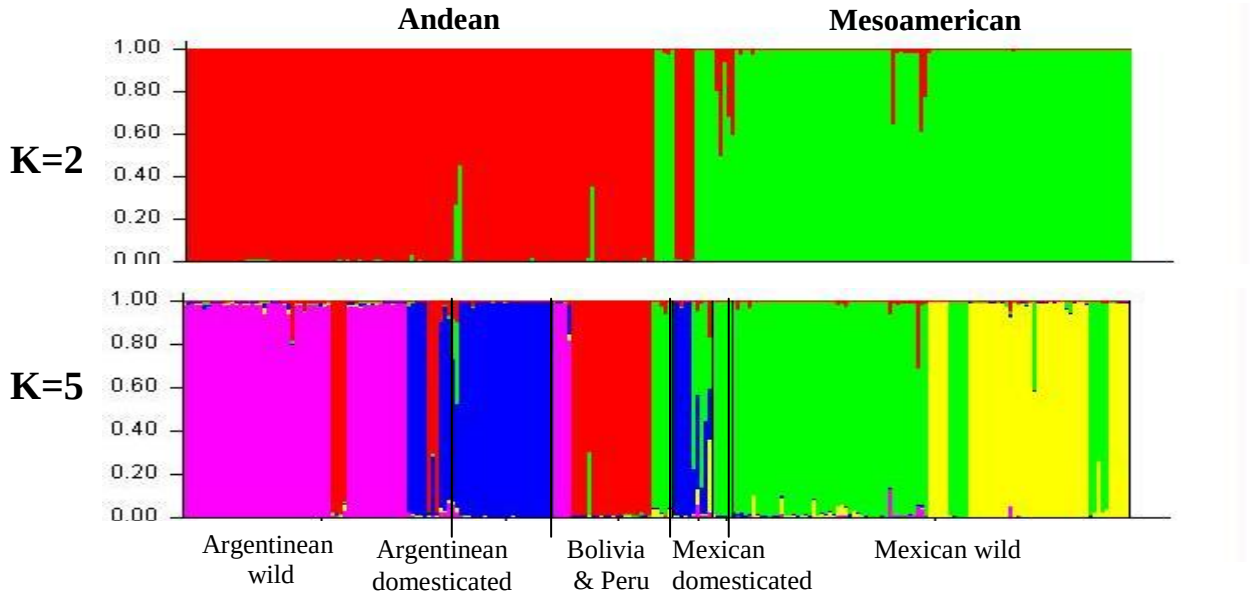

Figure 1. Hierarchical organization of genetic relatedness of 56 common bean accessions based on ten microsatellite markers and analyzed by the STRUCTURE program.

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SCREENING BEAN GENOTYPES FOR ENHANCED N FIXING ABILITY

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Thirty four dry bean genotypes were screened for their ability to fix nitrogen from the atmosphere. These genotypes were either elite breeding lines or commercial varieties. The genotypes studied represent important seed classes in Michigan along with a non nodulating check and a known high N-fixing check.

MATERIALS AND METHODS

After surface sterilizing and coating with *Rhizobium etli* strain UMR 1597 inoculant prepared in a peat carrier, three seeds were planted into plastic pots containing a 3:2 volume for volume ration perlite to vermiculite. Seedlings were thinned as they emerged to leave one seedling. At ten days additional inoculant was added to the top of the growing medium and gently watered in with tap water. Plants were grown in greenhouse conditions and fertilized twice weekly with a modified Hoagland's solution lacking nitrogen. Plants were watered as needed between fertilizer solution applications.

At first bloom plants were harvested by cutting the stem at the surface of the growing mix. Roots were removed from the perlite/vermiculite. Tissues were dried for several days and then ground to 40 mesh. Samples were sent to a lab for total nitrogen analysis.

RESULTS AND DISCUSSION

All genotypes studied developed nodules except R99 which was the non-nodulating check. Total nitrogen present in tissue ranged from just over 1% to nearly 4%. The non fixing check, R99, had the lowest total nitrogen in the study. The highest amount of total nitrogen was found in Puebla-152, which also had among the largest biomass.

Visual symptoms of insufficient nitrogen were obvious on R99, and were also noted on some nodulating genotypes. There appeared to be no correlation between grams of nitrogen fixed and the number of days to harvest as has been suggested may contribute to an increase in nitrogen fixation. Root mass was not correlated to biomass or mg N fixed.

CONCLUSION

Puebla 152 was identified as a high potential nitrogen fixer as was expected based on prior studies (Thomas et al 1983, Wolyn et al 1989, Bliss 1993). Puebla 152 could be a useful donor for enhanced nitrogen fixation in dry bean breeding programs. Sanilac was also identified as a low nitrogen fixer in the present study as in previous studies. There was no significant difference among Sanilac, Bunsi, or R99 in mg nitrogen. R99 is derived from Bunsi through EMS mutation (Park and Buttery, 2006). Among contemporary commercial dry bean varieties as well as elite breeding lines there is variability in nitrogen fixation potential. Further, nitrogen fixation is not maximized in these modern cultivars providing opportunity for future improvement in this characteristic. The use of a

nitrogen limited growing system can be an effective and simple means of identifying the nitrogen fixing ability of bean genotypes.

Table 1. Mean Total nitrogen content (mg) per plant of the 34 bean genotypes studied

Genotype	**Commercial Class**	**Mean Total N (mg)**	
Puebla-152	Black, High Fixer	123.02	A*
Zorro	Black	79.71	B
Sedona	Pink	72.12	BC
Santa Fe	Pinto	71.34	BC
TARS SR05	Small red	69.01	BCD
Buster	Pinto	66.12	BCDE
Matterhorn	Great Northern	64.28	BCDEF
Jaguar	Black	63.01	BCDEFG
T-39	Black	62.11	BCDEFGH
Red Hawk	Dark Red Kidney	54.91	CDEFGHI
Othello	Pinto	54.65	CDEFGHIJ
Merlot	Small Red	53.46	CDEFGHIJ
Montcalm	Dark Red Kidney	50.14	DEFGHIJK
USDK-CBB 15	Dark Red Kidney	49.85	DEFGHIJK
Chinook Select	Light Red Kidney	45.24	EFGHIJKL
Beluga	White Kidney	44.80	FGHIJKL
115-11M, Rhino	Black	42.23	GHIJKL
California ELRK	Light Red Kidney	41.84	HIJKL
Michelite	Navy	38.56	IJKLM
Vista	Navy	38.10	IJKLM
Condor	Black	36.66	IJKLM
Capri	Cranberry	33.83	JKLM
Seahawk	Navy	29.17	KLM
Bunsi	Navy	25.06	LMN
Sanilac	Navy	17.72	MN
R99	Non- Nodulating	7.95	N

*Means followed by a different letter are significantly different ($\alpha \leq .05$, LSD=21.0).

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PLANT GENETIC RESOURCES NETWORK OF INTA – ARGENTINA

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INTRODUCTION

The National Institute of Agricultural Technology (INTA) has implemented a Germplasm Bank Network (GBN) constituted by 9 Active Banks of Plant Genetic Resources and 11 field collections distributed in diverse ecological areas along the country in Argentina that preserves germplasm in short -medium term and a base bank that maintains backup duplicates from the collections of active banks and other institutions on request.

The INTA GBN gather economic interest introduced species and native and wild species of current or potential interest. The objectives of the GBN are to contribute to the conservation of agrobiodiversity and valorize plant genetic resources through morphological, genetic, biochemical and molecular characterization and evaluation, as well as documentation in order to make available the genetic diversity for research, the re-introduction into the regions of origin and plant breeding to contribute to food security and sustainability of biological systems.

RESULTS AND DISCUSSION

According to the Second National Report on the Status of Plant Genetic Resources in Argentina (www.pgrfa.org/gpa/arg/descrip.htm) INTA GBN conserves 93.5% of the germplasm available in official institutions in the country. It attempts to ensure institutional continuity in genetic resources and laying the foundations for the creation of the National Genetic Resources System which will include all actors and institutions involved in these activities.

GBN´s activities are conducted primarily through the Institutional Project Ex-situ conservation and evaluation of germplasm collections of Plant Genetic Resources Network of INTA belonging to the Strategic Area of Genetic Resources, Breeding and Biotechnology, together with other projects and agreements with universities, private companies, agencies and national, regional and International organizations.

Each bank and active collection has responsibility for certain species. Institutional responsibility for species / cultivars of banks and collections are: wild potato and Andean potato varieties, temperate forage and wild sunflowers (EEA Balcarce), native forage and introduced arid temperate forage (EEA Guillermo Covas) vegetables (EEA La Consulta), sunflower, peanut and sorghum (EEA Manfredi) wheat and soybeans (EEA Marcos Juarez), pome fruit: pears and apples (EEA Alto Valle), maize and temperate climate forage (EEA Pergamino), Cotton, subtropical and tropical forage legumes and native forest (EEA Roque Sáenz Peña), beans, tobacco, amaranth and native aromatics (EEA Salta), almond and wall-nut (EEA Catamarca), Dry land native shrubby forages (EEA Chubut), native and naturalized Forage of northern Patagonian (EEA Bariloche), herbaceous and semi-ligneous leguminous species of the Chaco region (EEA Santiago del Estero), beer and fodder barley, oats and triticale (EEA Bordenave), native forest: Balsam and amburana trees (Yuto

EEA), Citrus (EEA Concordia), sugarcane (EEA Famaillá), olive, grapevine, peach, plum and apricot (EEA Junín), yerba mate and tea (EEA-Cerro Azul), in vitro conservation of sweet potato , in vivo bank of aromatic natives (Base Bank-Castelar). The current collections comprise wild species and those wild relatives of crops, obsolete varieties, traditional varieties, landraces, modern cultivars currently in use, advanced lines and special genetic materials (mutants, aneuploid, genetic stocks) as a result of research activity.

National activities relating to genetic resources are carried out within the framework of initiatives such as the National Advisory Committee on Genetic Resources of the Ministry of Agriculture, Livestock, Fisheries and Food (CONARGEN), which advises both nationally and internationally, in subjects related to genetic resources, including development of legal rules to regulate access, exchange and protecting them.

At regional level, the GBN interact with REGENSUR, which is the PROCISUR Genetic Resources Network, which aims to promote the strengthening of technical and operational capacity of the institutions of the Southern Cone countries to conserve, enrich, assess, identify, characterize and use genetic resources to ensure the availability of germplasm and information. It is also an area of discussion and collaboration on issues of common interest of countries. Internationally it takes part in meetings of diverse organizations, treaties and conventions such as CBD, FAO ITPGRFA.

THE GERMPLASM ACTIVE BANK OF EEA-INTA AT SALTA

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The EEA-INTA germplasm active bank at Salta is a part of the Plant Genetic Resources Conservation Network of INTA. It is located in Cerrillos, in the Lerma Valley, province of Salta (latitude 24º 53' S; longitude 65º 28' W; 1240 masl).

It preserves seeds of beans, tobacco, native aromatics, tree tomato and amaranth. Bean is the most important collection that is composed of landraces of cultivated beans (*Phaseolus vulgaris* L) and wild populations (*Phaseolus vulgaris* var *aborigineus*. (Burk.) Baudet). Wild populations and landraces have been collected in different localities in the provinces of Salta, Jujuy, Catamarca and Tucumán, between 1000 and 3000 m.a.s.l. and between 22º y 24º S y 67º y 65º W , in natural formations of different phytogeographic regions, in the valleys, ravines, riverbanks, hillsides and rainforests growing on different species of trees and shrubs.

In the same regions in humid valleys were collected primitive varieties, grown in the fields of local farmers. At the present time the collection includes 561 entries, of which 400 have been characterized.

The collections are preserved to medium term as seed in climatic chambers with low humidity (temperature between 5-7ºC and humidity of the seed between 6-7%). These collections are monitored regularly to maintain samples in optimal conditions of health and high germination values.

Other activities also carried out are the regeneration and / or multiplication of the entries, to ensure availability of seeds and characterization and evaluation of germplasm, to know their characteristics and variability. These activities are conducted in sites agro-ecologically similar to places of collection.

The information obtained from the collection and all those generated during the characterization and evaluation is incorporated in a database created inside the INTA Plant Genetic Resources Network called DBEGERMO to make information accessible. Since 2006 studies have been initiated in order to establish guidelines for defining genetic reserves of wild bean populations. Morpho-agronomic characters were recorded to evaluate the variability between populations and select the most suitable for the establishment of reserves.

Molecular biology studies are been performed to estimate the variability with a complementary methodology. This will allow the transmission of information to the relevant government authorities with the need to take appropriate measures to establish nature reserves in the mentioned sites in order to avoid the loss of these valuable genetic resources.

The importance of preserves wild and primitive germplasm of bean is that these species contain high genetic variability that can be a source of useful genes for breeding programs. Studies with other working groups have identified accessions with resistance / tolerance to diseases. Currently wild populations and landraces of beans tend to disappear due to the advance of civilization, the change in

the habits and the replacement by commercial varieties, resulting in a gradual and irreversible loss of this variability therefore it is necessary to continue efforts to achieve protection and conservation effectively.

THE GENE CONTROLLING SLOW DARKENING IN PINTOS IS NOT *J*.

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Post harvest darkening of the seed coat in dry beans is an undesirable characteristic in certain market classes of dry bean, particularly pinto, cranberry and carioca and represents a problem for producers and consumers. Consumers associate darkened beans with the hard-to-cook effect so it leads to downgrading of the product and a lower price to the producer. It is accelerated by warm, humid storage conditions and is exacerbated by exposure to light (Junk et al., 2007). There are at least three post harvest darkening phenotypes: non darkening (ND), slow darkening (SD) and regular darkening (RD). The gene *J* has been associated with after-darkening in beans and *jj* beans tend not to darken with age (Prakken, 1970; Bassett, 1996). The SD phenotype is also controlled by a single recessive gene (Junk et al., 2008).

The objective of this study was to study determine if the gene controlling slow vs regular darkening is *J* by evaluating various populations derived from crosses between SD and ND genotypes.

MATERIALS AND METHODS

Genotypes used for test crossing included: PI608688 (Genetic Stock 41 *jjvv*; darkening phenotype unknown), 1533-15 (SD), KVxUI-1 (ND; *jj* pinto from J. Meyers, Oregon State University). F1 and F2 seed coats (seed of F2 and F3 generations) were darkened using UV-C light to phenotype (Junk et al. 2007).

RESULTS AND DISCUSSION

PI808688 is a purple-seeded line and the F1 of the cross with 1533-15 was brown making it difficult to phenotype for its darkening reaction. Also, only eight of the F_2 seed coats were of a pinto type that could easily be used for assessing the darkening phenotype. Results from this small set of progeny demonstrate clearly, however, that there is more than one gene controlling the darkening phenotype (Table 1). Crosses made more recently with a *jj* pinto confirmed this with the F1 seed coat phenotype being ND and the F2 segregating for all three darkening classes (Table 1).

Putative F2 genotypes were tested for segregation using a chi-square test for two loci and the assumption that *j* is epistatic to the second locus and all fit the ratios expected (Table 1). The segregation results also suggest the two genes are not linked. Further testing of segregating generation is continuing to confirm the F2 results for the 1533-15 x KVxUI-1 cross. Test crosses with other SD and ND pinto, bayo and cranberry lines is being carried out to determine if there are yet other genes that contribute to this darkening phenomenon.

Table 1. Phenotypes and putative genotypes of progeny from crosses between SD and ND genotypes.

cross	F_1 phenotype	F_2 pinto phenotypes (RD:SD:ND)	F_2 X^2 p-value	F_3 family phenotypes (RD:SD:ND)	Putative F_2 genotype	F_3 X^2 p-value
PI808688 x 1533-15	Brown	6:0:2	0.42(9:3:4)	21:3:0	*JJ Sdsd*	0.57 (3:1:0)
				9:4:4	*Jj Sdsd*	0.97 (9:3:4)
				7:5:4	*Jj Sdsd*	0.62 (9:3:4)
				15:10:6	*Jj Sdsd*	0.29 (9:3:4)
				27:0:7	*Jj SdSd*	0.95 (3:0:1)
				26:0:8	*Jj SdSd*	0.99 (3:0:1)
				All ND	*jj ??*	1.00 (0:0:1)
				All ND	*jj ??*	1.00 (0:0:1)
1533-15 x KVxUI-1	ND	16:4:7	0.96 (9:3:4)	n/a		

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PHENOTYPIC EVALUATION OF A DRY BEAN RIL POPULATION FOR RESISTANCE TO POTATO LEAFHOPPER

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INTRODUCTION

A genetic study examining resistance to the temperate potato leafhopper *Empoasca fabae* was conducted in Michigan using a dry bean RIL population generated from a single backcross to Matterhorn, a susceptible Michigan commercial variety, from a cross with EMP 507, a line developed by CIAT for resistance to the tropical leafhopper *E. kraemeri.*

The potato leafhopper (PLH), *E. fabae*, is currently the most abundant insect pest of dry beans in Michigan. In the 1980's, PLH replaced tarnished plant bugs and aphids as the major insect pest of dry beans and they continue to be an annual threat that reduce bean seed yield and quality. By evaluating PLH incidence in a genetic mapping population, PLH preferences can be assessed, and used to identify QTLs for resistance to this pest. By monitoring the differences in the number of PLH nymph numbers present, inferences can be made as to oviposition, egg-laying, and feeding preferences, antixenosis interactions, of the insects (Schaafsma et al., 1998). In addition, the number of nymphs can indicate antibiosis interactions where the bean genotype causes direct deleterious effects to the pest. Another method for assessing pest resistance is to evaluate plant damage caused by the pest. PLH causes a specific set of symptoms known collectively as "hopperburn" that are evaluated on a 1-5 scale for leaf curl and leaf burn symptoms (Murray et al., 2001). By looking for correlations between numbers of nymphs and damage scores, tolerance mechanisms may be identified.

MATERIALS AND METHODS

Plant Material: The recombinant inbred line (RIL) population examined in this study was originally developed by the USDA dry bean breeding program in Puerto Rico. The RILs were created through a wide cross between a putative resistant line, EMP 507, and a susceptible commercial MI great northern variety, Matterhorn. EMP 507 was originally developed at CIAT for resistance to *E. kraemeri,* the tropical leafhopper species. A single backcross was made to the commercial Michigan parent to improve recovery of agronomic and adaptive traits critical for testing in northern latitudes. From the population, 75 individual F6-8 RILs, the two parents (EMP 507, Matterhorn) and three check varieties (EMP 509, Sierra, and Santa Fe) were planted in the field in 2009.

Field Screening: An open choice test was conducted at Michigan State University, East Lansing, MI. Three replications were planted on 15 June 2009 in a randomized complete block design (RCBD), 8 inches apart in 18 foot single row plots of up to 25 plants per plot. The plants were evaluated for leaf curl and leaf burn at 78 and 79 days after planting (DAP) using the damage scale from 0-5 as described in Murray et al. (2001), where 0= no visible damage and 5= severe damage. PLH nymphs were counted at 51 DAP by counting nymphs present on 3 trifoliolates on each of 3 randomly selected plants per plot.

The Proc Mixed and Proc Corr procedures of the SAS statistical package 9.1 (SAS Institute, Cary, USA) were used to analyze the data.

RESULTS

All damage-related indices were found to be significantly affected by genotypic effects ($p<0.05$). PLH nymph counts were normally distributed in the RIL population with a mean value of 6.7 ±0.29 (SEM). Leaf curl (LC) was normally distributed within the population, however, leaf burn (LB) was found to be left-skewed with the majority of lines having low scores (0-1). The mean leaf burn score was 0.93 ±0.08. Parent leaf burn scores were within the standard error and found to be 0.33 (EMP 507) and 0.66 (Matterhorn). The mean leaf curl score was 2.6 ±0.08 with parental leaf curl scores being 2.6 (Matterhorn) and 2.0 (EMP 507). In addition, leaf burn scores were correlated to leaf curl scores with a Pearson correlation coefficient of 0.49837 ($p<0.0001$).

RILs were classified as resistant (R = LB≤2, LC≤3), susceptible (S = LB>2, LC>3), or differentially resistant if damage scores were resistant for one rating, but susceptible for the other. Sixty RILs were found to be resistant and 5 were found to be susceptible. The remaining 10 individuals were found to be differentially resistant (9 RILs were classified as LB=R, LC=S; 1 RIL was classified as LB=S, LC=R). These differential genotypes may indicate that resistance or susceptibility to leaf burn and leaf curl damage is controlled by separate genetic mechanisms. The resistant individuals were further evaluated to identify individuals with PLH nymph counts significantly different from the mean. Nine resistant individuals were identified that had significantly higher nymph counts than the population mean. These RILs indicate that tolerance may be another mechanism at work within the RIL population. An additional nine individuals were found to have significantly lower nymph counts than the population mean. This result is consistent with antixenosis and antibiosis resistance mechanisms, in that higher nymph counts are associated with higher damage scores and vice versa. Nymph counts on both parents were equivalent to the resistant family mean (R=6.3±0.3, Matterhorn = 6.0, EMP 507= 6.1). However, susceptible families had 1.7 times as many nymphs per plot than resistant families (S=10.8±0.73). The mean PLH nymph count means for resistant and susceptible classes were found to be significantly different from each other ($p<0.001$). Potato leafhopper nymph counts were found to be correlated to both leaf burn and leaf scores using the Pearson correlation coefficient. PLH nymph counts were correlated to leaf burn with a correlation coefficient of 0.25 ($p=0.0001$) and to leaf curl with a correlation coefficient of 0.38 ($p<0.0001$). When analyzed separately, RILs that were classified as resistant for either leaf burn (<0-2) or leaf curl (<0-3) had significantly lower PLH nymph counts than susceptible RILs. This study will be repeated in 2010 in Michigan and Puerto Rico to confirm if the resistant/susceptible phenotypes are maintained when selected under pressure from both *E. fabae* and *E. kraemeri* and under different environmental conditions.

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PROGRESS IN PYRAMIDING WHITE MOLD RESISTANCE FROM ACROSS *PHASEOLUS* SPECIES IN COMMON BEAN

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ABSTRACT

White mold (WM) is a severe disease of common bean (*Phaseolus vulgaris* L.). Partial resistance is found in Andean and Middle American gene pools and *P. coccineus* and other secondary gene pool (SGP) species of common bean. The objectives were to (1) pyramid resistance from diverse sources of germplasm, and (2) transfer high levels of resistance into pinto bean. Four WM resistant interspecific breeding lines (92BG-7, 0785.127.1.3, VCW 54, VRW 32) and four genotypes from Andean gene pool (A 195, G 122, MO 162, PC 50) were crossed among themselves for test of complementation. Also, two double-cross populations each comprising three diverse sources of resistance (Pop I = USPT-WM-1/CORNELL 601//USPT-CBB-1/92BG-7 and Pop II = Chase/I 9365-25//ABL 15/A 195) were used for gamete (GS) and recurrent (RS) selection for simultaneously pyramiding and transferring WM resistance into pinto bean. Crosses developed for complementation study are yet to be evaluated. Both selection methods were effective for pyramiding and introgressing WM resistance into pinto bean. However, selection gains were larger and GS produced more pinto bean genotypes with higher levels of WM resistance than RS.

INTRODUCTION

White mold is the most devastating and widespread disease of common bean in the USA and Canada. Common bean has low levels of WM resistance (Miklas et al., 1999). The highest levels of resistance occur in the SGP (e.g., *P. coccineus* L., Singh et al., 2009). The objectives of this study were to simultaneously (1) pyramid resistance from diverse sources of germplasm, and (2) transfer high levels of WM resistance into pinto bean.

MATERIALS AND METHODS

Two single-crosses each among Andean sources of WM resistance: A 195/G 122 and MO 162/PC 50 and interspecific breeding lines: 92BG-7/VCW 54 and VRW 32/0785.127.1.3 were made for complementation study. VRW 32 was derived from *P. costaricensis* and other three interspecific breeding lines were derived from *P. coccineus*. The F_1 resulting from single-crosses have yet to be evaluated and the two double-crosses and one eight-parent cross yet to be made and assessed for pyramiding WM resistance. In a separate study, two double-cross populations: Pop I = USPT-WM-1 / CORN 601 // USPT-CBB-1 / 92BG-7 and Pop II = Chase / I9365-25 // ABL 15 / A 195 were developed. GS from F_1 to F_4 and two cycles of RS were practiced in both populations. Thirteen breeding lines selected from each method and each population were compared in a randomized complete block design with three replicates in two greenhouse environments in 2007-2008. Each replicate consisted of 6 plants. Each plant was separately inoculated two times using the cut-stem method with two mycelial plugs each time. WM reaction was recorded on a single-plant basis at 33 days post-inoculation (DPI) and verified at maturity. A 1 to 9 rating scale, where 1= no disease

symptoms and 9= severely diseased or dead plants was used. Data were analyzed using a SAS package and the mean and LSD ($P \leq 0.05$) values were calculated.

RESULTS AND DISCUSSION

Both GS and RS were effective and the mean WM score of 13 selected families was significantly lower than the mean score for the four parents in both populations (Table 1). Furthermore, 20.6% gain was realized in Pop I and 18.6% gain in Pop II from GS. The gain in WM resistance from RS for Pop I was 10.7% and for Pop II was 5.1%. But, the selected family with the lowest WM score had significantly lower WM score than the best WM resistant parent only from GS in Pop I (Table 2).

Table 1. Mean WM score for four parents and 13 families derived from GS and RS for two double-cross populations at 33 DPI in two greenhouse environments in 2007-2008.

Selection method	Pop I		Pop II	
	Parent	Family	Parent	Family
GS	6.3‡	5.0	5.9	4.8
RS	6.5	5.8	5.9	5.6
LSD ($P \leq 0.05$)	0.2	0.1	0.2	0.1

Table 2. Mean for the parent and family with the lowest WM score derived from GS and RS in two double-cross populations at 33 DPI in two greenhouse environments in 2007-2008.

Selection method	Pop I†		Pop II	
	Parent	Family	Parent	Family
GS	4.8	4.1	4.5	4.5
RS	4.8	5.0	4.5	4.9
LSD ($P \leq 0.05$)	0.4	0.4	0.4	0.4

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GENETIC GAIN FOR SEED TRAITS IN SELECTION CYCLES IN RUNNER BEAN

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INTRODUCTION

The cross-pollinated scarlet runner bean (*Phaseolus coccineus* L.) is a climbing perennial crop but it is often grown as annual for its large dry seeds and as an ornamental climber also. It is of importance in some parts of Europe, although of minor importance in the United States (Mullins et al., 1999). The white seeded runner bean cultivars are often cultivated in Italy and Spain (Campion & Servetti, 1991) on a small scale. This species displays several useful agronomic attributes such as cold tolerance (Rodiño et al., 2007), lodging resistance due to thick stem bases, presence of a tuberous root system allowing a perennial cycle, long epicotyls and racemes, a high number of pods per inflorescence (Vanderborght, 1983), resistance to *Ascochyta* blight (Schmit & Baudoin, 1992) and resistance to *Sclerotinia sclerotiorum* (Gilmore et al., 2002). In order to grow runner bean in a commercial scale, uniformity in seed quality traits is required, that should be achieved by selection of breeding lines. The objective of this research was to display the genetic gain after a recurrent selection program of breeding lines within runner bean valuable landraces.

MATERIALS AND METHODS

Seven white seeded climbing runner bean landraces previously evaluated (Santalla et al., 2004) were chosen to select breeding lines by hand self-fertilizing and individually harvesting in each generation. Selection criteria were high seed production and large seed size. From each selection cycle, within the seven families, the following number of seeds were sown (when available): S_0 – 40; S_1 – 20; S_2 – 40; S_3 – 80; S_4 – 100. These materials were evaluated according to a randomized complete block design with two replications in 2008 in Pontevedra, Spain (42º 24' N - 8º 38' W; 40 masl; 14ºC mean temperature, 1600 mm yearly average rainfall). Morphological and qualitative data were recorded when the plants reached maximum vegetative development of the main stem and seed quality data were taken in dry seeds after harvest. The selection coefficient and the genetic gain were calculated for each generation in each family.

RESULTS AND DISCUSSION

The analysis of seed weight, one of the most important traits for the market, is shown in Table 1. The average variation in this trait was S_0=139.3 (g 100 seeds^{-1}), S_1=113.0 (g 100 seeds^{-1}), S_2=113.3 (g 100 seeds^{-1}), S_3=107.8 (g 100 seeds^{-1}) and S_4=179.07 (g 100 seeds^{-1}), resulting a genetic gain of 41 % in four generations of selection. All the families exhibited extra-large seed size, ranging in S_4 from 122.5 to 261.0 in family 311, which means a high value market since consumers demands this type of seed. In this family the genetic gain was positive in all the generations, scoring surprisingly the highest gain value as response to a low selection pressure. In the other six families, several values of the genetic gain in different generations were negative that could indicate the effect of inbreeding. Finally, only the 469 family had not genetic gain after the selection program. As a

conclusion, the runner bean breeding lines selected are appropriate for production and their genetic background in suitable for genetic improvement through recurrent selection.

Table 1. Effect of the selection on the dry seed weight (g 100 seeds^{-1}) in seven runner bean families in four generations.

	163*					**659**				
	Weight	s	∑s	∑Gs	%Gs	Weight	s	∑s	∑Gs	%Gs
S0	111.0					167.5				
S1	130.5	0.24	0.24	19.50	17.57	121.5	0.10	0.10	-46.00	-27.46
S2	64.0	0.16	0.40	-47.00	-42.34	134.0	0.15	0.25	-33.50	-20.00
S3	100.0	0.19	0.60	-11.00	-9.91	98.0	0.16	0.42	-69.50	-41.49
S4	160.5	0.07	0.67	49.50	44.59	191.5	0.09	0.51	24.00	14.33
	311					**1022**				
	Weight	s	∑s	∑Gs	%Gs	Weight	s	∑s	∑Gs	%Gs
S0	91.5					142.0				
S1	117.0	0.08	0.08	25.50	27.87	145.5	0.13	0.13	3.50	2.46
S2	107.0	0.18	0.26	15.50	16.94	127.0	0.06	0.18	-15.00	-10.56
S3		0.07	0.33			105.0	0.16	0.34	-37.00	-26.06
S4	261.0	0.20	0.53	169.50	185.25	155.5	0.10	0.44	13.50	9.51
	406					**1025**				
	Weight	s	∑s	∑Gs	%Gs	Weight	s	∑s	∑Gs	%Gs
S0	107.5					95.0				
S1	82.0	0.12	0.12	-25.50	-23.72	93.0	0.24	0.24	-2.00	-2.11
S2	113.0	0.17	0.29	5.50	5.12	110.5	0.19	0.43	15.50	16.32
S3	101.0	0.26	0.55	-6.50	-6.05	117.5	0.12	0.55	22.50	23.68
S4	122.5	0.16	0.71	15.00	13.95	172.0	0.07	0.61	77.00	81.05
	469									
	Weight	s	∑s	∑Gs	%Gs					
S0	172.5									
S1	101.5	0.50	0.50	-71.00	-41.16					
S2		0.05	0.55							
S3	137.5	0.13	0.68	-35.00	-20.29					
S4	125.0	0.14	0.82	-47.50	-27.54					

* families; s= coefficient of selection in each generation; ∑s= accumulated coefficient of selection; ∑Gs= accumulated genetic gain in grams; %Gs= accumulated percentage of genetic gain

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PHENOTYPIC AND GENOTYPIC EVALUATION OF COMMON BACTERIAL BLIGHT RESISTANCE IN A RESISTANT INTER-CROSS POPULATION OF PHASEOLUS VULGARIS

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INTRODUCTION

Common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli*, is a damaging widespread disease of *Phaseolus vulgaris L.* (common bean). In Canadian common bean germplasm, CBB resistance in navy bean has been introgressed from two distinct sources of *P. acutifolius* i.e., PI440795, from which OAC Rex (Michaels *et al.* 2006) was developed and PI319443, from which HR67 and HR45 (Park and Dhanvantari 1994) germplasm lines were developed. A major CBB resistance QTL, associated with the microsatellite marker J04555, was mapped on linkage group B5, accounting for 42% of variation in CBB resistance in an OAC Seaforth/OAC Rex $F_{2:4}$ population (Tar'an *et al.* 2001). A different major CBB resistance QTL, associated with the SCAR marker UBC420, was mapped on linkage group B6, accounting for 62% of variation in CBB resistance in an HR67/Envoy F_5 population (Yu *et al.* 2000). The purpose of this study is to examine the segregation of CBB resistance response in a resistant inter-cross population, derived from crosses between OAC Rex and HR45 and to investigate the effects of CBB QTL of chromosomes B5 and B6 and their interaction effects on CBB resistance.

MATERIALS AND METHODS

An $F_{4:5}$ recombinant inbred line (RIL) population of reciprocal crosses between OAC Rex and HR45 was evaluated for resistance to CBB and genotyped with molecular markers associated with CBB QTL. A field trial in 2009 was planted at Agriculture and Agri-Food Canada Research Centre near Harrow, ON in a 15 by 15 unbalanced square lattice design with two replications. Plant material included: 218 $F_{4:5}$ RILs, parental lines of the population, cv. Dresden (susceptible check), and 4 near-isogenic lines with different combinations of SU91 and UBC420, provided by Dr. P. Miklas. Plots were artificially inoculated and multiple evaluations of CBB severity in the field were conducted with one week intervals using a 0-5 visual scale (Yu et al 2000). The Area Under the Disease Progress Curve was estimated for each experimental unit as $AUDPC=\sum[(S_i+(S_{i+1})/2)(T_{i+1})-T_i)]$, where S is a measure of disease severity and T is days. The RILs included in the field trial were genotyped with UBC420 and J04555 (PV-ctt001). Statistical analyses were performed using PROC MIXED procedure in SAS (Littell et al. 1996).

RESULTS AND CONCLUSIONS

Frequency distribution of AUDPC in the RIL population had a continuous variation with population mean shifted towards resistance. 12% of the RILs had AUDPC estimates lower than OAC Rex and HR45 while 54% were above. These results suggest that in addition to the QTL associated with SU91 (Pedraza et al. 1997), which is common between the parental lines, OAC Rex and HR45 carry different QTL for resistance and that other small effect QTL may be involved in CBB resistance. The highest levels of disease resistance were conferred by RILs containing UBC420 (Figure 1),

which accounted for 29% of variation in AUDPC. In the presence of UBC420, the effect of J04555 was not significant. However, in the absence of UBC420, the effect of J04555 was significant ($P < 0.001$) and accounted for 7% of variation in AUDPC (Table 1). This, in addition to significant interaction effect of J04555 and UBC420 on AUDPC may point to an epistatic effect of UBC420 over J04555. Models with both UBC420 and J04555 explained up to 30.2% of phenotypic variation in AUDPC. Data obtained from the near-isogenic lines for UBC420 and SU91 confirm results from Vandemark *et al.* (2008) that the SCAR marker SU91 has an epistatic interaction with UBC420 (Figure 2). Further marker screening of the population is underway.

Table 1. Main and interaction effects of markers J04555 (J) and UBC420 (B) on the Area Under the Disease Progress Curve (AUDPC). Values are Lsmeans ± standard error.

UBC420	J04555			Main effect (UBC420)
	JJ	Jj	jj	
bb	22.6± 2.37	44.0±5.82	32.5±1.71	33.0±2.17
B_	7.7±2.18	6.3±2.63	8.8±1.46	7.6±1.24
Main effect (J04555)	15.1±1.16	25.1±3.19	20.7±1.12	

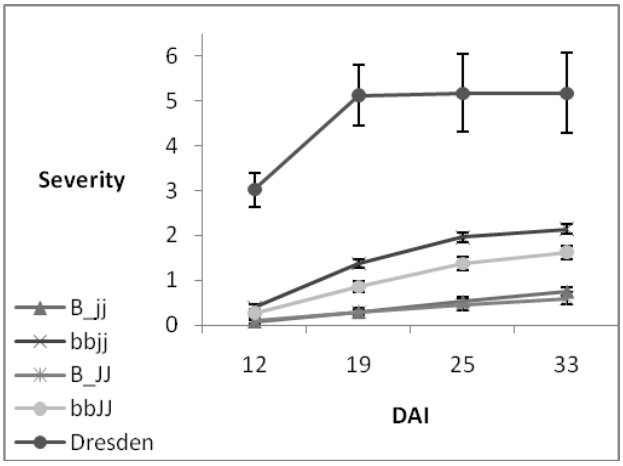

Figure 1. Disease severity at different days after inoculation (DAI) of various UBC420 (B) and J04555 (J) genotypic groups of RILs in the population.

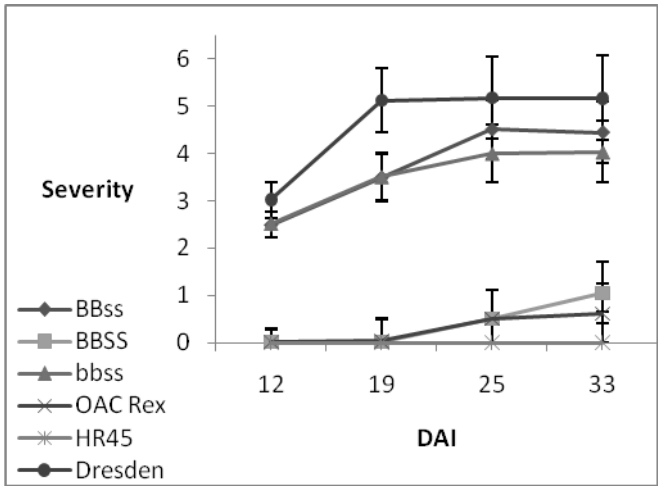

Figure 2. Disease severity at different days after inoculation (DAI) of three near-isogenic lines for UBC420 (B) and SU91 (S) and parental lines of the RIL population.

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MOLECULAR MARKERS LINKED TO ANGULAR LEAF SPOT RESISTANT GENES IN COMMON BEAN ACCESSIONS FROM EASTERN AFRICA AND BRAZIL

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INTRODUCTION

The angular leaf spot (ALS) caused by the fungus *Pseudocercospora griseola* (Sacc.) Ferraris is one of the most important common bean diseases. The use of host resistance to address the risk posed by the ALS disease is the most effective and practical strategy, especially for smallholder, resource-limited farmers (Mahuku et al., 2009). Genetic resistance to this pathogen occurs in genotypes from Andean and Mesoamerican origin. Six independent dominant genes (*Phg*) have been identified (Caixeta et al. 2005). The association of molecular markers with resistance genes has been frequently used for the common bean, not only for genetic studies, but also for marker-assisted selection (MAS). The objective of this work was to evaluate the presence of molecular markers linked to angular leaf spot resistance (ALS) genes in common bean germplasm from Brazil and Eastern Africa.

MATERIALS AND METHODS

This study was conducted in a greenhouse and at the Laboratório de Biologia Molecular from the Núcleo de Pesquisa Aplicada à Agricultura (Nupagri) of the Universidade Estadual de Maringá (UEM), Paraná, Brazil, and at the Crop Evolution Lab in the Plant Sciences Department at University of California, Davis. The seeds of thirty-one and ninety-three common bean accessions from Brazil and Eastern Africa, respectively, were sowed in the greenhouse. At the first trifoliolate stage, the leaf tissue of each cultivar was collected for DNA extraction performed according to the method described by Afanador et al. (1993), with modifications. DNA from leaf tissue of each cultivar was analyzed for the following SCAR markers: SH13 linked to the *Phg-1* gene (Queiroz et al. 2004), SMO2 linked to *Phg-ON* (Queiroz et al., 2004), and SNO2 linked to *Phg-2* (Nietsche et al. 2000). The microsatellite Pv-ag004 linked to the *Phg-G5686B* gene (Mahuku et al., 2009) was also analyzed.

RESULTS AND DISCUSSION

Marker-assisted selection requires at least a double, linked polymorphism between parents to be operative. First, the donor and recipient parents have to be phenotypically different (e.g., resistance vs. susceptibility, respectively). Second, the two parents have two show differences sequences linked to the phenotypic trait genes (e.g., presence or absence or size differences in a PCR amplification). In the work described here, we provide an assessment of the level of molecular polymorphism for ALS markers between donors of resistance and preferred target varieties in East African bean

breeding programs. Figure 1 shows that two markers (SH13 and Pvag-004) are relatively less abundant, one marker is moderately abundant (SN02) and a fourth marker (SM02) appears to be fixed in Eastern Africa and Brazil (confirmed by PCR under different annealing temperatures and sequencing of the amplicon). These results show that there will be more opportunities for introgression in these two germplasm pools for the first two markers and, to a lesser extent, for the third marker. For the fourth marker, alternative, linked markers need to be identified. As a reminder, the presence of the marker does not guarantee the presence of the resistance gene. Indeed, with one exception, these markers are located at a distance of several cM from their respective ALS resistance genes: SH13 – Phg-1 (5.6 cM; Queiroz et al. 2004), SN02 – Phg-2 (3.2 cM; Nietsche et al. 2008), SM02 – Phg-ON (5.3 cM; Queiroz et al. 2004), and Pvag-004 - Phg-G5686A (0.0 cM; Mahuku et al. 2009). Thus, without confirmatory evaluation, it is possible that genetic recombination may have separated the markers from their respective linked resistance gene in some lines.

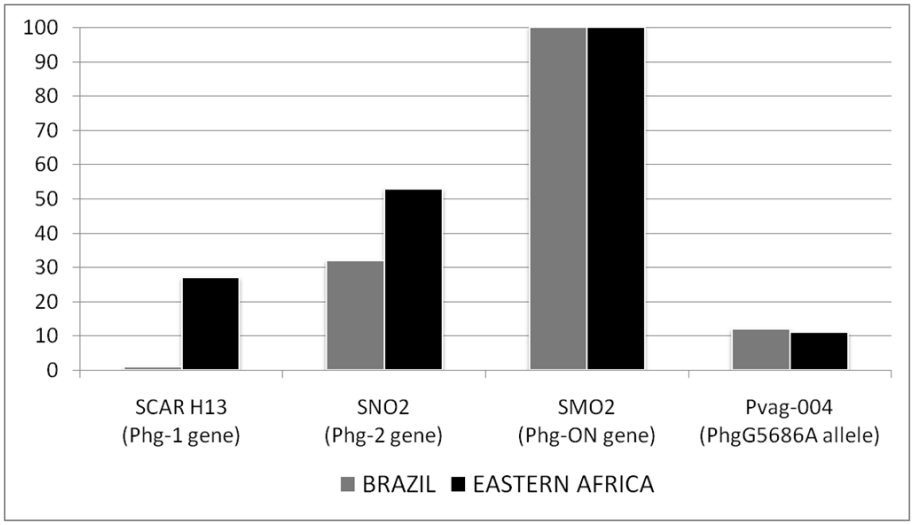

Figure 1. Frequency of amplified band for several markers tagging ALS resistance genes in Brazilian and Eastern African bean germplasm.

ACKNOWLEDGEMENTS

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YIELD EFFECT FOR TWO QTL CONTROLLING COMMON BACTERIAL BLIGHT RESISTANCE IN A NEAR-ISOGENIC DRY BEAN POPULATION

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Common bacterial blight (CBB) caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) is a serious seed-borne disease of common bean (*Phaseolus vulgaris* L.) worldwide. Major QTL conferring resistance to CBB have been successfully introgressed into susceptible market classes using traditional breeding methods and marker-assisted selection. The effect major QTL have on reducing disease severity is well documented, but the effect these QTL have on yield potential is relatively unknown.

This yield study tested near-isogenic lines (NILs) homozygous for presence/absence of two independent QTL (SU91 and BC420) backcrossed six times into 'Teebus' small white bean. The BC_6F_3 derived F_4 NILs (Teebus*6/XAN 159) were generated from a previous study (Vandemark et al., 2008) which showed 9:3:4 recessive epistasis between the QTL in the BC_6F_2 generation. The BC_6F_2 plants with both QTL present (9) were most resistant, with SU91 QTL only were intermediate resistant (3), and with BC420 only or no QTL were susceptible (4). The homozygous NILs were obtained from F_3 lines that were fixed for presence or absence of the QTL.

Four NILs with both QTL, four NILs with SU91 only, four NILs with BC420 only, four NILs with no QTL, and the parents, Teebus and an advanced line (Teebus*5/XAN 159) with high level of CBB resistance used for the last backcross, were tested. There was an inoculated versus non-inoculated treatment, thus 2 treatments x 18 genotypes arranged in an RCBD with four replications. Plot size was four rows (5 m length x 0.75 m spacing). The experiment was conducted in South Africa across multiple locations and years.

A mixture of *Xap* and *Xapf* isolates (Xf410, Xf260, X326) was used to inoculate four rows of each plot for the inoculated treatments. Plots were rated for CBB using a 1-9 scale (Van Schoonhoven and Pastor-Corrales 1987) with 1 being resistant (no disease present) and 9 being susceptible (dead plants). At maturity two rows of each plot were harvested and yield recorded. For this report, for each year, data were combined across locations and analyzed by General Linear Models (SAS). Only main effects for genotype and treatment, and the interaction between genotype x treatment across locations are included in this report.

Increased disease was observed for the inoculated plots (Figs. 1-4), which enabled comparison of yield under high vs. low disease pressure. For 2008 inoculated plots (Fig. 1), NILs with both QTL had lower disease score, SU91 only was intermediate, and BC420 only or no QTL were mostly susceptible which matches the recessive epistasis (9:3:4) interaction observed by Vandemark et al. (2008), where BC420 QTL in the absence of SU91 had no effect for reducing disease severity. The resistant NILs with SU91 QTL had higher yield than the susceptible NILs which lacked SU91. For 2009 high disease pressure (Fig. 2), results matched 2008, except BBss had less disease but not improved yield. For 2008 and 2009 non-inoculated plots (Figs. 3 and 4), there was still some disease

pressure because susceptible lines exhibited higher disease scores. Resistant lines had higher yields than susceptible lines in 2008, but slightly lower yields in 2009, although not significantly different.

In summary, no yield-drag effects were observed for either BC420 or SU91 QTL in the low disease pressure treatments, indicating the QTL can be deployed without harming yield potential in environments which lack disease. The SU91 QTL contributed a significant yield advantage under severe disease pressure. The BC420 QTL, by itself (BBss), exhibited some effect for reducing disease severity in the field, while earlier greenhouse inoculations showed no effect (Vandemark et al., 2008).

Fig. 1 Yield effect for CBB resistance QTL Severe disease pressure (2008)

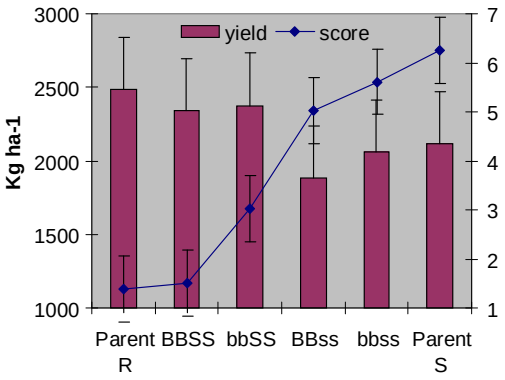


Fig. 2 Yield effect for CBB resistance QTL Severe disease pressure (2009)

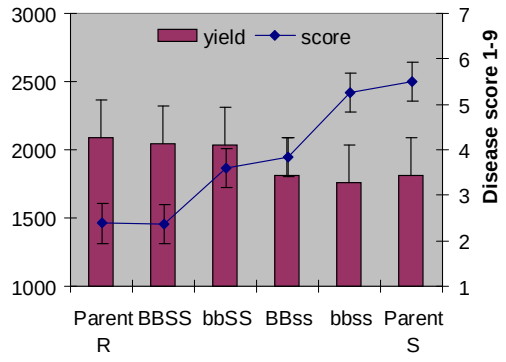


Fig. 3 Yield effect for CBB resistance QTL Low disease pressure (2008)

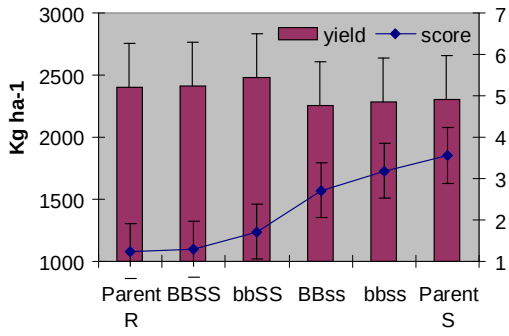
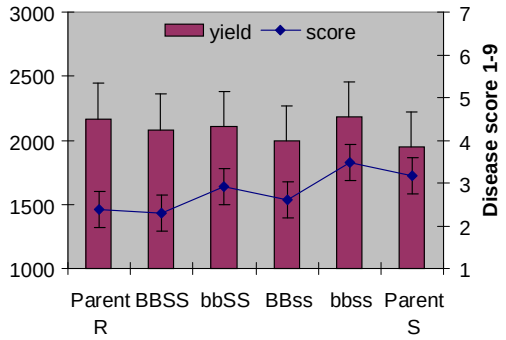


Fig. 4 Yield effect for CBB resistance QTL Low disease pressure (2009)



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MAPPING OF AN ANDEAN GENE FOR RESISTANCE TO ANTHRACNOSE IN THE LANDRACE JALO LISTRAS PRETAS

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INTRODUCTION

The characterization of new germplasm and the introgression of new resistance genes in improved cultivars is one of the most efficient and economic alternatives to control the anthracnose of common bean disease caused by *Colletotrichum lindemuthianum* (Vieira, 1983). To date, 13 genes that confer resistance to anthracnose have been described in the literature (Kelly and Vallejo, 2004; Gonçalves-Vidigal et al., 2009). Among these genes, only three originate in the Andean gene pool, *Co-1* (McRostie, 1919), *Co-12* and *Co-13* (Gonçalves-Vidigal et al., 2009). The Andean landrace Jalo Listras Pretas (JLP) which present the gene *Co-13* (Gonçalves-Vidigal et al., 2009) is one of the most important ones, once it confers resistance to eight races of *C. lindemuthianum* (Vidigal Filho et al. 2007). Unfortunately, among these Andean genes, only *Co-1* has been tagged and mapped.

The objective of this work was to identify RAPD molecular markers linked to resistant gene *Co-13*, present in Andean landrace Jalo Listras Pretas.

MATERIALS AND METHODS

The genetic and molecular analyses were carried out in an F_2 population derived from the cross between Jalo Listras Pretas (resistant to race 73) and Cornell 49-242 (susceptible to race 73) cultivars. Thirty four RAPD primers were assayed for linkage with the *Co-13* using the BSA (Bulked Segregant Analysis) approach (Michelmore et al. 1990). Two contrasting bulks of DNA were developed, one composed of DNA from six F_2 resistant homozygous plants (RB), and the other from six susceptible homozygous plants (SB). The marker that presented polymorphism among bulks and corresponding parents were tested in individuals of each bulk before phenotyping the entire population (116 F_2 individuals). Segregations for RAPD amplicons and resistance to the disease in the F_2 population were analyzed by a Chi-square test (χ^2). Estimations of recombination frequencies and genetic distances between markers and the *Co-13* resistance gene were obtained by the Mapmaker/EXP 3.0 program (Lander et al., 1987). The distance between the locus and the marker was calculated using the Kosambi's mapping function (Kosambi, 1944). Linkage group nomenclature follows Pedrosa-Harand et al. (2008).

RESULTS AND DISCUSSION

A total of 365 F_2 plants from the cross Jalo Listras Pretas x Cornell 49-242, inoculated with race 73 of *C. lindemuthianum*, provided a segregation of 275 resistant to 90 susceptible plants. The corresponding Chi-square for a 3:1 ratio was χ^2= 0.023 (p = 0.88), suggesting that resistance is conditioned by a single, dominant allele at the *Co-13* locus. The Bulked Segregant Analysis showed that, among 34 RAPDs primers analyzed, the marker $OPV20_{680}$ (5'-CAGCATGGTC-3'), present in JLP, was potentially linked to locus *Co-13*. The 680 bp amplicon was present in the resistant parent and was thus in coupling with the resistance allele. It was also present in all individuals of the resistant bulk but was absent from the susceptible parent and all individuals form the susceptible

bulk (Fig. 1). Further segregation analysis in an F_2 population (n = 116 individuals) inoculated with race 73 of *C. lindemuthianum* showed that the genetic distance between the marker and the resistance locus was 1.8 cM.

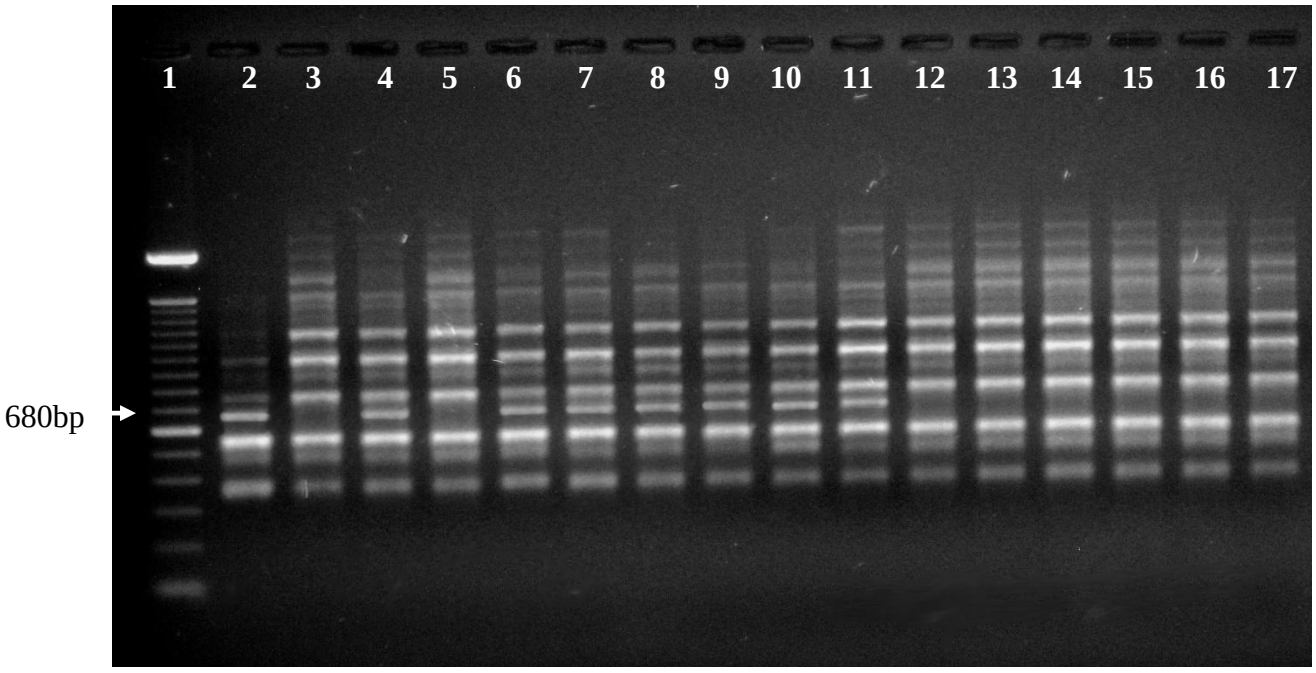

Figure 1 – DNA amplification of parents and F_2 plants from the cross Jalo Listras Pretas x Cornell 49-242 using primer RAPD $V20_{680}$. Lane (1) 100—bp DNA ladder, lane (2) Jalo Listras Pretas (Resistant parent), lane (3) Cornell 49-242 (Susceptible parent), Lane (4) resistant Bulk, Lane (5) Susceptible Bulk, lanes (6-11), F_2 resistant plants to race 73, lanes (12 – 17) F_2 susceptible plants. The arrow indicates a band linked to resistant loci.

The segregation analysis in the recombinant inbred population BAT93 x Jalo EEP558 (Freyre et al. 1998) showed that $OPV20_{680}$ segregated according to a 1:1 ratio of 1:1 and was linked to the *Co-13* gene on linkage group 3 of the common bean consensus map (Freyre et al. 1998). Marker $OV20_{680}$ has been previously described on linkage group 3 (Kelly and Vallejo, 2004), confirming, therefore, that the resistance gene found in JLP is located in that linkage group.

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DIFFERENTIATION OF APHID-TRANSMITTED VIRUSES IN SNAP BEANS USING REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION

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INTRODUCTION

A disease complex incited by aphid-transmitted viruses has been causing considerable yield and quality losses in snap bean production throughout the Great Lakes region of the United States since 2000. The increase in virus disease incidence has been associated with the appearance of a new vector, the Asian soybean aphid (*Aphis glycines* Matsumura). The predominant virus appears to be *Cucumber mosaic virus* (CMV) which has been detected through enzyme linked immunosorbent assay (ELISA) in the majority of infected plants in New York State. The sampling of affected fields has revealed the presence of a number of distinct viruses and virus species other than CMV that include *Alfalfa mosaic virus* (AMV), *Bean yellow mosaic virus* (BYMV), and *Clover yellow vein virus* (ClYVV) among others (Shah et al., 2006). The number of potential inciting viruses and the plurality and non-predictive nature of the symptoms make visual diagnoses insufficient. The detection and differentiation of these viruses using ELISA can also be difficult due to required threshold levels of virus concentration, and/or serological cross-reaction. Serological cross-reaction has been a problem in the ELISA based detection of the closely related potyviruses BYMV and ClYVV (Shah et al., 2006). Reverse transcription polymerase chain reaction (RT-PCR) is a much more sensitive and powerful technique for plant virus detection, and prior plant virology research has involved virus sequencing and primer design for detection of these viruses in other crop pathosystems. A number of primer sequences reported in the literature for use in the detection of these viruses were evaluated using positive control samples to determine their effectiveness in detection and exclusive differentiation of the viruses associated with the aphid-transmitted virus disease complex of snap bean. The primers were also evaluated to determine their most stringent respective annealing temperatures and clarity.

MATERIALS AND METHODS

Virus Isolates: CMV-Le – Field Isolate; Avon, NY; identity verified by bioassay and serology; **BYMV –** Field Isolate; Avon, NY; previously verified bioassay and serology; **ClYVV –** Field Isolate; Avon, NY; identity verified bioassay and serology.

Nucleic Acid Preparation: Total RNA was isolated from all tissue samples using the RNAqueous® RNA isolation kit (Ambion) according to the manufacturers instructions. **RT-PCR Conditions:** Reverse transcription and PCR reactions were performed sequentially (two-step). cDNA was generated from total RNA using the RETROscript® first strand synthesis kit for RT-PCR (Ambion) according to the manufacturers instructions. PCR amplifications were performed in a reaction mixture containing the following: 0.02 µl cDNA solution, 4µl modified PCR Buffer, 2µl 2mM dNTP mix, 2µl Taq Polymerase, 1µl 10mM each of the forward and reverse primers (Table 1), and 8µl sterile distilled water. PCR amplification was performed in a thermal cycler (Eppendorf Mastercycler Gradient or Stratagene Robocycler 96) with 40 cycles of denaturation at 94ºC for 1 min., annealing at primer specific temperatures (54-68ºC) for 1 min., extension at 72ºC for 2 min, and a final extension at 72ºC for 4 min. Commercially available 18 S rRNA primers (Ambion) and blank reactions were used as internal controls. PCR products were analyzed by electrophoresis on

2% agarose gels in TAE buffer with standard ethidium bromide staining and gel banding recorded on a gel-doc system (BioRad).

Table 1: PCR Primer Pairs

***Cucumber mosaic virus* (CMV)**				
Primer name	**Primer Sequence**	**Target**	**Exp. Size (bp)**	**Opt. Temp.**
CMV-1 F (*1)	5'-TATGATAAGAAGCTTGTTTCGCGCA-3'	CMV	500	67-69ºC
CMV-1 R	5'-TTTTAGCCGTAAGCTGGATGGACAACCC-3'			
***Bean yellow mosaic virus* (BYMV)**				
Primer name	**Primer Sequence**	**Target**	**Exp. Size (bp)**	**Opt. Temp.**
BYMV-4 F (*3)	5'-CTMCARATGGAGAAYCCYGC-3'	BYMV		
BYMV-4C R	5'-GRTAYGCTCTYTGRCCCCAMAC-3'		328	56-58ºC
***Clover yellow vein virus* (ClYVV)**				
Primer name	**Primer Sequence**	**Target**	**Exp. Size (bp)**	**Opt. Temp.**
CYVV-2 F (*2)	5'-TTGATGACAGCCAGATG-3'	ClYVV	844	64-66ºC
CYVV-2 R	5'-AATCGTGCTCCAGCAATG-3'			
***Alfalfa mosaic virus* (AMV)**				
Primer name	**Primer Sequence**	**Target**	**Exp. Size (bp)**	**Opt. Temp.**
AMV F (*1)	5'-CGTCAGCTTTCGTCGAACA-3'	AMV	288	66-69ºC
AMV R	5'-GCCGTCGCGCATGGTAAT-3'			

- Sequences of primer pairs that exclusively amplified their target virus (*references listed below)

RESULTS

A number of primer pairs (not listed in Table 1) were selected from the literature and tested in preliminary RT-PCR reactions to ascertain their respective abilities to amplify cDNA of the available virus isolates. All primers tested were capable of amplification of the target virus for which they were designed, and expected product size conformed with the literature. Optimum annealing temperatures were determined by temperature gradient PCR. A temperature gradient of 10ºC was applied to a 58ºC base annealing temperature, so that annealing temperatures varied from 51.2ºC – 68.5ºC. Optimum annealing temperature was determined by visual analysis for amplicon clarity and intensity, minimal unspecific amplification, and minimal formation of primer dimer. The optimum annealing temperatures determined are listed in Table 1. Each primer pair was evaluated for its specificity of amplification though the results are presented only in the context of the isolates used. The primers listed in Table 1 specifically and exclusively amplified their targets. The results presented are a preliminary effort to further develop the nucleic acid based detection resources for this virus disease complex. Further work is needed to test additional primers, particularly for some of the other viruses that may be associated with the complex that were not investigated here (BCMV, WMV-2, SMV, CYMV, PSV, TSV). The results presented here need to be confirmed through employment in a field-based survey with ELISA controls. In the future, the multiplexing of specific primers would enhance the ease and value of a nucleic acid based detection system.

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CONSTRUCTING A GUS-TAGGED INFECTIOUS CDNA CLONE OF *BEAN COMMON MOSAIC VIRUS*

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ABSTRACT
An infectious cDNA clone of *Bean common mosaic virus* strain RU1 was constructed, between the 35S promoter and NOS terminator, using overlapped fragments of the virus that were amplified with the primer pairs designed on the basis of previously posted sequence of RU1 (AY863025). To circumvent the toxicity of viral genome to *Escherichia coli* an intron was integrated into the viral genome and the whole cassette was introduced into *Agrobacterium tumefaciens*. Finally viral cDNA was tagged with the GUS gene for feasible study of BCMV-host interaction.

INTRODUCTION
Bean common mosaic virus (BCMV) and *Bean common mosaic necrosis virus* (BCMNV), both members of the genus *Potyvirus* (family *Potyviridae*), are the most destructive plant viruses affecting bean production worldwide. Both viruses have the genome structure like the other members of potyviruses consisting of ≈10,000 nucleotides that encodes a polyprotein, which is processed into 10 different proteins involved in virus infection. The 5' end is covalently attached to a virus encoded protein (VPg) and the 3' end is polyadenylated. Strains of BCMV and BCMNV are divided into seven and three pathogenicity groups on the basis of their interaction with *Phaseolus vulgaris* genotypes, respectively (Drijfhout, 1978). Despite the importance of both viruses to bean production, little is known on the *P. vulgaris*-BCMV/BCMNV molecular interactions. In the present study we describe our attempts to construct a GUS-tagged infectious cDNA clone of BCMV as a molecular approach to study BCMV-plant interactions and to develop a molecular tool for heterologous gene expression.

MATERIALS AND METHODS
BCMV-RU1 strain (Silbernagel *et al.*, 2001; Larsen *et al.*, 2005) was received in infected seeds of *P. vulgaris* cultivar BTS-II from USDA-ARS, Prosser, Washington. Seeds of susceptible cultivars DW and SGR were obtained from CIAT (Colombia). Total RNA was extracted from the infected tissues and viral cDNAs were amplified in overlapping fragments using enzymes high-fidelity reverse transcriptase (Roche, Mannheim, Germany), *Pfu* DNA polymerase (Stratagene, USA) and Expand DNA polymerase (Roche, Mannheim, Germany) according to the manufacturers' recommendations. Amplified fragments were cloned, sequenced and subsequently inserted into pAGUS1 vector (Skuzeski *et al.*, 1990) between the 35S promoter and NOS terminator using unique restriction sites. An intron of 189 bp was inserted within the viral genome to increase the viral stability in *E. coli* as recommended (Johansen and Lund, 2008). Finally the GUS gene and a protease site were introduced between the P1 and HCPro.

RESULTS AND DISCUSSION
The entire viral genome was amplified in six overlapping fragments. When these fragments were sequenced and compared to the published BCMV-RU1 sequence, more than 200 nucleotide differences were identified. The new RU1 sequence was submitted to NCBI (GQ219793). It was

also observed that after amplification in *E. coli*, cDNA clones covering the domains encoding the CI-P3 region had small nucleotide deletions. This instability suggested that these domains were toxic to *E. coli*, a phenomenon that is not uncommon (Johansen, 1996; Yamshchikov *et al.*, 2001). The instability was eliminated by insertion an intron within the CI domain at a position that was predicted to generate an efficient splice site (http://www.cbs.dtu.dk/services/NetGene2/). The cDNA fragments covering the complete virus genome were then assembled between the 35S promoter and NOS terminator in the vector pAGUS1. Manual inoculation of the full-length clone on two cultivars DW and SGR resulted in severe BCMV symptoms about a week after inoculation.

In order to visualize infected cells, the GUS coding sequence was inserted in the BCMV cDNA between the P1 and HC coding sequences. The GUS sequence was tailed with a sequence encoding the protease site found between NIb and CP. This should result in release of GUS from the virus polyprotein and minimize interference with the function of HC. Finally, the whole cassette was inserted into a binary vector (pCAMBIA) and *A. tumefaciens* were transformed with this construct to allow inoculation by agroinfiltration.

We are now using this infectious cDNA clone of BCMV-RU1 to study BCMV-plant interactions.

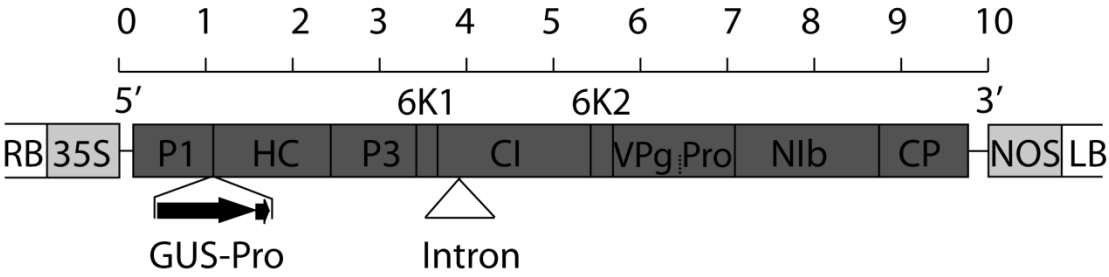

Figure 1. Schematic representation of BCMV-RU1 full-length cDNA clone. The scale bar shows approximate relative size of viral genes. The genome organization of BCMV with the viral open reading frame shown as black grey box with individual viral products and the approximate position of the intron within CI is shown with a triangle. The GUS gene and additional protease (Pro) site are shown with arrows. RB and LB are right and left borders of pCAMBIA plasmid, respectively. 35S promoter and NOS terminator are shown in grey boxes.

ACKNOWLEDGEMENTS

We are grateful to the Genetic Resources Unit of CIAT for the seeds of *P. vulgaris* cultivars DW and SGR, and to Dr. Richard Larsen, USDA-ARS, Prosser, Washington, USA for BCMV- RU1 strain.

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MAPPING AND QTL ANALYSIS OF AGRONOMIC TRAITS IN COMMON BEAN: VALIDATION OF A HALO BLIGHT RESISTANCE QTL IN BEAN BREEDING PROGRAMS

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INTRODUCTION

Selection of agronomic traits, like yield and disease resistance has been extensively used by bean breeders to develop cultivars with superior performance or to develop cultivars that are adapted to specific environments. However, complex inheritance patterns and strong environmental effects may limit the value of phenotypic estimates of these traits. The use of molecular markers will improve our understanding of the genetic factors conditioning complex traits since these factors can be located in specific regions of the genome, and their effects can be estimated individually. In addition, the use of molecular markers is expected to assist in the selection of superior genotypes.

The aims of this study are: i) Establish a genetic map of common bean based on a population highly relevant to the gene pool used by bean breeders, ii) Identify molecular markers linked to traits of agronomic interest in breeding programs in this genetic background and iii) Exploit these markers through Marker Assisted Selection (MAS).

MATERIAL AND METHODS

Population development: An F7 population of 188 recombinant inbred lines (RIL) from a cross between two commercial varieties: Magister and Clovis, was used in this study. Magister and Clovis are two dwarf beans with round pods destined to industry and fresh market, respectively. Magister is characterised by white seeds and flowers, pods diameter lower than 6.5mm and resistance to anthracnose, common blight and halo blight. Clovis is characterised by coloured seeds and flowers, pods with diameter between 9 and 10.5mm and susceptibility to anthracnose, common blight and halo blight.

Genotyping: 99 simple sequence repeats (SSR) and four sequence characterized amplified region (SCAR) markers were chosen based on their polymorphism between parents. For mapping, DNA was isolated from each line using a modification of the method of Edwards *et al*. (1991). Protocols for marker amplification and visualization were a modified method of that used by Tar'an *et al.* (2001).

Field and greenhouse screening: Twenty-six phenotypic traits of agronomic interest were evaluated. The RI population and parents were grown in an observation trial at La Ménitré (France) to record data on twelve traits on plants, four traits on seeds and six traits on pods. They were also grown in four greenhouse tests to evaluate disease resistance to anthracnose, common blight, halo blight and root rot.

Mapping and QTL analysis: The map was built using the Kosambi mapping function. Linkage groups were established with a minimum LOD score of 3.0. QTL detection was done with Multiple QTL Mapping method (MQM). The LOD threshold was evaluated by permutation test and the confidence interval defined by LOD decrease method of one point. Mapping and QTL analysis were performed using the programs JoinMap3.0 and MapQTL5.0, respectively.

RESULTS AND DISCUSSION

Mapping: The Magister x Clovis genetic map has 96 molecular markers associated in 10 linkage groups. It covers 289.5cM and represents approximately 21% of the bean genome. Limited map length is due to low polymorphism in this population due to the relatedness of the two parents which are members of the same gene pool (Mesoamerican) and belong to close commercial classes.

QTL detection: The QTLs analysis allowed detection of twelve QTLs mapped in five linkage groups. They are involved in ten phenotypic traits. The phenotypic variation explained by these QTL varies from 9.4 to 80.3% (Figure 1 and Table 1).

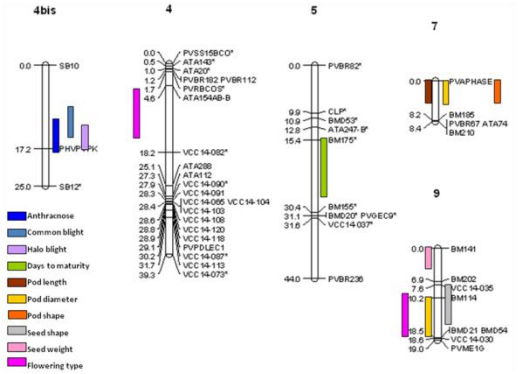

Figure 1: Linkage groups of the Magister x Clovis genetic map linked with QTL of agronomic interest

Table 1: Summary of the QTL analysis

Traits	QTL	Linkage Group	Marker linked	Marker position (cM)	QTL position (cM)	LOD score	R2 (%)	Confidence interval (cM)	Parent
Anthracnose	Ant	4bis	PHVPVPK	17,17	15,50	20,63	48,00	12.0 - 18.2	Magister
Common blight	GC	4bis	PHVPVPK	17,17	12,00	21,00	66,60	9.3 - 15.5	Magister
Halo blight	GH	4bis	PHVPVPK	17,17	15,50	48,32	80,30	13.5 - 16.9	Magister
Days to maturity	DM	5	BM175	15,44	20,94	5,61	20,20	14.9 - 27.0	
Pod shape	FGo	7	PVAPHASE	0,00	1,00	12,81	30,70	0.0 - 4.5	Magister
Seed shape	FGr	9	BM114	10,16	10,66	6,86	18,30	7.6 - 15.6	Magister
Pod diameter	FINES-1	7	PVAPHASE	0,00	2,00	12,50	28,40	0.0 - 4.7	
Pod diameter	FINES-2	9	BMD21	18,48	14,66	6,97	14,90	10.1 - 18.5	
Pod length	LONGGo	7	PVAPHASE	0,00	1,00	14,67	34,20	0.0 - 4.2	
Seed weight	PMG	9	BM141	4,28	4,28	4,28	10,70	0.0 - 4.0	Magister
Flowering type	TYPFlo-1	4	ATA154AB-B	4,60	10,10	4,33	16,20	4.8 - 14.1	Clovis
Flowering type	TYPFlo-2	9	BM114	10,16	14,16	3,70	9,40	8.4 - 18.2	Clovis

On linkage group 7, three QTL involved in pods characteristics (shape, diameter and length) were positioned in the same area. This organisation was also found for flowering type and pod diameter QTL on linkage group 9. These QTL grouping could be due to linked genes involved in these traits or genes with pleiotropic effects.

A major QTL linked to halo blight resistance was shown at 1.7cM from the PHVPVPK marker on linkage group 4bis. It explains 80.3% of the phenotypic variation. This genomic area seems to be rich in resistance genes or resistance gene analogues (Tar'an *et al.*, 2001), and has also been linked to a major resistance QTL to common blight and anthracnose (Figure 1 and Table 1). These data confirms results obtained previously by others authors (Tar'an *et al.*, 2001; Campa *et al.*, 2009).

QTL analysis improves our knowledge of bean genome organization and is a good molecular support to breeding programs. SSR marker PHVPVPK, closely linked to a major QTL involved in halo blight resistance is currently used to select for this resistance through MAS in our bean breeding programs.

In the future, in order to saturate the Magister x Clovis genetic map and improve QTL mapping, we plan to increase the number of mapped SSR through similar studies on others populations, building a consensus map. In a second step, we plan to develop and map SNP markers.

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GENERATION MEANS ANALYSIS OF AGRONOMIC AND SEED QUALITY TRAITS IN COMMON BEAN

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INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) experienced its domestication and diversification in America, existing two main genetic centres of domestication, Mesoamerica and South America, and genetic isolation between both centers during centuries. Mesoamerican varieties are more productive and resistant to biotic and abiotic stresses than Andean varieties, that present a larger seed size and a better commercial quality. In the XVI century Mesoamerican common bean germplasm was introduced in the Iberian Peninsula, and this fact gave rise to an increase of the genetic diversity. Occasional crossing, new environmental adaptation (temperature, humidity, photoperiod, soil fertility, diseases, insects and new cropping systems) and a strong selection, based on the European consumer preferences among the different type of seeds could have had an important role in the evolution of the new genetic variation in common bean observed in the Iberian Peninsula. Intermediate and recombinant natural forms adapted to these environments have been well documented (Santalla et al., 2002) and they might have appeared as a result of a initial recombination between the Mesoamerican and the Andean genetic pools.

The objetive of this work was to evaluate the potential of the new genetic variation of common bean found in the Iberian Peninsula, as an excellent resource for the genetic improvement of the species, since it can serve as bridging material for crosses between the Andean and the Mesoamerican genetic pools, overcoming the existing partial genetic barrier in the hybridization.

MATERIAL AND METHODS

The following simple crosses were carried out (Table 1): Cross with a Mesoamerican control: Matterhorn x PHA-0399 (line MBG-CSIC) (1), cross with an Andean control: Beluga x PHA-0399 (2), cross with a Mesoamerican control: PHA-0419 (line MBG-CSIC) x Matterhorn (1), cross with an Andean control: PHA-0419 x Beluga (2).

Table 1. Parental lines used in the crosses

Line	Commercial class	Origin	Grow habit	Size of seed	Resistance genes
Matterhorn (Kelly et al., 1999)	Great Northern	Mesoamerican	Indeterminate bush-II	35 g/100 seeds	BCMNV (I)
Beluga (Kelly et al., 1999)	Canellini	Andean	Determinate-I	60 g/100 seeds	BCMNV (I) Anthracnose (*Co-1*)
PHA-0399-102-02-05 (Santalla et al., 2004)	Great Northern	Mesoamerican-Recombinant	Indeterminate climbing-IV	90 g/100 seeds	
PHA-0419-111-03-02 (Santalla et al., 2004)	Great Northern	Mesoamerican-Recombinant	Indeterminate climbing-IV	80 g/100 seeds	

The crosses (1) would permit to study the favourable Mesoamerican genes from PHA-0399 and PHA-0419 to improve Mesoamerican germplasm, and the crosses (2) would permit to known the

favourable genes from PHA-0399 and PHA-0419 to improve Andean germplasm. A F6 recombinant line (RIL) was generated per each F2 plant by using the Single Seed Descent (SSD) procedure. The F2, F3, F4 and F5 lines and the parents (Matterhorn, Beluga, PHA-0399 and PHA-0419) were evaluated at the MBG-CSIC for agronomic and seed quality traits such as: days to first flower, weight of 100 seeds, seed length, width and thickness, number of pods or seed production.

RESULTS

The Figure 1 displays the histograms showing the relative frequencies for the characters seed length (a) and 100-seeds weight (b) of the segregant generations of the cross Beluga x PHA-0399. In both histograms, F5 generation presents a tendency towards the greater seed weight and greater seed length parental, namely PHA-0399. The relative frequencies of the agronomic traits for production of plants and days to first flower from the cross Beluga x PHA-0399 were plotted in Figure 2a and 2b respectively. There are a proportion of F5 individuals that surpasses the parental plant production, which implies a transgresive genetic improvement. Furthermore, a transgresive reduction in the days to first flower character is shown for the same cross.

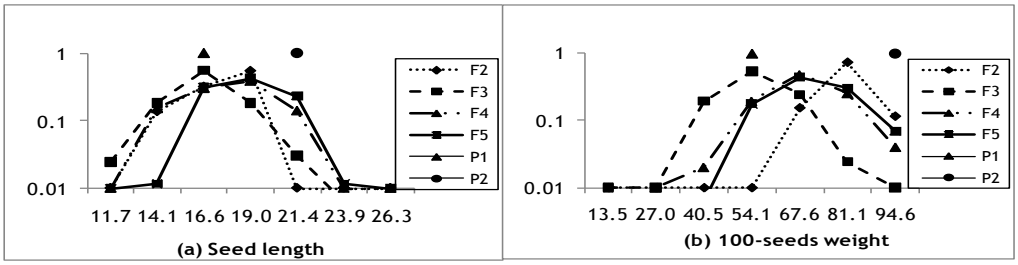

Fig.1. Histograms showing the relative frequencies for the characters seed length **(a)** and 100-seeds weight **(b)** of the segregant generations of the cross Beluga (P1) x PHA-0399 (P2).

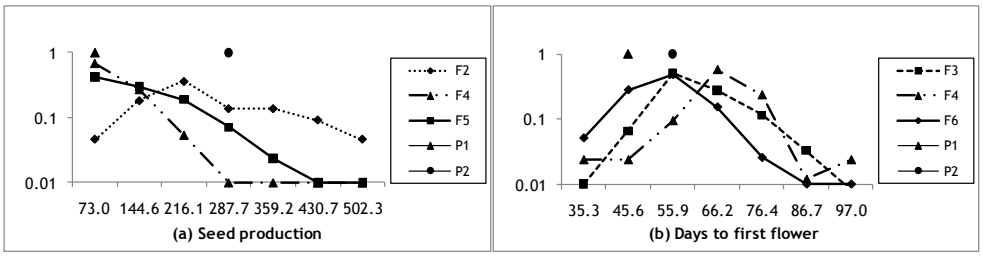

Fig.2. Histograms showing the relative frequencies for the characters seed production **(a)** and days to first flower **(b)** of the segregant generations of the cross Beluga (P1) x PHA-0399 (P2).

In light of these results, the cross Beluga x PHA-0399 can be used for bean genetic improvement, as it presents in the F5 generation improve for many characters, and therefore it could serve as a bridging material for crosses between the Andean and the Mesoamerican genetic pools. Other crosses in this study have also shown relevant results in some of the analyzed characters (data not showed).

ACKNOWLEDGEMENT

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COMPREHENSIVE LINKAGE MAP OF WHITE MOLD RESISTANCE QTL IN COMMON BEAN

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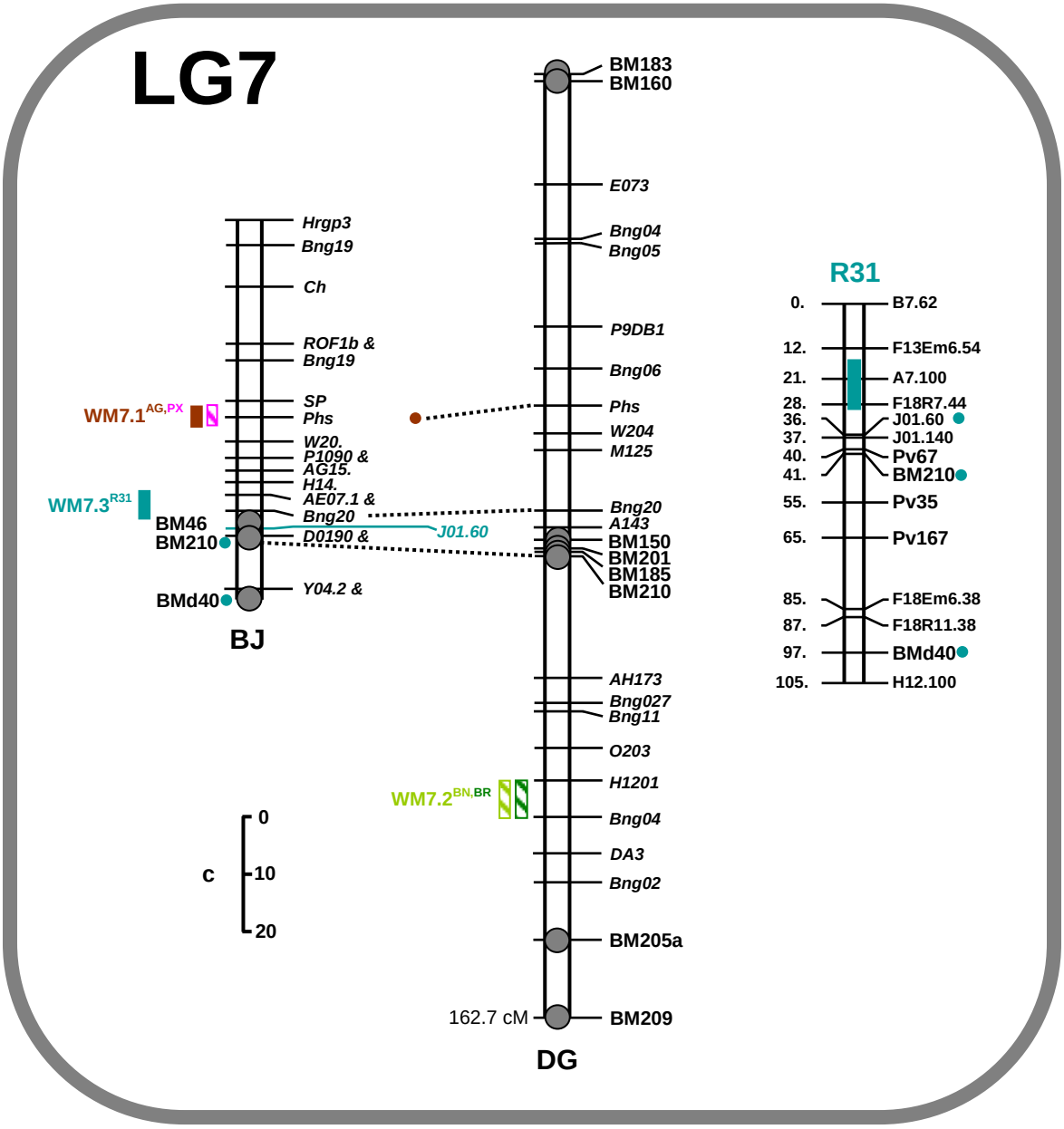
Sources of resistance to white mold (caused by *Sclerotinia sclerotiorum* Lib. de Bary) in the common bean gene pool (*Phaseolus vulgaris* L.) are limited. Multiple QTL conferring white mold resistance were identified in two dry bean germplasm lines, I9365-31 (R31 population) small black and VA19 (BV population) light red kidney. Five major and three minor QTL were detected in the R31 population (on LG2, LG4, LG5, LG6, LG7, and LG8), while in the BV population, one major and one minor QTL were discovered (on LG2 and LG8). Here we have integrated previously-identified white mold resistance QTL with the R31 and BV QTL onto a comprehensive core map and have inverted and named linkage groups according to Pedrosa-Harand *et al*. 2008. We also organized all existing QTL into discrete groups and applied new QTL nomenclature (Miklas, 2009).

A total of 38 QTL conditioning partial resistance to white mold were integrated on the core map. These 38 QTL coalesced into 21 regions (LG1[2], LG2[3], LG3[2], LG4[2], LG5[3], LG6[1], LG7[3], LG8[4], LG9[1]) across nine linkage groups. Ten QTL, one each on LG3, LG6, and LG9, two each on LG2 and LG7, and three on LG8, were identified in more than one population. Four of these QTL (on LG2, LG7 (2), and LG8) were further validated in marker-assisted selection studies (Miklas 2007; Ender *et al*. 2008). The QTL integrated on LG 7 are depicted in Figure 1 and Table 1. This map will provide a framework for integrating and interpreting future QTL studies and candidate gene analyses.

Table 1. List of QTL which integrated to LG 7 in four separate regions.

QTL on LG 7	RIL pop	Trait	Nearest marker	% variation explained
WM7.1^{AG,PX}	AG	Straw test	*Phs*	38
	PX	Straw test	J09.950	5, 9, 16
WM7.2^{BN,BR}	BN	Field	EaggMctt85	16.8
	BR	Field	EaacMctt223	14.7
WM7.3^{R31}	R31	Straw test	F18R7.440	52, 20

AG=A55/G122. PX=Pomp 50/XAN 159, BN=Bunsi/Newport, BR=Bunsi/Raven, R31=Raven/I9365-31.



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SINGLE NUCLEOTIDE POLYMORPHISM (SNP) DISCOVERY IN THE COMMON BEAN

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INTRODUCTION

Although the common bean (*Phaseolus vulgaris* L.) is the most important legume directly used as a human food, more efficient molecular tools for genetic studies in this species are still greatly needed. Single nucleotide polymorphisms (SNPs) – single DNA base differences between homologous DNA fragments and small nucleotide insertions or deletions (indels) – are highly desirable as molecular markers because they are an abundant form of DNA variation in eukaryotic genomes (Brookes 1999). SNPs can be used as biallelic and codominant DNA markers for a variety of tasks in crop improvement including genes and quantitative trait loci (QTL) discovery, assessment of genetic diversity, association analysis, and marker-assisted selection (Zhu et al. 2003). Another relevant characteristic of the SNP markers is that they can be transferred between closely related species and utilized for microsynteny analysis.

MATERIAL AND METHODS

SNPs were discovered in common bean via resequencing of sequence-tagged sites (STSs) developed by PCR primers designed to soybean shotgun and BAC-end sequences, to common bean genes and microsatellite flanking regions. DNA fragments harboring SNPs were identified in single amplicons from six contrasting *P. vulgaris* genotypes of the Andean ('Jalo EEP558', 'G19833', and 'AND277') and Mesoamerican ('BAT 93', 'DOR 364', and 'Rudá') gene pools. These genotypes are the parents of three common bean RIL mapping populations. The PCR primers were initially used to amplify the DNA of cultivar 'Jalo EEP558' at annealing temperatures of 58/48ºC (soybean PCR primers) or 54ºC (common bean PCR primers) followed by DNA sequence analysis of the resulting single amplicons. The two resulting sequence traces derived from opposite ends of each amplicon were analyzed and aligned with the aid of standard DNA analysis software Phred and Phrap. Resulting alignments and trace data were visually inspected in the Consed viewer. When good quality sequence data were obtained, the STS primers were then used to amplify the genomic DNA of the other five genotypes. The resulting PCR products were sequenced and analyzed for SNP discovery with the SNP-PHAGE software (Matukumalli et al. 2006).

RESULTS AND DISCUSSION

From an initial set of 1,880 PCR primer pairs tested, 265 robust STSs were obtained, amplified and sequenced in each one of the six common bean genotypes. In the resulting 131,120 bp of aligned sequence, a total of 677 SNPs were identified, including 555 single-base changes (295 transitions and 260 transversions) and 122 small nucleotide insertions/deletions (indels) (Tables 1 and 2). The frequency of SNPs was 5.16 SNPs/Kb and the mean nucleotide diversity expressed as Halushka's theta was 0.00226 (Table 1). This work represents one of the pioneer efforts aiming to detect SNPs in *P. vulgaris*. The SNPs identified are an important resource to common bean geneticists for quantitative trait loci (QTLs) discovery, marker-assisted selection and map-based cloning. They will

be also useful for diversity analysis and microsynteny studies among legume species. In the near future the SNPs developed in this work will be tested in the common bean germplasm using the SNP GoldenGate assay on the Ilumina BeadStation.

Table 1. Summary of results of SNP discovery in *P. vulgaris* DNA fragments generated by common bean and soybean-derived PCR primers

	Source of primers			Total
	Soybean STSs	Common bean genes	Common bean SSRs	
No. of tested primers[a]	1499	168	213[b]	1880
No. of single amplicons - STS (%)	128 (8.54)	66 (39.29)	71 (33.33)	265 (14.10)
Fragments with at least 1 SNP (%)	81 (5.40)	48 (28.57)	44 (20.66)	173 (9.20)
Sequence length (bp)	66,085	38,167	26,868	131,120
Mean STS length	516	578	378	495
No. of SNPs	277	237	163	677
SNP frequency (SNPs/Kb)	4.19	6.21	6.07	5.16
Nucleotide diversity (θ^c x 1000)	1.84	2.72	2.66	2.26

[a] The primer pairs were initially used to amplify the DNA of the common bean cultivar 'Jalo EEP 558' followed by DNA sequence analysis of the resulting amplicon. When high quality sequence data were obtained, the STS primers were then used to amplify and sequence genomic DNA of the other five genotypes that are parents of three mapping populations: 'AND277', 'BAT 93', 'DOR 364', 'G19833', and 'Rudá'. [b] Primer pairs producing a single band > 200 bp selected in a total of 758 tested common bean SSR primers to attend the requirement of the DNA sequencing platform utilized (ABI 3730). [c] $\theta = K / aL$; where 'K' is the number of SNPs identified in an alignment of 'n' genotypes, 'L' is the total length of aligned sequences in bp, and $a = \Sigma\ 1/(i - 1)$, with i = 2-to-n.

Table 2. Characteristics of SNPs identified in *P. vulgaris* DNA fragments generated by common bean and soybean-derived PCR primers

Source of primers	SNPs	Single-base changes				Indels[c]	
		Transitions[a]		Transversions[b]			
		No.	% of total	No.	% of total	No.	% of total
Soybean STSs	277	123	44.40	118	42.60	36	13.00
Common bean genes	237	109	46.00	88	37.13	40	16.87
Common bean SSRs	163	63	38.65	54	33.13	46	28.22
Total	677	295	43.57	260	38.41	122	18.02

[a] A↔G and C↔T. [b] A↔C, A↔T, C↔G, and G↔T. [c] Small nucleotide insertions and deletions.

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RESISTANCE TO HALO BLIGHT, COMMON BACTERIAL BLIGHT AND BACTERIAL BROWN SPOT IN SPANISH COMMON BEAN CORE COLLECTION

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INTRODUCTION

Halo blight (HB) caused by *Pseudomonas syringae* pv. *phaseolicola* (*Psp*), common bacterial blight (CBB) caused by *Xanthomonas campestris* pv. *phaseoli* (*Xcp)* and bacterial brown spot (BBS) caused by *Pseudomonas syringae* pv. *syringae* (*Pss)*, are among the major constraints of common bean (*Phaseolus vulgaris* L.) production in the North Central part of Spain (1, 2). Depending on environmental conditions of each year, these bacterioses can be found together or separately in this area.

The Spanish Plant Genetic Resources Centre hold the active bean collection which includes 2661 accessions collected in Spain. A core collection, based on seed morphology and passport data, which includes 211 accessions, has been stabilised (3). Different characterization and evaluation works has been carry out over this material (4).

The objective of this study was to evaluate the Spanish Core Collection against the three major bacterial pathogens in order to use it for breeding purposes.

MATERIAL AND METHODS

A total of 199 accessions included in the *Phaseolus vulgaris* Spanish Core Collection were screened separately against the HB, CBB and BBS pathogens. In the cases of HB and CBB the accessions were characterised in field, in two independent assays, for its resistance to the pathogens *Xcp* and *Psp*. For *Psp* a mixture of two isolates were used for inoculation, belonging to races 6 and 7, the two predominant races in the region (1). Two unreplicated rows per genotype were sown. Plants were inoculated by aspersion according to the method described by Beebe *et al*. (5).

With BBS the accessions were screened using a randomized design with 4 replications in controlled conditions (22ºC, photoperiod 12hr day/12hr night and about 80% humidity). Plants were inoculated in primary leaf by multiple needles, according to the method described by Andrus (6).The first evaluation was realized 10-15 days after inoculation.

The symptoms in all cases, for both leaves and pods, were visually evaluated using the 1 to 9 scale described by Schoonhoven *et al.* (7). Data were reported as an average severity for all plants, and was considering the plants with symptoms evaluated from 1 to 3 as resistant, 4 to 6 as moderately resistant and from 7 susceptible.

RESULTS AND DISCUSSION

Figure 1 show the level of bacterioses in 199 entries of Spanish Core Collection screened against the three bean bacterial pathogens, the 13% (25 accessions) showed some degree of HB resistance in leaf, and the 17,4% (32 accessions) in pod. In CBB only the 1,6% showed moderate resistance in leaf (3 accessions), and the 4,8% in pod (1 accession resistant and 8 moderately resistant). Regarding BBS is shown only moderate resistance to leaf, but with a higher percentage than in the case of CBB and HB, the 21,4% (41 accessions). Our results seem to confirm the difficulty reported by other authors (5, 8) to find adequate levels of CBB resistance in *Phaseolus vulgaris* germplasm.

Regarding the multiple resistance, a total of 8 entries were rated with some resistance to HB (leaf and/or pod) and BBS in leaf. Moreover, 5 entries were rated resistant to HB (leaf and/or pod) and to CBB (only pod). Other authors (9) have pointed out before the possibility of an association between these two characters. Finally, 8 entries were evaluated with some degree of resistance, only to HB, in leaf and pod together. All the resistant entries represent a wide range of bean market classes based on seed color and size,

and the majority of these material showed prostrate or semiclimbing growth habit (Table 1). These results would indicate the difficulties existing in finding common bean germplasm resistant to bacterioses, which combine morphological characteristics, as color and size seeds, and determinate growth habit.
These results will be submitted to INIA at the conclusion of the RF2007-00014-C04-03 and CC06-053 Projects, and will be showed in the CRF web site (http://wwwx.inia.es/webcrf).

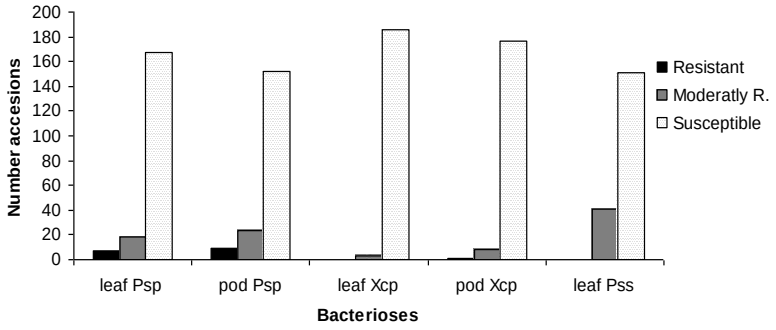

Figure 1. Bacterioses in the Spanish Bean Core

Table 1. Accession number, province of origin, growth habit, seed color, size and shape, *Psp*, *Xcp* and *Pss* reaction in leaves and/or pods, of 21 bean Spanish Core Collection accessions selected for showing some combination degree of *Psp*/*Xcp*/*Pss* or leaf/pod resistances.

CRF Accession[a]	Province	Growth habit[b]	Seed			Reactions[c]				
						Psp		Xcp		Pss
			Color	Weight (g)/100s	Shape	L	P	L	P	L
BGE022831	Cantabria	IV	purple	49	kidney	5	2	7	7	6
BGE002108	Cantabria	III	green	37	oval	6	6	8	8	5
BGE028940	Madrid	IV	bi-coloured (brown/gray)	62	kidney	5	6	9	8	6
BGE028960	Albacete	IV	white	25	truncated	5	6	9	9	6
BGE011037	Navarra	IV	white	42	rounded	7	1	7	9	6
BGE001472	Teruel	III	white	29	cuboid	8	5	7	7	5
BGE013972	Albacete	IV	white	40	cuboid	7	6	9	9	6
BGE013965	Albacete	IV	brown purple	39	cuboid	6	9	9	9	6
BGE029592	Salamanca	IV	mixture (ochre, white, bicolored)	29	cuboid	1	2	7	5	.
BGE003261	Asturias	IV	winy brown	62	cuboid	6	5	8	6	8
BGE003283	Asturias	IV	black	59	oval	9	1	8	3	7
BGE029569	Salamanca	IV	purple	38	oval	9	5	7	6	7
BGE005439	Asturias	III	yellow	53	rounded	6	.	7	5	8
BGE003562	Asturias	IV	black	44	oval	3	3	7	7	7
BGE003997	Soria	III	brown	44	truncated	3	3	8	8	8
BGE002189	Pontevedra	III	brown	31	truncated	1	5	9	9	7
BGE003700	León	III	bi-coloured (purple/cream)	46	kidney	3	6	9	8	7
BGE011736	Cuenca	I	black	28	kidney	4	2	8	9	7
BGE004435	Salamanca	I	yellow	35	oval	5	5	7	7	7
BGE011731	Cuenca	I	ochre	26	kidney	5	6	9	9	8
BGE028947	Madrid	I	green	22	kidney	6	4	9	8	7

[a] Spanish Bean Core Collection number (CRF)
[b] IV= indeterminate climbing, III= indeterminate, II= indeterminate upright, I= determinate upright.
[c] Mean bacterial blight score for each pathogen: resistant (1 - 3), moderately resistant (4 - 6), susceptible (7 – 9) (Schoonhoven and Pastor-Corrales, 1987).

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INHERITANCE OF RESISTANCE TO BACTERIAL WILT DISEASE IN EARLY ROSE AND PI 136725

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ABSTRACT

Bacterial wilt, a seed-borne disease of dry bean, is caused by yellow, orange, purple or pink variants of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (Cff). Since 1995, a resurgence of bacterial wilt disease has been observed in Canada and the USA. Development of cultivars with resistance to Cff is the most economical and environmentally sustainable means of control for this disease. Bean genotypes with resistance to Cff were identified. Preliminary results from inheritance studies indicate the pink bean cv. Early Rose and germplasm line PI 136725 each carry a dominant resistance gene. Results also indicate the resistance genes are non-allelic. Molecular markers linked to these genes, when developed may enable rapid detection of bacterial wilt resistant lines.

INTRODUCTION

Bacterial wilt of bean was first discovered in the USA (Hedges 1922) and was subsequently found in central Canada (Patrick 1954), Mexico (Yerkes and Crispin 1956) and many other countries during the early to middle part of the 20th century (Hsieh *et al.* 2004). The pathogen, Cff has broad geographic distribution and is listed as a quarantine disease in many countries (CAB International 1999; OEPP/EPPO 1982). Symptoms include stunted growth and wilting of bean plants and discoloration on seeds and seedlings. Recent reports indicate a resurgence of bacterial wilt of bean in North America after a prolonged absence, and the reports also suggest the pathogen continues to evolve, resulting in the presence of new colour variants (Huang *et al.* 2006; Harveson *et al.* 2006; Harveson and Schwartz 2007; Harveson and Vidaver 2008). Dry bean genotypes with resistance to three (yellow, orange and purple) variants of Cff have been identified (Hsieh *et al.* 2005; Huang *et al.* 2007). The objectives of this study were to determine the inheritance of resistance to Cff in Early Rose and PI 136725, and to determine allelic relationships among the two resistant genotypes.

MATERIALS AND METHODS

To determine the genetic control of resistance to Cff, reciprocal crosses between resistant genotypes Early Rose (pink) and PI 136725 (tan colour with dark red stripes and spots) and susceptible navy bean cultivars Morden003 and Kippen were made. To investigate allelism, resistant genotypes were crossed. The isolate of Cff used was YSB-2 (yellow variant). Seeds of parental genotypes, F_1 and F_2 plants were inoculated using the hilum injury/seed inoculation method of Hsieh *et al.* (2003). Fourteen days after inoculation, each seedling was rated for disease severity on a scale of 0 to 5. Plants with a rating of 0 were considered as resistant, and plants with rating of 1 to 5 were considered as susceptible. Data were subjected to Chi-square test of goodness of fit.

RESULTS AND DISCUSSION

Four F_1 seeds per cross combination were inoculated. The F_1 seedlings of R x S and reciprocal crosses were intermediate in disease severity rating due to absence of germination, seedling death or small sampling size (four seeds). The F_2 generation of R x S and reciprocal crosses (the exception being PI 136725 x Morden003) segregated in a 3 resistant : 1 susceptible ratio indicating a dominant

gene control of resistance to Cff in bean genotypes Early Rose and PI 136725 (Table 1). The F_2 plants of R x R crosses segregated in a 15 resistant : 1 susceptible ratio indicating Early Rose and PI 136725 possessed different resistant genes.

Table 1. Reaction [Resistance (R) and Susceptible (S)] to YSB-2 (yellow variant) of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* in F_2 populations and Chi-square tests of goodness of fit.

Cross	F_2 plants[z]		Ratio		
	R	S	tested	χ^2 value	*P* value[y]
	Resistant x Susceptible				
Early Rose x Morden003	222	66	3:1	0.667	0.41
Early Rose x Kippen	272	89	3:1	0.023	0.88
PI 136725 x Morden003[x]	87	9	3:1	12.5	0.00
PI 136725 x Kippen	171	46	3:1	1.673	0.20
	Resistant x Resistant				
Early Rose x PI 136725	162	6	15:1	2.057	0.15

[z]Includes reciprocal crosses

[y]*P*-value represents the probability that deviations from the tested ratio are due to chance alone. *P*-value greater than 0.05 indicate that observed values are not significantly different from the expected values.

[x]Only two F_2 families per cross combination were tested in this cross.

CONCLUSIONS

Preliminary results indicate a dominant gene for resistance in the pink bean cv. Early Rose and germplasm line PI 136725. The resistance gene in Early Rose and PI 136725 are non-allelic. Early Rose and PI 136725 may be used as parents in crosses to transfer bacterial wilt resistance. Molecular markers linked to resistance genes, when developed may enable rapid detection of bacterial wilt resistant lines. Additional F_2 populations and $F_{2:3}$ families of crosses listed in Table 1 will be screened to confirm the mode of inheritance of resistance to Cff.

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BIOLOGICAL CONTROL OF PHYTOPATHOGENIC FUNGI IN BEAN (*PHASEOLUS VULGARIS* L.) WITH *TRICHODERMA ATROVIRIDE* AND *TRICHODERMA VIRENS*

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important food crop in Iberian Peninsula, where the province of León is the main bean producing area (León produces > 40 % of total Spanish dry bean production). Phytopathogens fungi are major diseases affecting bean plant in the northwest plateau of Spain (Campelo *et al*., 2007). Seed treatment and sowing are fundamental operations because the initial stages of plant development are most susceptible to adverse environmental conditions and fungi diseases. Application of pesticides to seed and improved sowing technique improve the emergence and crop establishment (Valenciano *et al*., 2006). The European award of the Protection of Geographical Indication (PGI) in 2009 (Council Regulation (EC) No 510/2006) to the beans from La Bañeza-León is an important opportunity to increase the profitability of this product. The challenge is that consumers believe that beans form the PGI have an added value, that it depends in the actual situation on the following aspects: i) quality and healthy product; ii) that the techniques of production are safety for the consumers and friendly with the environment. *Trichoderma* spp. have been known since at least the 1920s for their ability to act as biocontrol agents against plant pathogens. Until recently, the principal mechanisms for control have been assumed to be those primarily acting upon the pathogens and included mycoparasitism, antibiosis, and competition for resources and space. Recent advances demonstrate that the effects of *Trichoderma* on plants, including induced systemic or localized resistance, are also very important. This fact was perhaps first conclusively demonstrated by Bigirimana (1997) who observed that the application of *T. harzianum* in the soil did that the leaves of plants of bean were more resistant to *B. cinerea* and *C. lindemuthianum*, though *T. harzianum* only was present in the roots of the plants.

MATERIAL AND METHODS

The antagonistic activity of species *Trichoderma atroviridae* and *Trichoderma virens* was evaluated on pathogenic isolates of *Botrytis cinerea*, *Fusarium* spp., *Sclerotinia sclerotiorum, Rhizoctonia solani and Trichothecium roseum*, which had been collected in bean fields and bean seeds from León. *Trichoderma atroviridae* and *Trichoderma virens* were grown on PDA medium on cellophane sheets and were incubated for 48 h at 28ºC. After this time, the membranes were removed and the pathogenic isolates were inoculated at the center of the plate. The pathogens were grown in parallel on PDA medium (control plates). Growth diameters were measured each 24 h. The percentage of inhibition that the metabolites and/or lytic enzymes, secreted to the medium by the *Trichoderma* strains, had on the growth of the different pathogens were calculated with the data obtained after 3 days of incubation.

RESULTS AND DISCUSSION

The inhibition percentages (Table 1) were 100% in all isolates of *B. cinerea, S. sclerotiorum, R. solani* and *T. roseum* by effect of *T. virens* and its inhibition percentage in *Fusarium* spp. was

higer 60% in 4 of 5 isolates tested. *T. atroviridae* showed lower control although the inhibition percentages were higher 50% in S. *sclerotiorum*, *R. solani* and *T. roseum* and one isolate of *B. cinerea* (B-015) and *Fusarium* spp. (FV-RV-004). Antifungal assays on dual cultures were also carried out. Thus, agar plugs of 6 mm diameter, of the *Trichoderma* strains and also of the different pathogens studied in this work, were placed on petri dishes containing PDA medium, or MEA medium for the *Botrytis* strains, maintaining a distance among the two strains of 5.5 cm. The plates were incubated at 28ºC and the results were observed after 5 days of incubation. Both biocontrol agents have shown good control of pathogenic fungi using *in vitro* conditions, but *T. virens* applied individually showed in general better control than *T. atroviride* with all tested pathogens.

Table 1. Growth diameters and inhibition percentages of fungi by effect of *T. atroviridae* and *T. virens.*

Fungi isolates	CL	*T. virens*	*T. atroviride*	*T. virens*	*T. atroviride*
	72 h (diámetro mm)			% inhibición 72 h	
F-001	39	15	33	61,5	15,4
F-002	33	-	21	100	36,4
F-003	33	12	20	36,4	39,4
F-004	42	10	19	76,2	54,8
F-005	40	10	28	75,0	30,0
B-015	31	-	11	100	64,5
B-022	30	-	17	100	43,3
S-009	51	-	-	100	100
S-013	52	-	12	100	76,9
R-006	85	-	13	100	84,7
T-008	37	-	12	100	67,6

F: *Fusarium* spp.; B: *Botritys cinerea;* S:*Sclerotinia sclerotiorum*; R: *Rhizoctonia solani*; T: *Trichotecium roseum*.

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THE INTERACTION OF CHEMICAL CONTROLS FOR ANTHRACNOSE IN DRY BEANS

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INTRODUCTION

Anthracnose (*Colletotrichum lindemuthianum*) is an serious seedborne disease in dry beans in Ontario. It can cause severe yield and seed quality issues, which dramatically reduces crop value. Control measures for anthracnose include resistance genes, pedigreed seed production, crop rotation and chemical controls (Tu 1988).

Seed treatments such as DCT have been used extensively for 30 years (Edgington and MacNeill, 1978), and have provided good control of seed infections. Azoxystrobin (trade name Dynasty) is a new seed treatment that was registered in 2008. There is little published work on Dynasty's effectiveness as a seed treatment for anthracnose control.

Quadris and Headline were registered as foliar fungicides in 2004. Conner et al. (2004) evaluated the application timing of Headline, and found that sequential applications at 40% and 80% bloom provided the best disease control and the highest yield. There is little published work on the efficacy of foliar fungicides applied alone or in combination with a seed treatment, on dry bean anthracnose.

METHODS

Two experiments were planted near Exeter ON in 2007 and 2008. The first experiment used infected seed with no visible lesions, while the second experiment used infected seed, with 30% with visible lesions. An RCBD design was used to compare metalaxyl/ fludioxanil/azoxystrobin (Apron Maxx + Dynasty) and diazinon/captan/thiophanate methyl (DCT) seed treatment combinations, azoxystrobin (Quadris) and pyraclostrobin (Headline) foliar fungicides, as well as the addition of crop oil concentrate (COC) to the foliar fungicides. The fungicides applied at mid flower stage of development (50% bloom). Ratings included % pod disease at harvest, seed yield and return on investment or ROI (seed yield x average price–dockage–pick).

Statistical Analysis:

All data were subjected to analysis of variance, using the PROC MIXED procedure of SAS (Ver. 8e, SAS Institute Inc. Cary NC). Data was combined where no experiment by treatment interaction occurred.

RESULTS AND DISCUSSION

Precipitation was below average in 2007 and above average in 2008. This resulted in low disease pressure in experiments 1 and 2 in 2007, and high disease pressure in experiments 3 and 4 in 2008. Compared to the control (Table 1), the seed treatments had 42% less pod disease. This resulted in a yield increase of 87%, but only under high disease pressure in one experiment. However, the seed treatments consistently provided a higher ROI (average of 51%). No differences were detected between the two seed treatments applied alone or in combination with a foliar fungicide. The foliar fungicides consistently had less pod disease (49%), higher yield (80%) and higher ROI (109.7%),

compared to the untreated control. Adding COC to Quadris increased yield by 14% and increased ROI by 20.1%, under high disease pressure in 2008. Headline outperformed Quadris under high disease pressure in 2008. Quadris had lower pod disease, but had lower yield (14.3%). In both years of the study, Headline outperformed Quadris for ROI with an average increase of 10.1%.

Table 1. Mean pod disease (%), seed yield (kg ha^{-1}) and return on investment ($ $acre^{-1}$) of contrasts for the interaction of seed treatments, foliar fungicides and crop oil concentrate in experiments at Exeter ON in 2007 and 2008. Treatment means followed by an asterick are significantly different, using Fisher's protected LSD (* = p<0.05, ** = p<0.01).

Contrast	Pod Disease	Seed Yield			Return on Investment		
	……..…..experiment……..……..						
	1-4	1-2	3	4	1-2	3	4
Seed Trt	8.6**	2300	958	817*	476**	153*	396**
Control	14.9	2168	699	435	363	110	215
MFA Seed Trt	8.4	2310	923	809	494	144	395
DCT Seed Trt	8.7	2290	993	825	459	161	396
MFA + Foliar	4.4	2315	1756	1378	504	399	675
DCT + Foliar	6.4	2388	1681	1322	517	383	646
Foliar Fung	7.3*	2361*	1584**	1198**	482**	329**	589**
Control	14.9	2168	699	435	363	110	215
Quadris+COC	5.5	2321	1717*	1240	493	380**	609*
Quadris	6.7	2321	1499	1031	486	320	502
Quadris	0.9*	2321	1608*	1135**	489*	350**	556**
Headline	3.1	2387	1751	1424	511	395	697

CONCLUSIONS

- The two seed treatments studied provided similar anthracnose control, under low and high disease pressure.
- The addition of COC to Quadris provides some benefit under high disease pressure.
- Headline was superior to Quadris, particularly under high disease pressure.

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GENETIC VARIABILITY OF POPULATIONS OF THE WEB BLIGHT PATHOGEN OF COMMON BEAN FROM CENTRAL AMERICA AND THE CARIBBEAN

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Web blight of dry edible beans (*Phaseolus vulgaris*) is an important disease in the tropical Americas. Chemical control can be used but it is costly and not always effective. Disease resistance has no grower cost and although breeding lines with lower web blight scores have been reported (Beaver et al., 2008), no web blight resistant varieties are currently available. The disease is caused by various subgroups of anastomosis groups of *Rhizoctonia solani* (teleomorph: *Thanatephorus cucumeris*). For resistance to be broadly effective, pathogen variation needs to be known. Isolates of newly described subgroups AG-1-IE and AG-1-IF are the most widespread in Central America and the Caribbean (Godoy-Lutz., 2008). Isolates within each of these subgroups are morphologically indistinguishable regardless of the country of origin. However, genetic variability of these isolates has not been examined despite variability in partial resistance observed in disease screening nurseries across regions. Thus, a study of genetic variability of populations of AG-1-IE and AG-1-IF isolates from Honduras, Dominican Republic and Puerto Rico was conducted using mycelial compatibility and molecular markers.

The study was on 90 *R. solani* isolates from subgroup AG-1-IE: 37 isolates from Puerto Rico and 24 from Honduras collected from 1994-2007 and subgroup AG-1-IF: 17 isolates from Dominican Republic and 12 from Honduras collected in a 1995 survey.

Markers tested were: microsatellites (Meinhardt et al., 2002); universal rice primers URP2R, URP6R, URP13R, and URP17R (Sharma et al., 2005) and Inter Simple Sequence Repeats ISSR-02 and ISSR-10 (Sharma et al., 2005). The data matrix was analyzed by the molecular variance (AMOVA) procedure. Initially the partition was established to estimate variation between regions with all isolate populations combined; subsequently the partitions were made between and within isolate subgroups. AMOVA and other measures of differentiation were obtained using Genalex software (Peakall and Smouse, 2006). Dendrograms were constructed using each of the three markers based on Nei's (1978) genetic distance among populations by the UPGMA (average linkage) method using TreeDyn 198.3 (www.phylogeny.fr)

Only three dominant markers (UPR2R, UPR6R and ISSR10) out of nine dominant or co-dominant markers showed polymorphism and were informative in differentiating between and within the four isolate populations. UPR2R was the most polymorphic for both subgroups**.** There was low variation between regions, but 67-85% for AG-1-IE and 70-90% for AG-1-IF of the variation resided within populations. The significant genetic variance within populations is an indication of sexual recombination ongoing in that population. Moderate to significant genetic heterogeneity was observed depending upon the molecular marker and the subgroup. Shannon's diversity index was under 0.5 for all markers which indicates that the isolate population within each subgroup was heterogeneous. UPGMA dendrograms (Figure 1- A,B,C) constructed from Nei's genetic distance supported subgroup clustering of the four populations with similar patterns of clustering no matter which molecular marker was used. Theoretical gene flow values ranging from 0.5-2.2 support

moderately low to high gene flow for both subgroups. Geographical distance, occurrence of sexual recombination and more seed exchange within than between populations are likely determinants in the population structuring.

Most of the paired isolates formed sclerotia along the area of contact or formed a line where no growth was visible, thus, interactions between isolate pairs were mostly scored as incompatible. This means that nearly 98% of the isolates were unique.

Genetic variability exists between and within populations of AG-1-IE and AG-1-IF. Dominant markers URP2R, URP6R and ISSR10 provide important baseline information for population structures. Further studies with species-specific markers will enhance our understanding of the genetic structure of *R. solani* AG-1-IE and AG-1-IF. Also virulence determination will be needed to select relevant resistance screening isolates.

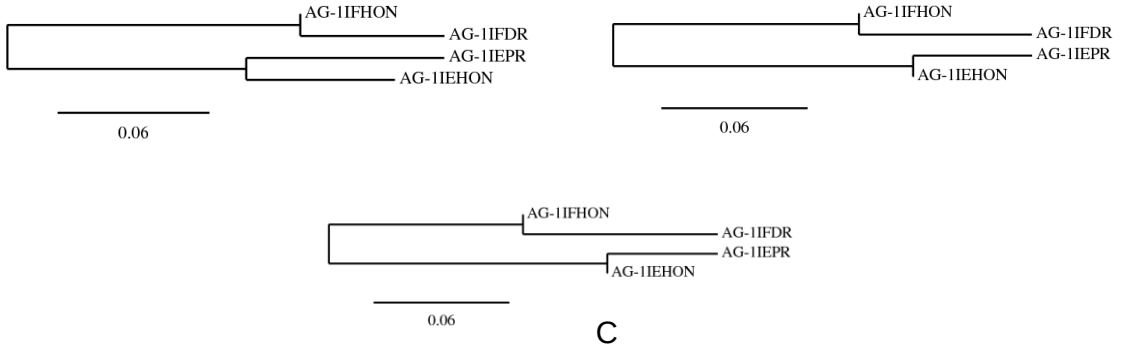

Figure 1. UPGMA dendrograms for marker: A) URP2R; B) ISSR10; C) UPR6R

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ALTERNATE HOSTS FOR THE DRY BEAN BACTERIAL WILT PATHOGEN IN WESTERN NEBRASKA?

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INTRODUCTION

Bacterial wilt of dry beans, caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, was a sporadic but often serious production problem in dry beans throughout the irrigated High Plains since first being reported from South Dakota in 1922. It was first observed in western Nebraska dry bean production fields in the early-mid 1950s, and continued to be economically important throughout the 1960s and early 1970s. The disease then only periodically appeared in seed, but has had little detectable effect on yields after the implementation of crop rotation and seed sanitation practices. The disease has now re-emerged and has been documented from more than 375 fields since 2004. It is not known why the disease has suddenly appeared again in dry bean production areas over the last four years to this extent, but it does warrant some concern.

Recently, bacterial isolates from soybean, corn and wheat plants have been identified in western Nebraska fields that exhibited the ability to cause disease on dry beans after artificial inoculations. Thus it is important to be able to document the distribution and incidence of the suspected wilt isolates found naturally occurring in production fields consisting of alternate crops grown in rotation with dry beans. Therefore we conducted a study during 2008-2009 to survey fields in western Nebraska to address this concept.

Survey Methodology

Between early July and mid-September 2008 and 2009, production fields were scouted for symptoms consistent with bacterial infections. The survey consisted of 212 and 233 fields for 2008 and 2009, respectively, and represented 11 counties in western Nebraska - Scotts Bluff, Morrill, Box Butte, Sheridan, Sioux, Banner, Kimball, Cheyenne, Keith, Perkins, and Duel.

From these fields, 270 (2008) and 466 (2009) symptomatic samples were collected and processed for identification of potential bacterial infections. Total number of samples from each crop or plant type included: alfalfa (5), bromegrass (11), camelina (5), chickpeas (1), chicory (8), corn (137), dry beans (286), eggplant (6), millet – proso and foxtail (19), forage peas (2), oats (2), pumpkins and gourds (4), soybeans (8), sugar beets (10), sunflowers (127), triticale (2), wheat (104), unknown grass weeds (6), and assorted other weed species (20).

All samples were either cultured on standard growth media or were incubated in a humidity chamber. As bacterial growth emerged from symptomatic tissues, they were re-streaked on new media and observed for colony growth characteristics and color. All recovered isolates were then tested by the Gram stain technique, which identifies the bacteria as either Gram positive or Gram negative. All Gram positive isolates were labeled and saved for later tests, as *Cff* is Gram positive.

Testing for Pathogenicity

A total of 73 Gram positive samples were identified in 2008, 38 of which were isolated from dry beans and identified as standard *Cff* isolates (39% of dry bean samples and 14% of all samples). For 2009, 113 Gram positive samples were identified, 58 of which were from dry beans (31% of dry bean samples and 12% of all samples).

Thirty-five additional Gram positive samples were identified from the remaining crop and weed plants in 2008 (20% of non-dry bean samples and 13% of all samples), with another 55 found in association with other crop and weed species (20% of non-dry ban samples and 12% of all samples)

All Gram positive isolates were then tested for pathogenicity on dry beans. Inoculations consisted of dipping sterile needles into bacterial colonies from 48-hour cultures and inserting into stems just below the first fully expanded trifoliolate, followed by incubation in lighted growth chambers with a 12-hour light/dark cycle and a temperature of 30ºC (94 F). Samples producing symptoms of bacterial wilt were identified with the Biolog® system

Only 5 of the 35 samples collected in 2008 were found to induce symptoms of wilt on dry beans and none from 2009 have been tested. However, since 2005, we now have identified about 25 isolates found in other crops (wheat, soybean, corn, and alfalfa) that have been determined to be *C. flaccumfaciens*. Many isolates were found associated with other bacterial infections.

CONCLUSIONS

The "re-appearance" of this disease after a long absence has been puzzling. Because of the historical seedborne nature of this problem, it raises the question of whether seed sanitation in areas of increase has been forgotten or ignored. However, it is also possible that this pathogen may behave in a different manner than previously thought, and has been able to adapt to other economic hosts or become able to survive unknowingly in fields on other plant species.

Over the last five years, the discovery of more than two dozen wilt-like isolates from fields grown with other crops suggests that the pathogen appears to be widely distributed throughout western Nebraska production fields. It is not known how this pathogen may affect corn, wheat, soybean, alfalfa, or potentially others as yet untested, but at the very least, these additional crops may serve as alternate hosts for the pathogen by serving as a survival mechanism and providing a source of inoculum for infecting dry beans when they are put back into the rotation.

POTENTIAL ABILITY OF BACTERIAL BLIGHT PATHOGENS TO MOVE BETWEEN SOYBEAN AND DRY EDIBLE BEAN

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INTRODUCTION

A complex of bacterial pathogens and their diseases affect the productivity of dry edible beans (*Phaseolus vulgaris* L.). These are primarily seed transmitted and highly regulated, making them a major threat to the dry bean seed industry; particularly in North Dakota (ND) which is the largest producer of dry edibles in the country. Soybeans (*Glycine max* (L.) Merrill) fit well in many North Dakota crop rotations, and acreage has recently been reported to increase by 3 to 4 million acres annually. Historically, soybeans and dry beans were grown in different parts of the state. However, in recent years these two crops are often grown in close proximity, and are not-infrequently planted in adjacent fields or rotated to the same fields. This is a potential concern for the industry as these crops can serve as alternate hosts for several pathogens including bacterial species. Such bacterial species include Pseudomonads which cause some of the major diseases in both these crops. *Pseudomonas syringae* pv. *phaseolicola* (*Psp*) and *Pseudomonas syringae* pv. *syringae* (*Pss*) are two major members of this group infecting dry beans. They cause halo blight and bacterial brown spot respectively. *Pseudomonas syringae* pv. *glycinea* causes bacterial blight on soybean plants and the epiphyte *Pseudomonas syringae* pv. *syringae* is of minor importance in this crop. Assessing the prevalence of these pathogens in ND and the ability of the isolates from this region to infect the alternate host is considered to be of great relevance for development of control measures. Therefore, the objectives of this study were to ascertain incidence of *Pseudomonas* species on dry bean and soybean in ND and to evaluate the cross-pathogenicity of the most prevalent *Pseudomonas* spp. on these hosts.

MATERIALS & METHODS

Bacterial isolation and pathogenicity tests: Leaves from soybeans and dry edible beans were collected during an annual disease survey in 2008. The bacteria were isolated by plating macerated leaf material onto King's B (KB) agar and Bacterial Blight Differential (BBD) medium. Colonies were streaked onto KB for green fluorescent pigment production. Fluorescence was observed under UV light. Bacterial cultures were also biochemically tested (Table 1). Pathogenicity of the bacterial isolates was determined on 'Mayflower', a navy bean cultivar and on the soybean cultivar 'Barnes'. Pathogenicity tests and disease evaluation were conducted according to standard protocols (http://www.css.msu.edu/bic/PDF/Halo_Blight.pdf).

Pathogen molecular identification by PCR: DNA was isolated from single colonies grown overnight in nutrient broth using the the Puregene DNA isolation kit (Gentra Systems). Primer pairs used for PCR amplification were B1- B2 for syrB+ *Pss* (Sorensen *et al.*, 1998); and primers 1-2 for detection of coronatine producing species such as *Psg* (Bereswill *et al.*, 1994). These yielded a 752-bp and 650-bp product size for *Pss* and *Psg* respectively. A multiplex PCR was used for detection of phaseolotoxin producting (tox+) and non-producing (tox-) *Psp* isolates. The primer pair P5.1-P3.1 amplified 0.5 kb fragment, specific for tox+ *Psp*, and the primer pair P3004L and P3004R amplified a 0.24 kb fragment that has been observed only in tox- isolates (Rico *et al.*, 2006).

RESULTS AND DISCUSSION

Of 39 dry bean fields sampled in 2008, *Pss* was isolated from 18 fields and *Psp* from 15. The soybean isolations from the 2008 survey of 40 fields led to the identification of *Psg* from 16 fields and *Pss* or intermediates from 17 fields. For dry beans, blight incidence in the field for *Pss* in 2008 ranged from 24-100% and for *Psp* from 19-87%. In the case of soybeans, bacterial disease incidence ranged between 44-100%.

Morphological and biochemical features of studied *Pss* and *Psg* isolates from dry beans were characteristic of the pathogen. However, 11 of the 33 potential *Psg* isolates tested were oxidase negative but did not hydrolyze

caesein like *Pss* or pectinase like *Psg* or *Psp* (Table 1). These isolates, hereby referred to as intermediates (IM), showed amplicons characteristic of coronatine producing *Pseudomonas* spp. including *Psg* in PCR reactions using specific primers (Bereswill et *al.*, 1994). These intermediate isolates were also tested using *Pss* and *Psp* specific primers. They did not show any amplification with the *Pss* primers. However, among these, seven isolates showed amplification of a band corresponding to the phaseolotoxin non-producing *Psp* isolates (0.24kb) and four isolates showed a multiple banding pattern with band sizes ranging between the toxin producing and non-producing *Psp* isolates (0.5-0.24kb).

In pathogenicity tests, all confirmed isolates *Pss*, *Psp* and *Psg* produced typical symptoms of bacterial blight on their respective hosts- soybean and dry beans, 10 days after inoculation. In cross reactivity tests as well, previously established reactions were observed. However, the IM isolates from soybean were observed to cause necrosis on dry bean leaves that spread and produced symptoms similar to those produced by *Pss* beans Fig.1.

Pseudomonas spp. have an overlapping host range, and there is a possibility of other genomic groups intermediate between pathovars (Schaad *et al.*, 2001). The isolates mentioned in this study could potentially belong to such group (s). According to the PCR results the IM isolates appear to have both the coronatine as well as the phaseolotoxin gene clusters (though the latter is possibly inactive in most isolates). Such rearrangements of plasmids carrying virulence genes could possibly influence their ability to cause the type reaction observed on dry edible beans. These and similar isolates are being evaluated further to confirm probable changes in pathogen populations and to discover possible factors resulting in the atypical disease reactions. However, this finding brings to light potential threats posed by development of pathogens with modified host ranges and increased pathogenicity created through changes in human cultivation practices.

Table 1. Typical Results of Biochemical tests for each of the *Pseudomonas* spp. and intermediates.

Pathogen	Gram reaction	Fluorescent on King's B	Levan formation	Oxidase reaction	Pectinase at pH4	Casein hydrolysis
IM	-	+	+	-	-	-
Pss	-	+	+	-	-	+
Psp	-	+	+	-	+	-
Psg	-	+	+	-	+	-

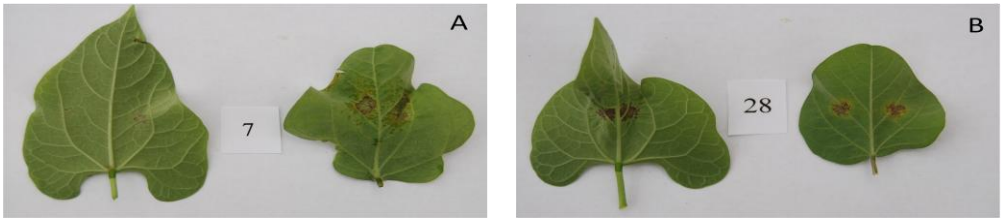

Fig 1A. Typical hypersensitive reaction shown by a *Psg* isolate on dry bean (left) and susceptible reaction on soybean (right). **1B.** Increasing necrotic lesion caused by an *IM* isolate on dry bean (left) and susceptible reaction on soybean (right).

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LEGUME ipmPIPE—A TOOL FOR DISEASE MANAGEMENT AND EDUCATION IN LEGUMES

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The Integrated Pest Management Pest Information Platform for Extension and Education (ipmPIPE) developed from the soybean rust (SBR) monitoring and communication system. It expanded to include soybean aphid (SA) in 2006 (1). In 2007, the Risk Management Agency (RMA) requested that ipmPIPE help determine causes of disease and insect losses in other legumes including fresh and dry peas and beans, chickpeas, lentils, lima beans, and cowpeas. Thus, the Legume ipmPIPE evolved with the objective of addressing diverse pathogens/pests on related legume hosts rather than a single crop (soybean) and microbial pathogen (SBR) or insect pest (SA) (2, 3). Legume specialists initiated sampling protocols, identified diagnostic procedures, and developed new diagnostic assays for three major legume groups of crops and four groups of legume diseases and insects. National mapping of this information on a public website began in 2008, extending the applicability of the system. Continued development has integrated additional pathogen information/images to the website (http://legume.ipmpipe.org) and a number of management and educational tools for researchers and stakeholders. This diversity of both pathogens/pests and hosts is uniquely suited to demonstrate the value of the Legume ipmPIPE as an interactive "one-stop shop" for legumes where educators and stakeholders can, within three easy links, obtain information on pathogens/pests identified in an area as well as relevant information on each pathogen/pest of interest.

The Legume ipmPIPE provides a dynamic system that combines pathogen/pest information provided by state coordinators into an IT platform to promote efficient and coordinated IPM decisions through the information and its products provided to extension educators and stakeholders. This system developed a network of extension educators, researchers and stakeholders who establish sentinel or mobile plots in target legumes to monitor pathogen/pests at research facilities and commercial fields in the US and limited areas of Canada and Mexico. Three groups of legume crops were organized with stakeholder's input. These are: Common bean: fresh and dry beans; Cool-season: fresh and dry peas, lentils, and chickpeas; and Warm-season: cowpeas and lima beans. Four groups of legume diseases and insect pests were selected by legume specialists and legume industry stakeholders. These include: soybean rust (*Phakopsora pachyrhizi*) and common rust (*Uromyces appendiculatus*); regionally prevalent diseases such as white mold (*Sclerotinia sclerotiorum*) or common bacterial blight (*Xanthomonas campestris* pv. *phaseoli*); viral diseases such as *Alfalfa mosaic, Bean pod mottles, Bean common mosaic, Bean yellow mosaics, Beet curly top, Cucumber mosaic,* and *Soybean mosaic viruses;* and soybean aphid (*Aphis glycines*) and other insects. Protocols were developed for sampling and diagnosis of selected pathogens/pests. High volume diagnostic plant viral assays are being developed and improved. State coordinators serve as the foundation of the monitoring network and lead by facilitating involvement of local cooperators, providing access to field diagnostic training, linking with State Diagnosticians (National Plant Diagnostic Network) to share information on disease/pest reports generated by the Sentinel Plot and/or other activities, establishing linkages

with the Legume ipmPIPE web site at http://legume.ipmpipe.org/cgi-bin/sbr/public.cgi, uploading weekly survey data and graphics (via a restricted web site) for release to the public on the Legume ipmPIPE public web site, and participation in national and regional conference calls for reporting new developments, writing new protocols, and promoting informed decisions. Specialists enter and update commentary on legume crops, pests, and diseases, scouting and management tools, and provide links to other resources such as crop and pest/disease models, pesticide recommendations, and other IPM products. Descriptive growth stages for legume crops or other resource links are available for the user as they devise specific pest management strategy. As specialists update files and displays, ZedX populates the public web site with constantly changing information. Public maps are customizable to the user by legume group and/or pathogen/pest group. Users query the map by positioning the cursor over a state/county/site with their computer arrow for highlights. The user can access state-specific information by selecting the state which then is displayed with county boundaries. Specific reporting information and commentary is provided by the specialist for that state or region. Digital images of legume crops and pests/diseases are being compiled on the Bugwood Network. Users of the public web site can directly link to images of disease symptoms associated with different pathogens and to insect pests and the damage they cause on legume crops. Future plans include development of a wiki resource.

In summary, Legume ipmPIPE accomplishments comprise a variety of programs and resources including: (a) legume sentinel or mobile plots in 27 states and in Canada and Mexico through collaborations with in-country scientists; (b) identified priority diseases for monitoring; (c) fungal and bacterial disease monitoring in plots; (d) protocols for sampling virus and kit-based high output immunoassays for BPMV, BYMV, SMV, CMV, BCMV, and AMV for use by NPDN labs; (e) communication between scientists specializing in legumes across the US; (f) data reporting to collate reports from across the US, (g) a web-based platform for access and information display to extension educators, research scientists, industry, and other stakeholders; and (h) a web-based portfolio of management and education tools. The ultimate goal of the Legume ipmPIPE remains identifying causes of loss in legumes and assisting producers in minimizing losses by implementing IPM of pathogens and pests.

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REACTION OF COMMON BEAN CULTIVARS AND LINES TO BACTERIAL BROWN SPOT IN SOUTH AFRICA

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INTRODUCTION

Bacterial brown spot (BBS) caused by *Pseudomonas syringae* pv. *syringae* is a seed-borne disease of dry beans (*Phaseolus vulgaris* L.) and results in serious yield and seed quality losses worldwide including South Africa (Franc, 1998; Jung *et al*., 2003). Planting of pathogen-free seed is the most important control method, however, it does not guarantee disease control. Copper based bacteriacides protect foliage against infection and secondary pathogen spread, but control is limited and resultant yield increases are minimal (Garrett and Schwartz, 1998). Plant genetic resistance is considered the most economically effective and environmentally friendly control measure (Jung *et al*., 2003; Petzoldt, 2007). The objective of this study was to evaluate bean genotypes for resistance to BBS, which could direct breeding strategies towards resistance against this disease in South Africa.

MATERIAL AND METHODS

Twenty-seven dry bean genotypes were evaluated for resistance to BBS in artificially inoculated field trials at Potchefstroom during the 2007/08 and 2008/09 seasons and at Delmas during the 2008/09 season. Genotypes were planted in 4 row plots, 5 m in length with 750 mm inter-row and 75 mm intra-row spacings. Trials were planted in a complete randomised block designs with three replications. Inoculum was prepared by mixing four 48 h cultures grown on King's B medium (Watson, 1980) in tap water and adjusting the suspension to 10^8 CFU/ml water. Trials were irrigated prior to inoculation to enhance disease development. The trials were inoculated at weekly intervals from 21 days after planting using a motorized backpack sprayer. Disease reaction was rated 10-14 days after the first inoculation on a 1-9 scale with 1 being resistant and 9 susceptible. Evaluations were repeated at flowering and at full pod set. Disease ratings and inoculation intervals were used to construct the area under disease development curve according to Campbell and Madden (1990). At maturity, plots of all genotypes were harvested manually and yield data were recorded. Data were analysed using analysis of variance (Statgraphics Plus 5.0) with disease rating and yield as variables and means were separated using Fischers LSD.

RESULTS AND DISCUSSION

Analysis of variance indicated genotype x locality/season interactions. Varieties BBSR 28, VAX 4, VAX 6, XAN 176, Jalo EEP 58, A 55, VAX 2, CAL 143, VAX 3, DOR 364 and BBSR 17 showed lower BBS ratings with BBSR 28 being the lowest (Figure 1). Disease reactions of BBSR 28, VAX 1, VAX 4 and VAX 6 were consistent throughout the different growing seasons and locations. Montana, Cerillos, Teebus and Tepary 4 were most susceptible irrespective of growing season and location.

Low AUDPC values were recorded in few genotypes including VAX 4, BBSR 28 and VAX 1 while Tepary 4, Bonus and Red Klout gave high values throughout the study (Figure 2). The AUDPC values also reveal that the disease was more intense during the 2008/09 season compared with the 2007/08 season. Statistical significant differences were identified among genotypes. High and

reliable resistance was identified, strengthening the prospects of using some of the genotypes for breeding for resistance against BBS in South Africa.

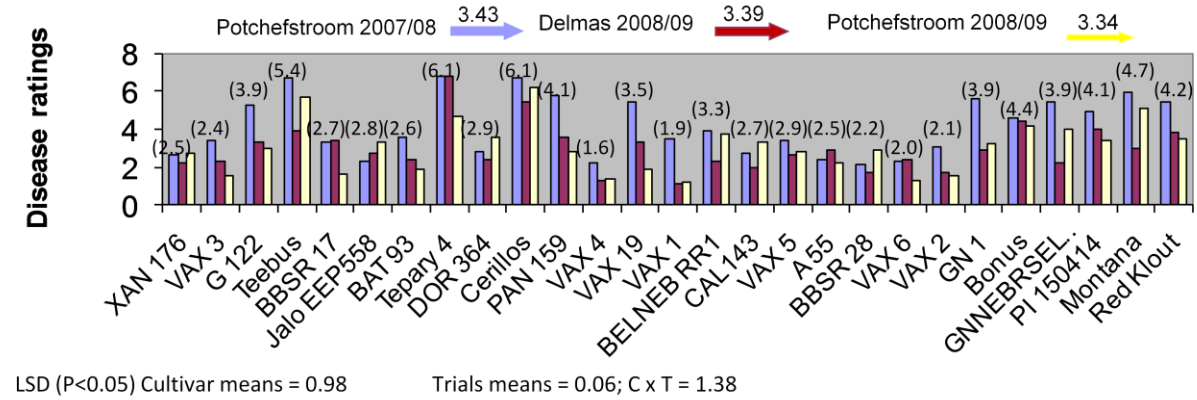

Fig. 1. Reaction of bean genotypes to bacterial brown spot (mean values provided above bars; trial means above legends).

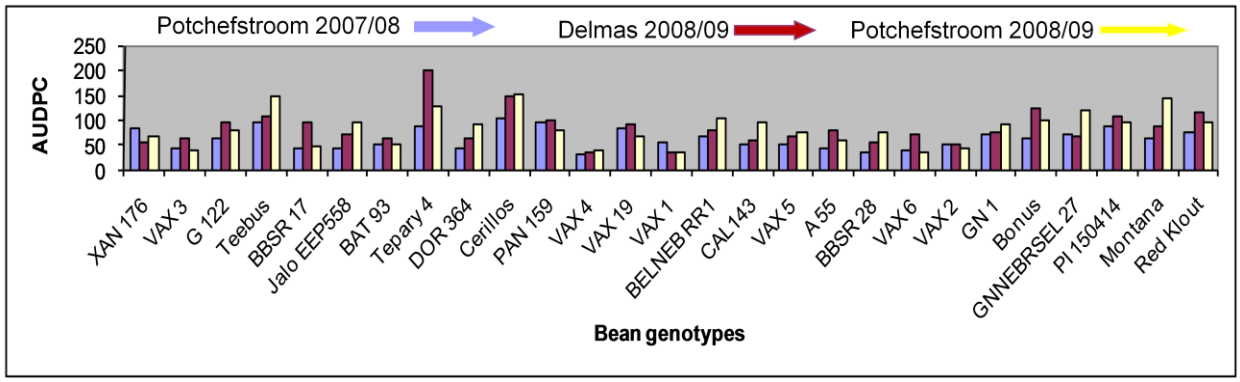

Fig. 2. Area under disease development curve of germplasm genotypes.

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COMPARING THE VIRULENCE OF NEW RACES OF THE COMMON BEAN RUST PATHOGEN FROM MICHIGAN AND NORTH DAKOTA

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INTRODUCTION

Host resistance is a cost-effective strategy for the control of the rust disease of common bean. The hyper variable rust pathogen is notorious for its capacity to recurrently produce new virulent strains, that when characterized are called races. Many races have been reported. In 2007, G. Varner in Michigan and in 2008, S.G. Markell and others in North Dakota, found rust on dry bean varieties with the *Ur-3* rust-resistance gene. This gene that had been widely used in the development of dry bean cultivars and had previously been rust-resistant. The newly found strains of the rust pathogen were initially characterized for their virulence (Wright et al. 2008 and by Markell). Isolates of these putative races were sent to ARS-USDA Beltsville for comparison and virulence confirmation. Here we compare side by side the virulence of the new MI and ND races, and discuss the reaction of additional sources of rust resistance to these races.

MATERIAL AND METHODS

Five isolates, one from MI and four from ND were sent to Beltsville, MD. Urediniospores of all isolates were increased on susceptible cultivars and then characterized by inoculating them on six Andean and six Middle American bean rust differential cultivars following established published protocols (Stavely, 1984). Ten plants per cultivar were inoculated and evaluated using published procedures.

RESULTS AND DISCUSSION

This study revealed two similar but not identical races. The MI isolate was characterized as race 22-2 and all four ND isolates as race 20-3 (Table 1). Both races infected Aurora (*Ur-3*), Golden Gate Wax (*Ur-6*), and Montcalm (unknown resistance genes). Neither race infected Mexico 235 (*Ur-3*+), Early Gallatin (*Ur-4*), Mexico 309 (*Ur-5*), Pompadour Checa 50 (*U-9*, *Ur-12*), PI 181996 (*Ur-11*), CNC (unnamed resistance genes), and PI 260418 (unnamed resistance genes). These races differed in their virulence; only MI 22-3 infected Redlands Pioneer (*Ur-13*) and only ND 20-3 infected Great Northern 1140 (*Ur*-7). Other sources of resistance were also resistant to both races. These included Ecuador 299 and NEP 2 (both with *Ur-3*+), PI 190078 (*Ur-11*). Dry bean cultivar 51051 was susceptible to both races. The virulence spectrum of race 22-2 was identical to that of races 48 and 62 maintained at Beltsville (Stavely, 1984 and Stavely et al. 1989). None of the races maintained at Beltsville were similar in their virulence spectrum to race 20-3. These results show that several rust resistance genes are very effective in controlling the new MI and ND races and suggest that the threat of these races to the U.S. dry and snap bean production does not appear to be as devastating as initially thought. However, these results demonstrably underscore the need for vigilance and are a reminder of the need to combine two, and preferably more effective rust resistance genes in the same bean cultivars for the effective management of *U. appendiculatus* and other highly variable pathogens.

Table 1. Reaction of six Andean and six Middle American bean differential cultivars to two new races of *Uromyces appendiculatus* from Michigan and North Dakota.

Differential Cultivars	Resistance Gene	Binary value	MI	Pheno type	Binary value	ND	Pheno type
Andean							
Early Gallatin	*Ur-4*	1	2,2^{+}	R	1	2	HR
Redlands Pioneer	*Ur-13*	2	4,5	S	2	f2,3	R
Montcalm	Unknown	4	4,5	S	4	4.5	S
PC 50	*Ur-9, Ur-12*	8	2	R	8	2	HR
Golden Gate Wax	*Ur-6*	16	5,4,6	S	16	5,4	S
PI 260418	Unknown	32	f2,3	R	32	f2,3	R
Differential Cultivars	Binary Value	22			20		
Middle American							
GN 1140	*Ur-7*	1	3,f2	R	1	5,4	S
Aurora	*Ur-3*	2	4,5	S	2	4.5	S
Mexico 309	*Ur-5*	4	f2,3	R	4	f2,3	R
Mexico 235	*Ur-3* +	8	f2,3	R	8	f2,3	R
CNC	Unknown	16	f2,3	R	16	f2,3	R
PI 181996	*Ur-11*	32	f2	R	32	f2	R
	Binary Value	2			3		
	Race	22-2			20-3		

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IDENTIFICATION OF SOURCES OF BACTERIAL WILT RESISTANCE IN DRY BEANS (*PHASEOLUS VULGARIS* L.)

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INTRODUCTION

Bacterial wilt caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* was one of the more problematic diseases of dry bean (*Phaseolus vulgaris* L.) throughout the irrigated High Plains (Colorado, Nebraska, and Wyoming) in the 1960s and early 1970s (Harveson et al., 2005).

As of 2006, the disease was detected in more than 300 fields in Nebraska, Colorado, and Wyoming. Affected fields were planted with dry beans from multiple market classes and seed sources, including yellow, great northern, and pinto beans. Seed quality was seriously affected, and in severely infected fields 10% of total yield was discolored. This pathogen is considered an A2 quarantine pest in Europe and is subject to phytosanitary regulations in some countries (Harveson et al., 2005). In addition to affecting seed movement between countries and even within the US, some health concerns could make it difficult for the Nebraska dry bean industry to commercialize those affected beans. Very few sources of bacterial wilt resistance have been reported. Emerson, which has a large bright white seed coat, was released in 1971 by the University of Nebraska and has some resistance to bacterial wilt, halo blight, brown spot, and bean common mosaic virus. One wild bean from the U.S. Dry Bean collection showed resistance to bacterial wilt (Urrea et al, 2008). The current cultivars Marquis, Orion, Beryl-R, Gemini, and La Paz are susceptible to bacterial wilt (Urrea et al, 2008), emphasizing the need to identify new sources of bacterial wilt resistance. The objective of this study is to screen the CIAT (International Center for Tropical Agriculture) *Phaseolus vulgaris, P. coccineus, P. acutifolius and P. dumosus* core collection for bacterial wilt resistance.

MATERIALS AND METHODS

A total of 1,700 accessions from the CIAT collection of dry beans are being screened for bacterial wilt resistance in the Panhandle Research and Extension Center dry bean greenhouse facilities (1,374 *P. vulgaris,* 42 *P. coccineus,* 244 *P. acutifolius and* 40 *P. dumosus*). Orion and Emerson are used as susceptible and resistant checks, respectively. The accessions are planted in an augmented block design. Each block consists of 32 entries plus 2 checks. Two seeds per accession are planted in each individual pot. The accessions have been planted two times. Ambient temperature is maintained at 27.8°C in the greenhouse.

A virulent bacterial wilt isolate originally found in a Nebraska great northern bean field is being used for testing accessions. Plants were inoculated at the V2 stage of development. One plant was punctured between the first and second node with a needle after dipping it into a 48-hour-old bacterial culture (Harveson et al., 2007). Negative controls consisted of plants being punctured with a sterile needle. Plants were evaluated every 7 days after inoculation for presence or absence of bacterial wilt symptoms. Koch's postulates were verified by re-isolation of the pathogen from symptomatic plants.

RESULTS AND DISCUSSION

Some of the results following the second inoculation were: The great northern variety Orion was susceptible in each of the two evaluations, and Emerson did not show symptoms in either of the two inoculations, suggesting a good level of bacterial wilt resistance. Fifteen hundred ninety-two accessions (93.6 %) were susceptible across the two evaluations. Twenty eight accessions (1.7 %) showed resistance in both evaluations and 80 accessions did not germinate which will be replanted. A third evaluation is in progress.

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ROW SPACING AND NITROGEN FERTILIZATION EFFECT ON SEED YIELD AND YIELD LOSS OF PINTO BEAN CULTIVARS UNDER DIRECT HARVEST

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INTRODUCTION

In North Dakota, the use of N fertilizer is recommended if the yield goal is greater than 2,200 kg ha^{-1}, or the residual soil-N level is greater than 56 kg ha^{-1} (NDSU, 2003). Studies showed that N increases the number of pods per plant, number of seeds per pod, seed weight, and resulted in increased yield (Fageria and Santos, 2008). Excessive N can increase lodging, inhibit nodule formation, delay maturity, and promote excessive canopy growth (Stevens and Belden, 2005). On average, North Dakota and Minnesota farmers apply 56 kg N ha^{-1} and some go beyond that (Knodel et al., 2008). Row spacing is another important factor affecting plant architecture. Farmers in North Dakota usually plant dry beans at a row spacing of 76 cm, which is adequate for type III cultivars, but seems to be too wide for type II cultivars (Grafton et al., 1988). Given the recent increases in fertilizer costs, it is important to find the optimum growing conditions that maximize yield and reduce production costs. The objective of this study was to evaluate the effect of N fertilization and row spacings on seed yield, and yield loss of pinto cultivars under direct harvest.

MATERIALS AND METHODS

This study was conducted at four environments (Carrington and Prosper, ND, in 2008, and Carrington and Hatton, ND, in 2009). The experimental design was a RCBD in a split-plot arrangement with three replicates. Row spacing was the main plot and the subplot was a factorial arrangement of nitrogen (N) levels and cultivars (the new Type II pinto bean cultivars Lariat and Stampede and an older Type III cultivar Maverick). The study had three row spacings: narrow, intermediate, and wide rows (30, 46, and 76 cm row spacing, respectively). Two N availability levels: 56 kg ha^{-1} N (residual soil N) and 112 kg ha^{-1} N (soil N + fertilizer N) were used with all row spacings and cultivars. The cultivars were planted in plots 7.62 m long at recommended seeding rates. A Hege 125B plot combine was used to direct harvest. Harvest loss was estimated by counting the seeds on the ground from two samples in each plot. Analysis of variance was performed within environments and then across the four environments (combined).

RESULTS AND DISCUSSION

The cultivar was consistently the most important in determining seed yield, yield loss, and seed weight. The cultivar Lariat produced the highest seed yield and 100-seed weight, and lowest seed loss, followed by a second tier group formed by Maverick and Stampede.

Increasing the N level did not have a direct effect on the seed yield, yield loss, yield potential, and 100-seed weight of the cultivars tested in this study. However, a significant interaction between row spacing, N level, and environment was observed (Table 1). The seed yield was significantly lower at Prosper (2008) due to adverse growing conditions. In the other three environments, the best response in seed yield was found at 46 cm row spacing but with different levels of N across environments: 56 kg N ha^{-1} produced the best seed yield in Carrington (2008), whereas in Carrington (2009) and Hatton (2009) the seed yield was greater with 112 kg N ha^{-1}.

There was a better condition for the establishment of plant stand and canopy development at early stages in 2009 than in 2008. Probably it contributed for an optimized shoot/root development that made it possible for the plants to use the available N in 2009, but not in 2008.

There was no statistical difference for yield loss among row spacing in Carrington (2008 and 2009) (Table 2). However, in the Red River Valley (Prosper and Hatton) the yield loss was greater at narrow row spacing (30 cm). It can be attributed to the stress caused by white mold (*Sclerotinia sclerotiorum* Lib. de Bary), since the disease pressure was significantly greater at narrow row spacing (Heard, 1990). More rapid canopy development resulted in higher moisture and lower airflow between rows, which promoted the development of white mold. At intermediate row spacing (46 cm), and using the current recommended plant populations, the conditions for disease development could be offset to a certain extent by the increased interplant spacing in the row. In summary, different recommendation of row spacing and N should be given across regions in North Dakota.

Table 1. Means of seed yield in a factorial of three row spacing and two N levels at four North Dakota environments.

Row	Available N level kg ha^{-1}	Carrington		Prosper	Hatton
		2008	2009	2008	2009
		Seed yield			
		----------kg ha^{-1} ----------			
30 cm	56 kg	1,750 bc	2,128 b	694.1 b	1,461 cd
	112 kg	1,980 ab	2,358 ab	801.7 ab	1,286 d
46 cm	56 kg	2,180 a	2,180 b	933.9 a	1,837 b
	112 kg	1,983 ab	2,434 a	922.8 ab	2,106 a
76 cm	56 kg	1,561 c	2,213 ab	938.4 a	1,636 bc
	112 kg	1,718 c	2,207 ab	1,002 a	1,488 cd
LSD (0.05) = 238.5					

Table 2. Means of yield loss in each row spacing averaged across cultivars and N levels at four North Dakota environments.

Row spacing	Carrington		Prosper	Hatton
	2008	2009	2008	2009
	Yield loss			
	---------- % ----------			
30 cm	11.0 a	21.5 a	27.6 a	32.8 a
46 cm	10.6 a	20.5 a	22.3 b	19.3 b
76 cm	13.8 a	18.2 a	21.5 b	17.0 b
LSD (0.05) = 4.39				

Only letters in the same column should be compared. If letter behind number is similar the numbers are not significantly different at $p<0.05$.

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HYDRATION PATTERNS VARY IN DIVERSE DRY BEAN MARKET CLASSES AND CULTIVARS

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ABSTRACT

Understanding the dynamic patterns of the dry bean hydration process is critical for rapid screening of bean lines for improved hydration capacity and seed hardness. Ten genotypes of navy, pinto, black, great northern and small red bean market classes were soaked for a period of 24 hours at room temperature, and 4 hours in boiling water. At room temperature, rapid water absorption occurred in the initial 10 hr in navy, black, pinto and great northern bean. But in small red bean, this process was much slower and peaked 22 hrs after soaking. Stone seed number remained high in small red bean at 16 hrs after soaking. In boiling water, the hydration process was more gradual in all genotypes, but saturated faster in navy and black bean. Significant variation among genotypes existed in the stone seed number and hydration coefficient at both 3-hr and 22-hr after soaking at room temperature.

INTRODUCTION

Dry bean market classes are diverse and may be classified according to seed size and color. The dry beans grown in Manitoba include navy, pinto, black, kidney (white, light red, and dark red), cranberry, great northern, pink and small red. Dry bean is consumed as a major source of protein in human diets. Dry beans can be consumed in a variety of pre-cooked canned products or can be cooked from dry-packaged beans. Major seed quality characteristics of concern for dry bean processing include hydration capacity, cooking time, and hard-shell 'stone seed'. In dry bean processing, enzymes, edible acids, and other chemicals are often used to accelerate the initial water uptake by seed (1). The hydration capacity and imbibition rate could be affected at any point of the process during water entering a seed from seed coat to cotyledon (2). Understanding the dynamic patterns of the dry bean hydration process in various market classes and cultivars is critical for rapid and efficient screening of bean lines in a breeding program. In this research, ten cultivars of five market classes were evaluated for their hydration patterns at room temperature and in boiling water treatments.

MATERIALS AND METHODS

Ten dry bean genotypes of five market classes were selected in 2007: Navy (Envoy, AC Cruiser), Pinto (AC Pintoba, Maverick), Black (AC Harblack, CDC Jet), small red (AC Scarlet, SR05-002), and great northern (AC Polaris, GN05-004). A sample of 100 seeds per genotype was used for each treatment and repeated three times. For the room-temperature (~23ºC) treatment, seeds were soaked in 1L tap water and drained each time at intervals during the 24-hr period. For the boiling-water treatment, seeds were soaked in boiling water in a constant temperature bath (Blue M, Blue Island, IL, USA), and drained at timed intervals during a 4-hr duration. Hydrated seeds were weighed. Stone seeds were picked, counted and weighed.

RESULTS AND DISCUSSION

At room temperature (RT), the hydration patterns varied in the five market classes. In navy, black, great northern and pinto bean, initial rapid water uptake occurs within approximately 10 hr after the soaking started. In small red bean, the seed hydration saturated 22 hr after soaking. The number of stone seed dropped significantly 16 hr after soaking in navy, black, pinto and great northern, but remained high in small red beans. At RT, the hydration patterns were similar between two cultivars used in black, pinto, great northern and small red bean. But in navy, AC Cruiser reached its full seed hydration capacity in almost three hours, while Envoy underwent a more gradual increase in seed hydration capacity. Soaking 3 hr at RT may be sufficient to distinguish genotypes with higher initial water absorption; 13 hr or longer soaking for navy, black, pinto and great northern, but 22 hr for small red bean may be needed to screen genotypes for their hydration capacity. However, higher initial water absorption does not indicate higher hydration capacity.

In the boiling treatment, the hydrated seed weight increased gradually during 240 min period in pinto, great northern and small red, but reached the maximum in approximately 100 min in most navy and black beans. The number of stone seed diminishes in approximately 1hr in most beans but persisted in AC Cruiser (navy) and AC Scarlet (small red) at 140 min. Significant variation was found in stone seed number ($P<0.0001$) and hydration coefficient (hydrated seed wt/dry wt) ($P<0.0001$) among genotypes at both 3-hr and 22-hr after soaking. Location also has significant effects on stone seed and hydration coefficient ($P<0.0001$).

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PERFORMANCE OF THREE MARKET CLASSES (PINTO, BLACK AND NAVY) ACROSS 24 YEARS IN MODERATE DROUGHT CONDITIONS IN THE NORTHERN PLAINS

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INTRODUCTION

Drought stress is a production problem worldwide not only for dry bean but also for any crop, especially now when water resources are becoming more scarce (Muñoz-Perea, et al. 2006). Declining ground water and diminished surface water supplies have exacerbated yield losses due to drought across the inter-mountain west and Great Plains over the past six years. In dry bean, reductions in seed yields can be as high as 90% depending on the variety and the severity of the drought (Muñoz-Perea, et al. 2006; Ramirez-Vallejo and Kelly, 1998; Singh 2007). The long term goal of the NDSU dry bean breeding for drought tolerance is the development of germplasm with improved field level tolerance under variable water conditions with the purpose of providing cultivars or lines adapted to the Northern Great Plains. In this report we describe the performance of three market classes across 24 years of collected data.

MATERIALS AND METHODS

Data was collected at the Carrington Research and Extension Center (REC) in North Dakota since 1981 from variety trials that had on average 3 black, 7 navy, and 9 pinto varieties, simultaneously grown under dryland and irrigated (central pivot) conditions. Each year, the entries were arranged in an RCBD with 4 replications. To assess the effect of drought on yield, data from these trials was purified (only cultivars planted at least 4 years were included), and was analyzed by market classes using the adjusted mean of each cultivar planted on each of both conditions (dryland and irrigated) per year. First, an ANOVA was performed for each experiment separately. Test for homogeneity was performed and then, statistical combined analysis was conducted using proc GLM from SAS. Common cultivars across years ('Othello' for pinto, 'Norstar' for navy 'T-39' for blacks), were used to compare between checks and conditions. The method described by Parate, 1961, and Pimentel-Gomes and Guimarães, 1958, was used to compare two cultivars in the same condition or from different conditions, respectively. F-protected LSD was calculated for both cases. Drought intensity index (DII), drought susceptibility index (DSI), and percentage reduction (PR) due to drought stress were calculated for each market class according to Fischer and Maurer (1978).

RESULTS AND DISCUSSION

Significant differences were found between stress condition (irrigated or dryland), market classes, and the year x condition interaction. Depending on stress conditions and severity, yield can be reduced up to 35.3% (1082 kg ha^{-1}) across all three market classes. Black beans were the most affected by drought stress with a yield reduction of 36.3% (1130.6 kg ha^{-1}), followed by navy and pinto beans with reductions of 35.5 and 32.4% (1045.3 and 1012.6 kg ha^{-1}), respectively (Figure 1). Pinto beans were the less affected by drought stress, confirming previous studies. Results showed that black and navy beans are more sensitive to drought. This is expected since they belong to race

Mesoamerica, which have been reported to be more sensitive to drought compared to Durango race (Muñoz-Perea et al., 2006; Singh, 2007). Black beans showed the highest drought susceptibility index (1.09), followed by navy and pinto beans with index of 1.01 and 0.93, respectively. The DII were lower than 0.4 for all market classes, which indicates moderate drought conditions across years. Looking at each one of the lines included in the variety trials from 1981 through 2008 under dryland conditions, the highest yielding pinto variety was 'Lariat', for navy beans, 'Norstar', and in the case of black beans, 'T-39' (Table 1).

Table 1. Lowest and highest mean seed yield of varieties within each market class under dryland conditions at Carrington REC, ND.

Market Class	Variety	Yield † (kg ha^{-1})
Black	Black Knight	1842.4c
	T-39	2349.3c
Navy	Seahawk	1146.3cd
	Norstar	2819.4b
Pinto	Rally	1513.0c
	Lariat	3834.9a

†Coefficient of Variance: 13.7%. Means with the same letter not significantly different at the 0.05 probability level.

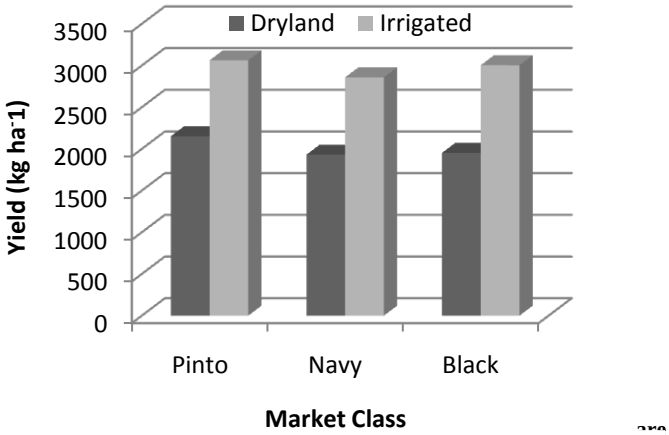

Figure 1. Mean seed yield of dry bean variety trials averaged across 24 years by market class in two conditions.

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MOLECULAR CHARACTERIZATION OF KEY GENES FOR FOLATE SYNTHESIS IN COMMON BEAN

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INTRODUCTION

Folates play an important role in preventing neural tube disorders in newborns as well as heart disease and cancer. Common beans (*Phaseolus vulgaris*) are an excellent source of dietary folates, but levels of these compounds can vary more than 3 fold among varieties. Previous results showed that high levels of folate content in bean varieties are correlated with high levels of expression of aminodeoxychorismate synthase (ADCS) and dihydroneopterin aldolase (DHNA) in the folate synthesis pathway (Y-S Shim et al. unpublished data). The objectives of this study were to screen two bean BAC libraries: cultivar OAC Rex (Perry et al. 2008) from the Mesoamerican gene pool and cultivar G19833 (Clemson University Genomics Institute) from the Andean gene pool for molecular characterization of ADCS and DHNA, and to develop new tools to select bean varieties with enhanced levels of folates.

MATERIALS AND METHODS

Common bean genomic BAC libraries of cultivar OAC Rex and cultivar G19833 were screened with the probes of ADCS and DHNA genes. The probes were prepared by PCR amplification using gene specific primers and DIG labeled dNTPs, hybridized to the membranes and visualized on X-ray film according to manufacturer instructions (Roche, Mannhein, Germany). Positive clones were verified by PCR using specific primers. The plasmids of positive clones were extracted using Large-Construct Kit (Qiagen, Mississauga, Canada), and sequenced at Plant Biotechnology Institute, Saskatoon, Canada. Sequences were aligned and a dendrogram was constructed using CLUSTALX (Chenna et al. 2003).

Fragments of gene ADCS were PCR amplified from two core map parents Bat 93 and Jalo EEP558 (Nodari et al. 1993) using primer sequences designed for conserved regions in Arabidopsis and other species. These fragments were cloned and sequenced. A single-nucleotide polymorphism (SNP) for ADCS was identified between Bat 93 and Jalo EEP558 (Y-S Shim et al. unpublished data). Two pairs of primers were designed based on the SNP for amplifying a specific band from each parent. A recombinant inbred population containing 70 individuals derived from a cross between Bat 93 and Jalo EEP558 was genotyped with this SNP marker. The gene position on the bean linkage map was determined using JoinMap (Stam, 1993).

RESULTS

With the DHNA probe, one and six positive clones were identified from the OAC Rex and G19833 libraries, respectively. The presence of the gene in the clones was verified by PCR (data not shown). All six clones from G19833 library belong to contig1808 in WebFPC: Phaseolus database (http://phaseolus.genomics.purdue.edu/WebAGCoL/ Phaseolus/WebFPC/). Full-length DHNA sequences were obtained after sequencing positive clones from the G19833 and OAC Rex libraries. There is one SNP, between OAC Rex and G19833, in the 393 bp coding region. The translated

sequences (130 amino acids) are identical between the two cultivars. The deduced amino acid sequence of DHNA in common bean is closest to a DHNA sequence (ACU16784) from soybean (Fig. 1).

The SNP marker of the ADCS gene was used to genotype the core mapping population with 70 individuals. ADCS gene was mapped on the long arm of chromosome 7 (Pedrosa-Harand et al. 2008) using Joinmap (Fig. 2).

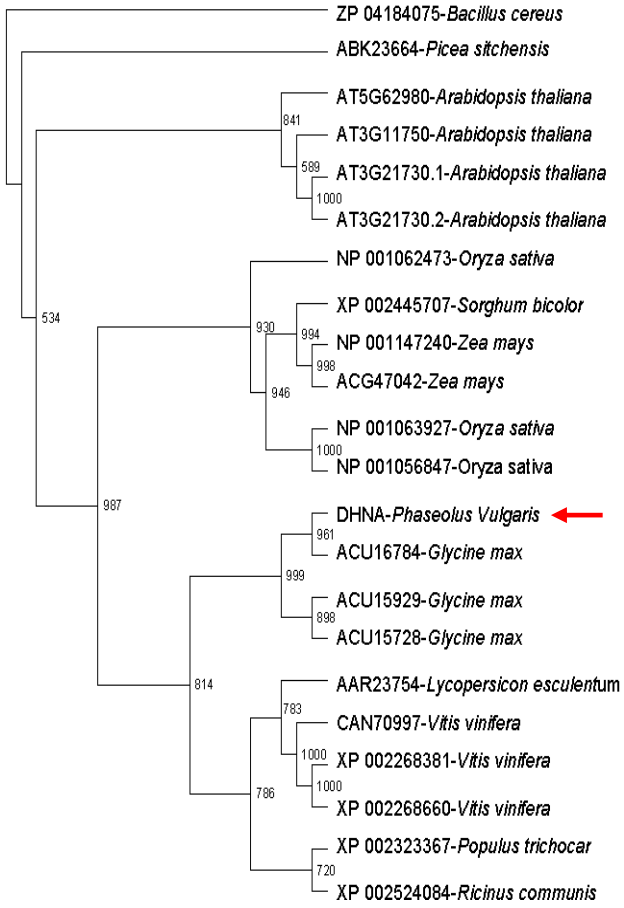

Fig. 1. Dendrogram of 21 DHNA homologs from different plant species

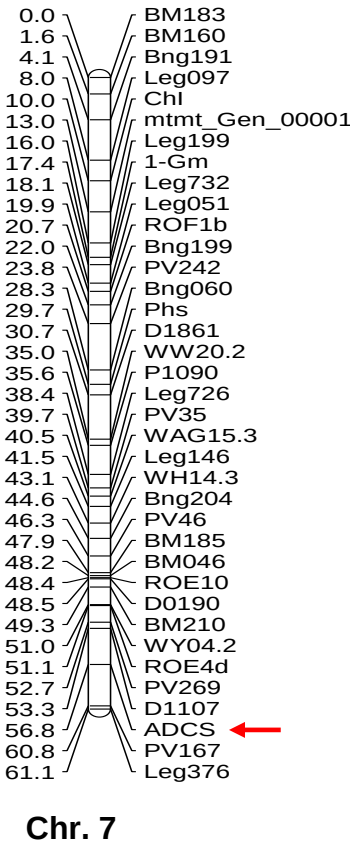

Fig. 2. ADCS was mapped on *P. vulgaris* Chromosome 7 by using a SNP marker

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WESTERN BEAN CUTWORM – THE PERSPECTIVE FROM THE GREAT LAKES REGION

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Monitoring and Distribution in the Great Lakes Region

Western Bean Cutworm, (WBC), *Striacosta albicosta* is native to Nebraska and remained there until 2000. A pest of both corn and dry beans, WBC then began to expand its range into the Midwest U.S. In 2006, WBC moths were captured in Michigan and Ohio, though levels were low and no damage was reported. In 2007, Ontario also began trapping for WBC but no moths were captured. Total moth counts increased slightly for Michigan and Ohio compared to 2006 and by the fall, crop damage and larvae were found in a few corn fields in NW Michigan.

Significant activity took place in 2008. WBC had successfully overwintered in Michigan. Ontario captured moths for the first time. By season's end, a total of 1760, 137 and 152 moths were captured in Michigan, Ohio and Southern Ontario, respectively. More cases of crop damage, including for the first time, feeding in dry beans were documented.

In 2009, WBC continued to spread further north and east into the Great Lakes Region. Trapping expanded to include New York, Pennsylvania and Southern Quebec. Trapping confirmed that WBC has now expanded as far north and east as southern Quebec, New York and eastern counties of Pennsylvania. An astonishing total of 28289 moths were captured in Michigan while Ohio, Ontario, Southern Quebec, Pennsylvania and New York captured 566, 1637, 8, 93 and 11, respectively. Four dry bean producing counties in central Michigan were advised to spray based on trap count numbers and signs of pod feeding. Several counties in both Michigan and Ontario have reported crop damage and larval activity, though no damage has been reported in dry beans in Ontario to date. Michigan reported that some counties (purple) experienced economic damage.

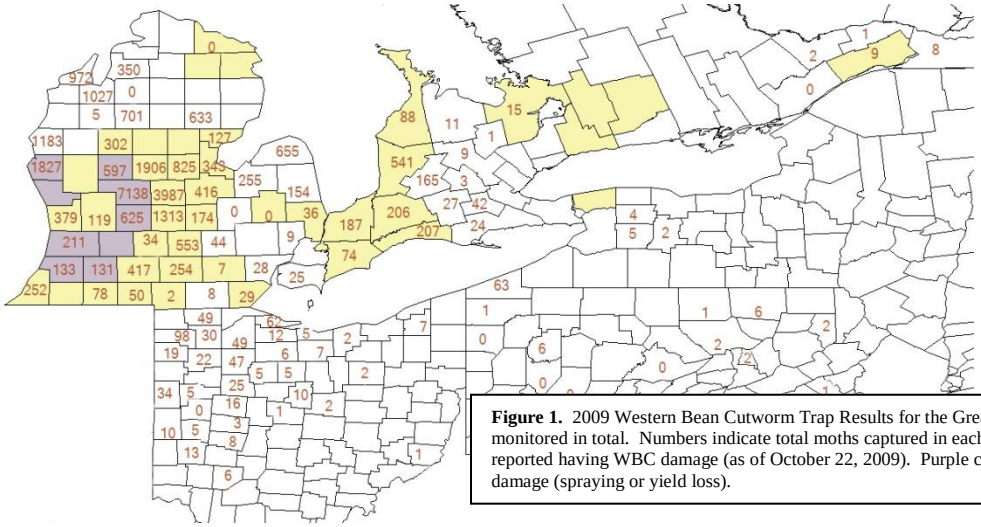

Figure 1. 2009 Western Bean Cutworm Trap Results for the Great Lakes Region. 252 traps were monitored in total. Numbers indicate total moths captured in each county. Shaded counties reported having WBC damage (as of October 22, 2009). Purple counties experienced economic damage (spraying or yield loss).

Damage and Impact

Damage begins as leaf feeding but once the larvae get bigger, they mine into the pods and feed directly on the seed causing yield loss (Figure 2). Entry holes on the outside of the pod can also promote pod diseases, which in turn impacts seed quality. Yield loss estimates are not well known. In 2008, a dry bean field in Michigan had 2-5% culls at harvest from WBC feeding, despite not reaching accumulated moth count thresholds set by Nebraska.

Figure 2. Photos of feeding damage caused by western bean cutworm larvae in dry beans in Michigan. Photo Credit: Chris DiFonzo, MSU

Scouting and Management

Scouting for WBC larvae in the dry beans is difficult. Most jurisdictions rely on an accumulated moth count threshold instead. Two milk jug pheromone traps per dry bean field are used, each on opposite ends of the field, in late June and monitored until early September to determine when peak flight takes place. Moth catch totals are accumulated over time until peak flight occurs. Pod feeding takes place approximately 3 weeks after peak flight.

Based on preliminary research in Michigan, environmental conditions and tillage practices are increasing the survivability and impact of WBC in the Great Lakes Region making thresholds established in Nebraska not effective at reducing yield loss and quality. Research is underway to determine the threshold for dry beans in the Great Lakes Region. Pyrethroid insecticides are the recommended chemical control option.

ACKNOWLEDGEMENTS

Funding for Ontario was provided in part by the Ontario Corn Producers' Association, OMAFRA through the Agricultural Adaptation Council's Ontario Research Development (ORD) Program and the Ontario White Bean Producers. We would like to thank additional collaborators including Michele Roy, MAPAQ, John Tooker, Pennsylvania State University and Keith Waldron, Cornell University. We would also like to thank all co-operators including growers, ag. industry reps, retailers and extension staff who monitored traps. A special thanks to the technicians and summer students involved including Katrina Schaafsma, Robyn DeBrouwer, Brianna Vyn, Steve Willis, Mike Jewett, and Chelsea Smith.

ANTHRACNOSE RESISTANCE LOCI IN COMMON BEAN ARE GENERALLY ORGANIZED AS CLUSTERS OF DIFFERENT RACE-SPECIFIC GENES

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Currently, thirteen anthracnose resistance genes, designated as *Co-* (*Co-1 to Co-13*) have been described in common bean. The *Co-* genes were identified as single genes conferring dominant resistance (except *co-8*) to several anthracnose races. However, in agreement with the cluster organization of families of resistance gene analogue sequences (RGAs) and/or resistance gene candidates (RGCs), mapping close to some of these genes, genetic analyses of joint segregations for resistance to different anthracnose races demonstrated that some of the *Co-* genes are organized as clusters of individual genes conferring race-specific resistance. Some of the anthracnose resistance genes or clusters described have been located in the integrated linkage map (Freyre et al. 1998; Kelly and Vallejo 2004): *Co-1* gene on linkage group B1, *Co-2* on B11, *Co-3* and *Co-9*, demonstrated to be allelic (*Co-3/Co-9*), located on B4, *Co-4* on B8, *Co-5* on B7, *Co-6* on B7, and *Co-10* on B4. Recently, an anthracnose resistance gene provisionally designed as *Co-u* has been mapped on B2 (Geffroy et al. 2008).

A broad genetic variability for *C. lindemuthianum* has been found worldwide, with more than 100 different races of the pathogen being described. Identification of anthracnose races has been internationally standardized based on the disease reaction of the 12 differential common bean cultivars, Michelite, Michigan Dark Red Kidney (MDRK), Perry Marrow, Cornell 49242, Widusa, Kaboon, Mexico222, PI207262, TO, TU, AB136 and G2333, and named based on a binary nomenclature system.

In this work we analyze the segregation for resistance to several races of anthracnose in the RILs proceeding from the cross between the breed line Xana and the anthracnose differential cultivar Cornell 49242, and in a population of F_3 families obtained from the cross between the anthracnose differential cultivars Kaboon and Michelite. Molecular marker analyses were carried out in these populations in order to map and characterize the anthracnose resistance genes or gene clusters present in Cornell 49242 and Kaboon. The results indicate that:

(i) One locus conferring resistance to anthracnose, located in linkage group B11, corresponding to the *Co-2* gene, is present in Cornell 49242. This locus is made up by a cluster of at least 9 different resistance genes conferring specific resistance to races 3, 7, 6, 19, 38, 39, 65, 357 and 449, respectively.

(ii) Two loci conferring resistance to anthracnose, located in linkage groups B1 (*Co-1*) and B4 (*Co-3/Co-9*), respectively, are present in Kaboon. In this differential cultivar, locus *Co-1* confers resistance to race 81, and locus *Co-3*/*Co-9* is a cluster including at least three different genes conferring specific resistance to races 3, 7, and 19, respectively.

This cluster organization of the anthracnose resistance genes is in agreement with previous results (Méndez-Vigo et al. 2005; Rodríguez-Suárez et al. 2007, 2008; Campa et al. 2007, 2009) obtained in several genetic analyses of joint segregations for resistance to different anthracnose races, in which the bean genotypes, Andecha, A252, Widusa, Mexico 222, TU, MDRK, and AB136 were involved. These results are summarized in Figure 1. It can be concluded that most of the *Co-* loci, previously considered as single genes conferring resistance to several anthracnose races, are made up of clusters of different genes conferring race-specific resistance.

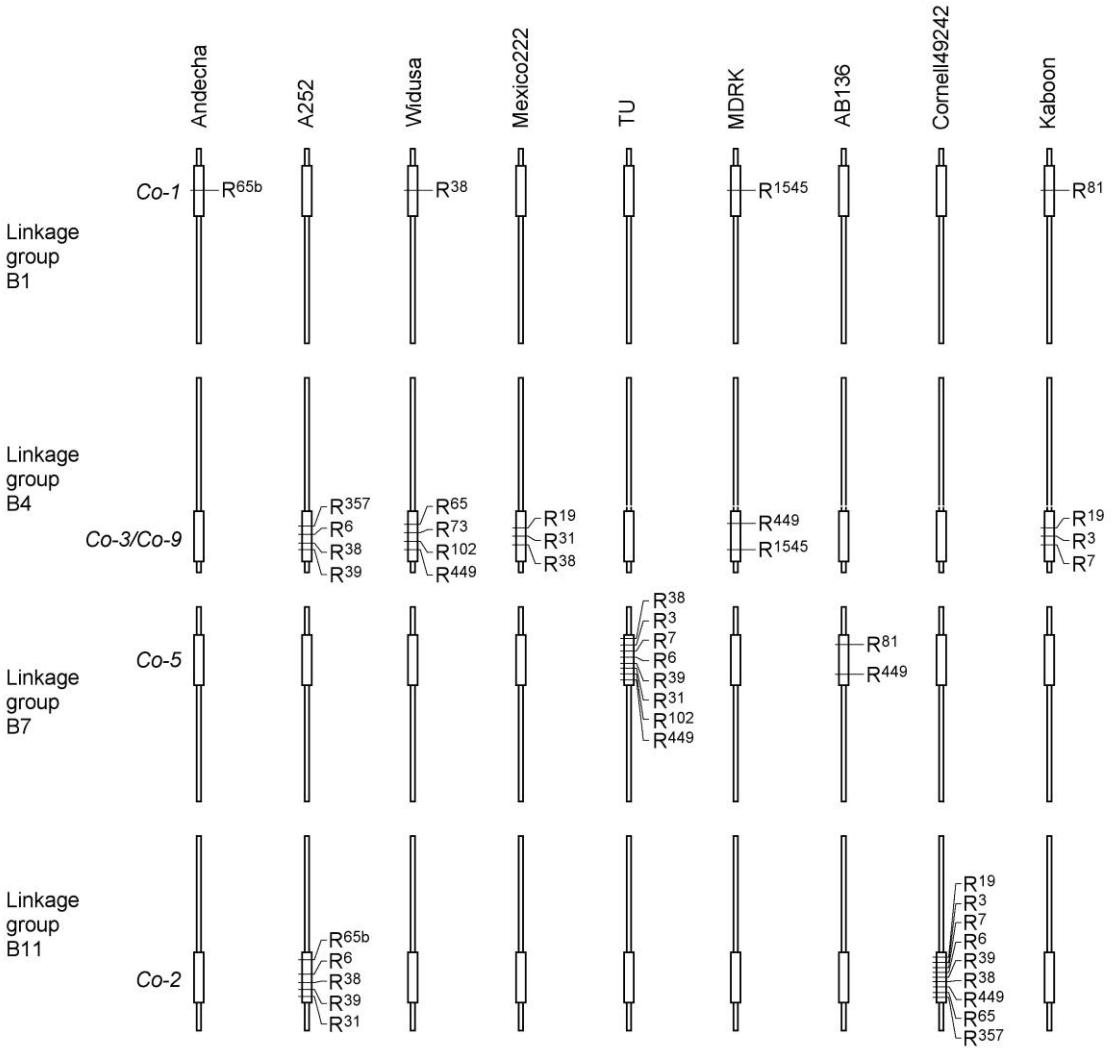

Figure 1. Anthracnose race-specific resistance genes present in the common bean genotypes, Andecha, A252, Widusa, Mexico 222, TU, MDRK, AB136, Cornell 49242, and Kaboon. In this figure the symbol R^X refers to a gene conferring specific resistance against race X.

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GENETIC VARIABILITY FOR PROTEIN AND MINERALS CONTENT IN COMMON BEAN LINES (*PHASEOLUS VULGARIS* L.)

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INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) has high nutritional value, with significant concentrations of protein and minerals. It represents the main source of protein for low income populations, especially in developing countries. The identification of lines with high levels of protein and minerals, adds value to the cultivars, without increasing the cost to consumers. Thus, breeding programs should be to associate great agronomic performance and nutritional quality in lines. Therefore, this study has as objective to evaluate the genetic variability for protein and minerals content of 100 common beans lines belonging to germplasm bank at Universidade Federal de Lavras (UFLA).

MATERIALS AND METHODS

The levels of protein and minerals (iron, phosphorus, potassium, calcium, magnesium, copper, manganese and zinc) of 100 common bean lines, differing in color, shape and size of the grains, belonging to germplasm bank of UFLA were quantified. Lines seeds, which have been stored in a cold chamber, were sown in February 2009 in experimental field of Biology Department at UFLA.

After the harvest, three samples of 50 grams of grains of each line were taken to determine the levels of protein and minerals. These analyses were carried out in Leaf Analysis Laboratory in Department of Chemistry at UFLA. Samples were grounded to obtain particles of size less than 1 mm in micro-mill. Nitro-perchloric digestion was carried out to determine levels of minerals content. Nitrogen content was determined using Kjeldahl method (Malavolta et al., 1997). Crude protein was obtained by formula: nitrogen content in seed x 6.25. Later this value was corrected to dry basis.

Variance analysis of data was carried out using a completely randomized experimental design with three replications. Heritability (h^2) was estimated using methodology presented by Ramalho et al. (2003).

RESULTS AND DISCUSSION

Significant differences were observed among lines by test F ($P \leq 0.01$) for protein and minerals contents. Average content of protein was 25%, ranging from 19.6 to 30.4%. However it is possible to increase protein content by common bean breeding. Interestingly, the occurrence of wide genetic variability was detected for iron, which is very important in human nutrition (Table 1). Iron content ranged from 54.2 to 161.5 mg kg^{-1}. However it is possible to raise iron content in common bean cultivars.

Wide variation was observed for the other minerals contents (Table 1), especially for zinc, which has important structural, enzymatic and regulatory functions in living cells (Cozzolino, 2007). The great genetic variability detected permit to infer that it is possible to increase by over 50% the zinc content in common bean grains. This is corroborated by the high h^2 estimate obtained for zinc content, as for other minerals and protein (Table 1). Therefore there is possibility of success in the selection of cultivars that present good adaptation and commercial higher nutritional quality grains.

Table 1. Mean protein and minerals contents and heritability (h^2) estimates obtained in evaluation of 100 common bean lines of germplasm bank at UFLA.

Nutrient	Mean and variation	h^2 (%)
Protein (%)	25.00 (19.60 – 30.40)	94.78
Iron (mg kg^{-1})	88.14 (54.20-161.50)	97.40
Phosphorus (g $100g^{-1}$)	0.52 (0.40 - 0.61)	98.29
Potassium (g $100g^{-1}$)	1.80 (1.45 – 2.06)	97.01
Calcium (g $100g^{-1}$)	1.43 (1.21 – 1.80)	96.82
Magnesium (g $100g^{-1}$)	0.25 (0.19 – 0.29)	94.41
Copper (mg kg^{-1})	11.30 (5.76 – 15.60)	98.40
Manganese (mg kg^{-1})	22.71 (9.19 – 36.78)	98.71
Zinc (mg kg^{-1})	49.24 (29.33 – 65.50)	97.86

ACKNOWLEDGEMENTS

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EFFECT OF THE ENVIRONMENT ON ZINC AND IRON LEVELS IN COMMON BEANS

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Biofortification is grounded in solid scientific principles: (a) there is a considerable and useful genetic variability in agriculture basic products; (b) breeding programs could easily manipulate nutritional quality traits, once they are inherited in high proportions and easily selected; (c) desirable characteristics are fairly stable in largely diverse cropping environments and (d) high nutrient content characteristics can be combined with agricultural superior quality characteristics and high yielding traits (Carvalho et 2005). The development of zinc and iron biofortified cultivars is an efficient tool to face iron deficiency anemia and to invigorate the immune system of underserved populations, especially in the Brazilian Northeast Region (CHIARADIA, 1997). Therefore, to characterize Zn and Fe high content promising bean lines concerning genotype x environment (GxE) interaction, those mineral contents have to be evaluated for a certain number of years in various locations and cropping seasons to get a reliable estimate of that interaction. That procedure would allow the identification of genotypes with high stability for those nutrient contents, giving more confidence to breeders when new biofortified cultivars are to be released.

Seventy two common bean genotypes from CIAT High Mineral Nursery (HMN) were evaluated. Trials were carried out in 2007/2008 cropping seasons in different places and under two watering systems (with and without water stress) in the following locations: [1] Porangatu Experimental Station - GO/irrigated, [2] Embrapa Rice and Beans (CNPAF) – Santo Antônio de Goiás-GO/irrigated, [3] CNPAF – Santo Antônio de Goiás-GO/water stress, [4] Ponta Grossa – PR/natural rain fall (without water control). Under no water stress condition, water was supplied when necessary (0.0325 MPa at 15 cm depth). Under hydrous stress, water was supplied 20 days after seedling emergence only. During water deficiency period, plants received approximately half the amount of water supplied to those under no water stress. In all assays the irrigation was controlled with tensiometers. The experimental design used was a completely randomized blocks design arranged in plots with two lines of two meter long, spaced 0.5 m, with 15 seeds per meter and three replicates. Cultural practices were the commonly used for the bean crop. Pods harvested were naturally sun dried and beans washed in distilled water, oven dried at 60°C for 48 h and grinded in a Zirconium ball mill (Restch MM200) to avoid contamination. To determine Zn and Fe contents, (2:1) nitro-perchloric acid digestion was used for organic matter oxidation (AOAC, 1995). The obtained extract was diluted and transferred to the air flame/acetylene atomic absorption spectrophotometer (Varian 50B) for reading. Laboratory tests were performed in triplicate and data submitted to individual and joint analyses of variance and means comparison performed by the Scott~Knott test at 10% probability using the SISVAR version 4.6 program.

Significant differences ($P<0.01$) in Zn and Fe levels were observed in all environments among the genotypes tested, indicating the existence of genetic variability for those traits. Environmental effect was also detected ($P<0.01$) as shown in Fig 1. As for Fe and for Zn CNPAF water stress environment had an effect on their mineral levels, with significant increases in their contents.

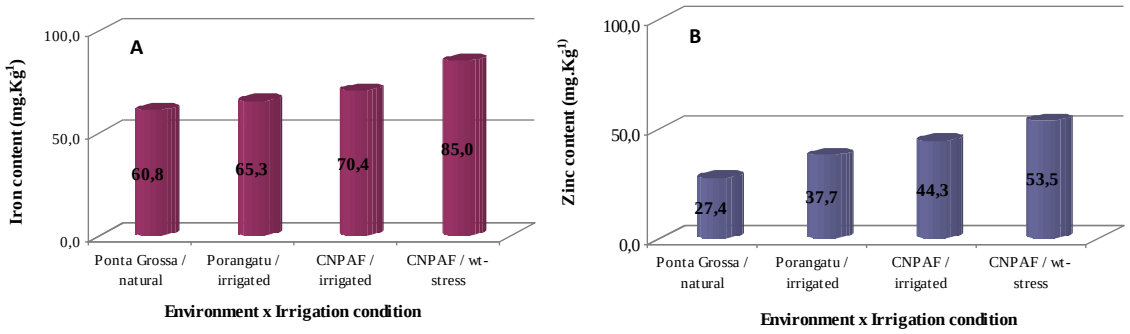

Figure 1. Fe (A) and Zn (B) average contents in common bean genotypes grains under different experimental conditions and environments.

Besides environmental effects, a significant interaction (P<0. 01) among genotypes and environments occurred, indicating a differential response in Zn and Fe contents, when environmental cropping conditions were modified.

Regarding experimental environments and conditions it was possible to observe that 12.5% of the genotypes tested showed Fe levels between 77 and 80.4 $mg.kg^{-1}$, differing significantly from the highest Fe level genotype tested (HMN-53: 85 $mg.kg^{-1}$). Regarding Zn, 31% of the genotypes presented levels between 43 and 49 $mg.kg^{-1}$, approximately.

Other bean genetic sources will be tested to achieve the research goals regarding beans with improved agronomic characteristics, good market quality and high levels of minerals.

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INHERITANCE OF SEED MINERAL CONCENTRATION IN COMMON BEAN

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INTRODUCTION

Micronutrients are essential elements needed in small amounts for adequate human nutrition and include the elements iron and zinc. Both of these minerals are essential to human well-being and an adequate supply of iron and zinc help to prevent iron deficiency anemia and zinc deficiency, two prevalent health concerns of the developing world. The objective of this study was to determine the inheritance of seed iron and zinc accumulation in a recombinant inbred line (RIL) population of common beans from a cross of low x high mineral genotypes DOR364 x G19833 using a quantitative trait locus (QTL) mapping approach. Results are compared to another CIAT mapping population AND696 x G19833.

MATERIALS AND METHODS

Experimental conditions: The experiments were carried out on the DxG populations across two field sites: first in Popayán, Cauca, Colombia (1,730 masl, 18ºC average yearly temperature, 2124 annual rainfall, Dystrudepts soil type, pH 5.6) and second in Darien, Valle de Cauca, Colombia (1400 m above sea level; 20ºC average yearly temperature, 1650 mm annual rainfall, Udand soil type, pH 5.6). Native, HCl and H2SO4 extractable mineral concentrations in the topsoil averaged 2.40 and 4.39 ppm for iron in the first and second sites, respectively; while soil zinc concentrations were 3.56 and 0.76 ppm. Total soil iron levels at lower profiles were 7.88 and 6.84 ppm at the two sites. Both experiments consisted in randomized complete block trials with two repetitions each.

Mineral Analysis: Two methods of mineral analysis were implemented: 1) Inductively Coupled Plasma – Optical Emission Spectrometry (abbreviated ICP) applied for both trials and 2) Atomic Absorption Spectroscopy (AAS) applied for the second trial. We were interested in validating the less expensive AAS method as an assay to replace the ICP analysis. Sample preparation for both techniques consisted of grinding 5 g of seed in aluminum chambers using a Retsch mill and aluminum grinding balls. Samples consisted of whole bean seeds that were surface cleaned with ethanol to remove soil and dust and oven dried before grinding. To determine the homogeneity of the sampling, two replicates were evaluated per technique based on subsampling of the ground powder described above. For the second trial, only ICP analysis was carried out on a single sample from each field replicate based on nitric/perchloric acid digested samples. AAS analysis was also based on nitric/perchloric acid digestion with samples read on a Unicam SOLAAR 969 mass spectrophotometer in the CIAT analytical services laboratory.

Data analysis: Analyses of variance (ANOVA) and Pearson's correlations between mineral averages of the RILs were carried out using the program Statistix version 8.0 (Analytical Software, Tallahasse, FL, USA). QTL were detected with composite interval mapping (CIM) analysis that was carried out using the software program QTL Cartographer v. 1.21. Population distributions were evaluated for normality with QTL Cartographer and LOD (log of the odds) thresholds for the individual QTL for each trait were determined by the generation of 1000 permutations of the data for that trait.

RESULTS

The parents of the population were contrasting in terms of mineral concentration in both the ICP and AAS analysis but the differences were greater for iron than they were for zinc. Correspondingly, the variability in seed mineral concentration among the lines was larger for iron (40.0 to 84.6 ppm) than for zinc (17.7 to 42.4 ppm) with significant correlations between trials, between methods and between minerals.

The two methods used for mineral analysis were reliable and gave similar results as shown by low coefficients of variation and highly significant correlations between methods. In terms of repeatability, coefficients of variation for ICP determination of iron and zinc averaged 5.8 and 11.2%, respectively; while reliability of the AAS method was also high with coefficients of variation for iron and zinc respectively averaging 6.9% for iron and 7.1% for zinc. Correlations between the ICP and AAS quantification methods for seed harvested in the second trial were highly significant both for iron concentration ($r=0.727$, $P= 0.0000$) and for zinc concentration ($r=0.828$, $P= 0.0000$). In addition, iron and zinc concentration measured with ICP analyses were correlated between the first and second trials ($r=0.681$, $P=0.0000$ and $r=0.594$, $P=0.0000$, respectively). The high correlations between methods showed the reliability of each method in determining iron and zinc seed concentrations.

Simple correlations were also calculated among mean mineral values for the RILs to reveal physiological relationships between the uptake of the two minerals and to evaluate similarity of the two techniques used for their measurement. Significant positive correlations were found between iron and zinc concentration from both the ICP analysis of the first trial ($r=0.3775$, $P<0.0006$) and the AAS ($r=0.602$, $P<0.0000$) and ICP ($r=0.7146$, $P<0.0000$) analyses of the second trial.

A total of 26 QTL were identified for the mineral x trial x method combinations of which half were for iron concentration and half for zinc concentration. Many of the QTL (11) for both iron (5) and zinc (6) clustered on the upper half of linkage group B11, explaining up to 47.9% of phenotypic variance, suggesting an important locus useful for marker assisted selection. These results suggest that some of the QTL for the accumulation of both minerals may be genetically linked or pleiotropic, controlling both traits at once. Other QTL were identified on linkage groups B3, B6, B7 and B9 for zinc and B4, B6, B7 and B8 for iron. The majority of the positive QTLs were associated with alleles from the high mineral parent, G19833; however this was reversed for the case of three QTL for iron (*Fe-ICPa4*, *Fe-AASb4* and *Fe-ICPa6* on linkage groups B4 and B6) and two for zinc (*Zn-AASb6.1* and *Zn-AASb6.2* on linkage group B6) where higher mineral concentration was derived from the DOR364 allele. These results are relevant for breeding common beans for micronutrient concentration as part of the biofortification program. Some of the QTL were found to be the same as in another CIAT mapping population, AND696 x G19833.

INHERITANCE OF SEED PHOSPHORUS AND SEED PHYTATE CONTENT IN A RECOMBINANT INBRED LINE POPULATION OF COMMON BEAN

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INTRODUCTION

Phytates are an important anti-nutritional component of legume seeds because they chelate mineral uptake in human digestion. Phytates can also bind certain charged proteins making them less digestible as well and the lack of phytase production in monogastric digestive systems prevents phytates from being hydrolyzed and utilized by humans. On the other hand phytates are important as a seed supply of phosphorus and as a health-promoting factor in some human populations susceptible to diseases such as heart disease and certain cancers. It is notable that phytate levels are often correlated with total seed phosphorus (P) and are the main storage form of P in plant seeds with phytates representing 65% or more of the P present in cereal or legume grain; and therefore both seed P and phytates are characteristics that should be considered jointly. From this perspective our goal has been to understand the inheritance of phytate content and its relationship with seed phosphorus in common bean seeds. The objective of this research was to evaluate quantitative trait loci (QTL) for seed phosphorus and phytate content in an inter-genepool (G2333 x G19839) recombinant inbred line population of common bean

MATERIALS AND METHODS

Plant Material: An inter-genepool recombinant inbred line population derived from the cross of G2333 (Mesoamerican, type IV climbing bean from Mexico) by G19839 (Andean, type III bush bean from Peru) and consisting of 84 $F_{5:8}$ lines was grown in two experiments in Popayán, Colombia in the 2004 growing season on soils that are inceptisols with a native P content of 2 ppm which is considered deficient. The two experiments differed in P fertilization: with a total of 200 kg ha^{-1} of 10-30-10 N-P-K fertilizer applied for a medium phosphorus treatment and 400 kg ha^{-1} applied for a high phosphorus treatment. The two levels of phosphorus fertilization were used since P supply is thought to influence seed phytate content. All other agronomic management except for P supply was the same for the two trials with plants grown on trellises and plot size consisting of double rows that were 3m in length and 2 m wide. Both medium and high P experiments were randomized complete block designs with two repetitions each and included the parents as control genotypes.

Seed P and Phytate analysis: Seed was hand harvested from each plot at full maturity, dried to 12% humidity prior to storage at 4ºC and used in seed phytate and total phosphorus analysis. Seed P and phytate content were quantified with spectro-photometric methods based on acid digestion with molybdenum blue and Wade reagents, respectively, and net seed P and net phytate content were calculated on a per seed basis using seed weights for each experiment.

Data Analysis: Analyses of variance were conducted for the seed phosphorus and seed phytate concentration traits in the RIL genotypes and parents across the two environments (medium P and

high P fertilization) using SAS with all effects considered random and each term assumed to be independent. The means for each genotype in each environment were used for quantitative trait locus (QTL) analysis with the probability of a QTL being present expressed in terms of LR (likelihood ratio) values.

RESULTS AND DISCUSSION

The molybdenum blue / Wade reagent method was found to be rapid as a quantification technique for total phytates, compared to more expensive, time consuming and multi-step analyses implemented for common beans with high pressure liquid chromatography (HPLC). In addition, the solid phase extraction column was found to be highly reproducible and coefficients of variation for the genotypes with this method were less than 5%. The analyses of variance showed significant differences between RIL genotypes for seed weight, total seed phosphorus, percentage seed phytate, net seed phytate and net seed phosphorous (Table 1). Calculations of net phytate and net P content were used to evaluate the amount of phytate or phosphorus per seed rather than on the percentage bases as described above. This was justified by the fact that we analyzed a Mesoamerican x Andean inter genepool population that segregated widely for seed size. .

Population histograms for percentage total seed phosphorus, percentage phosphorus, net phytate content, net seed P content and seed size in the G2333 x G19839 RILs were normally distributed in both environments and there was no evidence of kurtosis or skewing in any of the histograms. These results suggest that all of the traits measured were inherited in a quantitative manner. In each case, parental means tended to be less distinct than the lowest and highest seed P or phytate containing RILs suggesting transgressive segregation was important in the inheritance of the traits and that both parents contributed positive and negative alleles for the traits. A total of six QTL were found for total or net seed P while three were found for percentage or net seed phytates. In addition six QTL were found for seed weight. QTL for seed P and percent phytates were located independently. Meanwhile the QTL for net seed P or phytate content were related to seed weight QTL.

Table 1. Range for seed phytate content, total seed phosphorus (P), seed weight, net seed phytate and net seed P content in recombinant inbred line population G2333 x G19839 grown in two experiments in Popayán under high (HP) and medium phosphorus (MP) soil fertilization.

		RILs		
Trait	P level	Mean	Range	P_{RILs}
Seed Phytate (%)	HP	0.93 ± 0.31	0.29 - 1.78	*
	MP	0.94 ± 0.48	0.29 - 1.76	*
Total Seed P (g kg^{-1})	HP	4.22 ± 0.52	2.75 - 6.06	***
	MP	4.23 ± 0.45	3.11 - 5.95	***
Seed Weight (g)	HP	0.40 ± 0.08	0.24 - 0.67	***
	MP	0.39 ± 0.08	0.25 - 0.65	***
Net Phytate Content (mg $seed^{-1}$)	HP	0.37 ± 0.15	0.88 - 9.26	***
	MP	0.38 ± 0.22	0.87 - 8.26	***
Net P Content (mg $seed^{-1}$)	HP	1.69 ± 0.41	0.78 - 2.97	***
	MP	1.69 ± 0.39	0.94 - 2.90	***

* and *** , significance at probability levels of 95% and 99.9%. ns=not significant.

PHENOLOGY, YIELD, NUTRITIONAL QUALITY AND GROWTH HABIT OF SNAP BEAN (*PHASEOLUS VULGARIS* L.)

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INTRODUCTION

The increase in the population originates the necessity of rise in the food production of high quality (INEGI, 2004). The snap bean (*Phaseolus vulgaris* L.) is an important protein source (28.9%), carbohydrates (39.7%), fiber (22%), fat (0.88%), calcium (1.8%) and phosphorus (0.13%) (data in dry base, Salinas *et al*., 2008).Within the strategies to achieve these objective is the study of nutrimental content in snap bean cultivars, because studies suggest that the nutrimental content and fresh pod yield can vary with the genotype (Khah and Arvanitoyannis, 2003). The aim of this study was to determine the relationship between yield and nutritional quality with growth habits of snap bean.

MATERIALS AND METHOD

The study was realized in Montecillo, Mexico (19° 29' N, 98°53' O, to 2250 m of altitude), with climate BS1, (less dry of the arid), rains in summer, annual average temperature of 14.6 °C and rainfall of 558 mm (García, 2005). The cultivars: "La Palma", "Strike" and "Black Valentine" of determinate growth habits (DGH); "Hav-14", "Japanese" and "Oaxaqueño" of indeterminate growth habit (IGH) were planting at May 7 of 2008 to population density of 6.6 plants m-^{2}. The experimental design was a randomized blocks with four replicates. The nutrimental quality was evaluated through a chemical proximal analysis (Sosa, 1979). In order to determine humidity, the snap bean was put under an air forced stove to 55ºC until constant weight. The milling was realized in an electrical mill with sieve of 5 microns, to determine ashes (mineral), neutral detergent fiber (hemicellulose), protein, fat and dry matter. In addition was registered the phenology (Escalante and Kohashi, 1993) and the yield of snap bean (fresh weight pod, g m^{-2}).

RESULTS AND DISCUSSION

The snap bean phenology presented differences between cultivars. "La Palma" was the one of early cycle with 44 days of sowing to flowering (DSF) and 74 days to last harvest (DLH), followed "Strike" (47 DSF and 82 DLH), "Black Valentine" (51 DSF and 93 DLH), "Hav-14" (62 DSF and 106 DLH), "Japones" (84 DSF and 132 DLH) and "Oaxaqueño (112 DSF and 157 DLH) with the most longest cycle. The highest pod yield 1370 g m^{-2} was presented in "Hav-14", followed of "La Palma" with 1170 g m^{-2}, "Oaxaqueño" (1040 g m^{-2}), "Black Valentine" (870 g m^{-2}), "Japones" (530 g m^{-2}) and the lowest yield corresponded to "Strike" (480 g m^{-2}). One would hope that IGH genotypes by its longer cycle would show a yield higher, than those of DGH of shorter cycle. Nevertheless "Japones" presented one of the lowest yields, possibly it is related to differences in adaptation, because it proceeds from a warm climate. The results of table one indicate that the IGH cultivars produce pods of greater quality, for example "Oaxaqueño" has the highest ash content,

FDN, protein and dry matter with 9.7%, 34.2%, 23.1% and 90.7% respectively, followed of "Hav-14" and "Japanese". In the DGH, "Strike" presented lowest quality with 7.9%, 1.2%, 18.0% and 87.4%, respectively. Similar tendencies were reported by Esquivel *et al*. (2006) for protein contained.

Table 1. Chemical proximal analysis of snap bean cultivars. Montecillo, Texcoco, Estado de Mexico. 2008.

Nutriment	La Palma	Strike	B. Valentine	Hav-14	Japonés	Oxaqueño	Promedio
Ashes	8.3 cd	7.9 **d**	9.1 ab	8.8 bc	8.4 cd	9.7 **a**	8.7
Calcium	1.2 b	1.2 b	1.4 a	1.5 **a**	1.1 **c**	1.2 b	1.2
FDN	25.4 d	30.7 b	23.1 **e**	31.5 b	28.0 c	34.2 **a**	28.8
Protein	19.8 c	18.0 **d**	20.7 b	22.3 a	19.6 c	23.1 **a**	20.5
fat	1.5 c	0.5 **e**	1.2 d	1.7 b	2.1 **a**	1.8 b	1.4
Dry matter	87.0 c	87.4 c	87.2 **c**	88.6 b	87.5 c	90.7 **a**	88
Humedity	13.0 **a**	12.6 a	12.8 a	11.4 b	12.5 a	9.0 **c**	11.8

Averages with the same letter within rows are statistically equal (Tukey 0.05)
FDN = detergent neutral fiber; B. Valentine = Black Valentine. The collected data of the proximal analysis are expressed in %.

CONCLUSION

Under semi-arid climate, the snap bean cultivars present variability by phenology, yield and nutrimental content. The indeterminate growth habit cultivars present the highest nutrimental quality but not all the highest yield.

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DEHULLING CHARACTERISTICS OF DRY BEAN (*PHASEOLUS VULGARIS* L.) CULTIVARS

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ABSTRACT: The Tangential Abrasive Dehulling Device (TADD) was used to evaluate the dehulling properties of thirteen dry bean cultivars from five market classes. The yield or percent kernel removed was cultivar dependent and increased linearly (R^2 = 0.984 to 0.999) as dehulling time increased from 30 to 120 sec. Percent kernel removed was significantly different within cultivars of great northern and pink bean market classes while variation within black and pinto bean cultivars was minimal and insignificant. Bean seeds of the black market class were the hardest to dehull since the longest time (928 sec) was required to completely remove the hulls by abrading on average, 50% of the seed. Multiple regression analysis showed that dehulling parameters were not related to any seed characteristics (seed length, width, thickness and weight).

INTRODUCTION: The hull content of bean seeds range from 7 to 13 %, and specifically between 8-10% seed weight (1-6). The hull is rich in dietary fiber (7,8), minerals, particularly calcium (6), and phenolic compounds exhibiting strong antioxidant activity (3,4) essential for development of novel food products. Bean hull offers physiological health benefits crucial in fulfilling the increasing need and demand for a diversified functional food base. Bean hull extract supplementation has been demonstrated to reduce the incidence of azoxymethane- induced colon cancer in rats (9), and prevent DNA damage or liver injury in mice (10,11). Extracts of hulls obtained by abrasive dehulling of black beans were generally more effective than whole-seed extracts against colon, breast and prostatic cancer cell proliferation (5). While the benefits of bean hulls have been known for some time, their incorporation into foods is nonexistent, due to the lack of efficient hull extraction platforms. Therefore bean components must be separated efficiently to ensure their economic potential, particularly bean hull as a source of dietary fiber with demonstrated physiological benefits. Navy bean hulls have been prepared by cracking seeds in a disc attrition mill followed by aspiration (7). Abrasive dehulling of legumes has been evaluated with a dehuller (2). The AHI, defined as the time in seconds to abrade 1% of the kernel as fines ranged from 7.6 sec (mung bean) to 19.4 sec (kidney bean) and only 2 min residence time in the dehuller was required to remove over 90% of the hull for kidney bean (2). The TADD has been used in evaluating the large variability in dehulling quality of mung bean, cowpea, chickpea, and pigeon pea cultivars (12, 13). However, mechanical dehulling of dry bean seed has been limited. This investigation extends our previous study on bean pearling (3) and describes the application of the TADD in evaluating the dehulling characteristics of Canadian dry bean cultivars.

MATERIALS AND METHODS: Seeds of 13 dry bean cultivars grown at Lethbridge, AB in 2006 and 2007 were used. The cultivars included five market classes, black (AC Black Diamond, AC Black Violet, and CDC Jet), great northern (AC Alert, AC Polaris, and AC Resolute), pink (AC Early Rose, and Viva), pinto (AC Agrinto, CDC Minto, Othello, and Winchester), and small red (AC Redbond) bean. Seed dimensions (length, width and thickness) were determined from a randomly drawn sample of 25 seeds with a Digimatic Caliper. Abrasive dehulling was done on a model 4E-230 TADD with an eight-cup cover plate (14). Dehulling characteristics of bean cultivars were

determined using 25 g of bean in two of the cups in the eight-sample plate. After dehulling for a given time interval, seeds were removed from the sample cups using the vacuum aspirating device described previously (15). The dehulled seeds and hulls, separated by air aspiration, were weighed and the weight loss (%) was designated as the yield of hulls. Dehulling was performed for successive time intervals of 30 s for a total of 120 s to generate the dehulling curve. The dehulling process was continued until the seed coat was completely removed from the endosperm. Kernel hardness as rate constant was determined according to (16). The abrasive hardness index (AHI) was determined according to (2), greater abrasive hardness values indicating harder seeds. Dehulling was duplicated for each sample with three replicates per bean cultivar. Data were subjected to analysis of variance using SAS.

RESULTS AND DISCUSSION: The hull yield, defined as loss in weight during dehulling, did not reflect the actual amount of hull recovered as observed previously (14). Nevertheless, it is a quantitative measure of grain hardness, commonly referred to as pearling index. The hull yield ranging from 6 to 11% (AC Alert and Viva) upon dehulling bean cultivars for 120 seconds was similar to the 5 to 10.5% seed coat fraction observed for 67 common bean genotypes grown in north western Spain (17).

Complete hull removal was achieved at extended dehulling time varying from 600 to 1005 seconds for CDC Minto and Black Diamond, respectively. Cultivars requiring the longest time for complete hull removal such as Black Diamond, Black Violet and Alert had the highest (> 51%) amount of kernel removed. Similarly, Viva, Minto and Polaris with the shortest dehulling time (< 640 sec) for complete hull removal had the least amount of kernel removed (< 38%), or easily dehulled. Hulls (38-40% total seed weight) were completely removed in the shortest time (< 685 sec) for pinto, pink and small red market classes (AHI range 10 to 13). Black beans were the hardest to dehull since the longest time (928 sec) was required to completely remove the hulls by abrading on average, 50% of the seed.

Dehulling parameters were not related to any seed characteristics evaluated in this study, although high correlations (r^2= 0.786-0.967, $P < 0.005$) existed among seed length, width thickness and weight. However, percent kernel removed at 60, 90, and 120 sec was positively related ($r = 0.961$, 0.909. 0.863; $P < 0.0001$) to the percent kernel removed when seed coat was completely (100%) removed. Therefore, dehulling for only 2 min can be used to evaluate bean cultivars for ease of complete seed coat removal.

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CANNING QUALITY OF COMMON BEAN CULTIVARS PLANTED IN DURANGO, MÉXICO

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INTRODUCTION. Common beans (*Phaseolus vulgaris* L.) are grown in 272 000 hectares in Durango State—northern México— under rainfed conditions where 133 000 Mt of grain are annually produced (SAGARPA, 2010). Lower grain prices are commonly observed during high production years. Value added products could help to increase farmer's income. In Durango State, significant advances have been observed for cleaning common bean seed using air and sieving machinery. Beans are usually sold loose in open sacks or packed in clear plastic bags by domestic and national enterprises. Cooked beans, flour and extruded foods are also observed in local market, but canning industry is an unexplored option in Durango. Canning industry needs low cost and high yielding inputs in order to maximize earnings. Lack of canning quality tests have been observed in common bean cultivars planted in Durango, and their industrial use is limited. The aim of the study was to evaluate the canning quality of common bean cultivars produced in Durango, México.

MATERIALS AND METHODS. During 2008, an experiment including eight common bean cultivars was planted at three locations in Durango, México. Plants were harvested, mechanically threshed and the seeds stored at room temperature until the canning quality test was performed in 2009. The experimental unit consisted of a can (303 x 407, easy open lid and sanitary white glaze) with 100 g of common bean grains and 350 mL of brine, with two replicates per cultivar. Brine was prepared dissolving 12 g of sodium chloride (NaCl) per L^{-1} of preheated water (95°C), 1.2 % w/v of salt solution. The sterilization of the cans was made in an autoclave with boiling water during 45 min. After canning process evaluations were made for grain increments in volume and weight, broth volume and pH, and shattered grains percentage. A Randomized Complete Block Design was used to obtain the Analysis of Variance (ANOVA) and mean range test was performed using Least Significant Difference ($\alpha = 0.05$).

RESULTS AND DISCUSSION. Significant differences ($p<0.05$) were observed among locations only for broth volume and pH (Table 1). Location which showed highest broth volume was Victoria (154.5 mL) (Table 2) and this trait could be related to low water absorption by grains. Higher shattered grains percentage (37.7%) and broth pH (5.9) were registered in Victoria while higher volume increment was observed in Durango (263.5%) and highest weight increment in Madero (255.2%). Significant differences ($p<0.05$) were observed for broth volume among cultivars without location interactions. Bayo Victoria showed the highest broth volume (159.8 mL) which could be related to reduced water intake by the grains (Table 3). Highly significant difference ($p<0.01$) was observed among cultivars for shattered grains percentage, broth pH and increments in grain volume and weight (Table 1). Bayo Victoria showed the lowest shattered grains percentage (12.6%) and could be considered as an important option to produce entire canned beans. Intermediate value was observed for Negro Vizcaya (27.1%) and the other cultivars showed high percentage of shattered grains and could be used in refried beans prepared as mashed paste (Perez *et al*., 2004). Highest values for broth pH were observed in Pinto Saltillo (5.98) and Pinto Durango (5.91) and this trait could be related to carbohydrate releasing by cotyledons after grain shattering. Pinto Saltillo also showed higher grain volume (282.1%) and weight (257.5%) increments. Traits observed in Pinto Saltillo represent an important investment recovery since almost duplicate the grain volume and weight and then grain buying investments could be reduced more than fifty percent. Results suggest that selection can be made to offer productive options to farmers and to improve inputs quality to canning industry.

CONCLUSIONS. Diversity in canning quality among cultivars planted in Durango was found and important traits in canning quality selection could be water absorption capability, shattered grains percentage and increments in volume and weight.

Table 1. Mean squares of the analysis of variance for traits evaluated in eight bean cultivars planted at three locations in Durango, México.

Source of variation	Degrees of Freedom	Broth Volume (mL)	Shattered Grains (%)	Broth pH	Volume Increment (mL)	Weight Increment (g)
Replication	1	93.5	2.3	0.001	4.1	60.8
Location (L)	2	288.8*	129.1n.s.	0.10*	452.4 n.s.	484.0 n.s.
Error a	2	10.1	10.4	0.001	130.0	26.3
Cultivar (C)	7	319.4*	895.7**	0.03**	1341.8**	199.6**
L x C	14	191.3 n.s.	122.6**	0.011*	1160.8**	42.3**
Error	21	108.7	25.8	0.005	304.0	12.3
CV (%)		6.9	14.7	1.2	6.8	1.4

*= Significant (p<0.05); **= Highly significant (p<0.01); n. s. = not significant; CV= Variation coefficient.

Table 2. Location means observed for traits evaluated in canning characterization of eight dry bean cultivars planted at three locations of Durango, México.

Location	Broth Volume (mL)	Shattered Grains (%)	Broth pH	Volume Increment (mL)	Weight Increment (g)
Durango	152.4	32.3	5.8	263.5	249.6
F. I. Madero	146.3	33.6	5.8	257.1	255.2
G. Victoria	154.5	37.7	5.9	252.9	244.2
Mean	151.1	34.5	5.85	257.8	249.7
*$LSD_{0.05\ locations}$	7.9	8.0	0.08	28.3	12.7
CV (%)	6.9	14.7	1.20	6.8	1.4

*LSD= Least significant difference; CV= Variation coefficient.

Table 3. Means across locations observed for traits evaluated in canning characterization of eight dry bean cultivars planted in Durango, México.

Cultivar	Broth Volume (mL)	Shattered Grains (%)	Broth pH	Volume Increment (mL)	Weight Increment (g)
Pinto Saltillo	138.5b*	56.3a	5.98a	282.1a	257.5a
Pinto Durango	143.0a	32.9bc	5.91ab	242.2b	254.7ab
Pinto Colibrí	150.8a	37.0bc	5.83b	247.0ab	248.2bc
Bayo Victoria	159.8a	12.6d	5.90ab	244.1b	239.7d
Negro San Luis	157.3a	36.6bc	5.80b	249.7ab	244.7cd
Negro Vizcaya	153.0a	27.1c	5.80b	268.8ab	249.7bc
Frijozac N101	149.7a	34.6bc	5.80b	273.2ab	254.0ab
FM Anita	156.3a	39.3b	5.80b	255.7ab	249.0bc
Mean	151.1	34.5	5.85	257.8	249.7
**$LSD_{0.05\ cultivars}$	21.7	10.6	0.2	36.3	7.3
CV (%)	6.9	14.7	1.2	6.8	1.4

*Means sharing the same letter in columns are not significantly different from each other (LSD test; $\alpha = 0.05$). **LSD= Least significant difference; CV= Variation coefficient.

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COOKING TIME OF BLACK BEANS GENOTYPES EVALUATED IN DIFFERENT ENVIRONMENTS

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Among the many common bean types grown in Brazil. 430.000 t (FEIJÃO. 2010) of black beans are harvested annually, corresponding to 20% of the total beans output (Del Peloso & Melo. 2005). This type is mostly consumed in the states of Rio Grande do Sul. Santa Catarina, Paraná and Rio de Janeiro, although other states also produce it in smaller amounts. Lines developed by breeding programs are expected to have improved agronomical characteristics, good culinary quality such as environment affected cooking time.

Since the final quality tests of black bean lines from the Rice and Beans Research Center breeding program are carried out in a great number of environments. it is possible to determine the cooking time of lines tested in those environments and to verify the presence of genotype x ambient interaction.

In 2009 trials were carried out in five environments: Inhumas/Goiás/dry season (ENV1); Ponta Grossa/Paraná/dry season (ENV2); Santo Antônio de Goiás/Goiás/winter season (ENV3); Porangatu/Goiás/winter season (ENV4); and Senador Canedo/Goiás/winter season (ENV5).

A completely randomized block design arranged is plots with four meter rows and two replicates were used. Each trial comprised fourteen genotypes of common black beans (Table 1). Samples were collected from the two central rows and stored at room temperature for a maximum of 90 days. Cooking tests were performed according to the method described by Proctor and Watts (1978). Two replicated of whole seeds were placed in 100 ml of distilled water for 16 hours at room temperature. After that, 25 seeds were placed in a beaker at the Mattson cooking apparatus containing 1000 ml of boiling distilled water and cooking time recorded until the 13^{th} rod fell. Data were subjected to the analysis of variance and the Scott Knott test at 10% was used for mean comparison.

The joint analysis showed adequate precision (CV=11.7%) and significant differences ($P<0.01$) among genotypes. environment and genotype x environment interaction were detected. The average cooking time was 32.0 minute, varying from 22.5 to 47.1, depending on the environment the beans were tested (Table1). That range is related to the variations in the environmental conditions, harvesting method and storage time, since the tests were conducted after different storage periods. Samples with the longest cooking time were those from the dry season with drought spells and high temperature, and storage period longer than the samples from other environments.

Among the controls tested BRS 7762, Supremo showed the shortest cooking time (28.6 min), BRS Campeiro, IPR Uirapuru and BRS Esplendor were assigned to a third group along with five lines with cooking time similar to those cultivars already being formed. Those lines had an "acceptable" cooking time, although had not shown any advantage for that characteristic. Other four lines had cooking times superior to all controls tested. Line CNFP 11976 had the shortest cooking time among the genotypes tested (22.6 min); 6 min shorter than the best control (BRS 7762 Supremo-28.6 min) corresponding to 20% less time. Even in then environments with higher average cooking time that line did not show cooking time longer than 30 min. considered "standard".

Table 1. Average cooking time (CT) (minutes) of 14 genotypes of common beans commercial type black. Brazil. 2009.

GENOTYPES	CT	ENV1	ENV2	ENV3	ENV4	ENV5
CNFP 11976	22.6 a	29.5	29.5	17.0	18.0	19.0
BRS 7762 SUPREMO	28.6 b	30.5	44.0	20.5	24.0	24.0
BRS CAMPEIRO	30.7 c	31.0	44.0	25.0	25.0	28.5
CNFP 11973	30.8 c	37.5	48.0	22.0	23.5	23.0
IPR UIRAPURU	31.3 c	40.5	39.0	23.0	29.5	24.5
BRS ESPLENDOR	31.5 c	40.0	43.0	22.5	26.5	25.5
CNFP 11995	31.7 c	38.0	49.0	24.5	22.5	24.5
CNFP 11984	32.6 c	39.5	53.5	22.0	24.5	23.5
CNFP 11985	32.7 c	42.0	48.5	23.5	24.5	25.0
CNFP 11979	33.3 c	46.0	43.0	24.0	27.5	26.0
CNFP 11994	34.7 d	45.5	59.0	19.5	24.5	25.0
CNFP 11991	35.2 d	41.5	57.0	23.5	24.0	30.0
CNFP 11978	36.1 d	47.5	51.0	24.0	31.0	27.0
CNFP 11983	36.3 d	55.5	51.5	24.0	24.0	26.5
AVERAGE	32.0	40.3 c	47.1 d	22.5 a	24.9 b	25.1 b

[1]Means followed by the same letter do not differ by Scott Knott at 10% probability.

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IDENTIFICATION OF QUANTITATIVE TRAIT LOCI FOR ALUMINUM RESISTANCE IN COMMON BEAN

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INTRODUCTION

Aluminum (Al) toxicity is a major limiting factor of crop production in acid soils (pH ≤ 5.0), which are found mostly in developing countries of the tropics and sub-tropics. Common bean (Phaseolus vulgaris L.) is shown to be particularly sensitive to Al toxicity; for this reason development of genotypes with better performance in Al-toxic soils is a priority. The objectives of the present study were: (1) to physiologically assess root architectural traits in a recombinant inbred line (RIL) population for an Al susceptible x resistant cross (DOR364 x G19833) of common bean; and (2) to identify quantitative trait loci (QTL) controlling Al resistance.

MATERIALS AND METHODS

Plant materials: Recombinant inbred lines from the cross DOR364 × G19833 were used along with the parents of the population to screen for resistance to Al as described below. To begin the experiment we germinated 40 seeds on peat moss (pH 5.5) for 3 days. A total of 8 seedlings were then selected for uniform root elongation and suspended over the hydroponic solution. This was done by placing their hypocotyls through small circular foam pads held in place by multi-well floating plastic trays placed in tanks supplied with 20 L of a simple nutrient solution (5 mM ClCa2, 0.5 mM KCl, and 8 µM H3BO3 at pH 5.5) and continuous aeration.

Screening Method: Experiments were carried out in a greenhouse at CIAT with relative humidity of 72%, temperatures of 29 ºC and the maximum density of photon flow during the day of 1100 µmol ms–1. Two treatments were applied, one with 20 µM AlCl3 (+Al) and one without Al (-Al) considered as a control. To prevent growth inhibition through pH shock the seedlings were given a period of adaptation for 1 day at pH of 5.5 after which pH in the growth solution was decreased using 1 N HCl to pH 5.0 for 8 hours, and then to pH of 4.5 for the remainder of the treatment period. After 3 days, hydroponic solutions were changed and Al treatment was applied to the appropriate tanks for 48 h. Experimental design was a completely randomized design with 4 repetitions / treatment.

Data collection: To determine the tap root elongation rate, the roots were measured for tap root length at the beginning (li) and the end of treatment (lf) using a ruler with millimetric precision and calculated based on the formula TRE = (lf - li) / 48 h. At the end of the treatment, whole roots and aerial parts were collected. The roots were washed and scanned for analysis with WinRHIZO 2003b software to determine total root length (TRL), average root diameter (ARD), and number of root tips (NRT). Finally, both roots and aerial parts were dried in an oven at 65°C for 48 h and then weighed in an analytical balance to estimate root (RDW) and shoot dry weight (SDW) and to calculate the specific root length based on the ratio between TRL and RDW.

RESULTS AND DISCUSSION

A total of 28 QTL were found through composite interval mapping (CIM) analysis, 13 for traits under Al treatment (+Al), 8 for traits under control (-Al) treatment, and 7 for relative traits (ratio of +Al/-Al). Among the individual traits, 6 QTL were found for root length with most derived from G19833 under the relative condition of +Al/-Al. Number of rooot tips also had 6 QTL where all but one were derived from G19833 and three were expressed only under +Al. Specific root length was the next most frequent in QTL with 5 loci identified these being from both DOR364 and G19833 and this may be related to the fact that we found one QTL for root diameter from G19833 and the other from DOR364. Finally, there were 5 QTL fro root dry weight and 2 QTL for shoot dry weight with the former from both parents and the latter only from G19833. These results show that Al resistance in common bean is under polygenic control, each QTL contributing in a small degree to Al resistance, and indicate that multiple mechanisms of Al resistance might be operating simultaneously. Furthermore, some QTL were identified at the same location as QTL for tolerance to low phosphorous (P) stress, thus, suggesting cross-links in genetic control of adaptation of common bean to different abiotic stresses.

Table 1. Quantitative trait loci (QTL) for root architectural traits in nutrient solutions with (+Al, 20 µM) and without (-Al) aluminum treatments for the DOR364 x G19833 population. Values represent QTL significance (LR) and determination coefficients explained by each QTL (R^2 and TR^2).

Trait	**QTL name**	**LG**	**Treatment**	***Source***	***Significance***			**Additive effect**
					LR	***R^2***	***TR^2***	
Total Root Length (cm)	*Trl6.1*	6	- Al	G19833	19.55	0.20	0.51	36.95
	Trl8.1	8	- Al	DOR364	16.62	0.15	0.32	31.74
	Trl8.2	8	+ Al / -Al	G19833	14.81	0.12	0.37	2.43
	Trl9.1	9	+ Al / -Al	G19833	18.85	0.29	0.66	3.89
	Trl11.1	11	+ Al / -Al	G19833	16.86	0.15	0.39	2.79
	Trl11.2	11	+ Al	G19833	22.14	0.22	0.38	18.2
Average Root Diameter (mm)	*Ard6.1*	6	+ Al	G19833	25.66	0.18	0.47	0.02
	Ard7.1	7	+ Al / -Al	DOR364	29.15	0.28	0.5	2.08
Specific Root Length (m/g)	*Srl2.1*	2	+ Al	DOR364	23.68	0.15	0.55	6.03
	Srl5.1	5	- Al	G19833	25.17	0.22	0.55	11.09
	Srl7.1	7	+ Al	G19833	19.63	0.13	0.57	5.47
	Srl8.1	8	- Al	DOR364	17.6	0.13	0.47	8.88
	Srl9.1	9	- Al	DOR364	18.25	0.15	0.5	9.16
Number of Root Tips (n)	*Nrt3.1*	3	+ Al	G19833	15.46	0.14	0.52	23.19
	Nrt5.1	5	+ Al	G19833	25.41	0.16	0.47	23.22
	Nrt9.1	9	- Al	DOR364	17.29	0.14	0.4	65.72
	Nrt9.2	9	+ Al / -Al	G19833	14.31	0.12	0.4	3.34
	Nrt9.3	9	+ Al / -Al	G19833	19.61	0.16	0.4	4.25
	Nrt11.1	11	+ Al	G19833	21.36	0.15	0.47	23.04
Root Dry Weight (g)	*Rdw1.1*	1	- Al	DOR364	23.71	0.18	0.5	0.0019
	Rdw1.2	1	+ Al	DOR364	16.27	0.10	0.52	0.0011
	Rdw11.1	11	+ Al / -Al	G19833	18.36	0.17	0.44	3.88
	Rdw11.2	11	+ Al	G19833	18.51	0.13	0.54	0.0012
	Rdw11.3	11	- Al	G19833	14.74	0.11	0.48	0.0014
Shoot Dry Weight (g)	*Sdw2.1*	2	n.a.	G19833	30.30	0.23	0.45	0.0171
	Sdw7.1	7	n.a.	G19833	33.38	0.30	0.52	0.0185

EVALUATION OF RECURRENT SELECTION FAMILIES FOR TOLERANCE TO WATER DEFICIT IN COMMON BEAN

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INTRODUCTION

The adaptation of plants to stress environments is a challenge to modern agriculture. This requires understanding the behavior of plants in contrasting environments, with and without stress, and interrelation between them (Lizana et al., 2006). Among the various abiotic stresses, water deficiency is highlighted by the occurrence and extent of the reduction in productivity. It is estimated that 60% of the world's beans are produced in regions with water deficit. In Brazil, common bean (Phaseolus vulgaris L.) is grown in almost the whole country at various times of the year, which exposes it to a great climatic diversity. The objective of this work is to evaluate the adaptation to water deficit the families of recurrent selection $C_oS_{1:6}$ and $C_oS_{1:7}$, with carioca grain type of a base population obtained from a multiple crosses involving parents tolerant to water deficit.

MATERIALS AND METHODS

The experiments with and without water deficit, were conducted on an Oxisol at the SEAGRO Experimental Station in Porangatu-GO for two consecutive years, 2008 and 2009. We evaluated 25 families $C_oS_{1:6}$ in 2008, with carioca grain type, of a base population (Co) obtained from multiple crosses involving parents tolerant to water deficit and three tests genotypes, BRS Pérola, BRS Radiant and BAT 477. The latter is a tolerant line to water deficit from the International Center for Tropical Agriculture (CIAT). They were sown on 13/06/2008 in plots of two rows, three meter long and 45 cm spaced in a randomized block design with three replications. Of the families evaluated, in 2008 were selected 15 best-productive families in both water treatments, with and without water deficit and with better quality seed. The 15 families, plus the test genotypes used on the previous year, were reevaluated in 2009. Sowing was done on 23/05/2009 in plots similar and adopting the same agriculture practices of the previous year, but in rows spaced 40 cm. Two experiments were conducted in each year. The first was well irrigated throughout the crop growth and the other only up to 20 days after emergence, when it was applied the water deficit. Therefore, total irrigations were made in the first experiment and during the phase without water deficit in the second experiment. Irrigation water was applied when the potential of soil water to 0.15 m depth amounted to - 0.035 MPa (Silveira & Stone, 1994). During the period of water deficit was applied about a half the water irrigation used in the experiment without water deficit. We evaluated the effect of water deficit on yield and on flowering date.

RESULTS AND DISCUSSION

The water treatments significantly influenced bean yield in 2008, but did not affect the flowering date. Yield obtained was 536 kg ha^{-1} and 2259 kg ha^{-1} in the treatments with and without water deficit, respectively. It was also observed that genotypes yield differently from each other and responded with different levels of intensity to the effects of two water treatments, since it was observed a significant interaction between water levels and yield. The genotypes flowering date differed significantly, however responded with the same intensity to the effects of water treatments,

since it was not observed significant interaction between water levels and flowering date. In selecting for drought tolerance was considered the yield in both water conditions, with and without water deficit, since it is desirable that the genotypes present both good yield when rainfall is normal or when it does not. The genotypes were distributed into quartiles defined by the average yield in the treatments well irrigated and with water deficit. In 2008 the average yields in treatments without and with water deficit were 2259 kg ha^{-1} and 536 kg ha^{-1}, respectively. Genotypes were selected from quartile one. That included the families of recurrent selection, number 39, 191, 20, 118, 148, 113 and 150, because they yielded above average in both water levels. They yielded fine in the irrigated treatment and were less susceptible to water deficit. All of these lines showed flowering date between 43 and 45 days after sowing (DAS) and not significantly different, except the family number 39, which flowered at 47 DAS. In 2009 were re-evaluated the 15 selected families in 2008, adopting the best criteria for productive behavior in both water levels, with and without water deficit, and a better quality of grain, plus the same lines test used in the previous year, the varieties BRS Pérola and BRS Radiance and the line BAT 477. It was conducted a joint analysis considering the results of these genotypes in 2008 and 2009. It was found that the yield of the genotypes differed significantly between the years of conducting the experiments. The yield obtained was 1503 kg ha^{-1} and 1008 kg ha^{-1} in 2008 and 2009, respectively. Flowering date was also influenced by the ears of conducting experiments. The lines were earlier in 2009, influenced probably by the anticipation of 20 days in the sowing. A joint analysis of the effect of water treatments was similar to that observed in 2008, when it was observed that only the yield was significantly affected by water treatments. It was observed 400 kg ha^{-1} and 2111 kg ha^{-1} in the treatments with and without water deficit, respectively. The genotypes presented significantly different and also flowered at different times. However, all these components responded similarly to the effects of two water treatments, because there was no significance in the interactions between water levels and genotypes for yield and flowering date. In selecting for drought tolerance considering the yield of experiments conducted in 2008 and 2009, it was adopted the same methodology used in 2008. Genotypes were selected from quartile one. That included the families of recurrent selection, number 191, 118, 20, 148 and 150, because they have above average yield in both water levels, well produced in the irrigated treatment and were less susceptible to water deficit. All these families flowered under 43 DAS, remained the same productive behavior, with and without water deficit, observed in 2008 and were also higher yielding than the genotype BAT 477, tolerant to water deficit.

C**ONCLUSION**

The families of recurrent selection number 191, 118, 20, 148 and 159 were selected because they had good performance with and without water deficit in the two consecutive years of genotype evaluations and responded similarly to the effects of two water treatments.

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PHENOTYPIC EVALUATION OF A SUBSET OF THE *PHASEOLUS VULGARIS* CORE COLLECTIONS, THE *P. ACUTIFOLIUS* GERMPLASM COLLECTION, AND CULTIVARS FOR DROUGHT TOLERANCE IN NEBRASKA AND PUERTO RICO

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Drought stress is an important constraint to common bean production worldwide and is an increasing constraint on US production (Singh, 2007; Muñoz-Perea et al., 2007). To address this issue, exotic common bean and tepary bean germplasm from the NPGS and CIAT collections and from US and international breeding programs were evaluated for their response to drought stress.

MATERIALS AND METHODS

A total of 277 entries, 128 cultivars and elite lines and 149 accessions of *Phaseolus vulgaris* and *P. acutifolius* from the NPGS and CIAT core collections were screened under terminal drought stress conditions at Mitchell, NE (41°56.6′ N, 103°41.9′ W, 1240 m elevation) and at Fortuna, PR (18^o 01'N, 66^o 22'W, 21 m elevation) during 2008. The entries from the core collections were previously selected for insensitivity to photoperiod in 2006 and 2007 in Puerto Rico. The effect of drought using adjacent non-stressed (NS) and drought stressed (DS) blocks, with two replications in each environment, were evaluated as described by Terán and Singh (2002). Within each block, the selected lines were assigned to experimental units using an augmented block design. Beryl-R, Marquis, Orion, Poncho, and SEN 21 were used as reference checks. Each plot consisted of two 7.6 m rows spaced 0.6 m apart in Nebraska and single 4 m plots spaced 1 m apart in Puerto Rico. Targeted plant density was 200,000 plants ha^{-1} in Nebraska and 150,000 plants ha^{-1} in Puerto Rico. Both NS and DS blocks were irrigated until flowering to ensure good plant establishment and normal vegetative growth. Thereafter, the stressed block was not irrigated in Nebraska and was irrigated at half the non-stress rate in Puerto Rico.

Within each location, each replication was analyzed separately as an augmented block. Adjusted means from each replication were combined and analyzed as an RCBD. Homogeneity of the variances was evaluated using Barlett's test. Means were separated using an F-protected LSD. All tests were considered significant at $P \leq 0.05$. To evaluate plant response to water stress, yield (kg/ha), 100-seed weight (g), and the number of days to flowering and to maturity were determined. To quantify drought severity, the drought intensity index (DII), geometric mean (GM), the drought susceptibility index (DSI), and percent yield reduction (PR) were determined to predict the performance of a line under DS and NS conditions.

RESULTS AND DISCUSSION

In the combined analysis across the Nebraska and Puerto Rico locations, drought stress was moderate (DII = 0.34) with significant precipitation of 77.8 mm occurring at 53 d after planting at Mitchell, NE. Yield under NS and DS ranged from 800 to 2510 kg/ha, and from 596 to 2516 kg/ha, respectively. Under DS conditions, yield and 100-seed weight were reduced an average of 33.4 and 7.4%, respectively, relative to NS conditions.

Using GM as the major selection index, NE1-06-11 was found to be well adapted to both NS and DS environments in Nebraska and Puerto Rico and had the lowest PR (5%), smallest DSI (0.2)

and the largest GM (2456 kg/ha). Montrose and Lariat had the second and third largest GM (2082 and 2070 kg/ha, respectively) with a PR of 30.2 and 31.9%, respectively. In addition, the PR of CO23704 and USPT-CBB-6 was 24 and 11.4%, DSI was 0.7 and 0.3, and GM was 2036 and 1848 kg/ha, respectively.

Among the reference checks, Poncho had lowest yield reduction (19.3%), lowest DSI (0.6) and an intermediate GM (1880) compared to Orion (30.8%, 0.9, and 1758, respectively) and SEN 21 (33.2%, 1.0, and 1535, respectively). The yields of all of the PI accessions (*P. vulgaris* and *P. acutifolius*) were lower than the cultivars and germplasm tested under both DS and NS environments with the exception of PI 476751, which had a PR of 5%, GM of 1583, and a DSI of 0.15. The results illustrate that progress has been made in breeding for improved adaptation and drought tolerance in breeding programs.

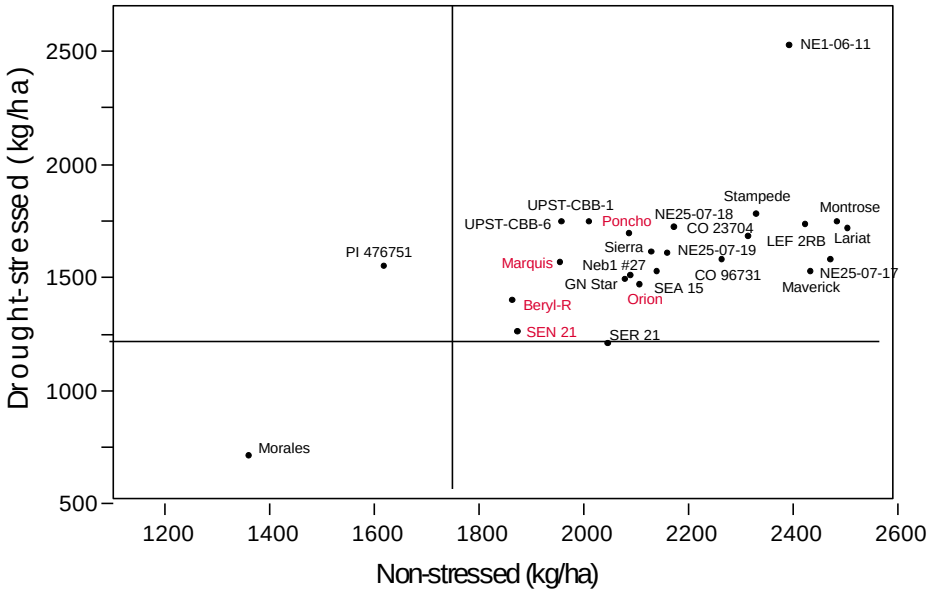

Figure 1. 20 Top yielding (kg/ha) accessions based on combined analysis of NS and DS environments in Mitchell, NE and Fortuna, PR.

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PARTIAL IDENTIFICATION AND SEQUENCING OF TWO PUTATIVE GENES ENCODING THE TREHALOSE 6-PHOSPHATE SYNTHETASE ENZYME IN COMMON BEAN

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Environmental conditions are severe limiting factors for growth and yield in plant crops. Drought stress is one of the main problem in beans (*Phaseolus vulgaris* L.) due has great economic importance particularly in developing countries (Subbarao *et al*., 1995). Other factors limiting production are salinity and extreme temperatures (Sunkar, 2004). Trehalose is widespread in nature and it is present in a large number of organisms including bacteria, yeast, fungi, insects, and plants (Elbein *et al*., 2003). It has different biological functions serving as energy and carbon source and signaling and it helps to express tolerance to cold, heat, osmotic, oxidative and dehydration stresses (Iturriaga *et al*., 2009). For a better understanding of trehalose role in *P. vulgaris* we designed a couple of primers to identify the homologous gene in common bean according to the sequence previously published at NCBI for trehalose 6- phosphate synthetase (TPS) gene from other plants.

We obtained 30 TPS enzymes protein sequences deposited at NCBI gene bank (http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide); then, it was performed an alignment of sequences using MegAling DNAstar suite laser gene program (Fig. 1) to design primers. Target genomic DNA was extracted from fresh leaves of common bean plants using the DNAprep kit (Promega©; Madison, WI). For amplification reactions we used the Gotaq enzyme (Promega) following the supplier instructions. The alignment temperature was 52ºC for PCR. The DNA fragments obtained were purified by using Gene Clean kit (Quiagen©; Hilden, Germany) and then visualized at Gel-Doc XR Digital imaging system (BioRad©; Hercules, CA) in order to determinate the concentration and then adjust itself to 20 ng μL^{-1} according with sequencing reaction (Abi Prism kit©; Singapore). Sequences were aligned and analyzed by homology using the identity score from BLASTx sequence analysis program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) in order to compare each one with other protein sequences.

The designed primers with better amplification were: TGGCCNYTNTTYCAYTA (Tm = 53ºC); ACNARRTTCATNCCRTCNC (Tm = 58) and identified as TPSF and TPSR, respectively. Amplified DNA fragments using these primers are shown in Fig. 2. The analysis of sequences indicated that two segments showed high identity with plant TPS proteins. Identity score obtained in both sequences were: one fragment with 63/118 amino-acids that corresponding to 53.5% of trehalose-6-phosphate synthase, putative *Ricinus communis* and the other 38/73 amino-acids that corresponding to 62% of trehalose-6-phosphate synthase from *Solanum lycopersicum*. Fragments were previously named *TPS1Pv* and *TPS2Pv* respectively because the total segment has not been fully sequenced yet. On the other hand our results were interesting because we can amplify two putative TPS enzyme genes using a single couple of primers. Currently, it is well known that several

members of these enzymes are presented in organisms. For example, *Arabidopsis thaliana* contains 21 putative trehalose biosynthesis genes (Ramon *et al*., 2009). This research is a good point for start works related to TPS gene implications on signal cascades under drought stress conditions in common beans.

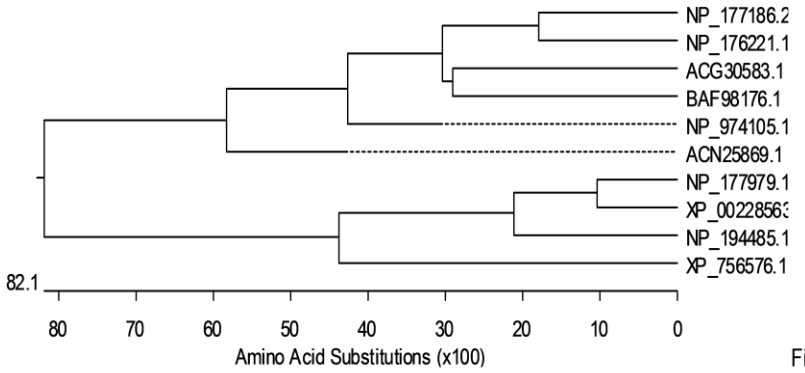

Figure 1. Alignments of TPS sequences from diverse plant species.

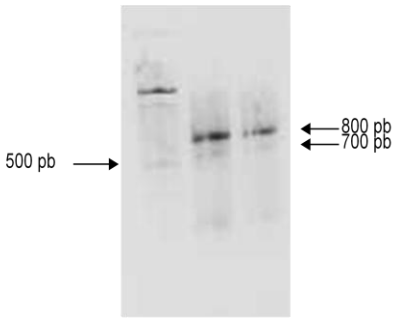

Figure 2. PCR products using TPSR and TPSF in beans. 1= Molecular ladder, 2 and 3 = Common bean.

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NODULATION AND GROWTH OF INOCULATED SNAP BEAN CULTIVARS WITH RHIZOBIAL CELL STRAINS

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INTRODUCTION: The snap bean (*Phaseolus vulgaris* L.) requires high amount of nutrients in short-term intense growth, mainly nitrogen (N) and potassium (K). The recommendations for fertilizing the crop are based on other common bean varieties, however, the snap bean differs from those inr size, leaf area, height, cycle, productivity and growth habits, mainly in the indeterminate growth cultivars. The inoculation of In Leguminosae crops with rhizobial strains can replace nitrogen fertilizers, in an economically sound way at lower cost, however for the snap bean culture there is no information in the literature about their response to inoculation. This work aimed to verify the behavior of the snap bean cultivars after inoculation with selected rhizobial strains.

MATERIAL AND METHODS: The work was carried out in $3dm^3$-vases, in a green house in the Soil Science Department, Federal University of Lavras-MG, Brazil. The design was 4-replicate-randomized blocks in 4x7 factorial scheme involving four commercial snap bean cultivars (Macarrão Rasteiro Conquista, Macarrão Favorito, Macarrão Preferido e Macarrão Atibaia) and seven treatments involving inoculation (five rhizobial stains) plus two non-inoculated controls, one with mineral $N\text{-}NH_4NO_3$, 500mg pot $^{-1}$ and other without mineral N. The cultivars were selected for showing good acceptance in the market and for the fact that two of them (M. Atibaia and M. Preferido) are less susceptible to root-knot nematode (FERREIRA *et al.*, 2010). The soil (P: 300mg/L; Zn: 5mg/L; Cu: 1,5mg/L; S: 40mg/L; Mn: 3.6 mg/L; K: 300mg/L; B: 0.8mg/L; Mo: 0.15mg/L; pH 4.9) was previously limed to increase the base saturation up to 60%. All the plots received the same phosphorus and potassium fertilization. The rhizobial strains were UFLA 02-100 (*Rhizobium etli*), UFLA 02-127 (*R. leguminosarum* bv. *phaseoli*) and UFLA 4-173, CIAT 899 and PRF 81 (*R. tropici*). The strains were grown in "79" semi-solid medium (Fred & Waksman, 1928) at 28ºC, for two days, under shacking. Before the sowing, the snap bean seeds had been surfaced disinfectedfor 30 seconds in alcohol and 2 minutes in H_2O_2 P.A., which was followed by six washings with sterilized distilled water. 1 mL of inoculant (around 10^8 bacterial cells) was added in each seed during the sowing (3 seeds/pot). On the 8th day after emergence, it was thinned to one plant per pot. Each plot contained two pots; in the first, shoot dry matter (SDM), the number (NN) and dry matter (NDM) of nodules were evaluated when the plants showed complete flowering; in the second pot, the number (NP), fresh matter (FMP) and dry matter (DMP) of green pods per plant were evaluated, when each cultivar presented more than 50% of commercial green pods. The data obtained were submitted to variance analysis (nodule dry mass was transformed to $\sqrt{x}$ and nodule numbers to $\sqrt{x+1}$. The averages were compared by Scott-Knott test at 5% probability.

RESULTS AND DISCUSSION: Variance analysis showed the cultivars had an effect over all characteristics and treatments involving inoculation on NN and NDM, and the interaction was meaningless. The treatments differed only to the NDM, where the UFLA 4-173 strain showed

smaller mass, not differing from the witness with N (table 1), which was expected, due to the well known mineral N inhibiting effect over nodulation.

Table 1. Average values of shoot dry matter (SDM), number of nodules (NN), nodule dry matter (NDM), average number of green pods (NP), fresh matter of green pods (FMP) and dry matter of green pods (DMP) after the inoculation with rhizobial strains, fertilization with mineral N (CN) and the control without inoculation and mineral N (SN) (averages from 4 cultivars)*.

TREATMENTS	SDM (g/plant)	NN (unit/plant)	NDM (g/plant)	NP (unit/plant)	FMP (g/plant)	DMP (g/plant)
SN	7.50 a	84.90 a	0.125 a	7.85 a	28.35 a	3.02 a
PRF 81	7.79 a	121.75 a	0.142 a	8.31 a	30.10 a	3.17 a
UFLA 02-127	7.61 a	133.94 a	0.185 a	7.81 a	28.26 a	2.90 a
CIAT 899	7.16 a	172.87 a	0.215 a	7.77 a	31.05 a	3.14 a
UFLA 02-100	6.71 a	225.75 a	0.230 a	7.98 a	36.65 a	3.59 a
CN	8.00 a	42.56 a	0.032 b	9.94 a	34.11 a	3.27 a
UFLA 4-173	7.73 a	81.94 a	0.080 b	7.62 a	24.43 a	2.55 a

*Averages followed by the same letter in the column, do not differ by Scott-Knot test at 5% probability.

In general, the SDM in the controls without mineral N as well as the inoculation did not differ among the inoculated strains, showing that native populations were highly efficient. It can be observed that the inoculation with the strains increased nodulation, however not statically significant (table 1), with average values of 147.25 nodules and 0.18 g of nodule dry matter per plant. The UFLA02-100, UFLA 02-127 and PRF 81 strains presented a performance like CIAT 899, the strain used in the inoculants applied in other varieties of common beans cropped in Brazil. M. Preferido cv. showed higher growth (SDM) and higher production of green pods per plant (NP, FMP and DMP). The same cultivar stood also in relation to NN and NDM, but without significantly differing from M. Atibaia cultivar (table 2).

Table 2. Shoot dry matter (SDM), number of nodules (NN), nodule dry matter (NDM), average number of green pods (NP), fresh matter of pods (MFP) and dry matter of green pods (DMP) from 4 snap bean cultivars (averages from seven treatments involving inoculation)*.

CULTIVARS	SDM (g/plant)	NN (unit/plant)	NDM (g/plant)	NP (unit/plant)	FMP (g/plant)	DMP (g/plant)
M. Preferido	9.14 a	182.25 a	0.227 a	12.31 a	37.88 a	4.19 a
M. Atibaia	7.92 b	168.28 a	0.194 a	7.52 b	26.73 b	2.66 b
M. Favorito	7.26 b	88.87 b	0.093 b	7.17 b	27.30 b	2.75 b
M. Rasteiro Conquista	5.68 c	54.14 b	0.063 b	5.74 c	29.78 b	2.76 b

*Averages followed by the same letter in the column, do not differ by Scott-Knot test at 5% probability.

We can conclude that: 1) M. Preferido and M. Atibaia cultivars, less susceptible to the root-knot nematodes, are the ones which present higher nodulation; 2) The M. Preferido cv. shows better agronomic behavior, with higher growth and green pods yields, which might be related to higher potential for nitrogen fixation.

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IDENTIFICATION OF HIGH NODULATION EFFICIENCY AMONG WILD GENOTYPES OF COMMON BEANS

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is widely grown in Brazil and used by the poorer population as an important protein source. This crop establishes symbiotic association resulting in spherical determinate nodules where N_2 fixation process takes place. However, many studies have shown a relative low efficiency of the N_2 fixation on common bean due to an easy association of this crop with indigenous rhizobia species (MOAWADE et al., 2004), which result in some difficulty to the introduction of more efficient species (VIEIRA et al., 1998). The screening for high N_2-fixing ability among wild genotype of common bean could provide genetic material of great interest for the common bean breeding programs.

MATERIAL AND METHODS

Aiming to evaluate the nodulation of 377 wild genotype of common bean, a greenhouse experiment was carried out at the National Rice and Beans Research Center of Embrapa, located in the county of Santo Antônio de Goiás, Goiás, Brazil. The experiment was performed on a randomized block design, in which 377 wild genotype of common bean, obtained from the active bank of genotype of the Embrapa Rice and Beans, were evaluated under sterile conditions. Two seeds of each wild genotype were planted in 3 L pots containing sterile sand and vermiculite (3:1). Seven days after emergence (DAE), plants were inoculated with a mixture of three strains of *Rhizibium tropici* (SEMIA 4077, SEMIA 4080 and SEMIA 4088), on a final concentration of 10^8 colony forming unit mL^{-1}. Ouro Negro was also inoculated with the *Rhizobial* mixture and used as a reference of good nodulating cultivar (BLISS et al., 1989). Once a week, 200 mL of Norris' solution were added per pot until harvest. Common bean plants were harvested 30 DAE and it were determined the number of nodules (NN) per plant, total nodule dry weight (TNDW) and relative nodule weight (RNW) as a relation of NDW/NN. These data were used to generate a Relative Nodulation Index (RNI=(RNW*1.3)+(TNDW*1.1)+(NN*0.6)/3). The parameters used to determine the RNI were multiplied by different factors due to the fact that there is a positive correlation between nodule mass and the amount of N accumulated by legumes (DOBEREINER, 1966), however, not necessarily the greatest number of nodules implies in high N_2-fixing ability (CARVALHO, 2002). Data of nodulation were submitted to a variance analysis and the means were compared by the Tukey´s test at 5% of significance.

RESULTS AND DISCUSSION

The analysis of variance had been shown differences among the wild genotypes of common bean regarding RNW, TNDW, NN and RNI (Figure 1). About 70%, 33% and 13% of the wild genotypes of common bean (blue columns) showed greater RNW, TNDW and NN, respectively than the reference cultivar (Figure 1A, B and C). Nevertheless, RNI (Figure 1D) indicates that about 45% of the wild genotypes of common bean have been shown great potential to be used as high N_2-fixing

source for the EMBRAPA's common bean breeding program. Among of then, 4 wild genotypes showed the best results since they figured among the greatest values of RNW, TNDW and NN.

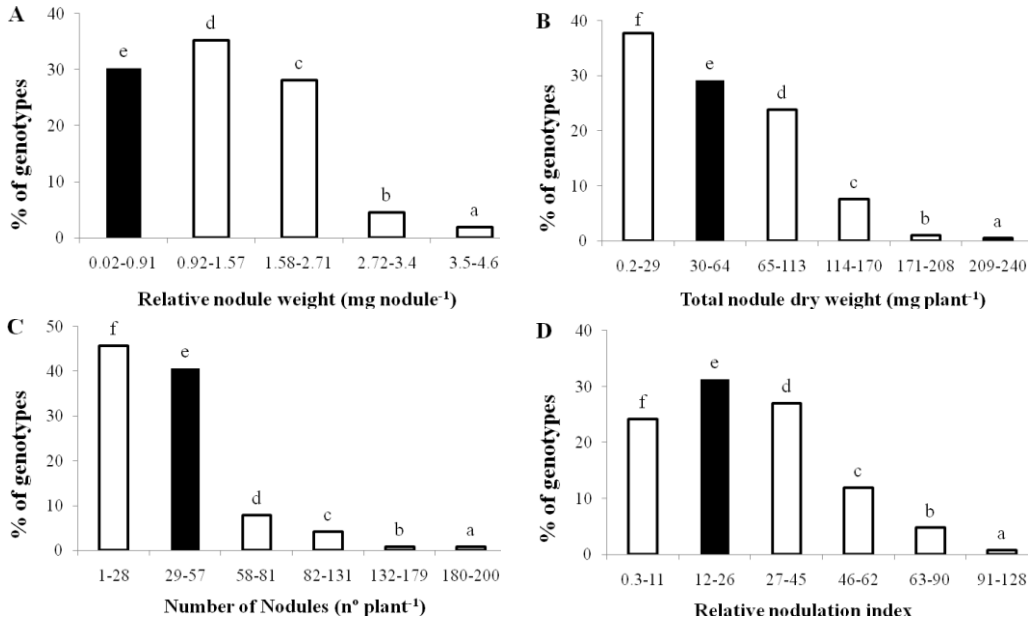

Figure 1 – Percent distribution of the wild genotypes of common bean according to the different classes of Relative nodule weight (A), Total nodule weight (B), Number of nodules (C) and Relative nodulation index (D). Black columns indicate the classes which comprise the reference cultivar (Ouro Negro).
Columns followed by the same letter are not different by the Scott-Knott test ($p>0.05$).

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NITROGEN FERTILIZATION AND INOCULATION WITH *RHIZOBIUM TROPICI* IN BLACK BEAN

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INTRODUCTION

Although being very important to the Brazilian society, the bean crop average yield is still far below its potential. Several factors in the production system of this legume have been contributing to this scenery. We can affirm that some factors, such as, the lack adapted genotypes to the different producing areas and the inadequate handling of nitrogen fertilization contributed for this situation. This work had as objective to evaluate the behavior of lineages of black bean plant submitted to nitrogen fertilization and inoculation with *Rhizobium tropici*.

MATERIAL AND METHODS

The experiment was carried out on the agricultural year of 2009 in Dourados, Mato Grosso do Sul state, Brazil (22º16'S; 54º49'W). The lineages of beans from the black group tested were: CNFP 11973, CNFP 11976, CNFP 11978, CNFP 11979, CNFP 11983, CNFP 11984, CNFP 11985, CNFP 11991, CNFP 11994 and CNFC 11995. The control treatments were the cultivars BRS Campeiro, BRS Esplendor, BRS Supremo and IPR Uirapuru. The experimental design was in randomized block with three replications in a 14x2 factorial arrangement, 14 cultivars (10 new lineages and 4 commercial cultivars) and 2 ways to supply nitrogen (N fertilization and biological fixation - BNF). For the treatments with nitrogen fertilization, 40kg/ha of N were applied on the sowing and also in coverage after 30 days after the germination. Urea was used as a source of N. For the treatment with BNF, the seeds were inoculated with *R. tropici* strains CIAT 899 and PRF 81, as described by Pelegrin et al (2009). The productivity data were transformed (square root of x +1) and subjected to analysis of variance. The means were compared by Tukey test at 5%.

RESULTS AND DISCUSSION

It was not observed significant difference to the average productivity (new lineages and commercial cultivars) between treatments with nitrogen fertilization and with *Rhizobium tropici* inoculation (figure 1). This result is similar to Silva et al. (2009) that did not find differences in dry matter production of aerial part of bean plants when compared with the same treatments. However, in the treatment with nitrogen fertilization there was no difference on the yield of the new lineages and also between the new lineages and the control (Table 1). This fact indicates that the lineages tested have a productive potential similar to the commercial cultivars. On the other hand, when the seeds were inoculated with *R. tropici*, the control and the lineages CNFP 11973, CNFP 11976 and CNFP 11995 presented a yield higher than the lineage CNFP 11979 (Table 1). In this case, it should be noted that the lines CNFP 11973, CNFP 11976 and CNFP 11995 showed highest yield to that obtained with commercial varieties represented by the control. This indicates for these beans lineages, the possibility to use the seed inoculated with *R. tropici* replacing the mineral nitrogen fertilization,

especially for the lineage CNFP 11973 that showed higher yield when inoculated. To confirm these results it is necessary to run new experiments.

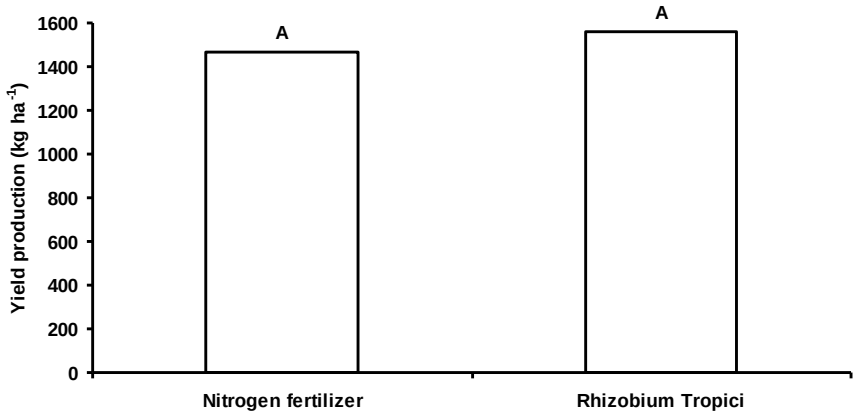

Figure 1. Average yield of bean grains from black bean group when supplied with mineral nitrogen fertilization and biological fixation with *Rhizobium tropici* (CIAT 899 e PRF 81). Equal letters on the bar indicates that the averages do not differ by Tukey test at 5% probability.

Table 1. Average yield of different bean lines of the black bean group fertilized with mineral nitrogen or inoculated with R tropici (CIAT 899 and PRF 81).

Lineage	Yield ($kg.ha^{-1}$)	
	Fertilization (Mineral N)	Inoculation (*R. tropici*)
Control*	1,735 Aa	2,042 Aa
CNFP 11973	1,174 Ba	2,291 A a
CNFP 11976	1,881 Aa	2,161 Aa
CNFP 11978	1,015 Aa	1,001 Aab
CNFP 11979	719 Aa	615 Ab
CNFP 11983	1,865 Aa	1,677 Aab
CNFP 11984	1,395 Aa	1,371 Aab
CNFP 11985	1,983 Aa	1,294 Aab
CNFP 11991	1,689 Aa	1,650 Aab
CNFP 11994	889 Aa	844 Aab
CNFP 11995	1,784 Aa	2,240 Aa

Averages followed by capital letter in the row and lower in the column do not differ by Tukey test at 5%,
* represented by four commercial cultivars (Campeiro, BRS Esplendor, BRS Supremo e IPR Uirapuru)

CONCLUSIONS

The bean lineage CNFP 11973 showed highest yield when the seeds were inoculated with *Rhizobium tropici*,

On the average yield of the black beans group there was no difference between mineral N fertilization and inoculation with *Rhizobium tropici*,

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BEAN PLANT LINEAGES SUBMITTED TO THE NITROGEN FERTILIZATION AND TO THE *RHIZOBIUM TROPICI* INOCULATION

Osmar Rodrigues Brito[1], Auro Akio Otsubo[2], Fabio Martins Mercante[2], Aline Érika Hori[1], Victor Hugo Nakase Otsubo[1] and Jenifer Aparecida Schnitzer[1]

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INTRODUCTION

The bean crop has great social and economic importance in Brazil, because besides guaranteeing income to the farmers, the bean is the major source of protein to the low income population. The crop yield is still low for lack of genetic materials of high productive potential and also due to the inadequate management of nitrogen fertilization. The objective of this work was to evaluate the yield of common bean lineages from Carioca group when submitted to the nitrogen fertilization and inoculation with *Rhizobium tropici.*

MATERIALS AND METHODS

The experiment was carried out on the agricultural year of 2009 in Dourados, Mato Grosso do Sul state, Brazil (22º16'S; 54º49'W). The new beans lineages (Carioca group) 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 and CNFC 11966. These beans lineages were compared to the commercial cultivars BRS Cometa, BRS Estilo, IPR Juriti and Pérola. The experimental design was in randomized blocks with three replications in a 17x2 factorial arrangement with 17 bean cultivars (14 new lineages and 4 commercial cultivars) and 2 ways of nitrogen supplying to the plant (mineral fertilization and biological fixation (BNF)). For the treatments with nitrogen fertilization, 40kg/ha of N were applied on the sowing and also in coverage 30 days after the germination, using urea as the N source. For the treatment with BNF, the seeds were inoculated with *Rhizobium tropici* strains CIAT 899 and PRF 81, as described by Pelegrin et al (2009). The characteristics evaluated were: number of pods/plant, number of grains/pod and grain yield.

RESULTS AND DISCUSSION

For all variables studied were not observed significant differences between mineral nitrogen fertilization and bean seeds inoculation with *R. tropici* (table 1). This result matches with the ones found by Valadão et al (2009).

Considering the increase in the average production of the four commercial cultivars (control) was observed difference of behavior between the tested lineages (figure 1). The most of the lineages fertilized with mineral nitrogen presented positive responses when compared to the controls, highlighting the CNFC11953, CNFC10429 and CNFC11966. However, when was used just the inoculation with *R. tropici*, the lineages that stood out were: CNFC 11966, CNFC 10429 and CNFC 11944. In the Brazilian breeding programs of common beans, the selection pressure has been to the

response to mineral nitrogen fertilization. In this work there is evidence that inoculation with *R. tropici* is a viable alternative that should be considered.

Table 1. Mean values of pods per plant, number of grains per pod and crop yield from different beans lineages (Carioca group) under mineral nitrogen fertilization or seeds inoculation with *Rhizobium tropici*. Dourados, MS, Brazil. 2009.

Treatments	Number of pods/plant	Number of grain/pod	Yield (kg ha^{-1} of grain)
Inoculated	12.64	3.03	2,327
Nitrogen fertilization	12.20	2.94	2,175
DMS	1.36 ns*	0.23 ns	269 ns
VC (%)	18	25	27

*ns= not significant by Tukey test at 5% probability. DMS=difference minimum significant. VC= variation coefficient.

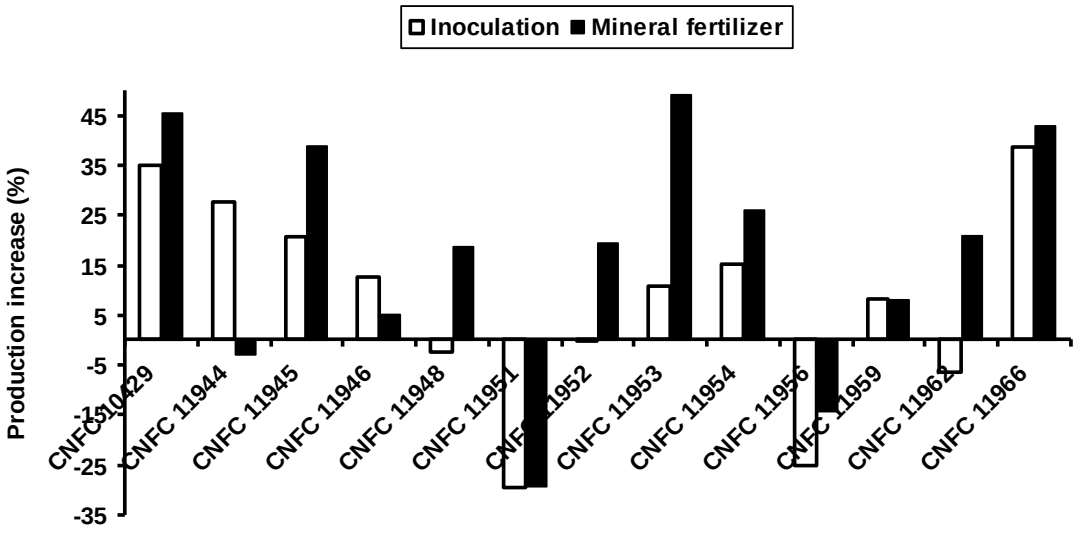

Figure 1. Mean values of the production of different lineages of common beans in relation to the average yield of four commercial cultivars (BRS Cometa, BRS Estilo, IPR Juriti and Pérola) used as control.

CONCLUSIONS

The bean lineages CNFC 11953, CNFC 10429 and CNFC 11966 showed potential answer to the mineral nitrogen fertilization.

The lineages CNFC 11966, CNFC 10429 and CNFC 11944 presented potential to answer to the biological nitrogen fixation.

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DETERMINATION OF GENE FLOW EVENTS IN NATURAL "WILD-WEEDY-CULTIVATED" COMPLEXES IN GENEPOOLS OF *PHASEOLUS LUNATUS* L.

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Gene flow events in natural populations of Lima beans have been reported along its broad range of distribution in the Americas (Mexico to Argentina) (Baudoin et al. 2004), as a result of its facultative reproduction system that allows varying levels of allogamy up to 48% (Maquet et al. 1996, and Zoro Bi et al. 2005). We were interested in testing evidence of gene flow events using weedy materials from six populations of Campeche (Mexico) (Debouck 1979) and ten populations from Cajamarca (Peru) (Debouck et al. 1987). The natural populations were chosen and analyzed using the same methodology as described by González-Torres et al. (2003, 2004) using morphoagronomic markers (Table). We evaluated the participation of nuclear genome through 18 microsatellite *loci* (Gaitan-Solís et al. 2002), and lectin patterns (Gutiérrez Salgado et al. 1995). In order to establish the direction of the gene transfer we studied 25 non-coding regions of chloroplast DNA using RFLPs (Fofana et al. 1999, Chacón 2001, and González-Torres et al. 2003). On the other hand, the concentration of HCN has been evaluated (Essers et al. 1993) as an antinutritional compound in order to assess some consequences of gene flow in these populations.

RESULTS AND DISCUSSION

The biochemical and molecular characteristics of the weedy materials indicated that they were indeed hybrids between cultivated and wild forms, and markers such as seed weight and color of seed testa help verify individual cases of gene transfer. Specific SSR alleles were found in each biological form among both genepools, and these were shared by weedy materials (Table). The direction of gene flow, using cpDNA haplotypes data, indicates that the movement of pollen occurs from wild populations towards cultivated forms, and in the other direction too. To confirm the direction we calculated the nuclear genome contribution of each biological form, using an admixture population analysis; the main direction in the Mesoamerican genepool was from wild pollen towards cultivated forms (1.3 times higher), in contrast with the Andean genepool, where the main direction was from cultivated to the wild form (3 times higher). Although the inheritance of HCN trait in *P. lunatus* is still unclear, yet suggesting a polyfactorial inheritance with dominance in the wild (Baudoin et al. 1991), this information could be used to infer gene flow events with other markers such as seed weight and lectins. The HCN concentration was found higher in wild types as compared to the cultivated forms, and at middle levels in weedy forms, in both genepools. We also found absence of relationship between color of seed testa and the cyanide content, confirming an early result (Baudoin et al. 1991). Additionally, we found evidence for the two major gene pools: different banding patterns such as M1 and A1/A4 in lectins, three defined SSR loci within each gene pool, and contrasted cpDNA haplotypes. The analysis of multiple correspondence using SSR data (Figure) shows similar trends about well defined forms of the complex "wild-weedy-cultivated" (a and c); however, Andean genepool exhibited a higher dynamics suggesting repeated gene flow events and backcrossings. The item (d) illustrates cases of outcrossing between Andean and Mesoamerican genepools surely due to seed migration by farmers and their selection work on the complex. These results provide additional evidence of simple or complex events of gene flow among the different biological forms of *P. lunatus*.

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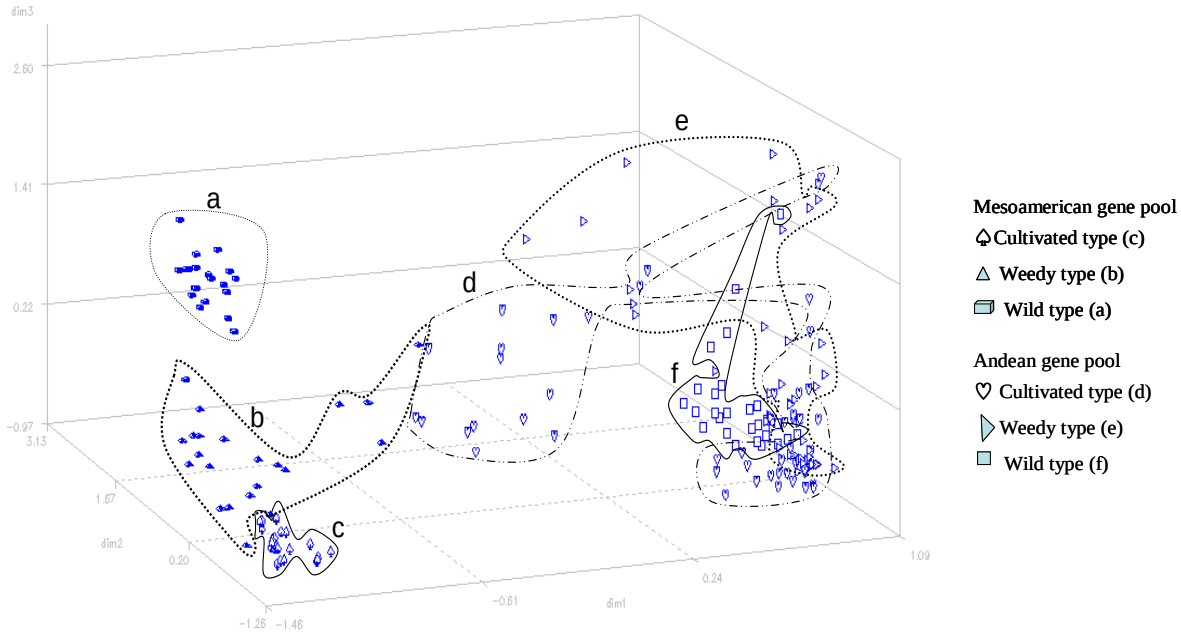
	Biological Status	Average seed weight (g) / color of seed testa	Lectin pattern	BM 140	BM 143	BM 170	BM 211	BM 156	BM 141	GATS 91	BM 183	BM 212	BM 146	AG1	BM 181	BM 202	HCN* (ppm)	H Cp
Andean Genepool	Wild N=60	13.6 (Wild type)	A2 A1	168 (21) 178 (18)	150 (14)	162 (28)	206 (48)	218 (54)	178 (58)	230 (42)	145 (50)	206 (48)					2978.5	H4
	Weedy N=116	47.4 (Wild type; colored)	A1 A4	165 168 178	147	160 162	194 206	224	178 (93)	240	152	206					870.3	H4
	Cultivated N=122	94.1 (colored)	A4 A6 A1	165 (84)	143 (23) 149 (27)	160 (24)	194 (61) 214 (24)	224 (31)	182 (25)	222 (29)	152 (22)	200 (32)					184.8	H4 H2
Mesoamerican Genepool	Wild N=30	13.83 (Wild type)	M1 M2 M4	164 (2) 168 (9)	150 (20)	172 (3) 180 (1)	210 (6) 220 (2)	218 (30)	182 (30)	222 (18)	150 (3)		272 (28)	151 (30)	174 (15) 178 (1) 182 (12) 184 (2)	152 156	2978.5	H4
	Weedy N=24	25.3 (Wild type; colored)	M1 M2 M8	164 168	150	172) 180	194 206	224	170 174 178	216	150 144		278 280 272	153	178	146 152 156	870.3	H4
	Cultivated N=45	43.4 (colored)	M4 M8 M1 M2	172 (36)	144 (44)	168 (42)	202 (8)	224 (37)	170 (4) 174 (20) 178 (9)	216 (2)	144 (45)		278 (38) 280 (3)	153 (45)	178 (34)	146 (37)	184.8	H4 H2



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GENETIC DIVERGENCE FOR PHYSICAL AND CHEMICAL CHARACTERS OF SEEDS IN LIMA BEAN (*PHASEOLUS LUNATUS* L.)

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INTRODUCTION

Lima bean (*Phaseolus lunatus* L.) seeds possess high nutritive value. Lima bean dried grain chemical composition (Bressani and Elias, 1980): carbohydrates 62.9%, protein 25.0%, 6.1% fiber, 3.9% ash and 2.0% ether extract. However, studies observed variation, Azevedo et al. (2003), carbohydrates ranged of 64.40 to 73.59%; crude protein ranged of 17.95 to 26.70%; fiber ranged of 2.27 to 4.59%; ash ranged of 3.06 to 4.10% and ether extract ranged of 0.88 to 1.42%. In breeding programs, crosses among genotypes of the same pattern should be avoided. The use of parents with the greatest possible divergence to maximize the heterosis shown in the hybrids, increase the probability of superior segregants in advanced generations and widen the genetic base. A multivariate analysis is powerful tool for parentals choice. This study aimed the physical and chemical characterization of the lima beans seed and evaluates the genetic divergence among samples.

MATERIAL AND METHODS

In this characterization, the genetic material consisted of 27 samples, from Lima Bean Germoplasm Active Bank from Universidade Federal do Piauí, cultivated in the 2006 year crop. The genetic divergence among the samples was estimated and the grouping by Tocher method, with the employment of the Mahalanobis distance, as measure of dissimilarity. The relative contribution of each trait for divergence was estimated.

RESULTS AND DISCUSSION

The seeds of these samples were evaluated regarding to chemical characters: moisture (8.26 to 11.25 g/100g), ashes (2.62 to 3.70 g/100g), ether extract (0.16 to 0.93 g/100g), crude protein (16.85 to 23.41 g/100g), non-nitrogen extract (62.24 to 70.07 g/100g), total dietary fiber (24.21 to 62.42 g/100g), insoluble fiber (5.65 to 11.88 g/100g), soluble fiber (15.82 to 53.11 g/100g) and cyanic acid (44.49 to 160.63 mg / kg). The physical characteristics that were evaluated in the seeds: length (9.66 to 18.52 mm), width (7.41 to 11.83 mm), thickness (5.33 to 6.90 mm) and weight of 100 seeds (27. 60 to 87.79 g), which permitted the classification as to form (spherical, elliptical and oblong / reniform), profile (flat and semi-flat) and size (small, medium, normal and great). Genetic distance among 27 samples ranged from 21.50 to 249.53. Ten groups were formed by Tocher method. Group I contained 6 genotypes; seven genotypes composed the group II; group III contained 4 genotypes;

groups IV, V and VI contained 2 genotypes, each; and groups VII, VIII, IX and X are composed of one genotype. The relative contribution of each trait (Table 1) indicated that the total dietary fiber (48.62%) and soluble fiber (46.03%) were those who most contributed the total divergence (94.65%) among the samples of lima beans evaluated.

TABLE 1. Relative contribution of each trait for genetic divergence among 27 lima bean genotypes, by method proposed by Singh (1981).

Character	Value (%)	Cummulative value (%)
Insoluble fiber	48.62	48.62
Soluble fiber	46.03	94.65
Total dietary fiber	2.96	97.61
Seed length	0.48	98.09
Ashes	0.45	98.54
Moisture	0.41	98.95
Crude protein	0.23	99.18
Ether extract	0.19	99.37
Non-nitrogen extract	0.17	99.54
Seed width	0.14	99.68
Cyanic acid	0.12	99.80
Weight of 100 seeds	0.12	99.92
Seed thickness	0.08	100.00

CONCLUSIONS

Crosses UFPI-491 x UFPI-282, UFPI-491 x UFPI-121, UFPI-491 x UFPI-220 and UFPI-491 x UFPI-229 are potentials to obtain segregating populations with high levels of protein and fiber and low content of hydrogen cyanide. The traits total dietary fiber and soluble fiber were those who most contributed the total divergence.

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GENETIC VARIABILITY AMONG LIMA BEAN (*PHASEOLUS* LUNATUS L.) LANDRACES COLLECT IN PIAUÍ STATE

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INTRODUCTION: The lima bean (*Phaseolus lunatus* L.) is one of the main legumes cultivated in tropical countries, performing better in the humid tropics and warm (RACHIE et al., 1980). According to Rodrigues et. al. (2002) there is a high genetic diversity in genus *Phaseolus*, and this can be used in breeding programs. The most important step in a breeding program is the choice of parents with good performance and wide genetic base. Genetic diversity is one of the criteria of parent selection in the hybridization program. Thus measures of the genetic divergence may help breeders to concentrate their efforts only on the most promising combinations. The availability of transgressive segregant in any breeding program depends upon the diversity between the parents involves. The divergence analysis has a definite role to play in an efficient choice of divergent parents for hybridization to exploit maximum heterosis. Some studies have permitted to establish genetic relationships among cultivars and landraces from different origins and also to link the diversity observed in different geographic regions and other elements of the physical environment. The aim of this work was to estimate the genetic divergence of 70 landraces of lima bean in Piauí State and correlation between genetic distance and geographical distance.

MATERIAL AND METHODS: The seeds of the 70 landraces of lima bean were collected in fields of production, trade and popular markets from counties Piaui State. The samples were grouping by counties of origin and characterized using descriptor list for lima bean published by International Plant Genetic Resources Institute (IPGRI, 2001). The dissimilarity was estimated through Average Euclidean distance and grouping was determined using UPGMA method. Correlation between genetic distance and geographical distance was estimated.

RESULTS AND DISCUSSION: Genetic distance among 70 samples ranged from 0.038 to 4.996 and average of 1.12. Geographical distances among groups ranged from 0.5 km to 516.3 km. Based on morphological traits evaluated, it is observed that there was a formation of seven distinct groups could be inferred that there is genetic divergence between the samples studied. The UPGMA grouping method from the Average Euclidean distance enabled the division of the 70 landraces into four groups (Figure 1). Group I contained 7 genotypes; group II contained 1 genotype; groups III contained 5 genotypes and IV contained 57 genotypes. The use of parents with the greatest possible divergence to maximize the heterosis shown in the hybrids, increase the probability of superior segregants in advanced generations and widen the genetic base. Therefore, the information in Figure 1 should be used for cross recommendation. Using the multivariate statistic as a base, a high degree of similarity could be expected to be found among the genotypes belonging to the same group. Thus crosses within the same group should be avoided. There was positive correlation of 0.4109 ($P<0.01$) between genetic distance and geographical distance showing limited exchange de lima bean among counties.

CONCLUSIONS: High variability among landraces occurred in lima bean from Piaui State, with tendencies for regionalization. The exchange of lima bean is limited among counties.

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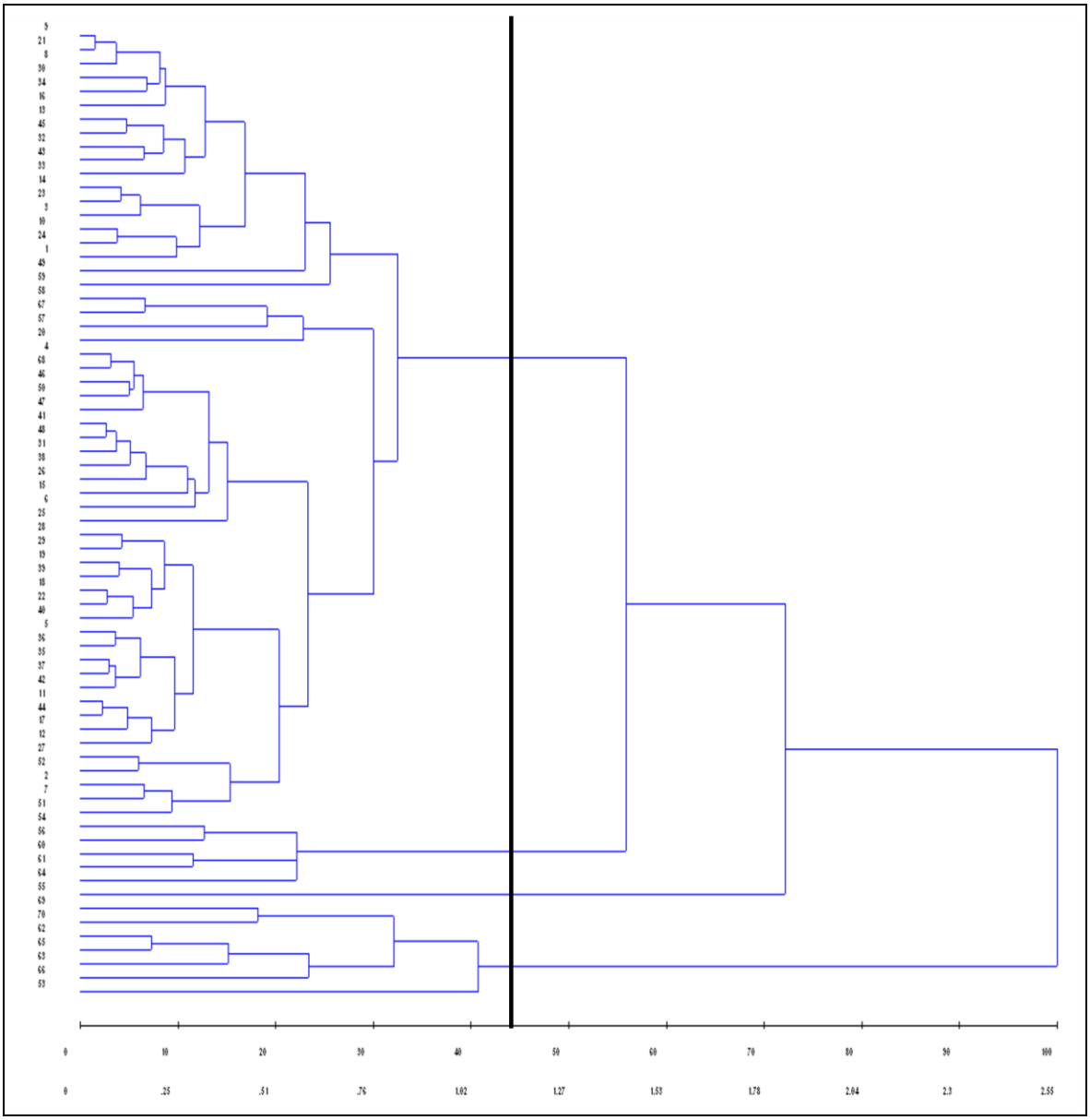

FIGURE 1. Dendrogram showing relationships among lima bean landraces, using UPGMA grouping method.

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GENETIC DIVERSITY ANALYSIS OF *PHASEOLUS COCCINEUS* L. FROM HUASTECO KARST OF MEXICO

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The 'ayocote' bean (*Phaseolus coccineus* L.) is a legume species originated from México and it is one of the domesticated and cultivated *Phaseolus* species (3). The species supplies proteins and minerals to daily diet of Mexican rural people, mainly (2). Currently, surface cultivated with beans has been reduced in Mexico and bean production shows reductions near 45 % during later 10 years due the most of beans grown under rainfed conditions with intermittent drought stress periods, the high incidence and severity of diseases, poor inputs used to bean production, and changes on diet by Mexicans.

Ayocote germplasm (117 accessions) from Huasteco Karst (northern Puebla, Mexico) plus five controls (Pinto Villa, Pinto Zapata, *P. glabellus*, *P. coccineus* var. *coccineus* and *P. coccineus* cv. Blanco Tlaxcala) were sown at Chapingo, México during 2008. Young leaves from each accession were collected during flowering and transported to Reynosa, Mexico for DNA isolation (1) and AFLP analysis based on Vos *et al*. (4). AFLP products were electrophoresed using a Li-Cor IR2 sequencing system (Li-Cor©, Lincoln, NE), bands visually scored and zero-ones matrices subjected to AMOVA, cluster analysis and diversity index (DI) calculations.

The four AFLP primer combinations produced 256 bands, 224 polymorphic (87.4 %) Cluster analysis based on geographical origin showed two clusters: one (A) included ayocote beans from eleven locations and the other (B) accessions from Ahuacatlán and Xochiapulco. Accessions of *P. vulgaris* (Pinto Villa and Pinto Zapata) as well as *P. glabellus* were genetically distinct to all *P. coccineus* from Huasteco karst (Fig. 1). Genetic variability was significant and accessions were separated based genotype and geographical origins. Germplasm from Market of Zacapoaxtla (56.8 %) and Ahuacatlán (50.5 %) showed the highest values of DI while accessions from Chignahuapan showed the lowest values (39.6%) (Table 1). High genetic differentiation among ayocote accessions indicates incipient reproductive isolation despite it is well known the constant seed exchange among farmers from the same or different locations. This is the first work where Mexican ayocote germplasm is subjected to molecular analysis, despite accessions from a limited geographical origin is used. Further works must take into account a broad collection of *P. coccineus* germplasm from all other agro-ecological regions where ayocote beans are growing and cultivated.

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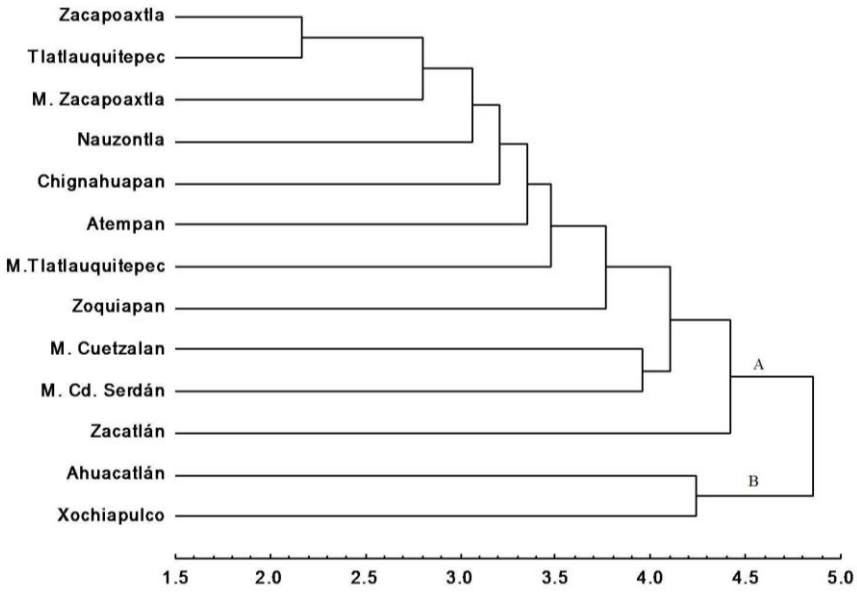

Figure 1. Dendrogram of ayocote germplasm from Huasteco karst of Puebla, Mexico based on AFLP markers.

Table 1. Genetic Diversity Indexes of ayocote beans from Huasteco karst analyzed with AFLP markers.

Location	EcoRI/MseI AFLP primer combinations				Mean
	AAG/AGG	AAG/ACC	AAG/ATG	AAG/AGT	
Zacapoaxtla	0.33	0.52	0.57	0.55	0.49
Zacatlán	0.24	0.52	0.47	0.44	0.42
Tlatlauquitepec	0.36	0.49	0.58	0.53	0.49
Nauzontla	0.34	0.48	0.59	0.52	0.48
Zoquiapan	0.36	0.46	0.46	0.62	0.47
Chignahuapan	0.24	0.48	0.43	0.44	0.40
Ahuacatlán	0.37	0.49	0.52	0.64	0.51
Xochiapulco	0.31	0.46	0.51	0.72	0.50
M. Zacapoaxtla	0.43	0.54	0.60	0.67	0.56
M. Cuetzalán	0.28	0.39	0.60	0.60	0.47
M. Tlatlauquitepec	0.35	0.50	0.53	0.59	0.49
M. Cd. Serdán	0.34	0.40	0.65	0.45	0.46
Atempan	0.29	0.47	0.53	0.53	0.46
Mean	**0.33**	**0.48**	**0.54**	**0.56**	**0.47**

EXTENSIVE SSR DIVERSITY IN A WORLD-WIDE COLLECTION OF POLE SNAP BEANS

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INTRODUCTION

Common bean can be grown as a grain crop (dry beans) or as a fresh vegetable (snap beans / green beans), both items being important in nutritional terms for providing essential minerals and vitamins to the diet. Snap beans are thought to be derived predominantly from dry beans of the Andean genepool and to be of a recent European origin; however the existence of Mesoamerican genepool characteristics especially in traditional indeterminate growth habit snap beans indicates a wider origin. Total world production of snap beans is around 9 million tons, with China, Turkey, India, Spain, France and the United States being among the biggest producers and consumers (FAOSTAT 2007). Marked preference and intense commercialization of snap beans occurs in developed countries of North America and Europe with many seed and food processing companies intensively involved in the product chain. In addition, the crop is of growing importance to developing countries, both as an export crop and as a local product. In terms of export, trade between Central America and the United States or East Africa and Europe produce important income streams for countries like Guatemala and Kenya. Meanwhile, as wages have gone up in countries such as Colombia or India, the markets for snap beans have also increased. The objective of this study was to evaluate genetic diversity within a set of indeterminate (pole type) snap beans using SSR (or microsatellite) markers. The genotypes were predominantly from Asia, Europe and the United States but included some varieties from Latin America and Africa.

MATERIALS AND METHODS

Plant material: A total of 127 genotypes were analyzed for this study of which 120 were snap beans, 5 were standard dry beans controls for each genepool that our laboratory regularly uses in diversity studies (Calima, Miss Kelly, G19833 for the Andean genepool, ICA Pijao and DOR364 for the Mesoamerican genepool) and 2 were wild accessions from Colombia and Guatemala (G21117 and G23441, respectively). Genotypes were from throughout the world with 3 from Africa, 45 from the Americas, 39 from Asia and 40 from Europe and most were either type III or IV growth habit. Heirloom snap bean varieties included 'Blue Lake', Kentucky Wonder', 'Genuine Cornfield', Golden Gate Wax', 'Romano Pole', 'Romano Bush' and 'Tendergreen'. All seeds except for those of UNAPAL Milenio were provided by the Genetic Resources Unit. DNA was extracted from fresh tissue with a CTAB extraction buffer and DNA quality was evaluated on a 1% agarose gel using ethidium bromide staining.

Microsatellite analysis: A total of 47 microsatellites were used for the study, 36 of these with fluorescently labeled primers and automated detection as described in Blair et al. (2009) while 11 of these were non-fluorescent microsatellites detected with silver staining. PCR amplifications were carried out as previously described After amplification, fluorescent microsatellites were mixed together in four color dye panels using 2 µL of each product and 10 µL of HPLC quality water, from which 0.5 µL was diluted 1:6 with water and submitted to an ABI3730 automatic fragment analyzer. Meanwhile, non-fluorescent markers were evaluated on silver-stained polyacrylamide gels as described in Blair et al. (2006).

Data Analysis: Fluorescent microsatellite alleles were called automatically and confirmed by manual observation of electropherogram peaks while non-fluorescent bands were called by eye. Allele size estimates were aided by an internal size standard in the case of the fluorescent microsatellites and by a 10 bp molecular weight ladder in the case of the non-fluorescent microsatellites. A matrix of allele sizes for all the successful

genotype x marker combinations was converted to a presence/absence and used in a principal coordinate analysis to derive a Euclidean distance matrix and to create a UPGMA dendogram.

RESULTS AND DISCUSSION

SSR polymorphism was very high averaging 95.3% for the 32 fluorescent and 11 non-fluorescent markers evaluated and total expected heterozygosity was higher for SSR markers (0.521) than for AFLP markers (0.209). SSRs efficiently grouped the genotypes into two genepools with Andean and Mesoamerican controls, respectively with the Mesoamerican group being predominant in terms of the number of genotypes assigned to this genepool.

The dendogram based on SSR marker analysis had 28 genotypes in the Andean group and 99 genotypes in the Mesoamerican group (Figure 1). The number of observed alleles and the number of expected alleles was higher for the Mesoamerican genepool genotypes than for the Andean genepool genotypes. For the SSR-based dendogram the two genepools separated at a Euclidean genetic distance of 0.5 to 0.75. Genetic distance within the Andean genepool ranged up to 0.35 compared to 0.50 within the Mesoamerican genepool. Genetic differentiation (Gst) between the genepools based on the SSR analysis was found to be 0.173 while geneflow (Nm) was found to be 1.192.

Phaseolin alleles were not tightly associated with genepool assignment indicating that introgression of this locus had occurred between the genepools, especially with phaseolin "S" in the Andean group (23.5%) and phaseolins "T" and "C" in the Mesoamerican group (12.2 and 8.2%, respectively). Growth habit was not very distinct between the genepools since mostly indeterminate climbing and semi-climbing beans were selected for this study (Table 3), but there was some tendency of type III beans to be clustered in the Andean group and type IV beans to be clustered in the Mesoamerican group.

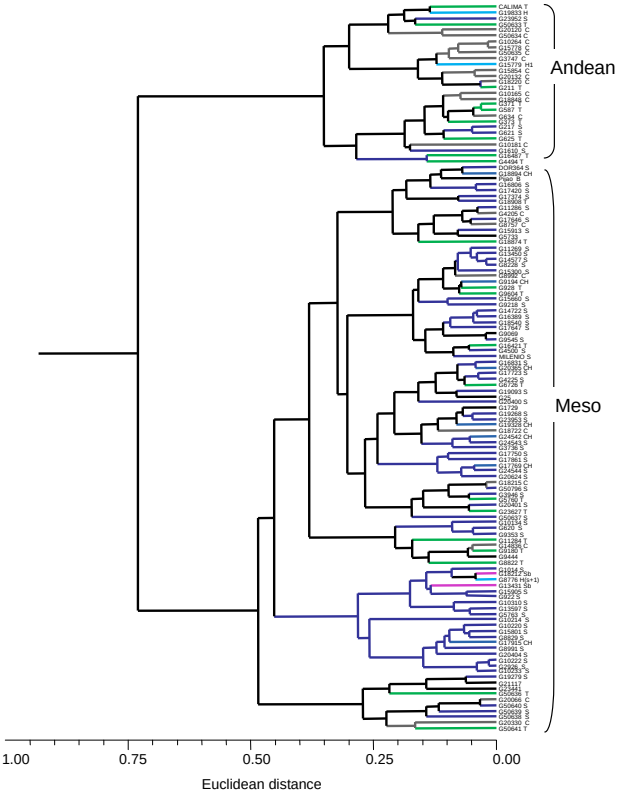

Figure 1. Dendogram of pole-type snap beans from the CIAT collection.

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GENETIC DIVERSITY AMONG SNAP BEAN ACCESSIONS BY USING MICROSATELLITES MOLECULAR MARKERS

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INTRODUCTION

For many years different plant breeding methods and genotypic identification have been implemented based on assessment of morphological and physiological characters. Nowadays, the use of molecular markers, especially the microsatellites, has demonstrated greater accuracy. They permit, among other aspects, the detection of more expressive genetic differences among the closest genotypes when compared with the use of morphological agronomic descriptors (Collard et al., 2005). The present work aimed to evaluate genetic diversity among snap bean accessions using microsatellite molecular markers.

MATERIAL AND METHODS

The genetic diversity between 32 snap bean accessions of the Gene Bank from Nupagri (Núcleo de Pesquisa Aplicada a Agricultura/Universidade Estadual de Maringá), was evaluated using microsatellite markers. The seeds of each accession were sown in pots with substrate and maintained in a greenhouse. At V_3 stage, a young leaflet of one plant of each accession was taken from the first trifoliolate leaves, placed in an Eppendorf tube and immediately deep-frozen for follow-up DNA extraction according to Afanador et al. (1991). The DNA fragments were amplified in a Perkin Elmer DNA Thermocycler, following the protocol by Williams et al. (1990). The unweighted pair-group method based on arithmetic averages (UPGMA) was used as clustering technique.

Figure 1 – Snap bean cultivars analyzed with microsatellite molecular markers (1, UEM/FVI-1; 2, UEM/FVI-2; 3, UEM/FVI-3; 4, UEM/FVI-4; 5, UEM/FVI-5; 6, UEM/FVI-6; 7, UEM/FVI-7; 8, UEM/FVI-8; 9, UEM/FVI-18; 10, UEM/FVI-29; 11, UEM/FVI-30; 12, UEM/FVI-32; 13, UEM/FVI-33; 14, UEM/FVI-35; 15, UEM/FVI-36; 16, UEM/FVI-37; 17, UEM/FVI-38; 18, UEM/FVI-39; 19, UEM/FVI-42; 20, UEM/FVI-43; 21, UEM/FVI-44; 22, UEM/FVI-45; 23, UEM/FVI-46; 24, UEM/FVI-47; 25, UEM/FVI-48; 26, UEM/FVI-49; 27, UEM/FVI-50; 28, UEM/FVI-51; 29, UEM/FVI-52; 30, UEM/FVI-53; 31, UEM/FVI-54 e 32, UEM/FVI-55.

RESULTS AND DISCUSSION

Twenty groups were formed by using the UPGMA method, and groups I, IV, VI, IX, X, XIV consisted of two subgroups (Figure 2). The accessions evaluated showed a genetic divergence index of 2 to 87%. The most similar accessions were UEM-FVI-36 and UEM-FVI-37, and the most dissimilar accessions were UEM-FVI-42 and UEM-FVI-49. Thus, UEM-FVI-42 and UEM-FVI-49 showed a high genetic viability potential for breeding program.

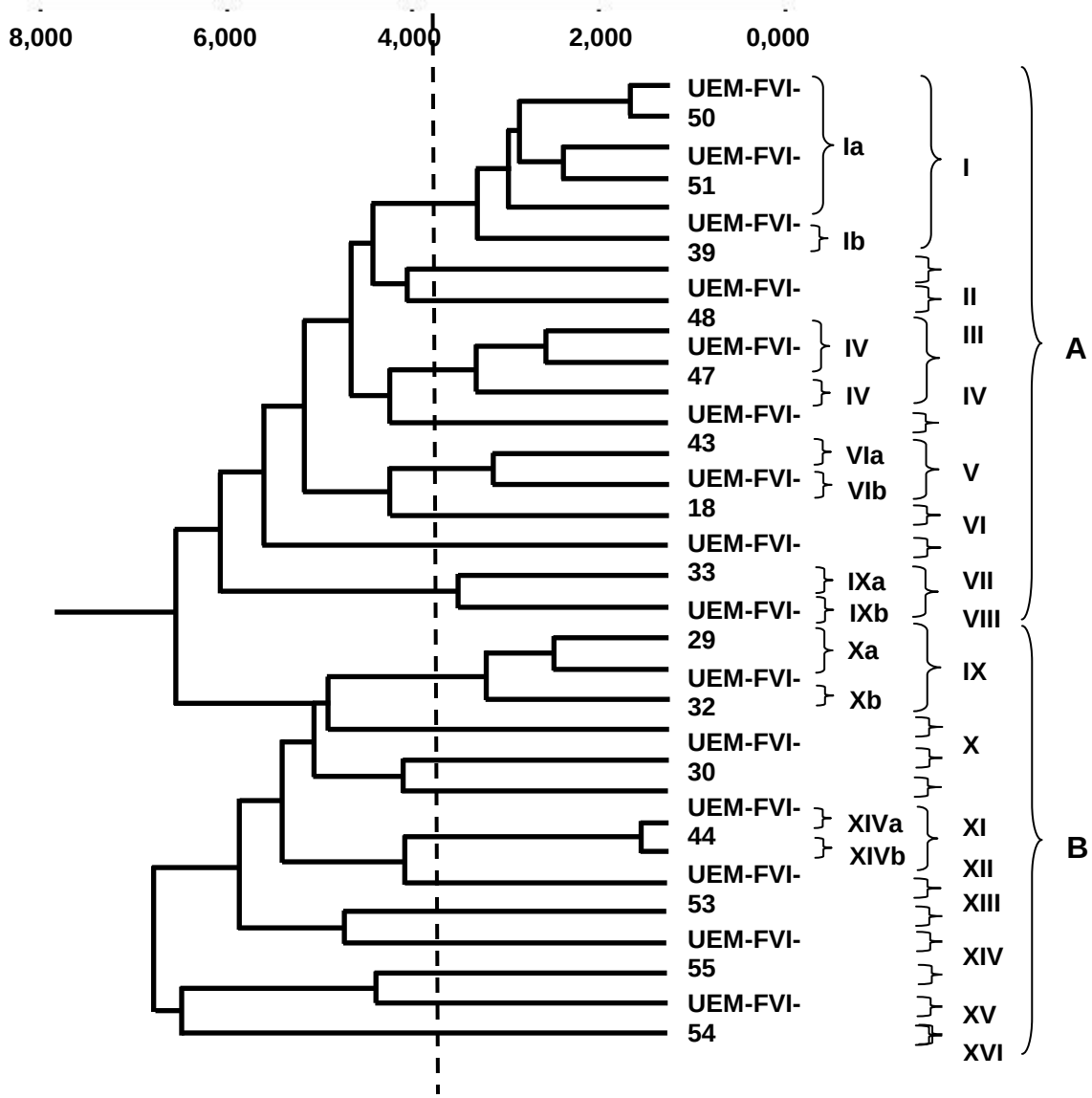

Figure 2 – Dendrogram of genetic divergence among the 32 snap bean accessions of snap bean, based on Nei's Minimum Distance (D_M), established through UPGMA method.

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GENETIC DIVERSITY IN COMMON BEAN GERMPLASM FROM BRAZIL USING MICROSATELLITE MAKERS

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INTRODUCTION

Common bean programs are based on genetic diversity to obtain and to select new cultivars with high yield potential. Thus, genetic variability is essential for development of better cultivars (Acosta-Gallegos et al., 2007), being also necessary to characterize it use in order to improve its use and/ or conservation. Nowadays, molecular markers, specifically microsatellites, have become an important tool for this type of analysis. Therefore, the present work had the objective to characterize genetic divergence presence among 40 common bean from NUPAGRI germplasm bank, Universidade Estadual de Maringá, Paraná State, Brazil, using microsatellite molecular markers.

MATERIAL AND METHODS

The seeds of each cultivar were sown in plastic containers, containing substrate. They were kept at the greenhouse until the emergence of the first trifoliate leaf (V3 stage). After this period, a young foliate of each plant was individually collected and stored in the freezer for later DNA extraction, according to methodology proposed by Afanador et al. (1993). Extracted DNA was utilized as strand for amplification reactions, which used 20 pairs of microsatellite primers designed by Blair et al. (2003) and Grisi et al. (2007). Genetic diversity among genotypes was carried out according to d^2 index of Smouse and Peakall (1999).

RESULTS AND DISCUSSION

From the 20 microsatellite analyzed, two of them were monomorphic (BMd-21 and BMd-31), thus they were not included in the statistical analysis. Based on the obtained results through analysis of 18 microsatellite *loci*, it was possible to identify five most similar combinations and five most dissimilar ones (Table 1). The two most genetic similarities occurred in commercial group Carioca (BGF 6 x BGF 16 and BGF 25 x BGF 26). On the other hand, the higher genetic dissimilarity was observed in genotypes BGF 27 x BGF 40 (d^2 = 3.39). It is important to point out that among the five most dissimilar genotypes, BGF 27 composed three combinations, and consequently, it was considered the most dissimilar one. Therefore, it is recommended combination in which this genotype can be used as parent, since the probability to obtain hybrids that provide higher segregation in recombination is higher.

Table 1 – Summary of genetic dissimilarity matrix obtained by index d^2 of Smouse and Peakall (1999), through analysis of 18 microsatellite *loci* in 40 common bean genotypes.

Combinations	Commercial Group	d^2
Between most similar accessions		
BGF 6 x BGF 16	Manteigão x Manteigão	0.22
BGF 25 x BGF 26	Carioca x Carioca	0.28
BGF 2 x BGF 3	Preto x Preto	0.44
BGF 3 x BGF 6	Preto x Manteigão	0.44
BGF 6 x BGF 11	Manteigão x Manteigão	0.44
Between most dissimilar accessions		
BGF 27 x BGF 40	Carioca x Manteigão	3.39
BGF 27 x BGF 41	Carioca x Manteigão	3.28
BGF 14 x BGF 34	Diversos x Preto	3.28
BGF 2 x BGF 27	Preto x Carioca	3.22
BGF 2 x BGF 30	Preto x Carioca	3.17

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GENETIC DIVERSITY IN COMMON BEAN CULTIVARS COLLECTED IN CHIHUAHUA, MÉXICO

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INTRODUCTION

Characterization deficiencies are the major challenge for systematic use of common bean (*Phaseolus vulgaris* L.) diversity in genetic breeding programs. In Chihuahua State—northern México—genetic diversity of common bean has been observed which need to be characterized in order to establish its importance in crop breeding. Classical methods to characterize genetic diversity in plants include the use of morpho-agronomic traits to establish genetic relations among commercial cultivars, landraces and wild relatives (Newbury and Ford-Lloyd, 1997). In Chihuahua significant advances have been observed in total area planted with Pinto Saltillo bred cultivar which may result in loss of genetic diversity in common beans. Some commercial classes in which genetic diversity has been lost are ojo de cabra (brown striped), canelo (clear brown) and bayo (cream). The aim was to assess genetic diversity present in a group of 61 landraces and cultivars collected in Chihuahua, México.

MATERIALS AND METHODS

Germplasm collections were made during 2008 in order to evaluate the degree of remaining genetic diversity in common bean cultivating areas from the Chihuahua State. In 2009 a nursery was planted with 61 landraces and cultivars collected during 2008. Cultivars were planted in a 5 m row with 0.80 m spacing in July 9th 2009 at Bachiniva, Chihuahua. Sowing was made in a Xerosol-luvic soil type with low moisture retention capability, reduced depth (20-30 cm), slope of 0 to 2 % and pH of 6.5. The climate is a semi-arid type with summer rainfall season [BS_1 Kw (w) (e)] (García, 1987). Cultivar characterization was made considering field and grain traits included in the *Phaseolus vulgaris* guidelines for the conduct of tests for distinctness, uniformity and stability (SNICS, 2001). Data obtained was used to perform Principal Component and cluster (grouping) analysis using statistical program Systat®.

RESULTS AND DISCUSSION

15 commercial classes were detected among seed collections and pinto (21) and ojo de cabra (goat eye-brown striped) (14) showed higher number of cultivars. Three main groups were detected (Figure 1), **Group I** included pinto cultivar with different color in background and strips of seed coat. Some cultivars could be considered as recombinants since showed similar traits to those observed in black seeded cultivars such as purple stems and flowers. Other recombinant cultivars (with intermediate traits among races) belonging to several commercial classes, such as pink flower, marbled and mottled (known as vaquita), were also found in this group. **Group II** included typical pinto cultivar brown-mottled and clear background. Ojo de cabra (goat eye) with brown-grayish background and brown striped seeds were also included in this group. **Group III** included several seed classes such as clear brown (canelo), red, purple, cream, yellow and pink. Some of cultivars included in this group could be also considered as a recombinant due to presence of traits similar among races and commercial classes. Outliers were also found in Figure 1, such as black seeded, sulfur (azufrado) and cranberry (cacahuate) cultivars, which were introduced to Chihuahua, since black cultivars with long branches mainly belongs to Jalisco race while sulfur and cranberry cultivars belongs to Nueva Granada race (Singh, 1991). Natural and man made genetic recombination between varieties mainly

belonging to races Durango and Jalisco favored formation of a gene complex which promoted formation of recombinant cultivars with intermediate traits among genetic races.

Productive specialization due to market demand for pinto seed class and slower darkening of seed coat observed in Pinto Saltillo caused significant reduction in area planted with other common bean commercial classes in Chihuahua (Ávila *et al*., 2009). Farmers living in sites located in the mountains, sowing different cultivars from several seed classes locally adapted, contributed to preserve common bean genetic diversity. In contrast in plain soils where market pressure is observed bred cultivars are considered as a better option. Area planted with pinto seeded bred cultivars (Pinto Villa, Pinto Mestizo, Pinto Saltillo, Bill Z and Montrose) has showed significant increments since an extinct government marketing company known as CONASUPO (Comisión Nacional de Subsistencias Populares) promoted production changes due to reduced consumer acceptation for brown striped seeded cultivars (ojo de cabra).

CONCLUSIONS

Reduction was observed in the genetic diversity of common beans planted in some areas of Chihuahua, caused by pressure of the common bean market. Small farmers growing traditional landraces in the mountains of Chihuahua contributed to conserve common bean genetic diversity.

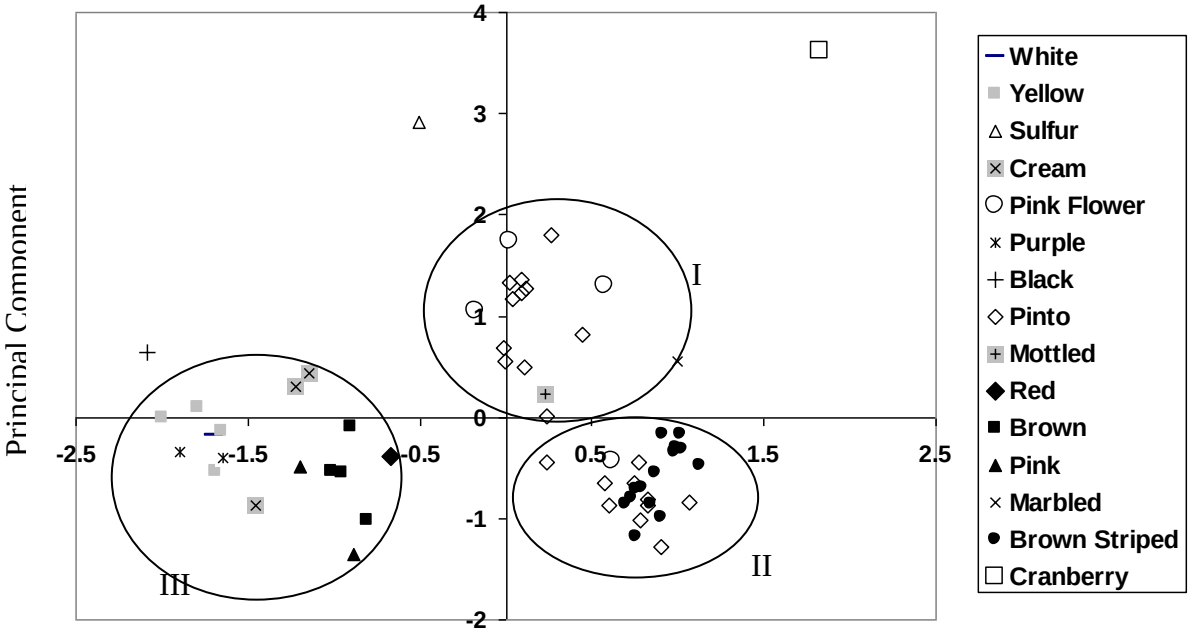

Figure 1. Main groups observed in the Principal Component Analysis obtained by using 45 morpho-agronomic traits evaluated in 61 common bean cultivars collected in Chihuahua, México.

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GENETIC DIVERSITY ANALYSIS OF ONE COLLECTION OF WILD X CULTIVATED BEAN ACCESSIONS FROM MEXICO

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Mexico is center of origin, domestication and diversity of *P. vulgaris* L. (3). Due beans have major economic, social, biological, food, and cultural importance some strategies for conservation and management of genetic diversity within cultivated and wild types have been designed. The characterization and evaluation of *Phaseolus* germplasm could help to improve the knowledge about genetic variability, diversity and differentiation patterns as well as the determination of genetic potential of genetic resources for breeding (2). The aim of this work was to analyze the genetic diversity and relations among accessions produced from wild x cultivated crosses of beans throughout Mexico.

The collection includes 175 accessions produced by the randomly crosses between cultivated x wild genotypes of *P. vulgaris* throughout Mexico. As out-groups, we included two *P. coccineus* (Blanco Tlaxacala, 'Variedad Tipo') accessions and two common bean bred cultivars (Pinto Villa, Pinto Zapata). Genomic DNA was isolated following the protocol of Saghai-Maroof *et al.* (9) and germplasm was subjected to AFLP analysis using four +3/+3 primer combinations (10). AFLP data were used to calculate diversity index (DI) (4) and to construct a dendrogram based on similarity coefficients (5). Dendrogram robustness was assessed by bootstrap analysis. In addition, we determined the genetic structure of bean populations using STRUCTURE 2.3.2 with K= 4 (7). Genetic relations were confirmed by principal coordinate analysis (PCoA).

AFLP analysis showed polymorphism up 95 % as been reported previously in wild Mesoamerican beans. DI were higher (0.22) compared to wild Andean beans (0.10) but similar to those reported in Mesoamerican germplasm (6, 8). Cluster analysis separated germplasm in four groups based on origins; the analysis was highly robust. Bayesian (data not shown) and PCoA analyses showed similar grouping (Fig. 1) despite no relation between groups and origins was found. We suggest that minimum genetic differences are present among wild *Phaseolus* parents due the number of loci was not enough to disscriminate accessions based on origin. Parameters used for statistical methods are based on genetic distances that take into account presence/absence of each locus (PCoA) while Bayesian analysis uses the frequency of alleles for each locus (1). Clusters produced by Bayesian analysis (data not shown) indicate the genetic admixtures within Mesoamerican gene pool due free and random crossing among wild, semi-wild and domesticated forms of *P. vulgaris*, since we found variable degrees of coancestry among them. Coancestry is produced and increased by genetic exchange due natural or artificial migration of *Phaseolus* germplasm throughout Mexico.

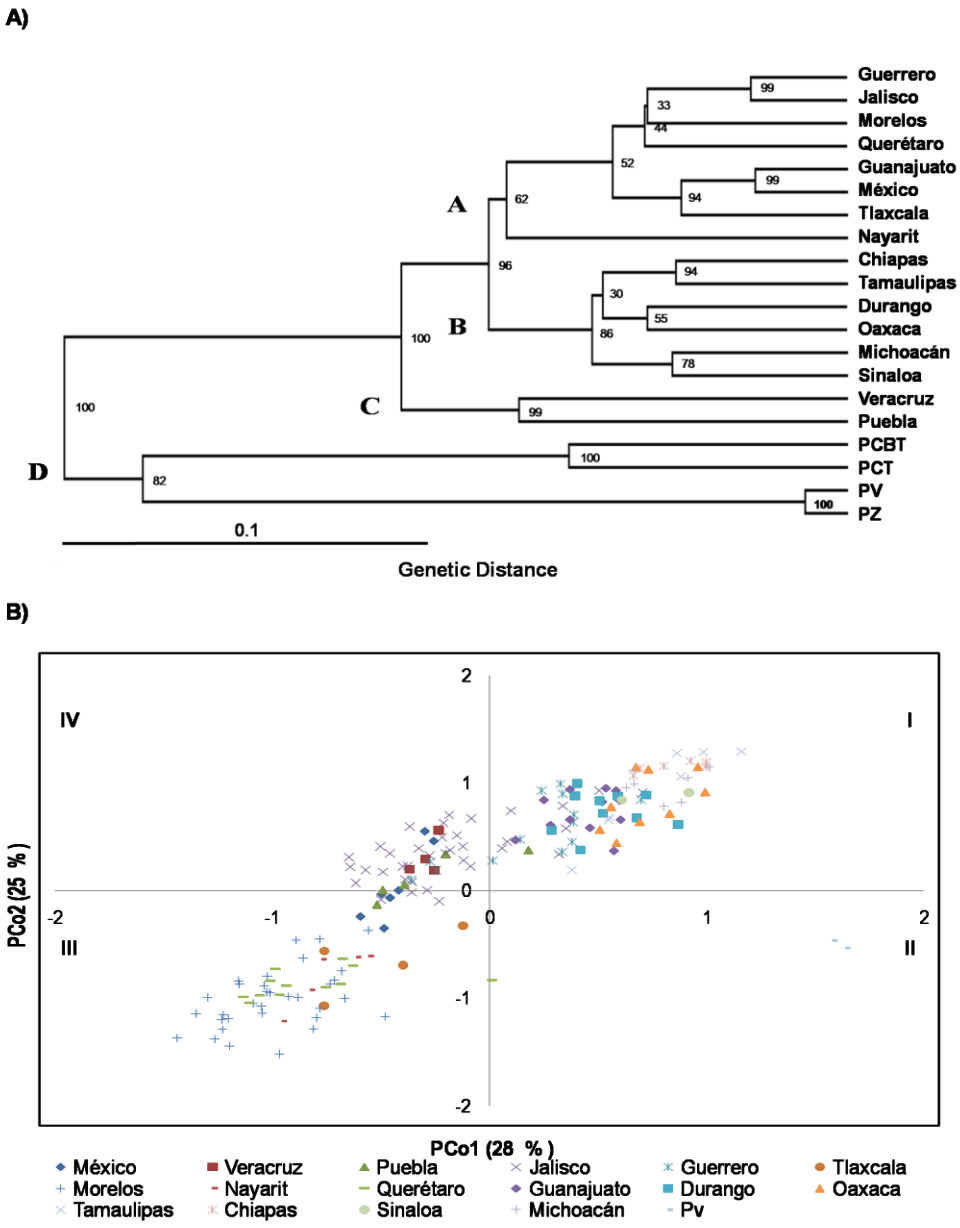

Figure 1. A) Dendrogram based on Nei and Dice distances and bootstrap values (1000 permutations) (PCTB= *P. coccineus* L. Blanco Tlaxcala, PCT= *P. coccineus* L. Tipo, PV= *P. vulgaris* L Pinto Villa, *P. vulgaris* L. Pinto Zapata). Numbers in each node indicate bootstrap values. **B)** PCoA based on origins and AFLP data.

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PHASEOLIN DIVERSITY IN COLOMBIAN COMMON BEAN GERMPLASM

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Colombia is located at one of the major crossroads of cultural and biological exchange in the Americas, and displays two interesting features for the common bean. First, it is a zone of contact between the two major gene pools (Chacón et al. 2007), and second, Colombia possesses a unique genetic diversity (Tohme et al. 1996; Chacón et al. 1999). Phaseolin was one of the markers used to reveal this unique diversity, as shown by Gepts and Bliss (1986), Beebe et al. (1997), who found the 'CH', 'B' and 'L' patterns, previously undescribed in common bean. More recently, Toro et al. (2007) described different phaseolin types, eight of which are unique for Colombian beans. Here we report a complete screening of phaseolin variability of the Colombian common bean collection held at CIAT, so to help us better understand its diversity.

MATERIALS AND METHODS. The 1,555 accessions reported here were obtained from the *Phaseolus vulgaris* L. world collection held in the Genetic Resources Program of CIAT (CIAT-PRG, 2010). A highly representative sampling was conducted to cover the full range of biological status of Colombian common bean collection (Table 1), where the analyzed sample was 55% of the total collection (especially for the wild form where the sample was 90%). Therefore the findings will be highly reliable for *Phaseolus vulgaris* L. from Colombia. The seed proteins that we analyze are globulins (phaseolins for common bean), which have narrow range of molecular weights (45-52 kD) and isoelectric points (5.6-5.8) (Brown et al. 1981). The samples were analyzed in ID-SDS-PAGE (Brown et al. 1981) and confirmed later in 2D-IEF-SDS-PAGE (O'Farrel, 1975).

RESULTS AND DISCUSSION. For the wild and Colombian weedy, the higher frequency of Mesoamerican phaseolin types (84% and 61 %, respectively) compared to the higher occurrence of Andean ones in the Colombian cultivated (54 %), may have resulted from a migration and subsequent selection of large seeded cultivars for Colombia, as proposed by Chacón et al. (2005), which excludes Colombia as a domestication scenario of these in South America. So far, several phaseolins have been found only in this country, such as L, CAR, Mu, Qui, LI, HE, TI1, and TI2, some of which are present in wild and weedy materials (L, Mu, and CAR). The high occurrence of the B and CH types in all phases of the biological state agrees with the hypothesis that Colombia is the origin center for these two patterns (Gepts and Bliss, 1986; Beebe et al. 1997). These facts reinforce the theory that this country is a genetic center of diversity of common bean (Tohme et al. 1996; Chacón et al. 1999). In Colombia, phaseolin distribution in all phases of the biological states, suggests an important gene flow between Mesoamerican and Andean materials. (Table 1). The different phaseolin types are unequally distributed over the bean-growing regions of Colombia. In the North Atlantic Coast the types (S and B) appear in the cultivated, and in the Eastern Andes, the Mesoamerican phaseolins dominate, especially in the wild form. Also in this region the Mu, Qui and L types appear. By contrast in the Western Andes, the Andean phaseolins dominate in all phases of biological state of beans of this region. The Car, LI, TI1, TI2, and HE patterns, are exclusively of the Western Andes (Table 2). In general, the high frequencies of the S, B, T, C and H1 types in Colombian cultivated bean, the presence of heterogeneous accessions with Mesoamerican and Andean phaseolins (for some accessions, we analyze more than one seed, see Table 1), as well as by the geographical gradient of this marker in this country, provide evidence of a zone of overlap of the two largest American gene pools in the Colombian geography. The very low frequency of native

Colombian phaseolins in all phases of the biological status, especially for the wild form where the frequency was 6%, reinforces the idea of making Colombia a priority for new germplasm collection activities.

Table 1. A comparison of phaseolin variability in all biological states of the Colombian common bean collection held in CIAT.

Number of Accessions (No. of seeds)[1]	**Biological Status/ Phaseolin type (Frequency)**		
	Wild	**Weedy[2]**	**Cultivated**
2834 total accessions	226 Accessions	185 Accessions	2423 Accessions
1555 Analyzed accessions (2207 seeds)	204 Analyzed accessions (295 seeds)	137 Analyzed accessions (248 seeds)	1214 Analyzed accessions (1664 seeds)
	B(103),C(16),Ca(1), CH(121),H(3),H1(2), H2(2),L(14),M1(1), M11(11),M13(1), M16(2),Mu(2),S(10), T(6)	B(60),C(38),Ca(2),Car(4), CH(42),H(4),H1(6), H2(2),L(12),M11(12), M15(1),M6(3),Mu(6), S(34),T(19),Tel(2),To1(1)	B(214),LI(2),C(202),Ca(9), Ca1(21),Car(112),CH(80), H(51),H1(118),HE(2),L(34), LI(3),M11(3),M6(1),Mu(3), P1(1),Qui(1),S(301),Sb(5), Sd(5),T(440),Tel(3),TI1(1), TI2(1),To1(27), H2(24)
Frequency of Phaseolin types	M: 249 (84 %) A: 30 (10 %) C: 16 (6%) Total: 295 (100%)	M: 152 (61 %) A: 74 (30 %) C: 22 (9 %) Total: 248 (100%)	M: 612 (36 %) A: 893 (54 %) C: 159 (10 %) Total: 1664 (100 %)

M: Middle America; A: Andes; C: Colombia

[1] For some accessions, we analyze more than one seed..

[2] The weedy materials are not typical cultivated or wild beans, they are intermediate genetically as result of gene flow events among cultivated and wild types (González et al. 2004).

Table 2. Geographical distribution of different phaseolin types in the bean-growing regions of Colombia.

Regions (Departments)	**Biological Status/ Phaseolin types**		
	Wild	**Weedy**	**Cultivated**
North Atlantic Coast (Bolivar, Atlántico, Magdalena, Guajira)			S, B
Eastern Andes (Cundinamarca, Boyacá, Santanderes)	B,CH,S,Mu,L,M1, M11,M13, M16	CH,B,S,L,To1,Mu, M6,M11,M15	S,Sb,Sd,M11,M6,B,CH,L,Qui, To1T,P1,C,H,H1,H2,P1,Tel,Mu
Western Andes (Antioquia, Caldas, Risaralda, Huila, Tolima, Valle, Cauca, Nariño)	C,Ca,T,H,H,1,H2,L	T,C,H,H1,H2,Ca,S,B, CH,Mu,L,Car,Tel	T,C,H,H1,H2,Ca,Ca1,S,,CH, Car,LI,TI1,TI2,HE

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A COMPARISON OF ROOT AND HYPOCOTYL XYLEM TRACHEARY STRUCTURES IN COMMON BEAN

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INTRODUCTION

The root of the bean plant (*Phaseolus vulgaris* L.) is tetrarch; the primary growth of the xylem vessels is composed of protoxylem (small vessels) and metaxylem (large vessels). Passioura (1982) stated that "the longitudinal resistance to flow in the main roots may influence the rate at which water in the subsoil can be transported by the roots through a dry topsoil to the shoot and that this character is related to the size of the main xylem vessel in the seminal axes of the wheat plants". Therefore, it can be accepted that a decrease of the xylem vessel diameter might reduce the water flow rate and contribute to save water and improve drought resistance. The objective of the present study was to determine if drought-tolerant cultivars have smaller diameter of root and hypocotyl xylem vessels than drought-susceptible cultivars.

MATERIALS AND METHODS

An outdoors experiment in large tubes (4" diameter and 0.5 m height) was carried out at Colegio de Postgraduados in 2006. Three drought tolerant cultivars [Flor de Junio (FJ) Marcela and Flor de Mayo (FM) Bajío (high-yielding) and (FM) Corregidora] and one drought-susceptible [(FM) RMC, low-yielding), and a landrace (Michoacán 128, low-yielding)], (all cultivars type III), were studied. A complete randomized block design with four replicates was used. Three 260 ± 5 mg individual seed weight were sown per tube. The whole seedling was harvested at 23 days after sowing; roots were thoroughly separated from soil and washed with tap water. Afterwards, one anatomical transverse section was obtained at each of three points of the main root axis: at one cm below the "nodal region" (B), at midpoint between the "nodal region" and the root apex (M), and at one cm above the root apex (A). A cross section of the hypocotyl, at one cm above the "nodal region" (H) was also collected. Cross sections images (Wilcox *et al.*, 2002) were used to determine data on anatomical root and hypocotyl dimensions. Statistical analysis of data was performed by using the SAS program version 9.1 for windows (SAS, 2007); LSD of Tukey ($P \leq 0.05$) was used for comparison of means.

RESULTS AND DISCUSSION

Root characters

Diameter of root (DR) and vascular cylinder (VC), root cortex thickness (RCT), xylem vessels diameter (XVD) and total number of xylem vessels (TNXV) at B were greater ($P \leq 0.01$) than that at M and A. Cortex thickness (CT) was greater ($P \leq 0.01$) at A than at M and B, since the vascular cylinder size was smaller than that of the RCT (data not shown). FM Corregidora, FJ Marcela and FM Bajío produced thinner main root axes than the drought-susceptible genotype (FM RMC) and the landrace (Michoacán 128) (Table 1). The smaller root diameter of these cultivars was reflected in a narrower vascular cylinder, cortex and xylem vessels diameter, excepting Marcela, whose xylem

vessels diameter was similar to those of the drought-susceptible cultivar and the landrace. The TNXV did not show a definite trend among cultivars (Table 1).

Table 1. Mean diameter of root (DR) and vascular cylinder (VC), root cortex thickness (RCT), xylem vessels diameter (XVD) and total number of xylem vessels (TNXV) for each cultivar at each of three points of the main root axis.

Cultivar	µm				
	DR	VC	RCT	XVD	TNXV
FM Corregidora (Drought-tolerant)	840.8	358.5	231.1	45.7	8.3
FJ Marcela (High-yielding)	785.1	365.2	209.9	53.0	5.8
FM RMC (Drought-susceptible)	976.0	373.2	301.4	48.6	5.2
FM Bajío (High-yielding)	824.3	347.3	238.5	45.6	6.9
Michoacán 128 (Low-yield landrace)	946.1	432.7	256.7	53.5	8.1
General mean	**874.4**	**379.4**	**247.5**	**49.3**	**6.4**
Tukey test (P≤0.05)	101.9	71.3	49.1	6.9	1.8

Hypocotyl characters

The hypocotyls of drought-tolerant FM Corregidora, FJ Marcela and FM Bajío cultivars were thicker than those of drought-susceptible FM RMC and landrace Michoacán 128. This difference was also observed for the diameter of the central hollow space (DCHS). Hypocotyl cortex (HC) and total number of hypocotyl xylem vessels (TNHXV) of Corregidora, Marcela, Bajio and RMC were higher than Michoacán 128, and there was not significance for hypocotyl vascular cylinder diameter (HVCD) among cultivars (Table 2).

Table 2. Mean diameter of hypocotyl (DH) and central hollow space (DCHS), vertical-horizontal hypocotyl pith diameter (HVCD), hypocotyl cortex thickness (HC) and total number of hypocotyl xylem rays (TNHXV) for each cultivar at one cm above the "nodal region".

Cultivar	µm				
	DH	DCHS	HVCD	HC	TNHXV
FM Corregidora (Drought-tolerant)	2610.1	2201.1	928.1	204.5	15.0
FJ Marcela (High-yielding)	2790.0	2347.1	762.5	221.4	13.2
FM RMC (Drought-susceptible)	2281.7	1920.7	1005.5	180.5	13.2
FM Bajío (High-yielding)	2578.6	2187.7	770.2	195.4	13.4
Michoacán 128 (Low-yield landrace)	2222.2	1903.4	696.7	159.4	12.4
General mean	**874.4**	**379.4**	**247.5**	**49.3**	**6.4**
Tukey test (P≤0.05)	356.1	339.8	488.5	51.8	2.4

CONCLUSION

The drought tolerant cultivars have smaller diameter of root and hypocotyl xylem vessels than the drought-susceptible cultivar and the low-yielding landrace Michoacán 128. Additionally, the results indicate that there is not a positive relationship between high yield and drought tolerance in these cultivars.

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IN SILICO EMBRYOGENESIS GENES IDENTIFICATION FROM COMMON BEAN (*PHASEOLUS VULGARIS* L.) ESTS

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INTRODUCTION

The systematic identification of genes with essential functions has been described for several plant species [1]. In flowering plants, essential genes may be required for gametogenesis, embryogenesis and seed development which play a central role in the life cycle of flowering plants. Many genes transcribed during embryogenesis have now been examined at the molecular level. Mutational analysis has been extensively applied to plant embryos to define the genes that specify many of different embryogenesis stages [2]. A large number of embryo-defective mutants was identified and analyzed, particularly in plant model Arabidopsis thaliana. Among the 27.000 genes estimated to be involved in functional plant development, a collection of 250 to 750 genes could be required for normal embryo development [1, 3]. Transcripts of these genes can be localized in the embryo proper, in endosperm, or in maternal tissues around the embryo. Interestingly, disruption of these genes affect both embryo and endosperm development. Some sets of genes collectively designed Homeobox genes [4], Heat shock protein genes [5], Lipid transfer protein genes [6], Pasticcino (PAS) genes [7], Leafy cotyledons (LEC) genes [8], Titan (TTN) genes [9] appear to be major regulators of a variety of embryonic stages. Despite recent advances in the functional genomics of model legume plants such as common bean (Phaseolus vulgaris L.), many genes remain unknown and uncharacterized. To identify and to examine the expression profile of genes involved in *Phaseolus* embryogenesis we have developed a strategy that involves a combination of in silico mining of new genes from expressed sequence tags (ESTs) databases and rapid determination of expression profile using RT-PCR and a panel of cDNA libraries derived from different embryo developmental stages.

MATERIAL AND METHODS

ESTs of *Phaseolus vulgaris* were obtained from dbEST (http://www.ncbi.nlm.nih.gov/dbEST/). The inputted EST sequences were compared with embryo genes of some model plants such as *Arabidopsis thaliana*, *Glycine max* and *Medicago truncatula*. A total of 22 *Phaseolus* ESTs were identified and selected. For each EST, the sequence was used to design primers for amplification of corresponding sequences from common bean. The wild-type of the cultivated *P. vulgaris* genotype BAT93 and its Ethyl Methyl Sulfonate (EMS) mutants were used as plant material. Total RNAs were extracted from developing seeds of wild-type and mutant EMS plants at different stages of embryo development (early globular stage, 3 DAP; globular stage, 6 DAP; heart stage, 8 DAP; torpedo stage, 9 DAP; cotyledon stage, 12 DAP). PCR reaction was conducted according to the following parameters: 94°C for 3min, 30 cycles at 94°C for 30s, primer-specific annealing temperature for 30s, 72°C for 1min. As internal control, 18s rRNA primers were used. The PCR experiments were repeated at least three times.

RESULTS AND DISCUSSION

Approximately 250 genes involved in embryo normal development in *Arabidopsis* were used to determine unknown *Phaseolus* embryogenesis genes. On the basis of homology analysis among these Arabidopsis cDNA clones and *Phaseolus* ESTs dataset, we identified 22 partial mRNA sequences (ESTs) encoding to corresponding genes during *Phaseolus* seed development. In this study we selected 6 genes for expression analyzes such as PASTICCINO (PAS2), TITAN (TTN5), Valyl-tRNA synthase (TWN2), Auxin resistant6 (AUXR6), Biotine (Bio2) and Auxin response protein (ARP7). We analyzed expression for the selected ESTs by RT-PCR from leaves, flowers, stems, roots, cotyledons and seeds to verify transcript abundance in different plant tissues. The relative transcript levels for these genes were at least several times higher in seed tissues than in vegetative tissues (Figure 1). These data suggested that genes selected are differentially expressed in the organs of common bean, particularly in seed tissues. Expression levels of the 6 genes (Figure 2) were compared between developing seeds of wild-type and EMS mutant plants at 3, 6, 8, 9, 12 days after pollination (DAP). EMS mutant plants showed deficiency in seed development; embryos fail to grow at globular, heart and cotyledon developmental stages. All tested genes seem to be affected in their expression (down regulated) in EMS mutant samples rather than in wild-type samples at different stages of seed development. The 6 genes are involved in the embryogenesis process and their regulation is altered in aborting seeds. In further experiments, these selected genes will be used as probes to follow the spatial expression pattern during *Phaseolus* seed development by using the *in situ* hybridization in order to characterize and localize selected protein expression in developing *Phaseolus* seeds.

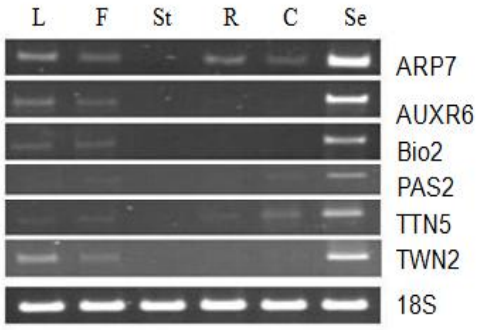

Figure 1. RT-PCR showing the expression of the selected genes from different tissue organs (L, leaves; F, Flowers; St, Stems; R, Roots; C, Cotyledons; Se, Seeds)

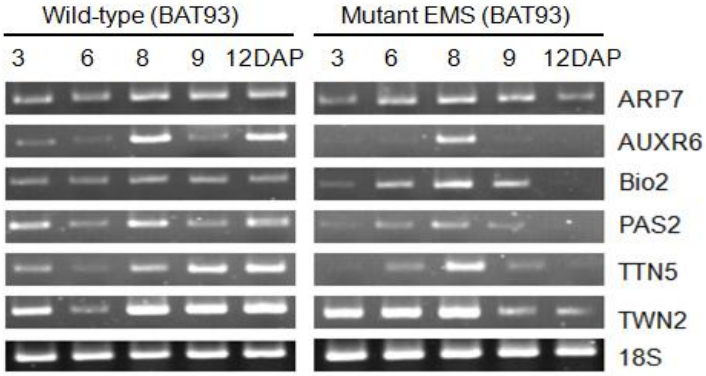

Figure2. RT-PCR showing the expression of genes selected during different stages of *Phaseolus* embryogenesis.

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DEVELOPMENT, TRANSFERABILITY AND MAPPING OF MICROSATELLITE MARKERS ON A REFERENCE BAT93 X JALOEEP558 POPULATION

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INTRODUCTION

Cultivated common bean (*Phaseolus vulgaris*) is a globally important crop. The advanced genomic studies of common bean can be successfully accelerated due to the development of new molecular tolls providing an opportunity for breeders to accelerate the development of varieties with valuable agricultural traits. The reduced genome size and the increasing pool of genetic resources is clearly a promising field with high potential to provide significant advances in research methodology useful for geneticists and breeders related to identification and elucidation of target genes. Codominant markers, such as microsatellites, are better suited for genome mapping because they are more informative and easily transferable. Microsatellite markers begun to be integrated into common bean linkage maps by Blair et al. (2003, 2006), resulting in a linkage map based exclusively on microsatellite markers mapped in the BJ population (Grisi et al. 2007) and, more recently, a new expanded version of the core linkage map also using the BJ population was released, which included markers with putative gene function (Hanai et al. 2009). The main objectives of the present work was: 1) to develop and to make available a set of SSR derived from express sequences (EST) of *Phaseolus vulgaris* obtained from the GenBank; (2) to genetically characterize a group of EST-SSRs and genomic SSR markers, 4) to examine the transferability of SSR markers among species of the Leguminosae family, 5) to integrate a set of new microsatellite markers into the core map for Bat93 x Jalo EEP558 population.

MATHERIAL AND METHODS

The EST sequences were obtained from the "*Phaseolus vulgaris* EST Project site" (**http://www.ccg.unam.mx/phaseolusest/**) and the Primer3 software used for primer design. The total number of 377 was synthesized, adjusted for PCR amplification and screened for polymorphism between the genitors BAT93 and JALO EEP558. The polymorphic markers were genotyped in a progeny consisting of 74 recombinant inbred lines (RIL) in the F_8 generation. The whole set of new segregant markers was integrated into a framework map composed of 123 SSRs markers, previously mapped in BJ population (Grisi et al., 2007). A total of 167 SSRs, being 107 previously published and 60 newly EST-SSRs, were selected for the analysis of transferability across 10 species of the Legumes genus, representative of four important tribes and one subfamilies of the Leguminosae family.

RESULTS

According to the criteria for the SSR containing sequence identification, a total of 9583 valid ESTs were screened for the presence of useful SSR sequences and 4764 sequences containing SSRs were identified. In the evaluation, out of the 377 EST-SSRs from *P. vulgaris*, 302 (80%) showed scorable

amplified product. while 24 generated non-specific products and 72 failed to amplify. Thus, a total of 315 markers were screened for the polymorphism in the BJ population, followed by the linkage analysis of the segregant markers. To access the transferability of SSR loci across Legumes species, the cross amplification of 167 primers (65 genic and 102 genomic- SSR) against 20 genotypes representing 10 species of the Leguminosae family was performed (*Medicago sativa, Phaseolus lunatus, Phaseolus coccineus, Phaseolus acutifolius, Vigna mungo, Vigna angularis, Vigna unguiculata, Glycine max, Arachis hypogaea* and *Dipteryx alata)*. From the 65 genic SSRs, a total of 61 (94%) amplified across, at least, one species and only four (6%) were specie specific. For the 102 markers tested derived from genomic libraries, 76 (75%) amplified across, at least, one species and 26 (27%) failed to produce an amplification product across the species. The ratio of transferable markers among the species ranged from 119 (71%) to three (1.8%), respectively, for *P. Acutifolius* and *A. hypogaea*, respectively, with a mean of 32% of cross amplified loci. As expected, the high index of interspecific cross amplification were observed for species within the genus *Phaseolus* (64%), followed for *Vigna* (26%), *Glycine* (20%), *Medicago* (10%) and *Dipterix* (6%). For the whole set of 167 SSRs tested, the mean PIC values was 0.50, from the 68 genomic SSRs the average value was 0.53 and among the EST- SSRs the mean PIC value was estimated in 0.47. Of the 315 newly SSRs screened for the polymorphism, 76 segregated in the BJ population, of which 72 were EST-SSRs and four anonymous SSRs. The integration of the SSRs into the reference linkage based exclusively in SSRs resulted in a dataset of 199 polymorphic markers, being 117 genomic SSRs and 82 EST-SSRs. Of these, a total of 180 (90%) markers was mapped and distributed in 13 chromosomes. The distribution of the EST-SSRs appeared to be relatively random and dispersed throughout the *Phaseolus* genome, of which every linkage group contained more than one EST-SSR marker. The comparative analysis, based on common SSR markers, performed between the current based SSR map and the based SSR map previously developed by Grisi et al. (2007) showed that all SSR markers (99%), but one (BM202), maintained their position in the same linkage group. A considerable degree of homology was observed in terms of marker order conservation (78%). Based on the present work a broad set of useful SSR markers for common bean derived from public EST databank was developed. Not surprising, the present results indicated that EST-SSRs are more transferable across the Legumes species than are anonymous SSRs and the level of EST-SSR polymorphism (0.47) was slight lower than that with SSR derived form genomic libraries. Despite the reduced level of polymorphism rates of the EST-SSR, these markers were very useful for genetic mapping of the BJ populations helping to increase the map coverage in the *Phaseolus* genome.

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GUIDELINES FOR COMMON BEAN QTL NOMENCLATURE

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Quantitative trait locus (QTL) analysis has become an important tool for the characterization and breeding of complex traits in crops plants, such as common bean (*Phaseolus vulgaris* L.). A standard system for naming QTL in common bean is needed for effective referencing of new and previously identified traits to more effectively differentiate QTL. A similar nomenclature system for chromosome identification in common bean was adopted (Pedrosa-Harand et al., 2008).

Although QTL for disease, abiotic, and pest resistance have been identified in common bean, the comparison of QTL across studies, populations, and locations is occurring more frequently with the proliferation of QTL studies. This QTL information serves to test the effects of loci across different genetic and environmental backgrounds, and thus to estimate GxE interactions. With this information available, those stable and consistent QTL can be used for marker-assisted selection in breeding programs. For example, a common bacterial blight QTL, linked to the BC409 marker, was shown to have significant effects across four different common bean populations and with three different *Xanthomonas axonopodis* strains, making it a broadly effective and stable QTL (Jung et al. 1999). Recent work with white mold resistance in common bean is another example whereby QTL with stable expression across environments and genetic backgrounds have been identified and used for marker-assisted selection (Kolkman and Kelly, 2003; Miklas et al., 2003, 2007; Ender et al., 2008; Miklas, 2009).

Considering the need for a common nomenclature, the Common Bean Genetics Committee approved the adoption of QTL nomenclature guidelines for use in future QTL publications, described below, during its 2009 Isabela, Puerto Rico, meeting. White mold QTL are used as an example for describing the nomenclature.

Guidelines for common bean QTL nomenclature:

1. To identify each trait, use capitalized letters in a 2-3 letter abbreviation. The capitalized trait name should not be italicized. For example, WM for white mold. A preferred list of abbreviations to use for common traits should be generated, and updated periodically.
2. Each QTL will have a linkage group designation directly after the 2-3 letter abbreviation. For example, WM1 indicates a QTL on linkage group 1.
3. QTL should be listed in chronological order. Thus, new publications on a specific trait will initially need to review and number previous QTL designations in order to arrive at a number for new QTL. For example the first QTL identified on linkage group 1 would be named WM1.1, and the second independent QTL on linkage group 1 would be named WM1.2, and so forth.
4. The population where the new QTL was identified should be indicated by an abbreviation in caps, and non-italicized, superscript after the linkage group designation. For example, the first QTL indentified on linkage group 1 for white mold resistance was in the A55/G122 RIL mapping population, and thus would be designated WM1.1^{AG}

5. To distinguish among QTL which co-localize or overlap in the same general region, subsequent population abbreviations should be separated by commas and listed in order of discovery. For example, the overlapping QTL identified first in A55/G122 and subsequently in John/Doe would be designated WM1.1^{AG,JD}, and so forth. The population abbreviations need only be cited in the first mention of the QTL in a publication. Thereafter, the shortened version, e.g. WM1.1, can be used.

Additional provisions:

6. If upon fine mapping in the future, two overlapping QTL are proven to be independent, then the subsequent QTL in the example above could be renamed WM1.1.1^{JD} to distinguish it from WM1.1^{AG}.
7. If two independent QTL (Ex: WM1.1^{AG,} and WM1.2^{JD} in the future are proven to co-localize, then the first QTL identified would retain its original name and the second QTL would be incorporated in the name of the first QTL: WM1.1^{AG,JD}. Note that if this occurs then the original number (2 in this case) representing chronological order is not used again.

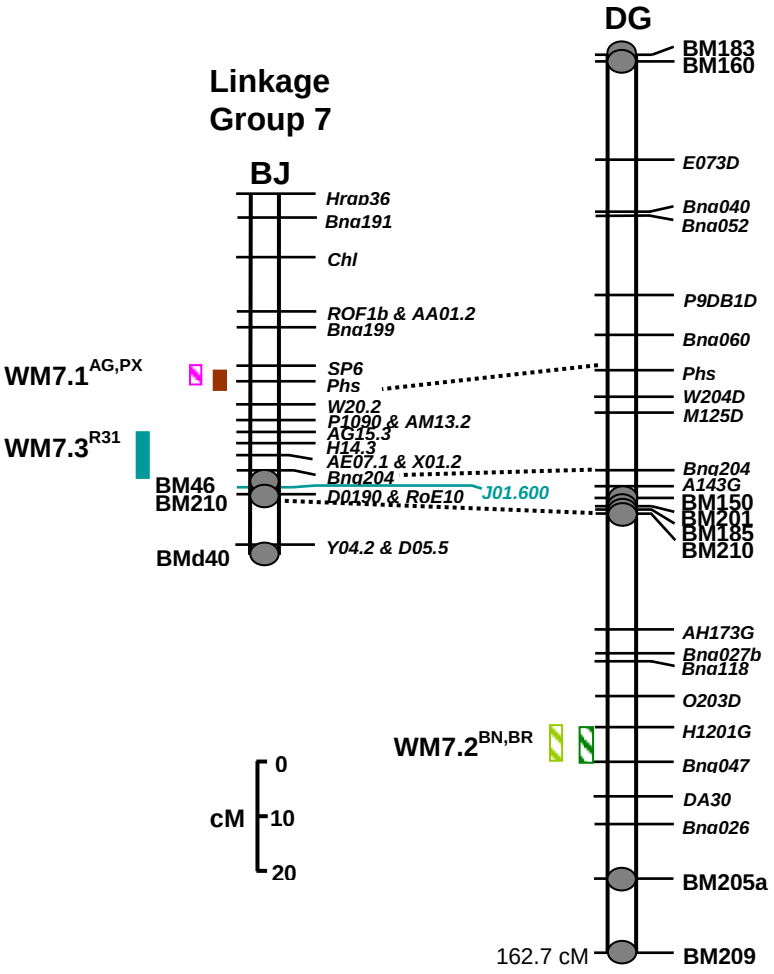

Figure 1. Comparative mapping in BJ-DG core maps (Blair et al., 2003) of QTL for resistance to white mold identified to date on linkage group 7 in different populations. PX, PC50/XAN159, Park et al., 2001; AG, A55/G122, Miklas et al., 2003; BN, Bunsi/Newport, Kolkman and Kelly, 2001; BR, Bunsi/Raven, Ender and Kelly, 2005; R31, Raven/I9365-31, Miklas et al., unpublished).

For example, the first QTL discovered on linkage group 7 (Fig. 1) was identified in the AG population (Miklas et al., 2001) near the Phaseolin seed protein locus (*Phs*). Subsequently, the same QTL was identified in population PX (Park et al., 2001); thus, this QTL is designated WM7.1^{AG,PX}. The second QTL found on linkage 7, near the Bng047 marker, was identified first in the BN population (Kolkman and Kelly, 2003) and subsequently in the BR population (Ender and Kelly, 2005); thus, this QTL is named WM7.2^{BN,BR}. In an unpublished study, an independent QTL in R31 was mapped to linkage group 7 near the Bng204 marker; thus, this QTL is named WM7.3^{R31}.

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IDENTIFICATION OF SSR MARKERS TO THE RESISTANCE ALLELES OF *PHASEOLUS VULGARIS* L. TO THE NEMATODA *MELOIDOGYNE INCOGNITA* RACE 1

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INTRODUCTION

The nematodes are outstanding among the most harmful diseases to the bean plant; the damage might be total, depending on the cultivar and the plant development, soil temperature and dampness, species/race and population density of the nematodes. Among the identified nematode species, the most common in these cultures in south Minas Gerais, are root-knot nematode *Meloidogyne incognita* and *M. javanica* associated ones (Lordello, 1988). However, the species which causes higher prejudice on the common bean culture is the *M. incognita*. In the case of snap bean, it is found in the literature that its susceptibility to *Meloidogyne* spp. is similar to common bean.

There are several strategies of control like crop rotation, application of nematicids and bare soil. However, the use of resistant cultivars is even the main alternative of control. Previous works have shown clearly (Omwega and Roberts, 1922) the resistant genetic control is monogenic and oligogenic, however, the character is difficult to be evaluated and too influenced by the environment. In a condition like that, the molecular markers can be useful for assisted selection. The resistance allele identification present on the Aporé cultivar, through microsatellite markers (SSR) can facilitate the breeder's work since hard steps like inoculation with pathogen and evaluation of number of eggs will be discarded.

That way, this work aimed to identify microsatellite markers to the resistance allele to the root-knot nematode *M. incognita* race 1 that might be used in assisted selection.

MATERIAL AND METHODS

A cross between 'Macarrão Rasteiro Conquista' – snap bean (susceptible, P_1) cultivar and 'Aporé' – common bean (resistant, P_2) cultivar was carried out. From generation F_1, 70 plants of generation BC_{11} were obtained. Fifteen days after sowing, the substrate was infected with *M. incognita* Race 1 eggs. The inoculum stems from tomato plant of Santa Clara cultivar, susceptible to *Meloidogyne* spp., held in greenhouse, in the experiment station, from the HortiAgro Seeds, Ltd Company, in vases with 10 dm^3.

The inocolum preparation was made according to Hussey and Barker methodology (1973), modified by Boneti and Ferraz (1981). After, there continued the eggs counting in Peter boxes (Southey, 1970), containing 1 mL rate, using stereoscopic microscopy. 10,000 eggs per vase were used, equivalent to 5 ml from the 2,000 mL^{-1} suspension, related to the nematodes initial population (P_i). The eggs distribution was made assisted by a veterinary syringe, perforating the soil beside each plant lap and applying the suspension with eggs.

Evaluations were done by 45 days after the infestation, in verifying great formation of galls and a mass of eggs on the tomato plant roots. The plants that presented number of eggs per gram of root, $\leq$ 6,500, were considered resistants and the ones that presented $\geq$ 6501 were considered

susceptible. The DNA from those plants was extracted according to the protocol used by Rodrigues and Santos (2006). For identifying the markers matched to the resistance alleles, a methodology known as Bulked Segregant Analysis (Michelmore et al., 1991) was used. This methodology consists in using DNA equitable mixtures of the 10 most resistant and susceptible plants for the constitution of the two contrasting bulks, one resistant and other susceptible. For verifying polymorphism between the bulks, 507 primes pairwise primers were evaluated. The polymorphic primers in bulks were used to genotype the segregant population. Markers data were used to evaluate the phenotype for estimating the distances through the maximum likelihood method using the software GQMOL (Cruz, 2008 available in www.ufv.br).

RESULTS AND DISCUSSION

From the inoculation results, 33 resistant and 37 susceptible plants in 1:1 rate were identified, as expected for the generation BC_{11}. Two polymorphic markers were identified on bulks (SSR BM164 e SR PVESTBR_72) and both segregate in 1:1 rate among the population, too.

The markers mapped themselves far from the resistance alleles, both at 46cM (± 6), though independent of that allele. This result suggests the two markers might be flanking the resistance allele. In this case, as those markers are codominants, they might be useful whether simultaneously evaluated, in an assisted selection, taking again the homozigotic plants for the two markers in population F_2. This way, the efficiency of the assisted selection will be about 65%.

A marker SRRXO4660 closer (36cM) to the nematode resistance allele was identified in common bean, using the same resistance source 'Aporé' (Alves et al., 2005). Unfortunately, that SSR was not polymorphic in segregant bulks, although it had been in progenitors. As in the present study the susceptible progenitor is snap bean, it must differ on common bean related to the distance of the marker and the resistance allele.

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CORRELATION BETWEEN EVALUATED PARAMETERS IN *PHASEOLUS VULGARIS* L. AS FOR THE RESISTANCE TO *MELOIDOGYNE INCOGNITA* RACE 1

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INTRODUCTION

Among the problems found in the bean plant culture, we highlight the event of root-knot nematode, sedentary endoparasites, represented mainly by the *Meloidogyne* genus which causes meaningful losses to the cultivation. The snap bean susceptibility to the root-knot nematode infection is similar to the common bean. The resistance evaluation is often carried out based on either capacity or nematode reproduction rate on tested plants. The reproduction is measured proceeding the nematode counting (juvenile and/or adult eggs, according to the genus involved) extracted from the root system and/or rizosphere. In improvement works, the environmental, phenotypic and genotypic parameter estimations assist the decision making about the choice of the improvement method, as well as the way to conduct and select plants and families. Other study of interest for selecting families is the environmental, phenotypic and genetic correlations. Those correlations allow the breeder to estimate the relationship between the characteristics, visualize the possible indirect selection and obtain selection gains from a hard estimation characteristic because of the selection itself, instead of other related characteristic. Therefore, this work aimed to estimate the association level between the resistance characteristics to the *M. incognita* race 1 root-knot nematodes in populations of 'Macarrão Rasteiro Conquista' and 'Aporé', aiming to assist improvement programmes for including resistance to this parasite.

MATERIAL AND METHODS

The experiment was carried out in greenhouse, in HortiAgro Seeds Ltd rooms, in Ijaci district, MG, Brazil. Cultivars 'Macarrão Rasteiro Conquista' (snap bean/susceptible/P_1) and 'Aporé' (common bean/resistant/P_2) were used as progenitors, beyond the F_1, F_2 and BC_{11} and BC_{12} obtained from these cultivars. After getting the generations the experiment was set totalizing 339 plants F_2, 40 plants F_1, 32 plants BC_{11}, 36 plants BC_{12}, 39 plants P_1, and 32 plants P_2. Three seeds were sowed in 3 liter-vases containing a sand-earth mixture as substrate, which were thinned out to one plant after germination and emergence. Fifteen days after sowing, the substrate was infested by *M. incognita* race 1 eggs. The inoculum was obtained from Santa Clara cultivar tomato plant infested by isolates, prepared according to Hussey and Baker methodology (1973) modified by Boneti and Ferraz (1981). 10,000 eggs were used in each vase (5ml out of 2,000 eggs ml suspension $^{-1}$). Forty five days after inoculation evaluations were done as for the nematode reproduction in all genotypes. The plants were removed from the vases and their root system carefully washed. Later they were weighted to determine the fresh root mass (in grams). The quantification of eggs was carried out as previously mentioned. The final number of nematode eggs per gram of root was estimated following Ferreira et al. (2010). The reproduction factor was estimated according to Oostenbrink (1966) and the reproduction index was calculated through Taylor (1967). The values obtained were used for the calculation of the correlation, which were obtained from the SAS programme (SAS, 2000).

RESULTS AND DISCUSSION

All the correlations were positive and significant, showing that the evaluated characteristic behavior was always in the same direction (table 1). The correlations between eggs and reproduction factor, number of eggs and reproduction index, reproduction factor and index, and number of eggs per gram of root and reproduction index per gram of root had l.00 value, that is, they were high and positive. These results show there is no difference in using number of eggs or number of eggs per gram of root because they are not statistically different. However, Silva et al., (2007) mention that the use of number of eggs per gram (or population when also counting juveniles) can express better the nematode reproductive rate, since it excludes one variation source, the root fresh matter production, which belongs to each genotype. As for the reproduction factor and index, we also verified that there was no significant difference, that is, both characteristics do not statistically differ among themselves, one can choose either one. It is worthwhile to emphasize that some methodologies are concerned only about damages caused to the host or the nematode reproduction as the gall and mass of eggs index, respectively. According to Cauto-Saénz (1985), the reproduction factor and reproduction index are more reliable criteria to evaluate plant reaction to root-knot nematode.

Table 1 - Correlations between number of eggs (NE), reproduction factor (RF), number of eggs per gram of root (NE/GR), reproduction index (RI) and reproduction index per gram of root (RI/GR). Federal University of Lavras, Lavras/MG.2010.

CHARACTERISTIC	NE	RF	NE/GR	RI	RI/GR
NE	-	1.00**	0.64**	1.00**	0.64**
RF			0.64**	1.00**	0.64**
NE/GR				0.64**	1.00**
RI					0.64**
RI/GR					

**Significant at 1% probability through Person correlation method.

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MAPPING OF SCAR MARKERS RELATED WITH *UR-13* GENE FOR RUST RESISTANCE IN COMMON BEAN (*PHASEOLUS VULGARIS* L.)

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INTRODUCTION

One of the major diseases of common bean (*Phaseolus vulgaris* L.) is rust caused by the fungus *Uromyces apendiculatus*, a pathogen that shows high levels of variability and that generates great losses in the crop (Correa *et al*., 2000 Crop Sci 40: 804-807). The R-gene *Ur*-13, is hypostatic to many other genes being used today and therefore provides resistance to many races of *U. appendiculatus* (Mienie *et al*., 2005 Theor Appl Genet 111:972-979). Genetic markers linked to disease resistance genes can be used in marker-assisted selection to identify resistant lines in an early stage of development. SCAR markers are generally allele specific and are generated as a dominant or co-dominant markers (Paran & Michelmore, 1993 Theor. Appl. Genet. 85:985-993.). The aim of this study was to locate three SCAR markers (KB126, KB85, KB4), reported by Mienie *et al.* (2005) as adjacent to the rust resistance gene *Ur*-13.on the DOR364 x G19833 and BAT93 x JaloEEP558 population maps so as to provide other potential markers for further evaluation of the locus.

METHODOLOGY

DNA extraction was performed according to the established CIAT methods. We used three SCAR markers (KB126, KB85, KB4) derived from AFLP markers designed by Mienie et al. (2005), for genetic mapping in the DOR 364 x G 19833 and BAT 93 x JaloEEP 558 mapping populations (Blair et al. 2003). The amplification conditions were determined empirically from the conditions published by Mienie et al. (2005). The amplifications were performed using 50ng of genomic DNA, 0.2 µM of each of the forward and reverse primers, 10 mM Tris-HCl (pH 7.2), 50 mM KCl, 2.0 mM MgCl2, 0.2 mM dNTP and 1 U of Taq polymerase to a final volume of 25µl. The amplification cycle consisted of strong denaturation for 5 min at 94ºC, 36 cycles denaturation at 92ºC for 1 min., annealing at 45, 57 or 60ºC for 1 min. according to the primers (KB7, KB126 or KB4 respectively), and extension at 72°C for 1 min. For the KB4 marker, a digestion was performed with enzyme *Hha*I (10u/µl) to a final volume of 20 ml at a temperature of 60ºC. The map was made with the program Mapmaker with the Kosambi mapping function and a minimum LOD of 3.0.

RESULTS

The markers KB4, KB85 and KB126 were previously mapped by Mienie et al. (2005) in the Bonus x Kranscop (BxK) population with two linkages (KB4 and KB85) to the BAT93 x JaloEEP558 (BxJ) population. In this study we mapped the third SCAR marker KB126 on the same reference map and extended the genetic mapping to another core reference map, DOR364 x G19833 (DxG). Polymorphisms were for the most part similar since all three populations shared an inter-genepool background. Genetic distances varied only slightly between the populations. For example Mienie *et al.* (2005) located KB4 and KB85 markers, at a distance of 23.0 cM and 20.8 cM in the BxK and BxJ, respectively, while we found that in DxG the distance between these markers was 23.4 cM.

In re-mapping within the BxJ population we were able to place the KB126 marker and found that is was 5.4cM from KB4 and 10.2cM from KB85. Therefore the total distance between the two markers mapped by Mienie et al. (2005) instead of being 20.8 cM could be as low as 15.6cM. The localization of KB126 is interesting as it is the marker most closely linked to the Ur-13 gene. In the DxG population was impossible to map KB126 marker by amplification problems.

Before this study only KB4 and KB85 were confirmed to be polymorphic outside the BxK population. As flanking markers both theses SCARs are useful for selecting the *Ur-13* gene, however our confirmation that KB126, the most closely linked resistance gene marker is within this interval makes it possible to more easily select lines with *Ur*-13 rust resistance without the need of inoculating to demonstrate line resistance or susceptibility. In addition, KB126 was a codominant marker that could be used to identify heterozygous genotypes, (Mienie *et al*., 2005). Finally, in this study we were able to more exactly place the Ur-13 gene on two saturated genetic maps since KB126 is known to be is located at a distance of 1.6cM from *Ur*-13 in BelNeb-RR x A55 and Bonus x Kranscop populations linkage maps (Mienie *et al.*, 2005).

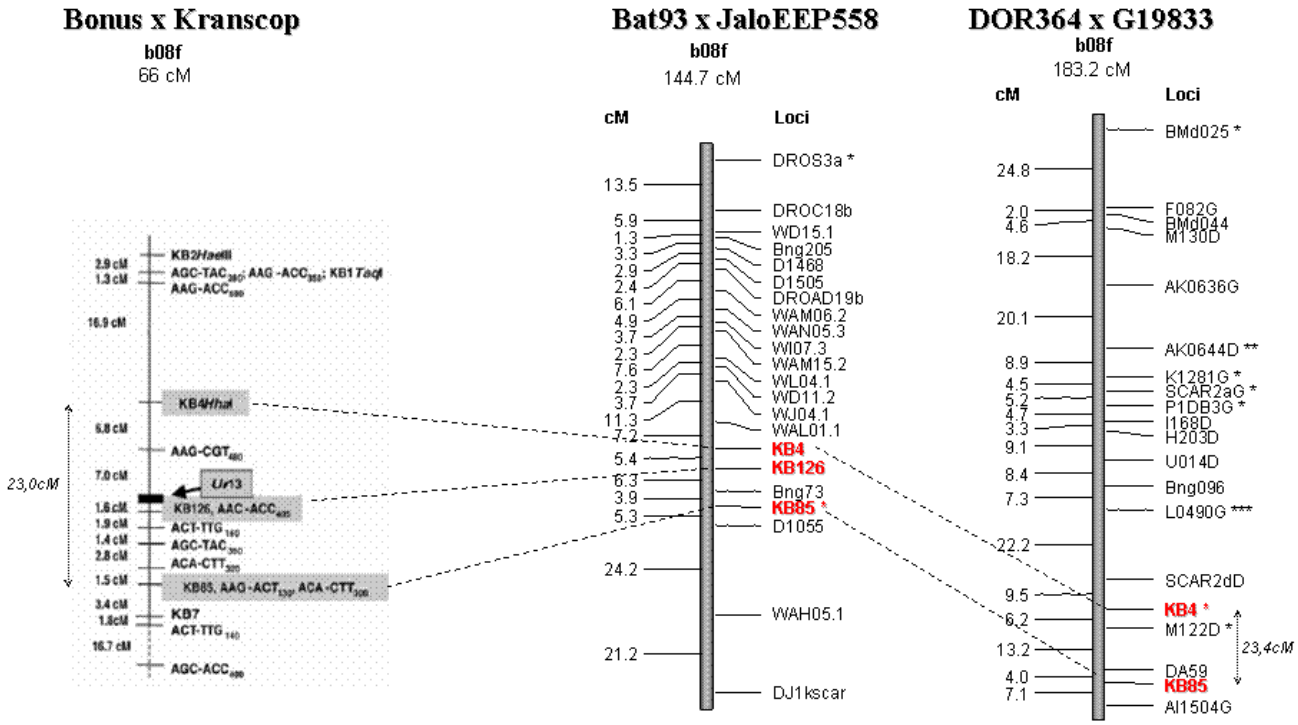

Figure 1. Comparison of B08 linkage group between Bonus x Kranscop (Mienie et al., 2005), DOR364 x G19833 and Bat93 x JaloEEP558.

DETECTION OF SCAR MARKERS LINKED TO RESISTANCE TO COMMON BLIGHT AND ANTHRACNOSE IN AYOCOTE BEANS

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Anthracnose [*Colletotrichum lindemuthianum* (Sacc. y Magn.)] and common blight [*Xanthomonas axonopodis* pv. *phaseoli* (Smith)] are two of major diseases of common beans throughout Mexico causing yield losses up 50 % (3). The use of resistant germplasm could be one cheap and appropriate strategy to reduce grain losses, since Mexican farmers not use pesticides for disease management. SCAR markers linked to both diseases have been identified in common bean germplasm, and they are reproducible and specific to identify genetic resistance to diseases (5, 6). Here we detected 10 SCAR markers previously reported in common beans linked to resistance genes to anthracnose and common blight in one collection of 'ayocote' bean (*Phaseolus coccineus* L.) germplasm from the state of Puebla, Mexico.

Ayocote bean collection was obtained through the region named 'Huasteco' karst which is included within the state of Puebla, Mexico and comprises 117 accessions. As out-groups we used two *P. vulgaris* cultivars (Pinto Villa, Pinto Zapata), one accession of *P. glabellus*, one of *P. coccineus* var. *coccineus* and cv. Blanco Tlaxcala (*P. coccineus*). Germplasm was sown in Chapingo Mexico on July 7th, 2008 and some traits related to phenology and pod and seed morphology were measured (4). Ten SCAR markers were amplified in ayocote beans: SAS13, SBB14, SAB3, and SH18 linked to anthracnose resistance genes *Co-4*, *Co-4^2*, *Co-5* and *Co-4^2*, respectively as well as common blight SCAR markers SAP6, BAC6, SU91, LG5, R7313, and R4865 located at linkage groups B10, B8, B10, B6, B8, and B8 respectively in common bean genome. ADN was isolated based on the protocol of Doyle and Doyle (2) and each SCAR was amplified based on data published by the Bean Improvement Cooperative (1).

Four SCARs for anthracnose disease were used but only two amplified in ayocote beans (SAS13 and SBB14) while five for common blight (SAP6, BAC6, SU91, LG5, R4865) were found. SAB3, SH18 and R7313 were not presented in ayocote bean collection (Table 1). The most frequent SCARs were SAS13 and SBB14 (anthracnose, 89 and 74%, respectively) followed by BAC6 and SU91 (74 and 42%) for common blight. Field confirmation of these results is needed and after the useful germplasm from Zacapoaxtla and Tlatlauquitepec could be used for crossing with susceptible germplasm or be used directly due accessions from both locations showed the highest frequencies of SCARs (five to six SCARs each one). No association between high frequencies of SCARs and morphological traits was found (Table 2). In conclusion, we found new sources of resistance to anthracnose and common blight diseases in ayocote bean germplasm from the state of Puebla, Mexico. Germplasm is available for further breeding of *P. coccineus* and/or *P. vulgaris*. This is the first reference of amplification of SCAR sequences in ayocote beans previously reported in common beans in Mexico.

Table 1.SCAR amplified in ayocote beans from Puebla, Mexico.

		Anthracnose				Common blight						
Location	n	SAS13	SBB14	SAB3	SH18	SAP6	BAC 6	SU91	LG5	R7313	R4865	Mean
Zacapoaxtla	38	34	30	0	0	0	25	17	2	0	15	12.3
Zacatlán	3	2	3	0	0	0	0	0	3	0	1	0.9
Tlatlauquitepec	18	17	10	0	0	0	11	6	2	0	5	5.1
Nauzontla	7	6	5	0	0	0	5	2	3	0	4	2.5
Teteles de Ávila Castillo	1	1	1	0	0	0	1	1	0	0	0	0.4
Zoquiapan	4	4	4	0	0	0	3	3	0	0	0	1.4
Huauchinango	1	1	1	0	0	0	1	1	1	0	1	0.6
Chignahuapan	6	6	5	0	0	0	3	1	0	0	0	1.5
Ahuacatlán	3	3	1	0	0	0	3	1	0	0	1	0.9
Xochiapulco	2	2	1	0	0	0	1	1	0	0	0	0.7
Market of Zacapoaxtla	14	14	13	0	0	1	12	5	2	0	6	5.3
Market of Cuetzalán	4	1	4	0	0	0	2	2	0	0	2	1.1
Market of Tlatlauquitepec	6	6	5	0	0	0	6	5	0	0	2	2.4
Market of Cd. Serdán	6	6	2	0	0	0	3	0	0	0	1	1.2
Texcoco	5	3	3	0	0	0	4	3	1	0	0	1.4
Atempan	4	3	3	0	0	0	2	4	0	0	1	1.3
Total	122	109	91	0	0	1	82	52	14	0	37	-

Table 2. Some morphological traits of ayocote beans with five or six SCARs linked to anthracnose and common blight.

Accession	Location	Days to flowering	Days to maturity	Seed color	Weight of 10 seeds (g)	Pod length (cm)
8449	Tlatlauquitepec	52	111	White	5.45	9.49
8210	"	77	131	Beige	4.70	-
8213	"	54	126	Dark purple	5.10	8.30
8446	"	39	101	White, violet, purple, black	7.46	9.66
8506	Zacapoaxtla	62	115	Beige	4.79	10.05
8762	"	46	139	Violet	5.60	8.40
8452	"	50	120	Beige, violet, white, purple	7.38	8.90
8104	"	46	126	Yellow	5.50	10.40
8193	Cd. Serdán	78	131	Beige	2.80	8.32
9237	Atempan	69	120	Dark purple	4.00	7.81

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DETECTION OF SCAR MAKERS LINKED TO RESISTANCE TO ANTHRACNOSE AND COMMON BLIGHT IN WILD X CULTIVATED BEAN COLLECTION

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Anthracnose [*Colletotrichum lindemuthianum* (Sacc. & Magn)] and common blight [*Xanthomonas axonopodis* pv. *phaseoli* (Smith)] are two of the main diseases of common beans (1, 4) due they can reduce grain yields up 95 %. One choice for disease management consists on the production of resistant cultivars using new sources of resistance to a broad spectrum of fungal/bacterial populations (4). New sources of resistance for bean breeding can be found in wild germplasm as well as landraces from major agro-ecological regions of Mexico. The identification of disease resistance genes can be fast and reliable discovered using SCAR (Sequence-characterized amplified region) markers previously reported (2, 8). The aim of this work was to detect ten SCAR markers linked to resistance to anthracnose and common blight in one wild x cultivated beans collection of Mexico.

The collection includes 187 accessions of wild x cultivated randomly crosses of beans which belong to Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) of México. Seeds of each accession were sown in greenhouse and young leaf tissue was collected 15 days after sowing. After, genomic DNA was isolated (11) and ten SCARs were amplified. The SCARs SAS13, SBB14, SAB3, and SH18 are linked to anthracnose resistance genes *Co-4*, *Co-4*^{2}, *Co-5* and *Co-4*^{2}, respectively while common blight SCAR markers SAP6, BAC6, SU91, LG5, R7313, and R4865 are located at linkage groups B10, B8, B10, B6, B8, and B8, respectively (2)

Only three SCARs for anthracnose resistance and four SCARs for common blight resistance can be amplified in the germplasm due some major genes or QTLs are presented in specific cultivars, races or gene pools (3, 5). SBB14 showed the highest frequencies of presence (64 %) while SAB3 exhibited the lowest frequency (24 %). In the case of SCARs linked to resistance to common blight, the most found SCAR was SAP6 (44.9 %) while LG5 showed presence near zero (0.004 %). Germplasm from Tlaxcala and Guanajuato scored high frequencies of SCARs. We can suggest that germplasm from both states have some alleles of *Co-4* and *Co-5* genes (6) since beans from Mesoamerican gene pool show 10 genes for anthracnose resistance (from *Co-2* to *Co-11*) while Andean germplasm shows three (*Co-1*, *Co-12*, *Co-13*), only. High frequencies of resistance genes in Tlaxcala and Guanajuato also suggest the broad pathogenic variability of causal agent of anthracnose, due the highest number of pathotypes have been reported in anthracnose isolates from north and central Mexico (10). We suggest that germplasm from north and central Mexico are subjected to higher selection pressure compared with other regions and need to generate new resistance genes for new pathotypes or races. One similar co-evolution between beans and common blight has been demonstrated (9). Our results must be taken with care before to propose new sources of resistance to anthracnose or common blight for bean breeding. Genomic distances between SCAR and resistance gene could generate false resistance by recombination events (7). In addition, the presence of each SCAR must be validated by classical pathogenicity test and trials under controlled

or field conditions. Evaluations of QTL stability throughout environments and genotypes are also needed (3). Finally, we must consider the genetic status of germplasm, due we have detected SCAR sequences in wild x cultivated germplasm and MAS uses highly endogamic lines. However, our results offer a new outlook about the availability of sources of resistance to two major diseases of common beans in Mexico. Special attention must be given to germplasm from central Mexico.

Table 1. Relative frequencies of SCAR markers detected in a wild x cultivated bean collection.

State	NA[†]	SCAR for:							Total[‡]
		Anthracnose			Common blight				
		SAS13	SBB14	SAB3	BAC6	SAP6	LG5	R4865	
Morelos	33	0.33	0.55	0.03	0.45	0.48	0.06	0.45	0.34
Nayarit	05	0.20	0.80	0.00	0.40	0.40	0.00	0.40	0.31
Querétaro	12	0.08	0.58	0.08	0.42	0.58	0.00	0.42	0.31
Tlaxcala	05	0.60	0.80	0.40	0.80	0.80	0.00	0.80	0.60
México	08	0.13	0.25	0.00	0.13	0.00	0.00	0.13	0.09
Veracruz	04	0.00	0.25	0.00	0.25	0.00	0.00	0.00	0.07
Puebla	05	0.40	0.40	0.40	0.20	0.20	0.00	0.20	0.26
Jalisco	44	0.27	0.61	0.30	0.43	0.55	0.00	0.27	0.35
Guerrero	15	0.53	0.67	0.13	0.67	0.80	0.00	0.20	0.43
Guanajuato	13	0.62	0.77	0.46	0.69	0.69	0.00	0.62	0.55
Durango	10	0.10	0.30	0.20	0.30	0.30	0.00	0.40	0.23
Tamaulipas	07	0.43	1.00	0.43	0.29	0.29	0.00	0.57	0.43
Chiapas	07	0.00	0.71	0.43	0.14	0.00	0.00	0.57	0.26
Michoacán	07	0.43	0.71	0.00	0.57	0.14	0.00	0.71	0.37
Sinaloa	02	0.00	1.00	0.50	0.00	0.00	0.00	1.00	0.36
Oaxaca	10	0.10	0.80	0.50	0.20	0.30	0.00	0.70	0.37
Total[‡]	187	0.26	0.64	0.24	0.37	0.35	0.004	0.47	0.26

[†] NA= Number of accessions.

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PRODUCTIVITY LOSSES AMONG DIFFERENT COMMON BEAN GENOTYPES CAUSED BY COMMON BACTERIAL BLIGHT

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INTRODUCTION

Productivity loses caused by plant diseases may reach 100% of the production, depending on the pathogen aggressiveness. Common bacterial blight (CBB), incited by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*), is among the diseases that may lead to significant losses of productivity to common bean (*Phaseolus vulgaris*), depending on the prevailing environmental conditions. This plant pathogen is widespread in almost all producing regions of Brazil.

MATERIALS AND METHODS

A field experiment was carried out to compare 52 common bean genotypes, inoculated at 30 days after sowing. Previous sand spraying was performed aiming to cause injury in the plants and, after that bacterial suspensions (10^8 FUC ml^{-1}) of four different *Xap* isolates were sprayed. The experiment was performed in a randomized block design and three replicates. Severity evaluation was performed in two lines of two meters at 30 days after inoculation, for which was applied a note scale varying from 1 to 9. Grain yield was performed for all treatments and the percentage of productivity loss was determined with basis on data of inoculated and non inoculated plants. Data were submitted to a variance analysis and mean treatments were compared by Scott-Knott test at 5% of significance by the software SISVAR.

RESULTS AND DISCUSSION

Among the non inoculated genotypes, BRS Pontal, CNFC 10762 and BRS Marfim showed the greatest grain yield with 1546, 1589 and 1604 kg ha^{-1} respectively, while BRS Executivo, WAF 75 and BRS Embaixador showed the lower ones, 487, 703 and 729 kg ha^{-1} respectively (Table 1). However, when it were compared loss of productivity and severity notes data of inoculated and non inoculated genotypes, it was possible to verify that BRS Pontal and CNFC 10408 genotypes were the most resistant ones, showing loss of productivity of 4.8 and 9.2%, respectively. On the other hand, BRS Supremo and BRS Embaixador were the most susceptible genotypes, showing loss of productivity of 20.7 and 40.7%, respectively. It was observed significant Pearson correlation (R^2= -0,33**) between the severity notes and grain yield, in which high severity notes indicates significant decrease in grain yield. In spite of the interesting results, field experiments should be repeated in several environmental conditions to ensure the reliability of our results.

CONCLUSIONS

BRS Pontal and CNFC 10408 genotypes showed the greatest resistance to common bacterial blight and, BRS Supremo and BRS Embaixador showed the lower ones.

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Table 1- Disease note, loss of productivity and grain yield of common bean genotypes inoculated and non-inoculated with *Xap.*

Genotypes	Note	Loss of productivity (%)	Grain yield of the inoculated plant	Grain yield of the non inoculated plant
BRS Pontal	3.7 b*	4.8	1556 a	1634 a
CNFC 10408	3.8 b	9.2	1384 b	1524 a
BRS Requinte	5.3 d	10.1	1383 b	1539 a
BRSMG União	4.6 c	10.1	862 b	959 d
CNFC 10762	5.4 d	11.2	1684 a	1896 a
BRS Expedito	5.2 d	12.8	1120 c	1285 b
WAF 75	7.2 e	17.2	703 e	849 d
BRS Marfim	5.0 d	19.0	1628 a	2010 a
BRS Valente	4.2 c	19.6	1155 c	1437 b
CNFC 10467	5.1 d	19.6	855 d	1061 c
BRS Executivo	4.2 c	20.6	487 e	613 e
BRS Supremo	5.6 d	24.7	858 d	1140 c
BRS Embaixador	6.8 e	40.7	729 e	1228 c

*- Values in the same column followed by the same letter are not different by Scott-Knott test ($p<0.05\%$)

CULTIVARS 'BESLET' AND 'DREZDEN' HAVE DIFFERENT GENES FOR RESISTANCE TO *COLLETOTRICHUM LINDEMUTHIANUM* ON LOCUS *CO-2*

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INTRODUCTION

Anthracnose, caused by the hemibiotrophic fungus *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams-Scrib., is the most serious disease on common bean worldwide due to its seed-borne nature and pathogenic variability. Genetic resistance is recognized as the most effective disease management strategy for the control of bean anthracnose. Most anthracnose resistance *Co*-genes, previously described as single major genes conferring resistance to several races, could be organized as clusters of different genes conferring race-specific resistance (Rodríguez-Suárez et al. 2007). The availability of information on the genetic construction of the source variety resistance would allow making the proper choice of parental components in breeding for durable resistance.
The aim of this investigation was to determine the genetic control of resistance to anthracnose in cultivars 'Beslet' and 'Drezden'.

MATERIALS AND METHODS

Plant material. Cultivars 'Beslet', 'Drezden', and 'Cornell 49-242' were investigated for the presence of a gene for resistance to anthracnose on the *Co-2* locus.
Inoculation procedure and disease scoring. Races 8 (kindly provided by Dr. Gonçalves-Vidigal) and 81 were cultivated on the medium of Mathur et al. (1950) at 18±1°C in dark for 10 days. The spore mass was washed with sterile distilled water and after filtration through gauze cloth the suspension was reduced to concentration 10^6 spores/ml.
Inoculated 10-day old plants were placed in a moist chamber for 96 hrs at 20±2°C, then transferred to greenhouse under the same temperature. The resistance reaction on the hypocotyls and the primary leaves was read 7-10 days after inoculation according to a 9-degree scale: 1- completely resistant; 9 – highly susceptible. Resistant phenotypes were considered grades 1 (no symptoms) and 3 (tiny black dots without sporulation) (Genchev, 1983).
Molecular-marker analyses. Genomic DNA was extracted from leaf tissue as described by Dellaporta at al. (1983). The presence of *Co-2* locus in cultivars 'Beslet', 'Drezden' and 'Cornell 49-242' was investigated using SCAR markers SCAreoli (Geffroy et al., 1998) and SQ4 (Awale et al., 2008). Amplifications were performed by PuReTaq™ Ready-To-Go™ PCR beads (GE Healthcare), according to the manufacturer's instructions. The PCR products were visualized on 1 % agarose gel.

RESULTS AND DISCUSION

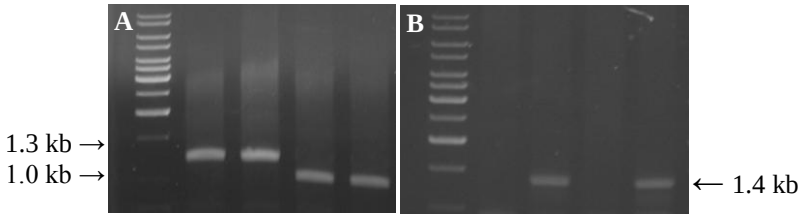
The new Bulgarian cultivar 'Beslet', cultivars 'Drezden' and 'Cornell 49-242' were investigated for the presence of *Co-2* locus. The locus is related with susceptible reaction to race 8 and resistant reaction to race 81 of *C. lindemuthianum*. In our experiments, the *Co-2* locus was found in varieties 'Beslet' and 'Cornell 49-242', according to the results of inoculation with race 8, and in cultivars

'Drezden' and 'Cornell 49-242', according to the results with race 81 (Table 1). The analysis with molecular marker SCAreoli showed the presence of *Co-2* in 'Beslet' and 'Cornell 49-242', but not in 'Drezden' (Fig.1A). Whereas molecular marker SQ4 determined the presence of *Co-2* locus in 'Drezden' and 'Cornell 49-242', but not in 'Beslet' (Fig.1B). Only in 'Cornell 49-242' the *Co-2* locus was confirmed by all four tests (races 8, 81 and both molecular markers). According to Park (1987) cv. 'Drezden' posses *Co-2* locus. The summarizing results for cultivar 'Beslet' were opposite of that obtained for cultivar 'Drezden'. Taken together our data suggest a cluster structure consisting of two genes for resistance in the *Co-2* locus and intra-cluster recombination. A cluster structure of locus *Co-2* has also been reported by Rodríguez-Suárez et al., (2007).

Table 1

Presence of the specific resistance gene Co-2 in cultivars 'Beslet', 'Drezden' and 'Cornell 49-242'

Physiological race or Molecular marker	Beslet	Drezden	Cornell 49-242
Race 8	+	–	+
Race 81	–	+	+
SCAreoli	+	–	+
SQ4	-	+	+



F**ig. 1** PCR amplification of SCAreoli (A) and SQ4 (B) SCAR markers cosegregated with the *Co-2* gene. (A/B) line 1 – 1 kb DNA Ladder; lines 2 – TO; line 3 – Drezden; line 4 – Beslet; line 5 – Cornell 49-242

The intra-locus recombination of the two resistance genes from locus *Co-2* of cultivars 'Drezden' and 'Beslet' is desirable for ensuring durable resistance.

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CHARACTERIZATION OF THE ANTHRACNOSE RESISTANCE GENE IN ANDEAN COMMON BEAN CORINTHIANO CULTIVAR

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INTRODUCTION

Anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. et Magn.) Scrib., is one of the most widespread and economically important fungal diseases of common bean (*Phaseolus vulgaris* L.). The use of resistant cultivars is the most efficient method to control common bean anthracnose, and it is important a continuing search for new resistance sources. Until now, ten resistant genes of Mesoamerican origin and three of Andean origin were identified. Among them, Andean genes (*Co-12, Co-13 and Co-14),* are those with greatest importance since they are present in common bean landraces in Paraná. Previous studies carried out at Nupagri-UEM, had shown that Corinthiano genotype, a landrace collected in small farms in Paraná State, is resistant to 2, 8, 23, 64, 65, 89, 73 and 2047 races of *C. lindemuthianum*. Therefore, mainly because of its resistance to 2047 race and for being an Andean cultivar, it is so important the genetic characterization of this cultivar for later inclusion in common bean breeding programs that search resistance sources against anthracnose. The present work aimed to characterize genetic resistance of Corinthiano Andean cultivar to races 8, 89 and 2047 of *C. lindemuthianum* through resistance inheritance study and allelism tests.

MATERIALS AND METHODS

The Andean common bean cultivar Corinthiano was crossed with Michelite, Michigan Dark Red Kidney (MDRK), Cornell 49-242, Mexico 222, PI 207262, TO, TU, AB 136, G 2333, Jalo Listras Pretas (JLP), Jalo Vermelho (JV), BAT 93, Ouro Negro, AND 277, H1 Line, Pitanga, and SEL 1308 to obtain F_2 populations. Parents, F_1 and F_2 of each cross, were spray-inoculated with standardized spore concentration (1.2×10^6 spores mL^{-1}), of each race of *C. lindemuthianum*, according to Cárdenas et al. (1964), using a De Vilbiss number 15 atomizer powered by an electric compressor. After inoculation, plants were maintained at high relative humidity (>95%) for 48 h at 21-23^{o}C. The inheritance test was conducted in F_2 population from cross between Corinthiano x Cornell 49-242 cultivars, inoculated with 2047 race. Allelism tests were applied to the crosses (R x R) where both cultivars resistance reaction to 8, 89 and 2047 races (Table 1), in order to evaluate the independence of the gene presented in Corinthiano cultivar from the other previously characterized. Symptom visual evaluation was done 10 days after inoculation, using a scale from 1 to 9 (Pastor-Corrales, 1991). Plants scoring from 1 to 3 were considered resistant, whereas 4 to 9 were susceptible. Genetic analyses of F_2 population were done by using Chi-Square test (χ^2).

RESULTS AND DISCUSSION

The inheritance study demonstrated a 3R:1S ratio in F_2 population from the cross between Corinthiano x Cornell 49-242 cultivars, inoculated with 2047 race. This fact indicates the presence of one resistant dominant gene in Andean cultivar Corinthiano. Allelism tests in the crosses involving Corinthiano with Michelite, Michigan Dark Red Kidney (MDRK), Cornell 49-242,

México 222, PI 207262, TO, TU, AB 136, G 2333, Jalo Listras Pretas (JLP), Jalo Vermelho (JV), BAT 93, Ouro Negro, AND 277, H1 Line, Pitanga, and SEL 1308 cultivars fitted a 15R:1S ratio, indicating the action of two dominant genes, one of them present in Corinthiano cultivar and the other in each one of the tested cultivars. The results of allelism tests indicated that the gene in Corinthiano is not allelic from those previously characterized.

Table 1. Allelism tests in F_2 populations from R x R crosses inoculated with races 8, 89 and 2047 of *Colletotrichum lindemuthianum*

Crosses	Race	Resistance Gene	Observed Plants		Expected Ratio	χ^2	P-Value
			R[a]	S[b]	R:S		
Corinthiano x Michelite	8	*Co-3*	93	6	15:1	0.006	0.94
Corinthiano x MDRK	8	*Co-1*	93	7	15:1	0.096	0.76
Corinthiano x México 222	8	*Co-3*	87	6	15:1	0.006	0.94
Corinthiano x PI 207262	8	*Co-4³; Co-3³*	92	7	15:1	0.114	0.74
Corinthiano x TO	8	*Co-4*	83	5	15:1	0.048	0.83
Corinthiano x TU	8	*Co-5*	94	6	15:1	0.011	0.92
Corinthiano x AB 136	8	*Co-6*	92	6	15:1	0.003	0.96
Corinthiano x BAT 93	8	*Co-3³*	92	9	15:1	1.220	0.27
Corinthiano x H1 Lineage	8	*Co-7*	94	6	15:1	0.011	0.92
Corinthiano x Ouro Negro	89	*Co-10*	81	5	15:1	0.028	0.87
Corinthiano x JLP	89	*Co-13*	93	6	15:1	0.006	0.94
Corinthiano x JV	89	*Co-12*	89	6	15:1	0.001	0.98
Corinthiano x G 2333	2047	*Co-4²*	238	18	15:1	0.267	0.61
Corinthiano x AND 277	2047	*Co-1⁴*	56	4	15:1	0.018	0.89
Corinthiano x SEL 1308	2047	*Co-4²*	94	6	15:1	0.011	0.92
Corinthiano x Pitanga	2047	*Co-14*	60	4	15:1	0.000	1.00

R[a] = Resistant; S[b] = Susceptible

CONCLUSION

It is concluded that Corinthiano cultivar is an important source of resistance against anthracnose, since it possesses a new dominant Andean gene. The allelism tests indicated that the dominant gene present in Corinthiano is independent from *Co-1, Co-1⁴, Co-3, Co-3³, Co-4, Co-4², Co-4³, Co-5, Co-6, Co-7, Co-10, Co-12, Co-13, and Co-14* genes. The authors propose that the anthracnose resistance gene in Corinthiano conferring resistance to races 8, 89 and 2047 be named as *Co-15*.

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NEW INSIGHTS INTO THE ANTHRACNOSE RESISTANCE OF COMMON BEAN DIFFERENTIAL CULTIVAR MEXICO 222

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INTRODUCTION

Anthracnose, caused by *Colletotrichum lindemuthianum* is one of the most important fungal disease of common bean (*Phaseolus vulgaris* L.), and has been shown to be particularly harmful in tropical and subtropical regions where temperatures are moderate to cold and the relative humidity is high (Vieira, 2005).

Mexico 222 is one of 12 diffential cultivars that were proposed to identify pathotypes of *C. indemuthianum* races, and presents the resistance *Co-3* gene on the linkage group B4 (Mendez-Vigo et al., 2005). Previous studies demonstrated the presence of a dominant gene in Mexico 222 conferring resistance to races 9 and 23 (Gonçalves-Vidigal and Kelly, 2006; Gonçalves-Vidigal et al., 2008). However, it was reported the presence of two independent genes in Mexico 222 conferring resistance to races 7 (Kelly and Vallejo, 2004; Sousa et al. 2009). Thus, the aim of this study was to investigate through allelism tests the independence of gene(s) presents is Mexico 222 and others cultivars.

Table 1. Resistance (R) and susceptibility (S) reactions of cultivar Mexico 222, MSU 7-1, H1 Line, and G 2333 to 7, 8, 23 and 64 *Colletotrichum lindemuthianum* races

Race	Mexico 222	MSU 7-1	H1 Line	G 2333
7	R[a]	R	R	R
8	R	R	R	R
23	R	-[c]	-[c]	R
64	S[b]	R	S	R

[a]R = Resistant; [b]S = Susceptible; [c] = No data available.

MATERIALS AND METHODS

The allelism tests were conducted with F_2 populations from the crosses Mexico 222 x Cornell 49-242, Mexico 222 x AB 136, Mexico 222 x H1 Line, Mexico 222 x PI 207262, Mexico 222 x BAT 93, Mexico 222 x MSU 7-1 under inoculation of race 7. Additionally, were carried out tests with the F_2 population derived from the crosses Mexico 222 x JLP with race 9, also Mexico 222 x G2333 inoculated with race 23, and Mexico 222 x H1 Line inoculating with race 64. Plants were evaluated for their disease reaction using a scale from 1 to 9 (Pastor-Corrales et al., 1995) 10d after inoculations.

RESULTS AND DISCUSSION

The allelism tests resulting of F_2 population derived from Mexico 222 x Cornell 49-242 and Mexico 222 x AB 136, both crosses inoculated with race 7 revealed the occurrence of segregation and independence of 3 dominant genes due to expected ratio of 63R:1S (Table 2). These results indicate that two genes are from Mexico 222 (*Co-3* and *Co-?*), while that one gene is present in Cornell 49-242 (*Co-2*) and AB 136 (*Co-6*).

Several lack of segregation were observed in F2 populations from the crosses between Mexico 222 with PI 207262, G 2333, MSU 7-1, H1 Line, and BAT 93, being these crosses inoculated with race 7. Additionally, other lack of segregation and susceptibility total was observed in F_2 individuals from Mexico 222 x H1 Line inoculated with race 64. Similar results over segregation not were found by Lima et al. (2008) studying F_3 population from Mexico 222 x H1 Line inoculated with race 8. Vallejo and Kelly (2009) working

with lines from MSU7 described that MSU 7-1 is more resistant than lines MSU7-3, MSU7-4, and MSU7-6 and only the MSU 7-1 carries the *Co-7* gene.

Results of F_2 population derived of Mexico 222 x G 2333 under inoculation of race 23 reveals segregation and suggests that there are three dominant genes, being two genes from G 2333, and one gene from Mexico 222 (*Co-3*). This explanation can be corroborated by similar results on presence of two dominant genes in G 2333 when inoculated with races 3 and 515 previously reported by Pathania et al. (2006).

Results obtained in F_2 population from the cross between Mexico 222 (*Co-3+Co-?*) with G 2333 ($Co\text{-}4^2$+ $Co\text{-}5^2$+*Co-7*) cultivar and H1 Line (*Co-7*), inoculated with race 7, did not show segregation. This fact suggests that genes denominated as *Co-7* present in cultivars G 2333, MSU 7-1 and H1 Line and a second gene in Mexico 222 confers resistance to race 7 of *C. lindemuthianum* are likely to be in the same locus. Gene *Co-3* in Mexico 222 that confers resistance to race 23 is not allelic to *Co-7*, since F_2 population from cross México 222 x G 2333, inoculated with this race segregated in a ratio of 63:1, suggesting the presence of a single gene in Mexico 222 (*Co-3*) and two genes in G 2333 ($Co\text{-}5^2$+$Co\text{-}4^2$). Similar results were obtained by Alzate-Marin et al. (2007) when the F_2 population from the cross Mexico 222 x PI 207262 was inoculated with race 23. Another strong evidence that Mexico 222 has an allelic gene to *Co-7* was observed when 100 F_2 individuals from cross Mexico 222 x H1 Line, inoculated with race 64, results only in susceptible plants. Therefore, based on these results, we propose that Mexico 222 carries the anthracnose resistance allele at the *Co-7* gene conditioning resistance to race 7, and it should be designated as $Co\text{-}7^2$.

Table 2. Allelic relationships of anthracnose resistance present in cultivar Mexico 222.

Crosses Mexico 222 with	Race	Resistance gene	Observed ratio		Expected ratio	χ^2	*P-Value*
			R[a]	S[b]	R:S		
Cornell 49-242	7	*Co-2*	98	2	63:1	0.124	0.72
PI 207262	7	$Co\text{-}3^3$/*Co-9*+$Co\text{-}4^3$	369	0	-	-	-
G 2333	7	$Co\text{-}4^2$+$Co\text{-}5^2$+*Co-7*	100	0	-	-	-
AB 136	7	*Co-6*	99	1	63:1	0.205	0.65
MSU 7-1	7	*Co-7*+*Co-5*	125	0	-	-	-
H1 Line	7	*Co-7*	100	0	-	-	-
BAT 93	7	*Co-9*	115	0	-	-	-
G 2333	23	$Co\text{-}4^2$+*Co-5*+*Co-7*	129	2	63:1	0.001	0.97
H1 Line	64	*Co-7*	0	100	-	-	-

[a]R = Resistant; [b]S = Susceptible.

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OCCURRENCE OF ANTHRACNOSE IN COMMON BEAN CULTIVARS COLLECTED IN THE STATE OF MINAS GERAIS – BRAZIL

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INTRODUCTION

Colletotrichum lindemuthianum (Sacc. and Magn.) Scribner is the causal agent of anthracnose in common bean. Anthracnose is one of the most severe diseases in this crop. Adoption of resistant cultivars is one of the main strategies to control this disease. However, durability of resistant cultivars could be unsuccessful due the great pathogenic variability exhibited by this phytopathogen. More than 100 races were described in the literature (Silva et al. 2007).

Knowledge about races prevalent in common beans regions producers can help breeders for a durable resistance to anthracnose. Therefore, the objective of this study was to investigate the incidence of anthracnose in bean-producing regions in State of Minas Gerais - Brazil.

MATERIAL AND METHODS

Forty two isolates from *C. lindemuthianum* were collected from 2008-2009 in three common bean-producing counties in Minas Gerais (Lambari, Patos de Minas and Lavras), Brazil. Small pieces of infected plant tissue were surface-sterilized and placed on Petri dishes containing M3 culture medium. Single conidia culture were obtained and maintained in M3 medium. Sixteen seeds from each of 12 differential cultivar proposed by CIAT (1990) were used for the pathogenicity test. Isolates were inoculated in bean pods culture medium and incubated at 22°C for 10-15 days in darkness to obtain high sporulation. Seedlings with fully expanded primary leaves were sprayed with the conidial suspension (1.2×10^6 conídios/mL). Inoculated plants were incubated in a humidity chamber at 22°C for 72 h with a 12 h photoperiod. After 7-10 days of inoculation, plants were evaluated using a scale from 1 to 9 (Schoonven & Pastor-Corrales, 1987). Plants with disease reaction scores from 1 to 3 were considered resistant, whereas plants that were scored 4 to 9 were considered susceptible. Identified races were assigned a value based on the binary nomenclature system proposed by Habgood (1970).

RESULTS AND DISCUSSION

Forty two isolates showed six different patterns of virulence when inoculated in the 12 differential cultivars (Figure 1a). Race 65 is the most frequent (35.7%) followed by race 81 (28.6%). These results confirmed previous results found by Silva et al. (2007) that showed higher percentage of races 65 (37.5%) and 81 (25%) in a population of 48 isolates collected in the same state in the past. Silva et al. (2007) and Ishikawa et al. (2008a) identified 10 and 12 different races, respectively. However for both studies the races, 65 and 81, were predominant. Although these races were the most frequent in the last years in this region, several studies demonstrated the existence of a great variability both genetic and pathogenic within races, especially, within the race or race 65 (Silva et al., 2007; Ishikawa et al, 2008b; Davide & Souza, 2009). Usually just one isolate from each race is inoculated and for this reason the selection of resistant cultivar becomes complicated. Other

interesting observation was obtained when analyzed the occurrence of races according the type of grain, carioca (Figure 1 b) or black (Figure 1c). For Carioca grain type was observed the predominance of races 65 and 81. However, to Black grain type a higher frequency of races 73 and 65 was observed. It is noteworthy that the samples collected from black grain type was lower (21% of total) compared to the Carioca type, since in this region there is a preference for the carioca grain type. But these results suggested that could be possible that a particular race is more often or not according of cultivars that are planted in the region. This work reaffirms the need for studies of pathogenic characterization of *C. lindemuthianum* in bean-producing regions to help breeding programs and to establish the best strategies and the main sources of resistance to be used.

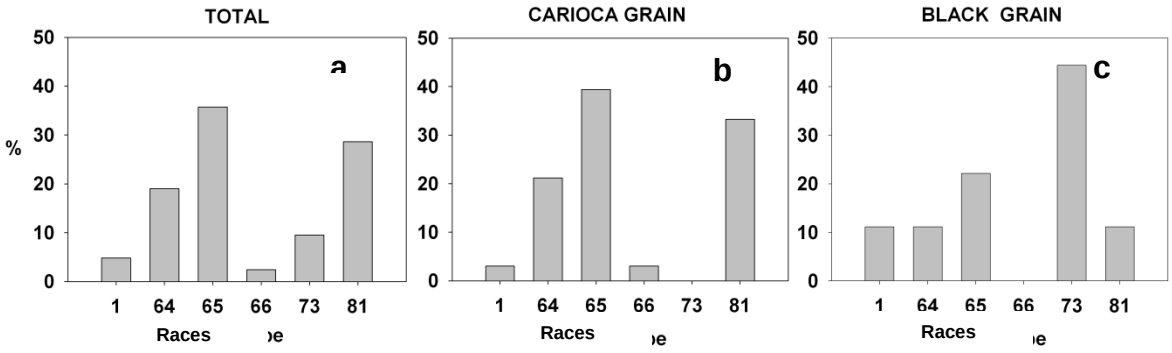

Figure 1. Percentage of *C. lindemuthianum* isolates belonging to different races identified in the State of Minas Gerais- Brazil. a) Total of characterized isolates; b) Isolates from Carioca type grain cultivars; c) Isolates from Black type grain cultivars.

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C. LINDEMUTHIANUM ISOLATE X COMMON BEAN GENOTYPE RELATIONSHIP ON RESISTANCE INDUCTION TO ANTHRACNOSE

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INTRODUCTION

One possible way of common bean anthracnose control is through plant resistance induction. Evidences on the efficiency of this methodology are available (Vieira et al. 1993; Campos et al. 2009). Insights on the relationship among *C.lindemuthianum* isolates and common bean genotypes on resistance induction are revealed in the present article.

MATERIAL AND METHODS

C.lindemuthianum Race 81 and isolates SC1 and SC5 from Santa Catarina State, Brazil, collected from common bean land races, as well as isolate ANT 03-09 obtained from the cultivar BRS Expedito, from location Sobradinho, Rio Grande do Sul State, at 2008 crop season, were the experimental material from *C. lindemuthianum* in this study. Twenty one genotypes with variated seed coat pattern constituted the common bean germplasm. The *C.lindemuthianum* genotypes were tested as resistance inductors against each of the other anthracnose genotypes on each of the common bean genotypes. Inoculum concentration was $1.2x10^6$spores.ml^{-1}. Eight seeds of each cultivar constituted individual plots according to the methodology designed by Ribeiro (2007). BOD growing chamber was the test environment. Experimental design was a RCD with four replications. Seedlings were inoculated with the avirulent *C.lindemuthianum* genotype four days after germination, followed by inoculation with the virulent genotype after 48h. At the 4th day after inoculation with the virulent genotype, seedling reaction was evaluated. Disease reaction was recorded through disease intensity scale application (Balardin & Pastor-Corrales 1990), and through the Disease Incidence Index (or McKinney Index), following Freire et al (1976). According to McKinney Index, values equal or above 0.5 represent a susceptible reaction whereas below 0.5 represent resistance.

RESULTS AND DISCUSSION

Results are shown in Table 1. The comparison of the reaction of individual common bean genotypes a) without and b) following previous inoculation with an avirulent genotype reveals that - for the race 81, the isolates SC1 and SC5 induced resistance in the cultivars Roxo Tavares, Preto 134. Preto Santa Rosa, Pintadinho Gostoso and Rosinha Precoce, SC1, additionally, induced resistance to race 81 in Pintadinho, Felipe and Balim Grosso; - for the isolate SC5, isolate SC1 and the race 81 were not able to induce resistance in the cultivars Macanudo and Pintadinho (on this cultivar. only the isolate SC1 has been evaluated); - for the isolate ANT 03-09. the isolates SC1 and SC5 induced resistance in the common bean genotype TB 98-20; the isolate SC1 and the race 81 induced

resistance in the cultivar BRS Expedito being the race 81 also able to induce resistance in the cultivar Macotaço.

Evidences of the not universal induction ability for a given group of avirulent *C.lindemuthianum* genotypes in relationship to a given group of common bean genotypes, are suggested.

Table 1 – Common bean genotype reaction to virulent *C.lindemuthianum* genotypes without and following inoculation with *C.lindemuthianum* avirulent genotypes (based on McKinney Index). Embrapa Clima Temperado. Pelotas. RS. Brazil. 2010.

Disease reaction					Disease reaction following inoculation with avirulent isolate[1]						
	C.lindemuthianum isolate										
Common bean genotype	RACE 81	SC1	SC 5	ANT 03/09	SC1 /81	SC1/ SC5	SC1/ ANT 03-09	81/ SC5	81/ ANT 03-09	SC5/ ANT 03-09	SC5/ 81
Macanudo	0.20	0.42	0.69	0.59		0.66	0.74	0.75	0.82		
Iapar44	0.28	0.29	0.43	0.78			0.64		0.58	0.59	
Macotaço	0.15	0.24	0.11	0.66			0.69		0.45	0.69	
BRS Expedito	0.16	0.29	0.24	0.70			0.42		0.32	0.67	
TB 98- 20	0.24	0.14	0.25	0.77			0.42		0.74	0.48	
Pintadinho	0.57	0.39	0.91		0.48	0.51					
Felipe	0.66	0.21	0.31		0.43						0.71
Balim Grosso	0.59	0.14	0.40		0.62						0.66
Mouro38	0.55	0.12	0.29		0.45						0.75
Roxo Tavares	0.76	0.17	0.36		0.32						0.38
Preto 134	0.80	0.11	0.36		0.29						0.33
Preto Santa Rosa	0.72	0.17	0.19		0.35						0.25
Pintadinho Gostoso	0.59	0.11	0.14		0.16						0.27
Rosinha Precoce	0.94	0.29	0.34		0.14						0.11

[1] Above line isolate represents the avirulent genotype

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PATHOGENICITY AND VIRULENCE STRUCTURE OF *COLLETOTRICHUM LINDEMUTHIANUM* ISOLATES

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INTRODUCTION

Anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.) Scribner is one of the most important diseases in common bean. *C. lindemuthianum* is known to vary greatly in its pathogenicity (Silva et al., 2007). Despite that, it is very difficult the development of durable resistance in common bean cultivars. Low durability of resistant cultivars leading investigations to detect the pathogenic diversity of *C. lindemuthianum* to establish the best method of disease control in the field. Therefore, the objective of this study was to analyze the pathogenic diversity and the virulence structure of *C. lindemuthianum* isolates in Lambari, Minas Gerais state, Brazil.

MATERIALS AND METHODS

Thirty-six isolates of *C. lindemuthianum* were collected in the experimental field in Lambari, Minas Gerais state, Brazil in 2008-2009. Small pieces of infected plant tissue were surface-sterilized and placed on Petri dishes containing M3 culture. Single conidia culture were obtained and maintained in M3 medium. Sixteen seeds from each of 12 differential cultivar proposed by CIAT (1990) were used for the pathogenicity test. Isolates were inoculated in pods culture medium and they were incubated at 22°C for 10-15 days in darkness to obtain high sporulation. Ten-day-old bean seedlings were sprayed with the conidial suspension (1.2×10^6 conidios/mL). Inoculated plants were incubated in moist chamber at 22°C for 72 h with a 12 h photoperiod.

Plants were evaluated, 7-10 days after inoculation, using a scale from 1 to 9 (Schoonven and Pastor-Corrales, 1987). Plants with disease reaction scores from 1 to 3 and 4 to 9 were considered resistant and susceptible, respectively . Identified races were assigned a value based on the binary nomenclature system proposed by Habgood (1970).

Phenotypic diversity was estimated using Simpson and Gleason indexes, according by Groth and Roelfs (1987) and the isolate complexity were determined according by Andrivon and Vallavieille-Poppe (1995) (Table 2).

RESULTS AND DISCUSSION

Thirty-six isolates analyzed in this study presented six patterns of virulence (Table 1). Estimates of diversity and complexity indexes are presented in Table 2. Ishikawa et al. (2008) analyzed 48 isolates of *C. lindemuthianum* collected in Minas Gerais state that were classified in nine different races. Simpson and complexity indexes were estimated for both populations and estimates were very similar. However, Gleason index estimate was higher in population analyzed by Ishikawa et al. (2008). These values were divergent because this index evaluates the different phenotypes number in the sample. Predominant races were same in both reports and there was not dominance of complex races. Ishikawa et al. (2008) identified races more complex, for example 329 and 337, but these

races were less often. In this present study, the races 73 and 81 were the most complex, because induced susceptible reaction in three differential cultivars. A probable hypothesis is that the stabilizing selection is carried out favouring races with less unnecessary virulence genes. These results confirm that *C. lindemuthianum* is pathogenically variable, in agreement with Silva et al. (2007). Therefore it is very important the knowledge of variability of this fungus to aid breeders in the choice of anthracnose resistance source.

Table 1. Races identification of *C. lindemuthianum* isolates collected in Minas Gerais State, Brazil, in 2008-2009.

Races	1	64	65	66	73	81
Number of isolates	1	7	14	1	4	9

Table 2. Formulate and estimates of Simpson, Gleason and complexity indexes of *C. lindemuthianum* isolates.

Indexes	Formula	Estimates	
		2008-2009	2004-2007*
Simpson	$Si = \sum [n_i (n_i -1)/N (N-1)]$	0.235	0.257
Gleason	$Gl = (r-1)/\ln (N)$	1.395	2.067
Complexity	$Ci = \sum (p_i \times v_i)$	2.139	2.128

n_i = isolates number of race i; r = Different phenotypes number in sample; N = Individuals number in sample, p_i = frequency of race i in population and v_i = virulence number of race
*Data obtained by Ishikawa et al. (2008)

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INHERITANCE OF RESISTANCE TO ANGULAR LEAF SPOT IN BEAN PODS

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INTRODUCTION

Angular leaf spot (ALS), caused by *Pseudocercospora griseola* (Sacc.) Crous & U. Braun, is one of the most important disease in common bean. The fungus shows great pathogenic variability which requires search and characterization of new sources of resistance. Knowledge of genetic reaction of bean cultivars to pathogen is essential in plant breeding programs for resistance. Usually, disease reaction is evaluated in the first trifoliate leaf. However, reaction has been shown to vary according to the organ plant studied. Previously, in Universidade Federal de Lavras the line ESAL 686 was obtained. This line is characterized by symptoms on the leaves but not on the pods. Thus, this work aimed to study genetic control of angular leaf spot reaction in pods and to estimate genetic parameters of breeding interest.

MATERIAL AND METHODS

ESAL 686 line (resistant) was crossed with Carioca MG cultivar (susceptible) to generate the populations F_1, F_2 and backcrosses, susceptible (BCs) and resistant (BCr). In dry season of 2009, parents and derived populations were evaluated in the field conditions under natural incidence of the pathogen. The number of evaluated plants was: 27 of ESAL 686, 38 of Carioca MG, 24 of F_1, 190 of F_2, 41 of BCr and 33 of BCs. Five evaluators used a diagrammatic scale to assess the severity on pods. From each plant were collected 4 pods randomly. Nine scale degree was based on: 1 – absence of pod symptoms; 2 - symptoms covered until 5% of pod area; 3 - symptoms covered 5-15% of pod area; 4 - symptoms covered 15-30% of pod area; 5 - symptoms covered 30-45% of pod area; 6 - symptoms covered 45-65% of pod area; 7 - symptoms covered 65-80% of pod area; 8: symptoms covered 80-90% of pod area; 9 - symptoms covered more than 95% of pod area. Average severity score of individual plants ≤ 3 were classified as resistant. It was analyzed the segregation of resistant and susceptible plants of F_2, as well as the genetic components of means and variance were estimated considering all populations. Model without epistasis was used for parameters estimate as described by Cruz et al. (2004).

RESULTS AND DISCUSSION

ESAL 686 line did not show symptoms of angular leaf spot on pods as expected. On the other hand, Carioca MG cultivar showed serious symptoms and was confirmed as susceptible. F_1 population was resistant like the resistant backcross (BCr) and ESAL 686. Susceptible backcross (BCs) showed higher severity (Table 1). The model used for estimating the mean components, containing only *m* (average of the contribution of the homozygous loci), *a* (the algebraic sum of the effects of the homozygous loci measured as deviations from the mean, additive effect) and *d* (deviations of the heterozygous from the mean, dominance effect), was sufficient to explain all observed variation. The estimates of the coefficient of determination (R^2) were higher than 99% and 85% for mean and variance components respectively (Table 1), indicating a well-fitting model. These results indicated that in the genetic control of angular leaf spot reaction did not occur epistasis (Ramalho et al., 1993).

Estimates of both additive and dominant effects were similar and important in the control of reaction on pods. Estimate of genetic effects were different from zero and the standard errors were low. Dominance effects (*d*) were in the direction of decrease the severity what is explained by negative estimate (Table 2). Additive variance ($\hat{\sigma}_A^2$) and dominant variance ($\hat{\sigma}_D^2$) estimates were higher than environmental variance ($\hat{\sigma}_E^2$). This result indicated the reaction on pods was less influenced by environment. Lower limit for the variance components were positives, thus estimates were different from zero (Table 2). Pods reaction heritability, in broad and narrow sense, was high. F_2 generation showed segregation of three resistant plants to one susceptible. Chi-square was not significant (Table 1). Segregation analyses suggest that one gene with dominant allele for resistance is involved in the control of character.

TABLE 1. Average severity of angular leaf spot (ALS) in bean pods, segregation analysis for reaction to *P. griseola* in the parents, ESAL 686 and Carioca MG, and in the populations derived from crosses.

Populations	ALS Severity	Expected ratio	Observed ratio	χ^2	P (%)
		R:S	R: S		
ESAL 686	1.0	1:0	27: 0		
Carioca MG	8.05	0:1	0: 38		
F_1	1.21	1:0	24: 0		
F_2	2.5	3:1	144: 46	0.06	80.16^{NS}
BCr	1.15	1:0	41: 0		
BCs	3.23	1:1	21: 12	2.45	11.72^{NS}

TABLE 2. Variance and mean genetical components, heritability in the broad and narrow sense estimates for ALS reaction in bean pods.

Mean components	Estimates ± Standard error	Variance components	Estimates
$\hat{m}$	4.46 ±0.04	$\hat{\sigma}_A^2$	1.57 [1.31; 1.91][1]
$\hat{a}$	-3.46 ±0.04	$\hat{\sigma}_D^2$	0.22 [0.17; 0.29]
$\hat{d}$	-3.28 ±0.07	$\hat{\sigma}_E^2$	0.15 [0.11; 1.21]
ALD[3]	0.95	*ALD*[3]	0.53
R^2	99.99	R^2	86.79
Heritability in broad sense	Estimate		
$\hat{h}_b^2$	0.92 ± 0.05[2]		
Heritability in narrow sense	Estimate		
$\hat{h}_n^2$	0.81 ± 0.30[2]		

[1]Lower and Upper Limits; [2]Associate error; [3]Average level of dominance.

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IMPROVEMENT IN SCREENING FOR RESISTANCE TO *SCLEROTINIA SCLEROTIORUM* IN COMMON BEAN THROUGH CHARACTERIZATION OF THE PATHOGEN

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White mold (*Sclerotinia sclerotium*) is a fungus that can persist in the soil for many years and has a host range of over 400 species. There are currently no sources of complete resistance to this fungus in common beans. Achieving repeatability of resistance expression in bean lines with putative sources of white mold resistance has been enhanced by screening in multiple locations using a white mold monitor nurseries in major bean production areas. The premise of screening these lines in multiple locations is to reduce variable screening results found in using only one location. One aspect of the variable results often recorded is how variable the pathogen is. To increase our understanding of the white mold pathogen variation in each location, isolates were collected from white mold field screening nurseries in eight states and two countries over 4 years and analyzed. The standard greenhouse house testing isolates used in nine states were also submitted. The genetic variation of the isolates was initially tested using mycelial compatibility groupings (MCGs). MCGs test the isolates for clonality by growing the isolates on a special medium. Two isolates are tested together on the media and if they grow together and form a continuous mycelial mat, they are considered compatible. If; however, the two isolates form a barrage line of dead cells where the hyphae met, the isolates are considered incompatible. The original 146 screening nursery isolates as well as the nine greenhouse isolates plus a control isolate 1980 (isolate sequenced by the Broad Institute) were tested using MCGs and a total of 64 MCGs were identified.

To increase our knowledge of pathogen variability, an additional 84 isolates were collected in 2007 from bean grower fields in Washington, North Dakota, Nebraska and Colorado. These isolates were tested against isolates in the previous 64 MCGs and an additional 22 MCGs were found for a total of 86 MCGs from 240 isolates.

Aggressiveness of the isolates was tested using a straw test developed by Petzolt and Dickson (1996). The spread of the pathogen from the infected stem was measured after 8 days and rated using a scale where 1 was least aggressive and 9 was most aggressive (Teran et al, 2005). The eight most aggressive MCGs with an average rating of 6 or higher came from 2 locations – North Dakota with 7 MCGs (highest straw test mean = 7.8) and Minnesota with 1 MCG (highest straw test mean = 6.2). The eight least aggressive MCGs came from Washington (2 MCGs with straw test mean of 3.8 and 3.2), Oregon (3 MCGs with straw test means of 3.7 to 2.9) and California (3 MCGs with means of 3.7 to 3.3). Isolates within an MCG did not differ significantly in aggressiveness, however; isolates in different MCGs were significantly different in aggressiveness.

To genetically characterize the population genotypes of these 240 isolates, a set of four microsatellite primers taken from a set of 25 developed by Linda Kohn (2000) were selected. The PCR products produced from these primers were sequenced. The microsatellite repeats were analyzed for number of alleles found at each locus (Table 1) and a set of 67 haplotypes were created using the allelic number at each loci.

The molecular variance (AMOVA) results confirmed the earlier findings from the MCGs that there is more variation within populations than between populations (Table 2). Sixty eight of the 86 MCGs had a single allelic heliotype and ten of the remaining 17 MCGs were only different in the 106 locus which had 20 observed alleles (Table 1). Analysis of the genetic diversity of these populations is continuing.

Table 1. Number of observed alleles at each locus.

Repeat motif	Locus	Number of observed alleles
$(GA)_{14}$	7	7
$(CT)_{12}$	12	4
$(CATA)_{25}$	106	20
$(TATG)_{9}$	110	3

Table 2. AMOVA* results

Source of variation	d.f	Sum of Squares	Variance components	Percentage of variation
Among groups	2	17.276	-0.01463 Va	-1.05
Among populations within groups	8	50.209	0.34479 Vb	**24.71**
Within populations	187	199.237	1.06544 Vc	**76.34**
Total	197	266.722	1.39559	

*****ARLEQUIN was used for calculating haplotype frequencies and for a hierarchical analysis of molecular variance (AMOVA) between and within populations

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MARKER ASSISTED SELECTION OF COMMON BEAN PLANT IN BACKCROSS POPULATIONS FOR WHITE MOLD RESISTANCE

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INTRODUCTION

One constraint of backcross method is the long time required for selecting the improved lines, when the recurrent parent may have become obsolete. The molecular markers may speed up the selection through the identification of plants with higher proportion of the recurrent genome, as well as those bearing the target alleles like QTLs for white mold resistance (Bouchez et al. 2002). The objectives were to transfer the QTL for white mold resistance identified by the *Phs* SCAR from the line G122 and associate with the favorable alleles of the line M20.

MATERIALS AND METHODS

The lines G122 and M20 were crossed. G122 is a line from Andean origin and has partial resistance to white mold. M20 is a line of carioca grain type, plant type II, and adapted to Southeast part of Brazil. There were generated 267 F_1 plants of backcross 1 (BC_1) and 113 plants of BC_2. DNA was extracted from the parents and each BC plant, and used for obtaining the SSR (simple sequence repeat) polymorphic markers (Pereira et al 2007). The relationship of BC plant and the recurrent parent was estimated based on the Sorensen-Dice genetic similarity (gs_{ij}) and the proportion of the recurrent genome (prg) in each BC plant (Benchimol et al. 2005).

RESULTS AND DISCUSSION

Polymorphic bands from 25 pairs of SSR primers in the 267 F_1BC_1 plants and the M20 recurrent parent were obtained. Results of genetic similarity and the proportion of the recurrent genome in the BC_1 plants were similar ($r_{(gsij,org)}$=0.99), indicating the average of 75% of alleles of the recurrent parent in the BC_1 population. The eight plants selected with higher proportion of the recurrent genome had 93% of the SSR alleles, and is similar to the average recurrent alleles proportion of BC_3 which is 93.75%. The 25 SSR primers amplified DNA fragments from 9 out of the 11 common bean chromosomes indicating that those estimates were efficient for accessing the relationship of the BC_1 plants and the M20 recurrent parent. Therefore the eight selected plants implied in a gain of two backcross generations.

Only 23 SSR primers detected polymorphism among the 113 F_1BC_2 plants and the M20 line. The average genetic similarity and average proportion of recurrent SSR alleles of the BC_2 population were similar ($r_{(gsij,org)}$=0.99) to the expected proportion for these generation of 87.5%. Although the BC_2 plants were not derived from those eight selected BC_1 plants, the five most related to the M20 line have an average of gs_{ij} = 94.8% and prg = 95.2%, which is similar to the average proportion of recurrent alleles of BC_4 (96.88%).

Concerning the selection of plants by the SCAR marker *Phs*, it showed up in four plants F_1BC_1 most similar to the recurrent parent, and in two of F_1BC_2. The F_1BC_1 selected plants have an average of

88% of the alleles from the recurrent parent, similar to the BC_2 generation. The two F_1BC_2 selected plants have an average of 91% of the alleles from the recurrent parent.

Considering the cost and time for generating the markers it would worth using them only in BC_1 because the selected plants are already very close to the recurrent parent. Unless in case of a very unadapted parent, like a wild donor, the marker assisted selection could be used in a more advanced BC.

ACKNOWLEDGEMENTS

CNPq and FAPEMIG

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PHYSIOLOGICAL RESISTANCE OF COMMON BEAN CULTIVARS AND LINES TO WHITE MOLD BASED ON OXALIC ACID REACTION

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INTRODUCTION

White mold caused by *Sclerotinia sclerotiorum* is an important common bean disease mainly in irrigated fields under intense cultivation in Brazil. Due to the prevalence of the fungus sclerotia in the soil for long time, and the existence of many weed and cultivated species susceptible to the pathogen, resistance is an important measure for helping to control the disease.

Measuring the physiological resistance of genotypes under natural infection is confounded by environmental factors, plant avoidance mechanisms like upright plant type, and pathogen variability. An indirect way of evaluating the physiological resistance is based on the reaction of the genotypes to oxalic acid which is fast and related to field resistance (Kolkman and Kelly 2000).

MATERIALS AND METHODS

Seventy eight cultivars and elite lines were used, i.e., 15 of black seed, 60 of carioca seed type, two from Andean origin, and the white mold resistance source G122 used as check. Twenty-one days seedling had its root system cut off and placed in a plastic tank with 20mM oxalic acid solution, with pH 4.0, for 15 to 20 hours mostly overnight at room temperature. Eleven experiments were set up, using seven or eight genotypes in each in 2009. The plot had 10 plants and replicated three times using the randomized completely design. The seedlings were rated for wilting symptoms using a 1 (no wilting) to 6 (plant death) scale (Kolkman and Kelly 2000). The average performance of the genotypes was grouped (P=0.05) using the Scott Knott (1974) procedure.

RESULTS AND DISCUSSION

Wide genetic variation was observed among the genotypes based on the combined analysis of variance (Table 1). The coefficient of variation was 13.7% and the heritability was 92.7%. Among the 18 most resistant genotypes are the check G122, 11 elite lines, two cultivars of carioca seed type, one black seed cultivar and two Andean lines (Table 1). The check G122 and the nine most resistant cultivars/lines to oxalic acid were also resistant to white mold after inoculation of the pathogen using the straw test (Singh and Terán 2008).

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Table 1. Average reaction of common bean cultivars and lines to oxalic acid, grouped (P=0.05) according to Scott and Knott (1974).

Cultivar/line	Average	Cultivar/line	Average	Cultivar/line	Average
CNFC 9506	4.83 a	CNFC 10722	3.03 d	RCII-14.27	2.37 e
CNFP 8096	4.23 b	CNFC 9504	3.00 d	VC-3	2.33 e
MAIV-18.264	4.20 b	CVIII-1	3.00 d	VP-23	2.30 e
CNFP 10798	4.10 b	CNFP 7994	2.97 d	RP-1	2.30 e
MAIV-15.204	4.10 b	RCII-2.21	2.97 d	Campeiro	2.30 e
CNFP 10773	4.07 b	Valente	2.93 d	CVIII-5	2.23 e
Pioneiro	3.83 c	BRS 8000	2.90 d	FP 3.47	2.23 e
MAIV-18.259	3.80 c	BP-31	2.90 d	FP 5.9	2.17 e
CVIII-39.24	3.67 c	MAIV-15.203	2.90 d	CVIII-3	2.07 f
CNFP 7966	3.67 c	RCII-6.14	2.90 d	MAIII-9.91	2.07 f
P1-103	3.67 c	P18-171	2.80 e	VC-15	2.07 f
Ouro Negro	3.67 c	RCII-2.2	2.77 e	BRS 9461	2.03 f
CNFC 10764	3.63 c	MAIII-17.185	2.77 e	G 122	1.97 f
MAII-2	3.63 c	Carioca	2.73 e	RP-2	1.97 f
Pérola	3,57 c	VP-21	2.70 e	MAII-22	1.93 f
CNFP 10802	3.53 c	CVIII-119.4	2.70 e	MAIV-18.266	1.90 f
MAIV-18.524	3.53 c	P18.163	2.63 e	CVII-85.11	1.80 f
CNFC 10720	3.43 d	VC-13	2.60 e	Cometa	1.80 f
CVIII-6	3.43 d	RCII-14.22	2.60 e	VC-16	1.77 f
VP-2	3.27 d	CVIII-2	2.60 e	CVIII-4	1.77 f
CNFC 9500	3.27 d	MAII-16	2.50 e	CVIII-7	1.77 f
VP-20	3.23 d	RCII-2.19	2.50 e	Supremo	1.70 f
VC-14	3.17 d	FP 5.3	2.47 e	Talismã	1.50 g
P5-7	3.13 d	MAIV-8.102	2.43 e	Majestoso	1.43 g
Carioca MG	3.10 d	RCII-10.26	2.43 e	CNFRJ 10564	1.30 g
MAIII-17.179	3.03 d	CNFP 9328	2.37 e	ESAL 550	1.03 g

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SEED YIELD OF PINTO SLOW DARKENING BREDING LINES IN CHIHUAHUA STATE, MÉXICO

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INTRODUCTION

In Chihuahua State —northern México— significant advances has been observed for total area planted with Pinto Saltillo bred cultivar in response to domestic bean market (Ávila *et al*., 2009). Based on farmers demand INIFAP´s breeding program developed several slow darkening improved lines, similar to Pinto Saltillo, but with larger seed size (> 36g/100 seeds). Validation is needed in order to evaluate adaptation of pinto bred lines across dry bean producing areas from Chihuahua. The objective was to select pinto improved lines based on earliness, disease tolerance, seed yield, slow darkening and seed size.

MATERIALS AND METHODS

During 2009, validation plots were planted at six locations in Chihuahua including five slow darkening pinto bred lines. Due to seed availability lines PT08033 (Pinto Bravo), PT08034 (Pinto Centenario) and PT08036 (Pinto Coloso) were used only at two locations (Table 1). Three improved cultivars were also included as the checks (Pinto Saltillo, Pinto Durango and Pinto Mestizo). Plots were planted in farmer´s fields in rows separated 0.8 m and a similar fertilization dose (25-35-00) was applied in all the plots. Planting date varied from June 3 (La Marta, Chih.) to July 26th (Nuevo Casas Grandes) according to water regime (irrigated or rainfed). Delayed plantings were caused by irregular rains observed during July and August. Data were taken for days to flowering, disease reaction and days to physiological maturity (CIAT, 1987). At maturity five equidistant field samples were taken consisting in two 5 m rows for yield and 100 seed weight determination. A Randomized Complete Block Design was used to obtain the Analysis of Variance (ANOVA) with five replications and mean range test was performed using Least Significant Difference ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Highly **s**ignificant differences ($p<0.01$) were observed among locations and lines for days to flowering, days to physiological maturity, seed yield and 100 seeds weight. Significance was also found for the line x location interaction. Higher seed yield was observed under irrigation at Baje de Agua (2,772 kg ha^{-1}) while lowest yield mean value was registered under rainfed conditions in Carbajal de Arriba (488 kg ha^{-1}). Highest yield value was observed for PT08004 (3,706 kg ha^{-1}) which surpassed the check Pinto Saltillo (3,387 kg ha^{-1}). Lowest yield value was observed for PT08034 in Carbajal de Arriba (329 kg ha^{-1}) due to delayed sowing date (July 17) caused by low rains. Pinto Saltillo registered the highest average yield across locations (1,617 kg ha^{-1}) and outstanding lines were PT08004 (1,542 kg ha^{-1}), PT08019 (1,361 kg ha^{-1}) and PT08018 (1, 309 kg ha^{-1}). According to farmers opinion selected lines (PT08004) also showed traditional taste present in pinto class, contrasting with Pinto Saltillo with light flavor and lower broth thickness.

Variations in seed size (100 seeds weight) were also detected among locations and higher values were observed in La Marta where PT08013 registered 36.5 g, contrasting with Pinto Saltillo (27.2 g). Similar seed size was observed among lines under rainfed conditions, then in Carbajal de Arriba PT08013 showed 26.3 g per 100 seeds and Pinto Saltillo 25.7 g. Seed size reduction in low water availability locations and years is an undesirable trait in pinto seed class in which higher seed size (> 36 g/100 seeds) is preferred. Lines show early flowering (35-47 days), maturity (84-95), and larger seed size (with maximum values greater than 36 g/100 seeds) than Pinto Saltillo (days to flowering: 41-60; Days to maturity: 90-117 and 25-27 g for 100 seeds weight). Compared to Pinto Saltillo, all the bred lines also showed similar slow darkening and disease tolerance. Some lines need to be used in further commercial plots plantings to establish its importance in improving Chihuahua State dry bean productivity.

CONCLUSIONS

Some improved pinto lines showed early maturity, high seed yields and larger seed size and could be recommended as an option to respond to farmers and market demand. Lines PT08004, PT08018 and PT08019 were selected for further yield evaluations in Chihuahua State.

Table 1. Combined analysis over locations for pinto bred lines planted in different locations of Chihuahua State. 2009.

Line/Cultivar	Bachiniva	Baje de Agua	La Marta	Nvo. Casas Grandes	Carbajal De Arriba	Benito Juárez	**Mean**
PT08004	1675	3706	1478	1057	618	716	**1542**
PT08013	1447	3448	1218	928	352	453	**1308**
PT08018	1900	2647	1355	743	443	765	**1309**
PT08019	1800	2734	1495	1052	509	576	**1361**
PT08034*	1553[b]	2290[c]	1394[c]	1055[a]	329[b]	736[a]	**1226**
Pinto Saltillo	1572	3387	1288	1614	859	981	**1617**
Pinto Durango	1483	2407	467	1363	407	864	**1165**
Pinto Mestizo	1533	1559	1121	1283	392	747	**1106**
Mean	**1620**	**2772**	**1227**	**1137**	**488**	**730**	
$DMS_{0.05\ among\ lines}$	275						
$DMS_{0.05\ among\ locations}$	151						
CV (%)	16.7						

*PT08033[a], PT08034[b] or PT08036[c].

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PINTO SALTILLO ADOPTION EFFECT ON DRY BEAN YIELDS IN DURANGO, MÉXICO

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INTRODUCTION. Significant increments in total area planted to Pinto Saltillo bred cultivar have been observed in recent years, in response to market demand for pinto beans. The large area planted to Pinto Saltillo explains yield fluctuations among locations and years. In 2009, a reduction in dry bean imports, led to an increase in the area planted to Negro San Luis black seeded cultivar (shiny rounded seeds) produced in Durango and Zacatecas. The objective was to evaluate the effect of Pinto Saltillo adoption on average yield and area planted to other seed classes in Durango, México.

MATERIALS AND METHODS. Randomized samples (size n= 31 to 38) of dry beans were taken in 2006-2009 in Durango´s main producing areas. Sampling plots were located in Los Llanos, north of Durango and Guadiana, Canatlán and Poanas´ Valleys. Sampling plots were randomly established each year as georeferenciated points across dry bean producing areas. Plots were visited after sowing, in order to avoid influence on cultivar selection, and then contact was established with farm owners. Weekly field trips were performed trough crop season and morphological and agronomic traits were registered to establish cultivar identity. Four equidistant samples, consisting in two rows with 5 m of length and 76 cm apart, were harvested at maturity in each plot to estimate seed yields. Cultivar characterization was made considering field and grain traits according to the *Phaseolus vulgaris* technical guide to conduct tests for distinctness, uniformity and stability (SNICS, 2001). Cultivar identification was made comparing plant and seed traits with those observed in main cultivars grown in Durango such as: Pinto Saltillo, Pinto Villa, Pinto Nacional, Negro San Luis and Negro Querétaro. When cultivar identification was difficult, seed commercial class (e.g. flor de mayo, bayo and canario) was used as a cultivar grouping trait. Frequency and seed yield for each cultivar and commercial class were then identified.

RESULTS AND DISCUSSION. Significant increments were observed in Pinto Saltillo frequency from one observation in 2006 to 21 in 2008 and 20 in 2009 (Table 1). Maximum frequency was observed for Pinto Saltillo in 2008, by the contrary, a significant reduction was observed for Pinto Villa frequency from 2006 (12) to 2009 (0), caused by the accelerated seed coat darkening that led to a rapid grain price decrease. Black seeded cultivars (Negro San Luis and Negro Querétaro) showed fluctuations among years in response to market demand. Other cultivars and seed classes observed in the sampling period were: canario (small-rounded, yellow seeds), Pinto Nacional, flor de mayo (pink seeds), flor de junio (pink striped seeds) and bayo (cream). Pinto Saltillo showed high yield variation among locations and years: from 89 kg ha^{-1} in Peñon Blanco in 2008 (due to low rains) to 2,062 kg ha^{-1} in Pánuco de Coronado in 2007, favored by rains and high input use.

Variation was also observed for Pinto Saltillo average seed yield among years: from 582 kg ha^{-1} in 2008 to 1,086 kg ha^{-1} in 2007. Higher seed yields were obtained by Pinto Saltillo in 2006-2009, compared to the average yield reported in Durango before 2006 (470 kg ha^{-1}) (SAGARPA, 2010). Pinto Saltillo high seed yield variation can be explained because marginal lands were also included. Another high yielding cultivar was Negro San Luis, planted mainly in locations with high annual rainfall records (450-500 mm), such as Cuauhtémoc and Guadalupe Victoria. The highest average yield for Negro San Luis (1,299 kg ha^{-1}) was observed in 2009, ranging from 338 to 2,079 kg ha^{-1}. Due to heavy rains, flooding, and maturity delay observed in several plots during 2008, its overall mean (440 kg ha^{-1}) was lower than those observed in 2006 (1,047 kg ha^{-1}), 2007 (898 kg ha^{-1}), and 2009 (745 kg ha^{-1})

Pinto Saltillo showed higher yields in some locations and has been preferred by consumers in the domestic and external markets. Considering market classes as a grouping criterion, pinto was the most popular seed type planted in Durango during 2006-2009. The main cultivar found in this class during 2008-2009 was Pinto Saltillo. Some commercial classes traditionally planted in Durango (such as bayo) were also found in 2009 samples.

CONCLUSIONS. The demand in domestic and external markets promoted rapid adoption of Pinto Saltillo bred cultivar and changes in the area share planted to different commercial classes of beans in Durango. Pinto Saltillo showed favorable influence increasing average dry bean yields in Durango. Despite market pressure, persistence of some landraces was still observed in traditional farm fields.

Table 1. Dry bean yields for different seed classes and cultivars in Durango, México, 2006-2009.

Year	Seed Class/Cultivar	Frequency and sample size (n)	Yield (kg ha^{-1})		
			Minimum	Maximum	**Average**
2006	Pinto Villa	12	372	1761	878
	Negro	13	309	2304	1,309
	Flor de Mayo	3	1,059	1,923	1,404
	Canario	3	945	1,582	1,216
	Flor de Junio	1	483	483	483
	Pinto Saltillo	1	993	993	993
	Average	**n = 33**	**694**	**1508**	**1,047**
2007	Pinto Saltillo	11	214	2,062	1,086
	Negro	10	476	1,982	1,120
	Pinto Villa	5	806	1,474	1,081
	Flor de Mayo	4	170	830	580
	Pinto Nacional	3	1,044	1,428	1,177
	Canario	2	555	1,126	841
	Flor de Junio	1	399	399	399
	Average	**n= 36**	**523**	**1329**	**898**
2008	Pinto Saltillo	21	89	1,599	582
	Negro San Luis	4	399	855	569
	Pinto Nacional	3	107	582	350
	Canario	2	90	211	151
	Flor de Mayo	1	547	547	547
	Average	**n= 31**	**246**	**759**	**440**
2009	Pinto Saltillo	20	112	1,558	800
	Pinto Nacional	2	869	1,084	976
	Pinto	1	479	479	479
	Negro San Luis	9	338	2,079	1,299
	Negro Querétaro	1	709	709	709
	Flor de Mayo	1	607	607	607
	Canario	3	212	818	460
	Bayo	1	628	628	628
	Average	**n= 38**	**494**	**995**	**745**

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ADOPTION AND ECONOMIC IMPACT OF PINTO SALTILLO IMPROVED BEAN CULTIVAR IN NORTH-CENTRAL MEXICO

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INTRODUCTION. North-Central is the main common bean (*Phaseolus vulgaris*) producing area in Mexico. Considering 1997-2008 average, the common bean planted area under rainfed conditions was mainly concentrated in the States of Zacatecas (666,191 ha), Durango (272,118 ha) and Chihuahua (140,063 ha) (SAGARPA, 2010). Risks of producing beans in this region sum 15.6%, reaching 167,707 ha damaged by several factors such as drought, floods and plant diseases. The state of Chihuahua shows the highest risk losing 23.6% of total area planted, followed by Zacatecas (16.1%) and Durango (10.2%). Region average for seed yield (597 kg ha^{-1}) and annual production volume (533,794 MT) are low, reaching a total production value of US $ 214,236,167. Pinto Saltillo bred cultivar was developed by INIFAP in order to reduce grain price losses caused by accelerated seed-coat darkening registered in traditional pinto cultivars. Pinto Saltillo also showed disease and drought tolerance, high grain yield and adaptation in several States included in the Semiarid Highlands of Mexico (Sanchez *et al.*, 2006). The objective was to determine adoption level and economic impact of planting Pinto Saltillo bred cultivar in North-Central Mexico.

MATERIALS AND METHODS. A survey was conducted for data collection using a questionnaire completed in a face-to-face interview. Sample size was determined with the equation proposed by Rojas (1982):

$$n = \frac{\left[\frac{Z^2 q}{E^2 p}\right]}{1 + \frac{1}{N}\left[\frac{Z^2 q - 1}{E^2 p}\right]}$$

Where:
n= sample size
Z= confidence level (1.96 for 0.95 %)
qp= variability (0.5)
E= precision level (0.08)
N= population (45,000)

Estimated sample size was n=450 (150 in each State) and random samples were taken for SAGARPA´s register of the farmers whom planted beans during 2008. Field work was carried out from September 2008 (Chihuahua and Durango) to March 2009 (Zacatecas).

RESULTS AND DISCUSSION. 65.9% of the farmers planting beans in the states of Chihuahua, Durango and Zacatecas used Pinto Saltillo bred cultivar and 34.1% sowed other bean cultivars and landraces. Most of the planted bred cultivars have been generated by the INIFAP´s common bean breeding program, such as Pinto Villa and Flor de Mayo Sol. High adoption level was observed for Pinto Saltillo varying from 93.3% in Durango, 82.8% in Chihuahua and 13.3% in Zacatecas (Figure 1). An important seed yield increase (averaging 68 kg ha^{-1}) was observed between Pinto Saltillo (764 kg ha^{-1}) and Pinto Villa (696 kg ha^{-1}) (Table 1). Difference in grain price was US $ 0.16 per kilogram: Pinto Saltillo 0.79 US $/kg versus 0.63 US $/kg for Pinto Villa. Considering seed yield and price, the production value per hectare for Pinto Saltillo (US $ 605) an increase of US $170 ha^{-1} was observed compared to Pinto Villa (US $435). Multiplying this increase in production value by the number of hectares sown with Pinto Saltillo in Chihuahua, Durango and Zacatecas

during 2008 (458,274 ha), an estimated increase of US $ 77,906,580 was observed for the regional income obtained for planting beans.

CONCLUSIONS. Pinto Saltillo showed an important adoption level in North-Central Mexico, mainly in Durango and Chihuahua States. Yield increments, preferential grain prices and market acceptance induced farmers to plant Pinto Saltillo bred cultivar. Outstanding economical impact was observed for Pinto Saltillo adoption.

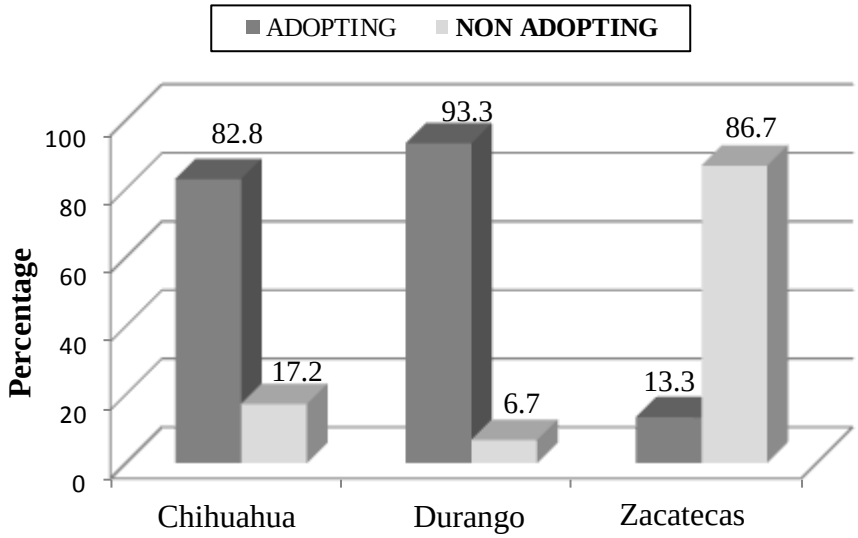

Figure 1. Percentage of farmers adopting and non adopting Pinto Saltillo bred cultivar in three States from North-Central Mexico.

Table 1. Estimated impact of adopting Pinto Saltillo common bean bred cultivar in North-Central Mexico. 2008.

	Common Bean Cultivar	
Component	Pinto Saltillo	Pinto Villa
Yield (kg ha^{-1})	764	696
Yield Increase (kg ha^{-1})	68	---
Production Value (US $ ha^{-1})[a]	605	435
Production Value Increase (US $ ha^{-1})	170	---
Planted Area (ha)[b]	458,274	90,291
Increase in the Value of Regional Production (US $)	77,906,580	---

[a] Average sale prices in 2008: Pinto Saltillo 0.79 and Pinto Villa 0.63 US $/kg
[b] Planted area 1,078,372 ha (average 1997-2008 according to SAGARPA, 2010).

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ECONOMIC EVALUATION OF THE INVESTMENT IN GENETIC BREEDING OF COMMON BEANS IN NORTH CENTRAL MEXICO

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is grown in 1.27 million hectares under rainfed conditions (June-October) in México, where 0.69 million MT, on average, are produced a year mainly of pinto, black, and flor de mayo seeded cultivars (SAGARPA, 2010). In the Mexican market, near to 0.4 million MT of pinto beans are annually demanded and due to yield variations caused by semi-arid climate, in many years there is a deficit in the domestic supply (Sánchez *et al*., 2001). Due to their market demand and favorable morpho-agronomic traits (such as higher yield, slow seed-coat darkening, and drought tolerance), the area planted to pinto cultivars has been increasing in the states of Chihuahua, Durango and Zacatecas, located in north central México. As a result of the investment in agricultural research made by the Mexican government through the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), specifically in genetic improvement of common beans, several bred cultivars has been released. Given the importance of agricultural research for economic development and of doing a proper use of scarce public resources, the objective was to make an economic evaluation of the investment in genetic improvement of common beans to produce, the pinto bred cultivars Pinto Villa, Pinto Mestizo, Pinto Bayacora, and Pinto Saltillo, released during 1990-2001, for the climatic conditions of north central México.

MATERIALS AND METHODS

The economic evaluation of research investment to generate new varieties of pinto beans in México, was carried out calculating the economic indicators commonly used of the net present value (NVP) and the internal rate of return (TIR). Data were obtained for releasing year, total adopting area and average yield and grain price. The NPV was estimated by discounting to the current moment (by means of a certain interest rate `k') all the future cash flow of the investment to generate each cultivar. From this value, the amount of the initial of investment is subtracted to obtain the net present value of the investment. The interest rate `k' selected was k= 9.5 %, taken as reference the fixed rent rate. If the NPV>0 the investment is profitable, since it would produce gains greater than the fixed rate `k'. If NPV<0 the investment is not profitable, since it would produce gains smaller than the fixed rate `k'. The TIR is an indicator of the profits of an investment, to greater TIR, greater profits. The TIR of an investment is defined as the interest rate to which the VPN is equal to zero.

RESULTS AND DISCUSSION

Pinto Villa bred cultivar was released in 1990, Pinto Bayacora and Pinto Mestizo in 1996 and Pinto Saltillo in 2001 (SNICS, 2010) (Table 1). Highest adopting area was observed for Pinto Saltillo (253,773 ha) and lowest value was observed for Pinto Bayacora (12,139 ha) (Gonzalez-Ramirez, 2003; Avila *et al.*, 2009). Lower yield and differences in growth habit (upright with short branches) compared to traditional pinto cultivars (prostrated type III) influenced adoption for Pinto Bayacora and Pinto Mestizo. In contrast adaptation, traditional growth habit (prostrated type III) and political situations caused rapid adoption of Pinto Villa and Pinto Saltillo cultivars. Pinto Villa planted area showed significant increase since the extinct government marketing company CONASUPO (Comisión Nacional de Subsistencias Populares) promoted production changes in Chihuahua State due to reduced consumer acceptation for brown striped seeded cultivars known as ojo de cabra (goat eye). Pinto Saltillo was also promoted to be planted in Durango State by government programs (Ávila *et al.*, 2009) to reduce economic losses caused by seed coat darkening. Estimated commercial yields for Pinto Villa was higher (697 kg ha^{-1}) compared to other bred cultivars such as Pinto

Saltillo (684 kg ha^{-1}), Pinto Mestizo (671 kg ha^{-1}) and Pinto Bayacora (655 kg ha^{-1}). Pinto Villa average yield was favored by drought tolerance and maturity adjustment observed in this cultivar. In contrast lower grain prices MXN $5.80 per kg (Avila *et al.*, 2003) and accelerated seed coat darkening caused disadoption of Pinto Villa in 2008 and 2009. Pinto Saltillo adoption was favored by higher grain prices paid to farmers (MXN $9.50 per kg with an interval from MXN $7.00 to $13.00 per kg). Consumer acceptance and improved market and cooking quality also influenced increase in Pinto Saltillo planted area in Chihuahua, Durango and Zacatecas. Economic analysis (Table 2) showed increase in yield (39-53 %) and grain prices (MXN $1.00 to $2.00) caused by improved bean quality and then benefits were obtained by farmers. Higher NPV were observed for Pinto Saltillo (MXN $2,118 million) and Pinto Villa (MXN $1,110 million). In spite of low NPV for Pinto Mestizo (MXN $57 million) higher value for IRR was observed in this cultivar (30.2 %) compared to Pinto Saltillo (29.0 %), Pinto Villa (27.3 %) and Pinto Bayacora (18.6 %).

CONCLUSIONS

Seed yield, market prices and maximum planted area were the main factors causing differences among cultivars for net present value and internal rate of return. Bean breeding program in north central México has been developing important and profitable technology.

Table 1. Traits observed for pinto common bean cultivars developed in north central México.

Cultivar	Releasing year	Adopting area (ha)	Average yield (kg/ha)	Average grain price ($/kg)
Pinto Villa	1990	212,800	697	5.80
Pinto Bayacora	1996	12,139	655	6.40
Pinto Mestizo	1996	22,664	671	6.40
Pinto Saltillo	2001	253,773	684	9.50

Tabla 2. Economic indicators of four pinto common bean cultivars released in north central México.

Variedad	Yield increase (kg)*	Price increase ($/kg)	Net present value (Million MXN)	Internal rate of return (%)
Pinto Villa	53	1.0	$1,110	27.3
Pinto Bayacora	39	1.5	$23	18.6
Pinto Mestizo	39	1.5	$57	30.2
Pinto Saltillo	40	2.0	$2,118	29.0

* Compared to Pinto Nacional.

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ASSESSING THE SOCIOECONOMIC IMPACT OF PARTICIPATORY PLANT BREEDING OF BEANS IN TWO REGIONS OF HONDURAS

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Common beans are Honduras' second most important basic grain crop after maize. As in all countries in Central America, the Honduran diet is based mainly on corn and beans, the major source of protein for poor households. During the past decades, bean farmers have been offered several improved varieties (IVs) to address biotic and abiotic stresses that reduce yields. However, while studies indicate that these varieties are planted on about 46% of Honduras' bean planted area, many small farmers, especially farmers producing in marginal areas, have not adopted them. It is estimated that small farmers account for about 40% of total bean production. Thus, if they adopted IVs, the impact would be substantial. Because of this, agricultural scientists have identified participatory plant breeding (PPB) as a strategy for increasing adoption and thereby extending the benefits of IVs to more farmers.

A study was conducted to: (1) examine the strengths and weaknesses of the PPB projects in two regions of Honduras; (2) empirically estimate the benefits and costs of PPB to farmers; and (3) generate recommendations to successfully scale up the PPB methodology in the country, if economically viable.

The study was conducted in two regions, which included the departments of Yoro (first region, called Yoro region), and Comayagua and Santa Bárbara (both in the second region, called Yojoa Lake region). Five communities in the first region and four in the second region were studied. The PPB project was implemented with farmers who were members of local agricultural research committees (CIAL). Thus, in these communities, all PPB-participants were CIAL members. Within each community, 50-100% of PPB-participants (N=60) were randomly selected for interview [and] a similar number of non-participants (non-CIAL) farmers were selected. Of the 120 farmers initially proposed for inclusion in the study, only 115 were interviewed and 108 surveys were valid (half in each region). The data were collected in 2006.

To evaluate the effect of PPB, both descriptive and econometric approaches were used. The surveyed farmers were disaggregated by PPB-participation (i.e. participants and non-participants) and by region (i.e. Yoro and Yojoa Lake). A single-equation linear regression model was estimated to evaluate factors associated with differences in farmers' yields. Conceptually, yields at time *t* were estimated using the following function:

$$Q_t = f(X_t, K_t, U_t, F_t, Z_t)$$

where yields (Q_t) depend on production-related variables, X_t, a number of project-related variables, K_t, socioeconomic variables, U_t, financial-related variables, F_t, and several quasi-fixed variables, Z_t. In addition, a net present value (NPV) analysis was done to determine whether investing in PPB was profitable.

The socioeconomic differences between PPB-participants and non-participants were non-significant for most variables. However, on average, heads of participant households were more

educated (3.7 yrs vs. 2.9 yrs of education; 10% significance level, SL) and had more access to credit (85% vs. 51%; 1% SL). Access to credit was higher for PPB-participants because they had access to credit through their respective CIAL or ASOCIAL, one of the benefits from participating in these farmer groups.

Contrary to the above, there were greater socioeconomic differences between regions. Households in the Yojoa Lake region were larger (5.5 vs. 4.7 members; 10% SL) and had almost one more adult with more than three years of education (1% SL). In addition, the share of adults with more than three years of education was higher (64% in Yojoa Lake vs. 41% in Yoro; 1% SL). In contrast, farmers in the Yoro region had more access to credit (76% vs. 61%; 10% SL)—possibly because communities in the Yoro region were closer to the closest small town, compared to communities in the Yojoa Lake region (9 km vs. 13 km, respectively).

Participant farmers, NGOs' staff, and scientists reported several strengths and weaknesses of the PPB project. The most important strengths were: (1) many varieties have been released and adopted through the communities, (2) farmers felt their expectations have been fulfilled, (3) capacity has been built, and (4) the time required for adoption of new varieties was reduced and adoption was increased. Similarly, the most important weaknesses were: (1) the need to increase direct contact with farmers, (2) seed of the new PPB varieties was not always available, (3) PPB was still in the experimental stage, and (4) PPB was dependant on the traditional breeding program.

The PPB project has benefitted farmers in many ways. However, we focus our discussion on three quantitative benefits: PPB knowledge acquisition, yields, and adoption levels. PPB-participants learned a great about the breeding process and this knowledge was spreading throughout the communities--many non-participant farmers learned about the breeding process perhaps from PPB participants. The yield regression showed that PPB varieties yielded, on average, 208 kg ha^{-1} more than landraces. In addition, conventionally bred varieties (i.e. varieties released by the breeding programs or IVs) yielded 621 kg ha^{-1} more than landraces. However, the adoption of IVs was very low in these areas: only 4% of the bean area was planted to IVs, while 32% was planted to PPB varieties. Thus, the impact of PPB varieties was greater than the impact of IVs. Low adoption of IVs was expected, since PPB was implemented in these communities because few farmers had adopted IVs. Furthermore, farmers in the Yojoa Lake region harvested 305 kg ha^{-1} more than farmers in the Yoro Region, which was expected since rainfall is higher in the former.

Finally, using the above yield results and assumptions about the bean price (\$0.68 kg^{-1}), CIAL size (10 farmers), costs of PPB and CIAL activities (PPB: \$305-\$1,995 per year in years 1-5; CIAL: \$700 per year), and discount rate (15%), it was estimated that the NPV of the PPB initiative was a little over \$5,100 per CIAL. Thus, it is profitable to invest in PPB.

The implications of these results are two-fold: *First*, the PPB methodology should be scaled up to other regions of the country because: (a) the PPB varieties yielded more than landraces; (b) adoption of PPB varieties was high; and (c) PPB was profitable (NPV>0). *Second*, to successfully scale up PPB, organizations should: (a) target marginal communities with different environmental conditions (efficient use of resources) and (b) additional funds should be made available for PPB--without available funding, PPB can not be scaled up (a weaknesses of PPB is that it requires more visits to farmers; therefore, if more farmers are added, the problem will be exacerbated).

GROWTH ANALYSIS, PHENOLOGY, HEAT UNITS AND GROWTH HABIT IN BEANS (*P. VULGARIS* L.)

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INTRODUCTION

Growth analysis has been a valuable tool in the quantitative analysis of crop growth (Hunt *et al*., 2002). This knowledge is of importance for the opportune agronomic handling and so to get a maximum crop production. In Mexico, the reports on growth analysis in dry bean (*Phaseolus vulgaris* L.) based on the time have been presented by Escalante and Kohashi (1982), Despite that studies have demonstrated the usefulness of temperature indices, like growing degree days or heat units, for predicting crop growth and development, its uses there are not been generally extend to growth analysis (Russelle *et al*., 1984). The aim of the study was that under conditions of semiarid climate to determine in dry bean of different habit growth: a) the heat requirement in each phenogical stage b) the curve of accumulation of dry matter, and c) the absolute rate of growth based on the temperature index.

MATERIALS AND METHODS

The study was realized under conditions of field and rainfed in Montecillo Méx (semiarid climate) in a clay soil. The cultivar Cacahuate 72 of determinate growth habit (DGH) type I of pink flower and grain extended to cream with red rays and Michoacán 12-A-3 (Michoacán) of indeterminate growth habit (IGH) bush of mulberry flower and black grain color, were seeded at May 8, 2006 to 13.3 plants m^{-2} and 100-100-00 of NPK. The phenology was registered as: V0 = germination; V1 = seedling emergence; V11 = ten trifoliolate leaves; R6 = opening of the first flower; R7 = pod growth beginning; R8 = seed filling; R9= physiological maturity. Every 10 days from seedtime until the physiological maturity three plants by experimental unit were harvest to determine the dry matter (DM) total and the seed yield (8% of humidity). The crop growth rate (TCC, g m^{-2} ºC d) was calculated with the equation: W2-W1/HU2-HU1, where W2 and HU2, W1 and HU1, are DM and HU (heat units, ºCd) in time 2 and time 1, respectively. The temperature base was 10ºC.

RESULTS AND DISCUSSION

Both cultivars only showed differences in the phenological stages after of V11 stage. Thus, from V0, the V1 stage it happened to 7 days after seedtime (DDS), V3 and V11 to 38 and 44 DDS with 60, 320 and 370 ºCd, respectively. For Cacahuate and Michoacán, the R6 stage happened to 45 and 56 DDS with 379 and 471 ºCd, R7 to 49 and 59 DDS with 412 and 496 ºCd; R8 to 64 and 74 with 538 and 622 ºCd and R9 to the 98 and 120 DDS with 790 and 1009 ºCd, respectively. In figure 1, it is observed that in both genotypes the DM accumulation in relation to HU followed a sigmoid tendency. The CGR of Cacahuate vegetative stage went slightly superior to Michoacán. In contrast, the CGR of Michoacán reproductive stage was highest that Cacahuate genotype (figure 2). To physiological maturity (R9) the Michoacán DM (308 gm^{-2}) went 27% superior to Cacahuate (280 gm^{-2}). Similar tendency was observed in the seed yield, which was of 148 g m^{-2} in Michoacán and 109 g m^{-2} in Cacahuate.

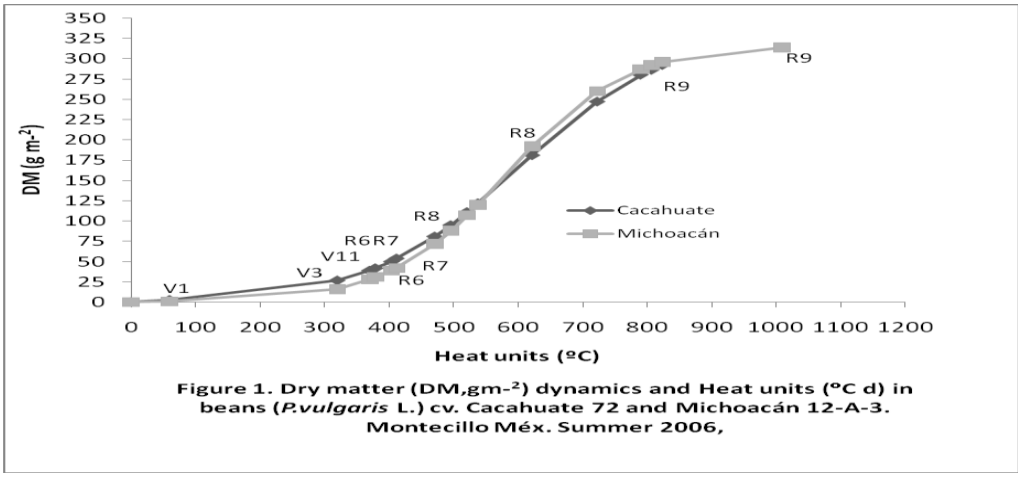

Figure 1. Dry matter (DM,gm-²) dynamics and Heat units (°C d) in beans (*P.vulgaris* L.) cv. Cacahuate 72 and Michoacán 12-A-3. Montecillo Méx. Summer 2006,

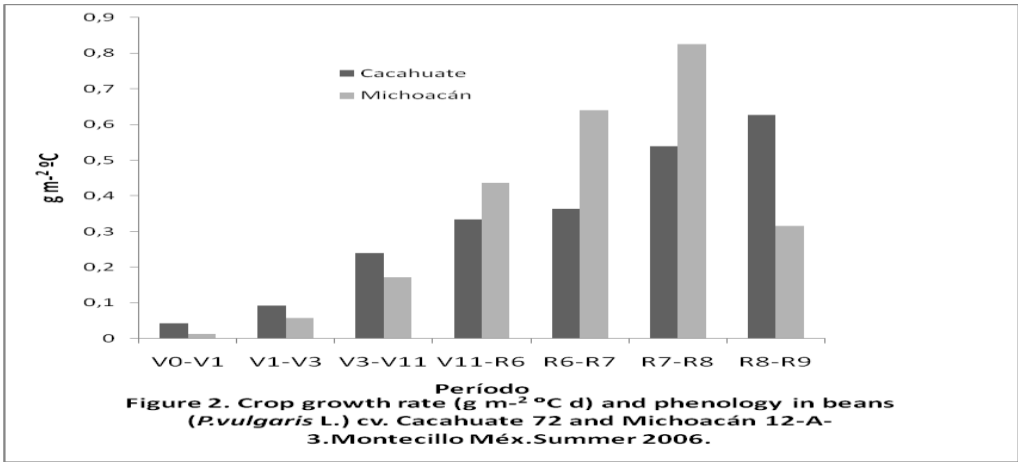

Figure 2. Crop growth rate (g m-² °C d) and phenology in beans (*P.vulgaris* L.) cv. Cacahuate 72 and Michoacán 12-A-3.Montecillo Méx.Summer 2006.

CONCLUSIONS

Exist differences by growth habit of dry bean in the occurrence of phenological stages, heat accumulation, crop growth rate, dry matter production and yield. The Michoacán bean of indeterminate habit presents higher crop growth rate in the reproductive period. The Cacahuate bean of determinate habit presents higher crop growth rate in the vegetative period. The biomass and seed yield is highest in Michoacán genotype of indeterminate habit.

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AGRONOMICAL PERFORMANCE OF COMMON BEAN CROPPED UNDER AGROECOLOGICAL MANAGEMENT SYSTEM

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) plays key social and economic roles on the Brazilian society, since it is an important source of protein for the poorer population (SILVA and DEL PELOSO, 2006). On common bean cropping, green manures may reduce or eliminate the use of N fertilizers, ensuring the conservation of the natural resources and reducing production costs. Besides, the use of green manure associated to conservative management practices increase soil organic matter, which contribute to a more sustainable agriculture.

MATERIALS AND METHODS

Aiming to evaluate the effect of soil management and cover crops on common bean, a field experiment was carried out at the National Rice and Beans Research Center of Embrapa, located in the county of Santo Antônio de Goiás, Goiás, Brazil. Common bean was cropped after Sunn hemp (*Crotalaria junceai)*, Velvet bean (*Mucuna aterrima*), Pigeon pea (*Cajanus cajan*), Sorghum (*Sorghum bicolor* L.) and Fallow (spontaneous plants) under conventional tillage (CT) and no-tillage (NT) management. Three common bean plants were randomly collected per plot at the V4 stage. To determine the number of nodules (NN) they were separated from the roots and counted. Thus, nodules were cut to determinate the percentage of active nodules (%AN) with basis on the presence of leghemoglobin. Stem fresh (SFW) and dry weight (SDW), leaf fresh (LFW) and dry weight (LDW) and the leaf area index (LAI) were also evaluated by determining the fresh and dry mass of the aerial plant parts and, grain yield (GY) was determined at 13% of humidity.

RESULTS AND DISCUSSION

Soil management systems showed significant effect on the NN, %AN, SDW and GY, although it had not been observed effect of the cover crops for SFW and GY. For the NN and %AN significant effects of the cover crops were only observed within NT, in which Velvet bean showed greater NN than Pigeon pea and, greater %AN than pigeon pea and Sorghum (Table 1). There were observed many significant correlations among the agronomical attributes of common bean, however, GY was only affected by NN and %AN (Table 2). These results partially corroborate the findings of CARVALHO and AMABILE (2006), in which any factor affecting plant growth will influence the biological nitrogen fixation, and vice versa since the association is a symbiotic system in which both partners are interdependent. Although many reports have been shown a positive correlation between nodules mass and fixed N, great number of nodules will not necessarily result in great amounts of fixed N and grain yield (CARVALHO, 2002).

Table 1: Number of nodules (NN – nº plant^{-1}), percentage of active nodules (%AN), stem fresh weight (SFW – g plant^{-1}), stem dry weight (SDW - g plant^{-1}), leaf fresh weight (LFW - g plant^{-1}), leaf dry weight (LDW - g plant^{-1}), leaf area index (LAI – m^2 m^{-2}) and grain yield (GY – ton ha^{-1}) of common bean cropped under different cover crops and soil management systems.

Soil management	Cover crops	NN	%AN	SFW	SDW	LAI	LFW	LDW	GY
CT	Sunn hemp	17.8 a	10.75 a	6.06 a	0.54 a	2.44 a	9.89 a	1.33 a	1.40 a
	Pigeon pea	4,3 a	6.25 a	6.69 a	0.56 a	2.81 a	11.30 a	1.45 a	1.48 a
	Velvet bean	15.8 a	10.00 a	7.73 a	0.60 a	3.38 a	13.64 a	1.72 a	1.82 a
	Sorghum	17 a	7.50 a	6.17 a	0.54 a	2.78 a	10.68 a	1.39 a	1.51 a
	Fallow	18.5 a	2.50 a	8.77 a	0.80 a	3.66 a	14.88 a	2.05 a	1.45 a
	Mean	14.7 B	7.40 B	7.08 A	0.61 B	3.01 A	12.07 A	1.58 A	1.53 B
NT	Sunn hemp	56.3 ab	47.50 ab	7.72 a	0.81 a	3.40 a	12.82 a	1.86 a	1.94 a
	Pigeon pea	24.5 b	17.00 b	6.64 a	0.68 a	2.88 a	10.32 a	1.47 a	1.96 a
	Velvet bean	71.3 a	57.50 a	9.23 a	0.96 a	3.41 a	14.80 a	2.11 a	2.07 a
	Sorghum	33 ab	17.50 b	7.36 a	0.72 a	3.16 a	12.58 a	1.81 a	2.09 a
	Fallow	49.8 ab	40.00 ab	6.62 a	0.61 a	2.76 a	10.10 a	1.34 a	2.07 a
	Mean	47 A	35.90 A	7.51 A	0.75 A	3.13 A	12.12 A	1.71 A	2.03 A

Values in the column, within soil management, followed by the same lower case letters and, mean of soil management followed by the same upper case letters, are not different by the Scott-Knott test ($p<0.05$).

Table 2: Pearson correlation coefficients among agronomical attributes of common bean cropped under different cover crops and soil management systems.

	NN	%AN	SFW	SDW	LFW	LDW	LAI	GY
NN	1							
%AN	0.97**	1						
SFW	0.50^{ns}	0.40^{ns}	1					
SDW	0.74*	0.65*	0.88**	1				
LFW	0.31^{ns}	0.21^{ns}	0.95**	0.75*	1			
LDW	0.47^{ns}	0.35^{ns}	0.96**	0.89**	0.95**	1		
LAI	0.33^{ns}	0.23^{ns}	0.90**	0.75*	0.94**	0.92**	1	
GY	0.74*	0.72*	0.29^{ns}	0.49^{ns}	0.15^{ns}	0.28^{ns}	0.26^{ns}	1

** - significant correlation ($p<0.01$); * - significant correlation ($p<0.05$); ^{ns} – non significant correlation.

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RESPONSE OF COMMON BEAN TO DIFERENT DIAZOTROPHIC BACTERIA AND COVER CROPS

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INTRODUCTION

Nitrogen is a key limiting nutrient for agriculture. The association among diazotrophic bacteria and leguminous plants, such as common bean (*Phaseolus vulgaris* L.) allied to the use of green manures are important technologies on the basis of the sustainable agriculture. Many rhizobial strains have been indicated as common bean inoculants, however, their N_2-fixing efficiency are relatively low since the amount of the fixed N is not enough to plant growth (SILVA AND DEL PELOSO, 2006). In this way, the use of green manure may supply this lack of N necessary to reach the complete plant growth.

MATERIAL AND METHODS

Aiming to evaluate the effect of the inoculation with a commercial common bean inoculant and of a rhizobial strain from Embrapa Agrobiology, a field experiment was carried out at the National Rice and Beans Research Center of Embrapa, located in the county of Santo Antônio de Goiás, Goiás, Brazil. P-enriched (Pe) and P-non enriched (Pne) seeds of common bean, cv. BRS Ouro Negro, were inoculated with commercial common bean inoculant (BR 520 + BR 322) and Embrapa Agrobiology strain (BR 293) and planted after sunn hemp (*Crotalaria juncea*) and fallow (spontaneous plants). Three common bean plants were randomly collected per plot at the V4 stage to determinate the number of nodules (NN), the percentage of active nodules (%AN) with basis on the presence of leghemoglobin, the number of pods (NP), the number of grain per pod (NGP), the 100 grain weight (100GW), the leaf index area (LAI) and grain yield (GY) was determined at 13% of humidity.

RESULTS AND DISCUSSION

Common bean cropped after sunn hemp showed greater LAI, %AN, NP, 100GW and GY than after fallow (Table 1). According to STONE AND MOREIRA (2001), the use of sunn hemp as green manure can increase LAI and GY. Besides, greater GY could be also a result of a greater activity of the nodulation (%AN), although under green manure the contribution of the biological nitrogen fixation in relatively low (RONDON et al., 2006). This assumption was corroborated by the results of G under sunn hemp, in which it were not observed significant differences among seed treatments (Table 2). Under fallow, the inoculation of P-enriched seeds of common bean with the rhizobia strain BR 293 resulted in significant difference to Ni treatments, while commercial inoculant (BR520+BR322) did not show significant difference from these ones. Since BR329 was only different from Ni treatments when inoculated on P-enriched seeds, P-enrichment can be considered an efficient strategy to promote best N_2-fixing results.

Table 1. Stand (plants m^{-1}), leaf index area (LAI – m^2 m^{-2}), Number of nodules (NN – nº $plant^{-1}$), percentage of active nodules (%AN), Number of pods (NP - nº $plant^{-1}$), Number of grains (NG - nº $plant^{-1}$), 100 grain weight (100GW - g) and grain yield (GY – kg ha^{-1}) of common bean cropped under different cover crops and inoculants.

	Stand	**LAI**	**NN**	**%AN**	**NP**	**NG**	**100GW**	**GY**
Sunn hemp	8.50 a	0.80 a	45.46 a	67.96 a	7.28 a	3.85 a	25.77 a	2344.83 a
Fallow	8.39 a	0.55 b	37.80 a	54.44 b	3.70 b	3.88 a	22.38 b	1535.13 b
CV (%)	**11.83**	**27.11**	**30,03**	**24,44**	**27,36**	**11.83**	**9.62**	**22.34**

CV (%) – Coefficient of variation.
Values in the column followed by the same letter are not different by the Tukey´s test ($p<0.05$).

Table 2. Interaction of the cover crops and inoculation on the grain yield of common bean.

Cover crops	**Seed treatments**	**Grain yield (kg ha^{-1})**
Sunn hemp	BR293+Pe	2705.67 a
	BR520+BR322+Pe	2516.17 a
	BR520+BR322+Ni	2485.64 a
	BR293+Ni	2256.83 a
	Ni+Pe	2241.17 a
	Ni+Pne	1863.72 a
Fallow	BR293+Pe	2330.67 a
	BR520+BR322+Pe	1748.25 ab
	BR520+BR322+Ni	1703.33 ab
	BR293+Ni	1694.00 ab
	Ni+Pe	1011.75 b
	Ni+Pne	722.75 b
CV (%)	**22.34**	

Ni – non inoculated, Pe – P-enriched, Pne – P-non enriched, CV (%) – Coefficient of variation. Values in the column, within cover crops, followed by the same letter are not different by the Tukey´s test ($p<0.05$).

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EFFECT OF GREEN MANURE SPECIES AND THEIR SOWING DATES ON COMMON BEAN CROP

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INTRODUCTION

Nitrogen is the most required nutrient by common bean (*Phaseolus vulgaris* L.). Although common bean can obtain this nutrient from atmosphere by the biological nitrogen fixation (BNF) process, the fixed amounts are not enough to supply all plant necessities (SILVA AND DEL PELOSO, 2006). Under agroecological production systems, part of the required N could be supplied by green manures as an alternative to fertilizers. However, many green manures species show distinct behavior to day length (photoperiod), which causes significant shifts on the phytomass production (CARVALHO AND AMABILE, 2006).

MATERIAL AND METHODS

Aiming to evaluate the effect of different sowing dates (November 28th 2007, January 2nd and February 26th 2008) of green manures (*Crotalaria juncea*, *Crotalaria ochroleuca*, *Cajanus cajan*, *Canavalia ensiformis* and *Mucuna aterrima*) on common bean crop, a field experiment was carried out at the National Rice and Beans Research Center of Embrapa, located in the county of Santo Antônio de Goiás, Goiás, Brazil. Common bean was planted after green manures flowering. The experiment was performed on a randomize block design with three replicates. For each green manure sowing date it were evaluated dry mass (DM) of green manures, number of pods (NP), number of grain per pod (NGP), number of grain per plant (NGPl), 100 grain weight (100GW) and grain yield (GY).

RESULTS AND DISCUSSION

Among the green manures, *C. juncea*, *C. ochroleuca* and *C. cajan* showed greater DM on the first sowing date, *M. aterrima* on the third one and, no effect of sowing date was observed for *C. ensiformis*. *C. juncea* and *C. ochroleuca* showed greater DM production than *C. cajan* and *M. aterrima*, however, GY of common bean had not a direct relation with green manure DM (Figure 1). According to HUXHAM et al. (2005), differences in grain yield may not be directly related to the effect of green manures on nitrogen availability or weed suppression, but to their impact on soil structure. Green manure sowing dates did not affect NP and NGPl, while NGP, 100GW and GY were greater on the second sowing date as compared to the other two ones. Significant Pearson correlations were observed among the studied parameters of common bean crop, however, only NP and NGPl showed a significant and positive correlation with common bean Y (Table 1).

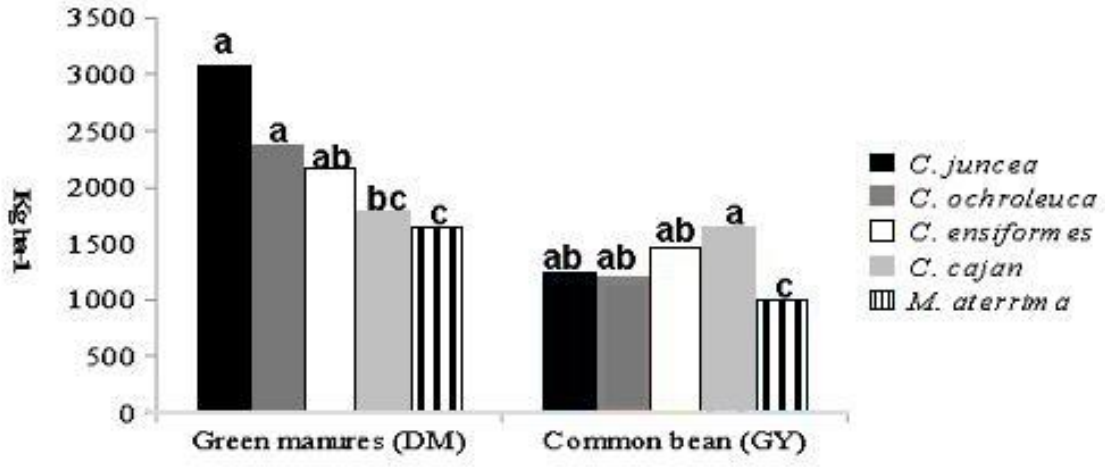

Figure 1: Green manure dry mass (DM) production and grain yield (GY) of common bean cropped after *Crotalaria juncea*, *Crotalaria ochroleuca*, *Cajanus cajan*, *Canavalia ensiformis* and *Mucuna aterrima*.

Table 1: Pearson correlation coefficients among agronomical attributes of common bean.

	NP	NGP	NGPl	100GW	GY
NP	1				
NGP	-0.05 ns	1			
NGPl	0.79**	0.56**	1		
100GW	0.01 ns	0.12 ns	0.06 ns	1	
GY	0.33*	0.11 ns	0.33*	0.17 ns	1

** - significant correlation ($p<0.01$); * - significant correlation ($p<0.05$); ^{ns} – non significant correlation.

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RESIDUAL EFFECT OF CORN FERTILIZATION ON BEAN CROP PRODUCTIVITY

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INTRODUCTION

The crop bean plant (*Phaseolus vulgaris* L.) is explored in different areas of Brazil. It is an important protein source, especially for the poorer population. The national bean production in 2006 was 3.5 million tons, and the Paraná state participated with 23.7% of this production (IBGE, 2007). In Brazil, the use of residues (prunes of the trees) *in natura* or composed in the soil organic fertilization is a frequently practice used because provides the liberation of nutrients to the plants without causing great environmental impacts. This work was carried out with the objective of evaluating the residual effect of the corn fertilization on bean crop productivity.

MATERIAL AND METHODS

The experiment was carried out in the experimental area of the State University of Londrina, Londrina, Paraná, Brazil. The experimental design was in randomized blocks with three repetitions. The treatments resulted of a factorial arrangement 4x2x2, where the factors were four doses of organic residues (0, 15, 30 and 45 Mg ha^{-1}), two fertilization levels (without and with fertilization (80, 50 and 30 kg ha^{-1} of N, P_2O_5 and K_2O, respectively) and two Carioca bean varieties (IPR-Colibri and IPR-Eldorado). Before the bean cultivation, the experimental area was used for corn cultivation. The evaluated variables were: mass of grains per plant and productivity. The obtained data were submitted to variance analyses and the averages were compared by Tukey test at 5% or adjusted to regression equations.

RESULTS AND DISCUSSION

The mass of grains/plant and the productivity of the bean culture was influenced by the interaction among fertilization type and bean variety (Table 1). The highest values were always obtained with organic mineral fertilization use and always for variety IPR-Colibri. The obtained productivities were higher than the medium productivity of the State of Paraná, that is 1.38 kg ha^{-1} (IBGE, 2008) but it was below to the potential productive of evaluated cultivate. In this case, 3.9 kg ha^{-1} for IPR Colibri (IAPAR, 2004) and 2.9 kg ha^{-1} for IPR Eldorado (IAPAR, 2007).

One of the causes of this difference was the antracnose incidence, mainly in cultivating IPR Eldorado. These results are in agreement with those presented by several researchers (Andrade et al. 2004; Almeida et al., 2000 and Arf et al., 1999). Andrade et al. (2004) verified that the application of doses of N, P and K in three varieties of bean plant increased significantly the mass of 1000 grains, the number of beans per plant and the productivity of the culture. The smallest productivities obtained in the treatments with organic fertilization are in agreement with Muchovej and Obreza (1996), which observed that organic residues not always substitute mineral fertilization completely. In addition, depending of the applied dose, do not totally supply the plants nutrients requirement and reduce the productivity. On the other hand, studies accomplished by Carvalho and Wanderley (2006) indicated to be possible to produce bean in a totally organic system, reaching productivities similar

to the obtained in a conventional system. For the area in Brasília-DF, Brazil, the authors obtained for different varieties cultivated in organic system, higher productivities than to the regional average (2,700 kg ha^{-1} in irrigated crop and 2,300 kg ha^{-1} in without irrigation crop).

Table 1. Medium values for grain mass per plant and productivity in function of interaction among fertilization types and bean variety.

Varieties	Fertilization types	
	OMF	OF
	---------- Grains mass per plant (g) ----------	
IPR Colibri	11.6 Aa	5.8 Ba
IPR Eldorado	7.2 Ab	4.1 Bb
MSD	1.39	
	---------- Productivity (kg ha^{-1}) ----------	
IPR Colibri	3,645 Aa	2,306 Ba
IPR Eldorado	2,475 Ab	1,745 Bb
MSD	309.4	

Averages followed by same small letter in the column or capital letter in the row do not differ to each other by Tukey test at 5%. OMF = organic mineral fertilization; OF= organic fertilization. MSD=Minimum significant difference

CONCLUSIONS

- The grains mass per plant and the productivity of carioca bean was highest in the treatments with organic mineral fertilization and for variety IPR Colibri.
- For the two evaluated varieties, the obtained productivities were higher than medium productivity of Paraná state, but they were below to the potential productive of each one of them.

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RESPONSE OF BEAN CULTIVARS (*PHASEOLUS VULGARIS* L.)TO SILICON FERTILIZATION

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INTRODUCTION

The cultivation of bean has attracted in recent years the attention of researchers and technicians to increase productivity and grain quality, because the Brazilian grain productivity is low (around 800 kg ha $^{-1}$). The study and use of new technologies to improve production processes, such as the use of silicon fertilization, especially in varieties not traditionally sown in the region, but has a promising market, can help to achieve good levels of productivity and increased grain quality. The objective of this work was to evaluate the alternatives cultivars of beans in response to the application of Si in the productivity components.

MATERIALS AND METHODS

The experiment was carried out at Fazenda Experimental de Iguatemi (FEI) that belongs to the Universidade Estadual de Maringá in 2008/2009.

The experimental design used was the randomized complete blocks in factorial outline 3X5, with four repetitions. The treatments involved the combination of three cultivars of bean (BRS Radiante, Bolinha and Vermelho 2157) and five doses of Silicon (potassium silicato - 23% Si): (T1 – 0.0 ppm of Si; T2 – 8.5 ppm of Si; T3 – 17.0 ppm of Si; T4 – 25.5 ppm of Si and T5 – 34.0 ppm of Si).The plots consisted in four lines of 5.0 m of length, spaced with 0.45 m, and the sowing density was 14 plants per meter. Ten plants were used to determinate the number of pods per plant (NPP), the number of seeds per pod (NSP) and mass of 100 seeds (MS), besides the determination of the productivity. The data obtained for each variable, they were submitted to the variance analysis (F Test) and when significant, being submitted to the regression analysis in the case of the doses for each to cultivar and test of average among them you cultivate (Banzato e Kronka, 2006).

RESULTS AND DISCUSSION

The foliate application of the doses of Si in the experiment presented effect in the variable number of pods per plant in the cultivar "Bolinha", and the absence of Si decreased the number of fruit.

The genotype "Vermelho 2157" differed from other cultivars at the level of 5% of probability in the number of seeds per pods. However, in the mass of 100 seeds with the biggest proportion was the cultivar "BRS Radiant"

The productivity wasn't influenced by the silicon fertilization.

The absence of answer of the agronomic characteristics in the genotypes to the different doses of Si used it can be related with the amount of Si applied and/our influenced by environmental conditions. In that way, later other doses and sources of Si should be studied with the same ones cultivars of interest.

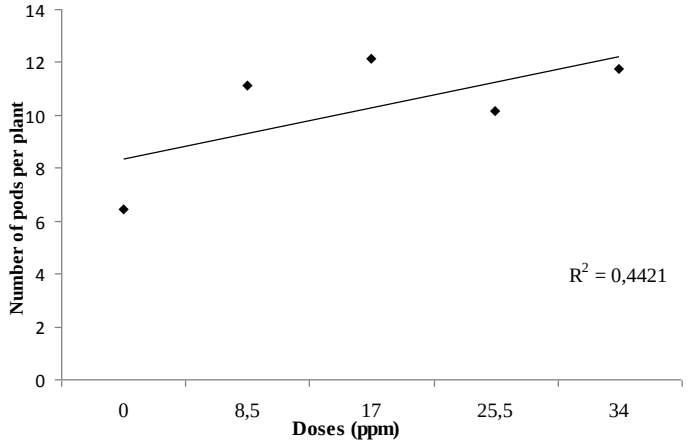

Figure 1. Regression analysis of number of pods per plant in relation the different doses of Si applied in the cultivar "Bolinha" in the crop of 2008/2009.

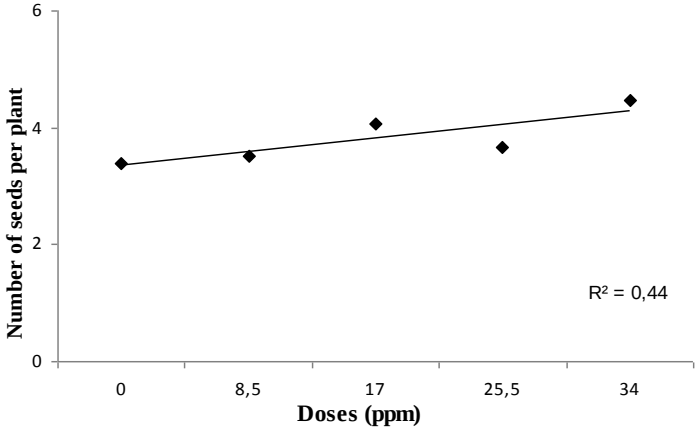

Figure 2. Regression of seeds per pod in the cultivar "Vermelho 2157"according to doses of Si in the crop 2008/2009 in the Fazenda Experimental de Iguatemi in Maringá – Brazil.

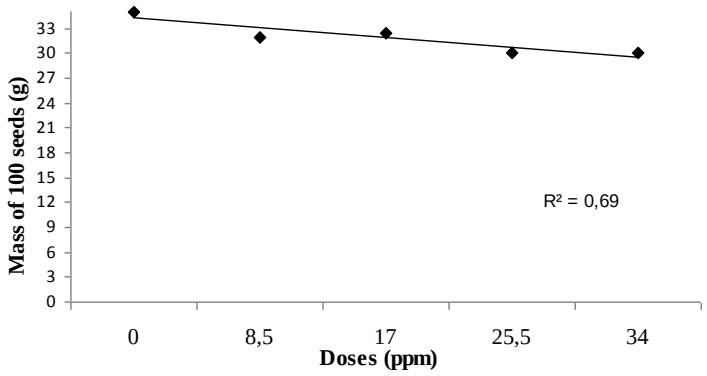

Figure 3. Regression analysis of mass of 100 seeds in function of the doses of Si in the cultivar "BRS Radiant" in the harvest 2008/2009 in Maringá – PR, Brazil.

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COMMERCIAL QUALITY OF BLACK BEAN GENOTYPES

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The Brazilian black beans production is around 430,000 tons per year, corresponding to 20% of the total consumed by the Brazilian population (Del Peloso & Melo, 2005). It is the second largest consumed common bean type. Breeding programs are working to supply cultivars with improved agronomical characteristics, such as yield, disease resistance, and upright plant, among others. More recently other characteristics related to commercial quality that could contribute to increase the acceptance of a new cultivar are also being assessed such as market preference, and 100 seed mass; therefore, the objective of this work was to assess the commercial quality of common black bean lines.

In 2009 trials were carried out in eight environments in the states of Goias (dry and winter seasons) and Paraná (dry season). The environments were Ponta Grossa/dry season; Araucária/dry season; Prudentópolis/dry season; Inhumas/dry season; Santo Antônio de Goiás/winter season; Urutaí/winter season; Anápolis/winter season and Senador Canedo/winter season. The experimental design was a completely randomized block design arranged in plots with four rows four meters long and three replicates. Each trial consisted of 14 bean genotypes, commercial group black (Table 1). Data for yield were collected in the two central rows, and 300 g samples were drawn from each plot and passed through a 2.25 mm sieve. Seeds kept in the sieve were weighed to obtain the percentage of standard commercial beans – PGPC. A sample was also drawn to obtain 100 seed mass. Data from the three characteristics studied were subjected to the analysis of variance followed by joint analysis. Scott Knott test at 10% was used for mean comparison.

A high experimental precision was obtained with CV varying from 16% to 9% and 4% for yield, percentage of marketable beans and 100 seed weight, respectively. The mean separation test was used to assign the genotypes in two groups with small variability among them (Table 1). Genotypes yielding best were: CNFP 11984, BRS Esplendor, CNFP 11985, CNFP 11979, CNFP 11995, IPR Uirapuru, CNFP 11973 and CNFP 11978, with similar average yield and superior to the two controls (BRS 7762 Supremo and BRS Campeiro).

Significant differences were observed ($P < 0.01$) for percent commercial standard beans, among genotypes, among environment and for genotype x environment interaction, evidencing great variability for that trait, also observed in the mean test that divided the genotypes in six groups. Beans kept in the sieve averages varied from 56.3 to 88.3, and genotype yielding higher was CNFP 11995, with 88.5% of sieve retention, higher than all controls (Table 1). Widely cropped cultivar IPR Uirapuru had the second highest average (74.3%). Significant differences were also detected ($P < 0.01$) for 100 seed mass among genotype, environments and genotype x environment interaction. Mean comparison test divided genotypes in seven groups (Table 1). Genotypes with the highest 100 seed mass were BRS Campeiro, CNFP 11985 and CNFP 11976 with an average 25g/100 seeds. Genotype CNFP 11983 with 19.9g/100 seed weight ranked lower (5g) than the best performers, indicating a great variability for that trait.

The best performing genotype for all traits evaluated was CNFP 11995 line, with a bean yield of 2,157 kg ha^{-1}; the highest sieve retention average, and the highest 100 bean mass; superior to all controls in the general analysis for all three traits tested; becoming a promising breeding line.

Table 1. Means yield (PROD) (kg ha^{-1}), percent of commercial standard beans (PGPC) and 100 seed mass (M100) of 14 common Black bean genotypes evaluated in eight environments in the states of Goiás and Paraná, Brazil in 2009.

GENOTYPE	PROD	PGPC	M100
CNFP 11984	2263 a	65.3 d	21.5 f
BRS Esplendor	2202 a	56.3 f	21.0 f
CNFP 11985	2200 a	61.5 e	22.6 d
CNFP 11979	2161 a	57.9 f	24.3 b
CNFP 11995	2157 a	88.3 a	25.1 a
IPR Uirapuru	2105 a	74.3 b	23.4 c
CNFP 11973	2096 a	67.3 d	23.6 c
CNFP 11978	2088 a	65.2 d	23.4 c
BRS 7762 Supremo	2054 b	70.3 c	22.8 d
CNFP 11983	2026 b	60.8 e	19.9 g
CNFP 11994	2011 b	70.1 c	23.1 d
CNFP 11991	1920 b	64.0 d	22.2 e
BRS Campeiro	1912 b	62.6 e	25.3 a
CNFP 11976	1906 b	64.5 d	25.0 a
AVERAGE	2079	66.3	23.1

[1]Means followed by the same letter do not differ among themselves (Scott Knott at 10% of probability).

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EVALUATION OF COMMON BLACK BEANS IN 2007 AND 2008 IN BRAZIL

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Among the many bean types cultivated in Brazil, black beans are the second most produced with 430,000 t per year (FEIJÃO, 2010), corresponding to 20% of the total national output (Del Peloso & Melo, 2005). These beans are cultivated and consumed mostly in the south of Brazil (States of Rio Grande do Sul, Paraná, Santa Catarina), and Rio de Janeiro, but are also cropped in other states in smaller amounts. The evaluation of Embrapa Rice and Beans breeding program lines are carried out in a network of national trials in the states representing 76% of the total national production (Goiás, Distrito Federal, Mato Grosso, Paraná, Santa Catarina, Rio Grande do Sul, São Paulo, Sergipe, Bahia and Alagoas). The final evaluation is performed in national trials network carried out in a great number of environments, representing the environmental conditions the new cultivars will be subjected. The release of new cultivars has been contributing to increase the national average yield of 1,223 kg ha^{-1} (FEIJÃO, 2010); therefore the search for new improved cultivars must be permanent.

In 2007 and 2008 trials were conducted in 85 environments in the States of Goiás, Distrito Federal, Mato Grosso, Paraná, Santa Catarina, Rio Grande do Sul, São Paulo, Sergipe, Bahia and Alagoas, at the winter, dry and wet cropping seasons. The experimental design was a completely randomized block design arranged in four meters long four row plots, with three replicates and data collected in the two central rows. Each trial comprised 14 black bean benotypes (10 lines and four controls: BRS Valente, BRS 7762 Supremo, BRS Grafite, and IPR Uirapurú) (Table 1). Field evaluations were performed for plant architecture, resistance to disease and lodging through a 1 to 9 ranking scale: grade 1 for ideal and 9 for undesirable phenotypes. In the laboratory 100 seed weight was determined. Beans yield data were subjected to the analysis of variance, followed by data joint analysis, using the Scott Knott at 10% for mean comparison.

The joint analysis showed adequate experimental precision (CV=14.2%) and significant differences ($P<0.01$) among genotypes, environment, and genotypes x environment interactions were detected, which was expected, considering the great variability present among environment trials. Average yield was very high (2,170 kg ha^{-1}). Genotypes performing the best for each trait evaluated were: CNFP 10794 and CNFP 10793 for yield; BRS 7762 Supremo, for plant architecture and lodging; BRS Valente for angular leaf spot resistance; CNFP 10794 for bacterial common blight, and CNFP 0221 for anthracnose. When traits data were analyzed jointly, two promising lines were identified: CNFP 10794 and CNFP 10793 (Table 1). These lines yielded higher than all controls and were 16% more productive than the most yielding control: BRS Valente. They also performed similarly between themselves for other characteristics, which could be attributed to the crosses they originate from: (POT51///ICAPIJAO/XAN170//BAC16/XAN91) and (POT51///OAC88-1/A429//OAC88-1/RM35) respectively. These lines ranked medium for plant architecture and superior in the general trial average, but similarly to the control BRS Grafite. For lodging they performed slightly inferior to the trial general average, but were similar to the control BRS Valente. Concerning disease resistance they were susceptible to angular leaf spot, ranking higher than the control; however, for common

bacterial blight reaction, they ranked the lowest and similarly to the best control – IPR Uirapurú. Regarding anthracnose, both lines were graded lower than BRS Valente and BRS Grafite, but CNFP 10794 showed higher resistance, with grade 3.1 versus 9 attributed to CNFP 10793. Besides, the maximum grade for CNFP 10794 was 7, versus 9 attributed to CNFP 10793, suggesting a total susceptibility of that line to some of those environments tested. Those lines also presented beans lager than the average (24g/100 seeds), similar to BRS Grafite. Based on those observations line CNPF 10794 will be released as a new cultivar.

Table 1. Mean yield (kg ha^{-1}) (PROD), 100 seed weight (M100) and average[(1)] and maximum[(2)] grades for plant architecture (ARQ), lodging (ACA), common bacterial blight (CBC), angular leaf spot (MA) and anthracnose (AN), of 14 genotypes of black common beans , evaluated in 85 environments in Brazil, in 2007 and 2008.

Genotype	PROD	ARQ	ACA	MA	CBC	AN	M100
CNFP 10794	2537 a	4.3[(1)] – 7[(2)]	3.5 - 6	5.2 - 8	2.6 - 7	3.1 - 7	25
CNFP 10793	2500 a	4.2 - 7	3.3 - 7	5.0 - 8	2.7 - 5	3.7 - 9	24
CNFP 10807	2239 b	4.0 - 6	4.1 - 8	5.3 - 8	2.7 - 6	2.3 - 9	22
CNFP 10806	2216 b	3.8 - 6	3.8 - 7	4.5 - 7	3.0 - 6	2.8 - 7	20
BRS VALENTE	2180 c	3.8 - 6	3.5 - 8	3.7 - 8	4.2 - 7	5.2 - 9	20
IPR UIRAPURU	2166 c	3.6 - 6	3.7 - 7	4.0 - 7	2.8 - 6	3.6 - 7	21
CNFP 10214	2140 c	4.5 - 6	4.5 - 8	4.9 - 8	3.3 - 7	3.3 - 9	23
CNFP 10800	2137 c	3.4 - 6	3.8 - 8	5.1 - 9	3.6 - 7	4.2 - 9	21
CNFP 10805	2128 c	3.7 - 7	3.8 - 7	4.4 - 8	2.8 - 7	2.4 - 8	20
BRS 7762 SUPREMO	2088 d	2.9 - 7	2.3 - 5	4.3 - 7	3.1 - 6	2.6 - 9	21
BRS GRAFITE	2068 d	4.3 - 6	4.4 - 7	4.4 - 8	3.0 - 6	5.2 - 9	24
CNFP 10799	2054 d	3.6 - 7	2.7 - 5	4.3 - 7	4.0 - 6	2.4 - 9	21
CNFP 10025	2015 e	4.1 - 7	3.6 - 7	4.9 - 9	3.8 - 7	3.0 - 7	19
CNFP 10221	1911 f	4.6 - 6	5.1 - 8	5.2 - 8	3.4 - 7	1.5 - 7	18
AVERAGE	2170	3,9	3,7	4,7	3,2	3,2	21

[1]Means followed by the same leter do not differ among themselves (Scott Knott at 10% probability).

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YIELD GENETIC GAIN AT NATIONAL LEVEL OF CARIOCA COMMON BEANS FROM THE EMBRAPA BREEDING PROGRAM - 1993 TO 2008

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The continuous demand for improved bean cultivars, with high yield and resistance to restrictive production factors have guided Embrapa breeding program in Brazil. In the last 25 years 48 cultivars of various commercial types, with an average of 1.9 cultivars per year, were released. The assessment of genetic breeding programs carried out in species of economic importance through the estimate of the genetic progress is a method used by breeders to measure intended goals. Some research work had been carried out to estimate common bean genetic gains at state level, but never at national level. After more than 20 years from the beginning of that program, an estimate has not been done yet. Those estimates are important to assess the efficiency of the program, and to organize data base banks to support further strategic planning. Therefore, the objective of this work was to estimate the genetic gain of the commercial type carioca dry beans breeding program carried out by Embrapa Rice and Beans Research Center in a period of 16 years, from 1993 to 2008.

Data were obtained from the national trials network denominated 'VCU' from Embrapa beans breeding program, carried out in the most common planting seasons: "wet", "dry" and "winter", among 1993-2008. Each cycle of VCU comprises two years tests with lines selected in the previous intermediary trials in such way that the group of lines tested is changed every two years. Data used were from yields ($kg.ha^{-1}$) of 104 genotypes selected in the previous 16 years in 532 trials. The experimental design used was a completely randomized block with three or four replicates arranged in four meters long plots with four meters long rows, spaced 0.5 m, with 12 plants per meter. All individual trial data were submitted to the analysis of variance and to the corresponding analysis of residues, aiming to detect data discrepancies using PROC GLM – SAS. Furthermore, joint analyses were performed including all trials data within each biennial cycle, using the PROC GLM – SAS procedure. To calculate genetic gain estimated it was used the genotypes general mean of each evaluation cycle for the proposed Breseghello's (1995) weighed method, who used the Weighed Minimum Squares (WMS) to estimate environmental deviations. The method involving balanced weigh uses matrix V, where variance and covariance coefficients are based on the number of observations in each mean which participated in the calculation and use of the Mean Squares of the annual Error. The genetic gain estimate was obtained using the computer program MATLAB version 6.5, 2002. The annual program genetic gain estimate and its percentage for the referred period was calculated regarding the average of the first trial cycle.

On Table 1 are shown the biennial genetic gain estimates and the percent accumulated regarding average of the genotypes tested in the first trial period (93/94). The biennial genetic gain varied considerably, oscillating from -2.34 to 242 $kg.ha^{-1}$/cycle. Those negative gains in some cycles showed a setback in yield due to change of genotypes based on yield associated to other desirable traits. Negative and positive values also indicated that gains obtained in one cycle hardly ever occur in the following cycle, suggesting stabilization in the posterior cycle. The estimated genetic gain of the Embrapa Rice and Beans breeding program was 12 $kg.ha^{-1}$ $.year^{-1}$ (0.67% per year), not significant by t test, indicating absence of yield genetic gain in that period for carioca grain type.

However, according to Melo et al. (2007) there was significant improvement in other characteristics, such as erect plant architecture and resistance to lodging, that contributed to harvest losses reduction; improved grain quality that favored consumer preference; and better disease resistance, that reduced production costs.

Table 1. Estimate of annual average genetic gain regarding the average of genotypes tested in the first biennial cycle, and accumulated genetic gain between pairs of biennial cycles.

Cycle pairs	Biennial genetic gain ($kg.ha^{-1}$)	Gain/ mean deviation	Biennial genetic gain (%)	Accumulated biennial genetic gain (%)
93/94-95/96	-2,34	-0,23	-0,12	-0,23
95/96-97/98	50,56	4,48	2,59	2,36
97/98-99/00	-100,24	-8,38	-5,13	-2,77
99/00-01/02	242,00	17,43	12,40	9,63
01/02-03/04	-90,24	-6,40	-4,62	5,01
03/04-05/06	16,84	1,91	0,86	5,87
05/06-07/08	43,97	4,32	2,25	8,12
Biennial genetic gain ($kg.ha^{-1}$)				12,32
Biennial genetic gain (%)				0,67 n.s.[1]

[1]not significant by t test.

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COMMERCIAL QUALITY OF CARIOCA COMMON BEAN GENOTYPES

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Among the many grain types of beans cultivated in Brazil, commercial type "carioca" stands out as the most consumed, representing 70% of the Brazilian dry beans market (Del Peloso & Melo, 2005). Breeding programs have been releasing new cultivars with improved characteristics, contributing to yield increases. Besides agronomic traits, other characteristics for consumer acceptance such as those related to commercial quality like 100 seed weight and grain size are of great importance. Cultivar Pérola meets those parameters, becoming a standard in the Brazilian dry beans market. During trials of new genotypes it is important to evaluate commercial characteristics, by comparing with the standards adopted, aiming to increase the chance of acceptance of new released cultivars. The objective of this work was to evaluate the quality of "carioca" commercial bean genotypes.

In 2009 trials were carried out in eight environments in the states of Goias (dry and winter seasons) and Paraná (dry season). The environments were Ponta Grossa/dry season; Araucária/dry season; Prudentópolis/dry season; Inhumas/dry season; Santo Antônio de Goiás/winter season; Urutaí/winter season; Anápolis/winter season and Senador Canedo/winter season. The experimental design was a completely randomized block design arranged in plots with four meter rows with three replicates. Each trial consisted of 16 bean genotypes, commercial group carioca (Table 1). Data for bean yield were collected in the two central rows, and 300 g samples were drawn from each parcel and passed through a 2.25 mm sieve. Seeds kept in the sieve were weighed to obtain the percentage of standard commercial grains in each sample – PGPC. A sample was also drawn to obtain 100 seed mass. Yield, PGPC and 100 seed mass data were subjected to the analysis of variance followed by the trials joint analysis and the Scott Knott test at 10% was used for means comparison.

The joint analysis for yield presented good precision (CV=18%) and significant differences (P<0, 01) among genotypes, environment and genotype x environment interaction were detected. Two eight genotype groups were formed by the mean test (Table 1). Genotypes Pérola, IPR Juriti, CNFC 11946, CNFC 11948, CNFC 11951, CNFC 11954, CNFC 11959 and CNFC 11966 yielded most. Regarding PGPC, the joint analysis also detected significant differences (P<0.01) among genotypes, environments and genotype x environment interaction. Environments average varied from 50% to 88% with a low 7% CV, evidencing a great variability among genotypes for that characteristic. Nine genotypes had average equal or higher than cultivar Perola, used as standard. Among those, genotypes BRS 9435 Cometa, CNFC 11948, BRS Estilo, CNFC 11946, CNFC 11962, CNFC 11944 and CNFC 11945 performed better than Pérola. The joint analysis for 100 seed mass also detected significant differences (P<0.01) among genotypes, environments and genotype x environment interaction. CV was low (4%) and genotypes average varied from 21.7 to 27.4, corroborating the existence of a great variability. Environments average varied from 23.6 to 26.7, corroborating the importance of the environment on the expression of those characteristics. Cultivar Pérola average was 27.2 and any genotype surpassed this value. CNF 11948 average was statically identical to Pérola, and four genotypes had 100 seed mass lower (above 25.6).

Among the outstanding lines, CNF 11948 and CNF 11946 presented high PGPC (85.6 and 85.5), yielding high commercial value beans. Besides, CNFC 11948 had an acceptable 100 seed mass (27.4), similar to Pérola (27.2) and line CNF 11946 had a 100 seed mass, a little lower than Pérola (25.6).

Table 1. Average yield (PROD) (kg ha^{-1}), percentage of commercial standard beans (PGPC) and 100 bean mass (M100) of 16 common bean carioca type genotypes, evaluated in eight environments in the states of Goiás and Paraná (Brazil), in 2009.

GENOTYPES	YIELD	PGPC	M100
CNFC 11954	2338 a	77.9 c	24.2 d
CNFC 11959	2208 a	78.5 c	22.0 e
CNFC 11966	2179 a	60.6 e	24.8 c
CNFC 11948	2133 a	85.6 b	27.4 a
PEROLA	2117 a	80.1 c	27.2 a
IPR JURITI	2074 a	68.8 d	24.3 d
CNFC 11951	2057 a	55.6 f	25.8 b
CNFC 11946	2039 a	85.5 b	25.6 b
BRS ESTILO	1948 b	85.5 b	26.0 b
CNFC 11962	1945 b	84.7 b	21.7 e
BRS 9435 COMETA	1915 b	90.3 a	24.5 c
CNFC 11952	1885 b	49.4 g	26.0 b
CNFC 11956	1883 b	46.6 h	23.9 d
CNFC 11953	1802 b	39.2 i	25.1 c
CNFC 11945	1797 b	84.0 b	24.7 c
CNFC 11944	1797 b	84.3 b	25.1 c
MÉDIA	2007	72.3	24.8

[1]Means followed by the same letter do not differ among themselves (Scott Knott at 10% probability).

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EVALUATION OF IRRIGATED BEAN LINES IN THE REGION OF PORANGATU-GO

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INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is one of the main food of the Brazilian peoples diet. It is grown in almost all country and in different growing seasons, and therefore has extensive soil and climate adaptation (Buratto et al., 2007). This is due to its genetic diversity. Ramalho et al. (1993) added that the study of genotype x environment interaction, where different environmental conditions occur, takes role in the process of new cultivars recommendation, and it is necessary to minimize this effect through the selection of cultivars with greater phenotypic stability. Therefore, the objective of this study was to evaluate inbred lines of carioca and black grain types common bean of the Embrapa breeding program under irrigated conditions and in the region of Porangatu-GO.

MATERIALS AND METHODS

Two irrigated experiments, Ψ_s of – 0.035 MPa at 15 cm depth, were conducted, one in 2007 and another in 2008 at the Experimental Station of SEAGRO, Porangatu-GO, located at 13 º 18 '31" South and 49 º 06' 47" West, with an altitude of 391 m, Aw climate, tropical savanna, megathermic, in an Oxisol. Sowing was done in 08/06/2007 and 13/06/2008, in plots of four rows with five meters in length each. The seeding rate was 15-18 seeds per meter. The experimental design was a randomized block with three replications. We evaluated the yield, in kg ha^{-1}, at the two years and the flowering date in days after sowing (DAS) in 2008 only, of the 14 black grain type lines, and 17 of the carioca grain type lines.

RESULTS AND DISCUSSION

Results showed that bean lines of grain types, black and carioca, produced significantly different yield in the two years of experimentations. The black grain type lines produced, 986 kg ha^{-1} and 1858 kg ha^{-1} in 2007 and 2008, respectively. While carioca grain type lines produced 1764 kg ha^{-1} and 2268 kg ha^{-1}, respectively. It was also observed that the lines of grain types, black and carioca, produced significantly different grain yield from each other. Additionally, it was found that the effect of years was different on the black grain type lines, since the lines x years interaction was significant. The same was not observed in the carioca grain type lines. Flowering date was evaluated only in experiments conducted in 2008 and it was observed that the lines flowering date of grain types, black and carioca, differed significantly among each other (Table 1). The more productive lines of black grain type in 2007 were: BRS Supremo, CNFP 10214 and CNFP 10806, which yielded 1350 kg ha^{-1}, 1350 kg ha^{-1} and 1356 kg ha^{-1}, respectively. Two other lines, CNFP 10800 and CNFP 10807, produced grain yield of 1081 kg ha^{-1} and 1047 kg ha^{-1}, respectively. These lines did not differ significantly from previous lines, however presented yields not differed from the second group which was more productive. The line CNPF 10221, with only 650 kg ha^{-1} was the least productive in 2007. However not differ significantly from the lines BRS Valente, IPR Uirapuru, CNFP 10025,

CNFP 10793, CNFP 10794 and CNFP 10805. The lines in 2008 were more productive and also differed significantly among each other. The most productive group of the black grain type lines was composed by the BRS Valente, BRS Grafite, BRS Supremo, IPR Uirapuru, CNFP 10214, CNFP 10793, CNFP 10794, CNFP 10800, CNFP 10805, CNFP 10806 and CNFP 10807, which produced 1756 kg ha^{-1} to 2352 kg ha^{-1} grain yield. They flowered from 43 to 49 DAS. Of these, only line BRS Supremo, CNFP 10214, CNFP 10800, CNFP 10806 and CNFP 10807 participated in the most productive group in the two years of experimentation. The line CNFP 10221, with 1289 kg ha^{-1} and flowering at 47 DAS, followed the pattern of 2007 by failing to present good yield performance.

Table 1. Summary of the analysis of variance for yield in 2007 and 2008, and for flowering date, in 2008, for the black and carioca grain types of bean lines.

Source of variation	D.F.	Mean square error	
		Yield (kg ha^{-1})	Flowering Time (DAS)
Black grain type bean			
Year (Y)	1	15 977 657.44**	
Error (a)	4	249 238.94	
Lines (L)	13	258 080.03**	**8.07****
Y x L	13	162 641.36**	
Error (b)	52	75 410.58	**2.22**
CV (%)		**19.31**	**3.26**
Carioca grain type bean			
Year (Y)	1	6 474 888.24**	
Error (a)	4	14 922.19	
Lines (L)	16	503 112.07**	**7.25***
Y x L	16	217 797.56^{ns}	
Error (b)	64	136 474.97	**3.11**
CV (%)		**18.32**	**3.81**

ns-F not significant at 5%,*-F significant at 5% e **-F significant at 1%.[1]DAS–days after sowing

The effect of years did not affect the productive performance of carioca grain type bean lines therefore it was discussed the productivity average of the lines in the two years of experimentation. It was found that the lines CNFC 10721, CNFC 10729, IPR Juriti, CNFC 10762, CNFC 10716, CNFC 10758, CNFC 10753, CNFC 10733, CNFC 10703, CNFC 10757 and BRS Pontal do not differ significantly in terms of productivity and classified into more productive group. They also presented similar flowering behavior, 46-47 DAS. These lines produced on an average of 1944 kg ha^{-1} to 2443 kg ha^{-1} during the two years of experimentation.

CONCLUSION

The black and carioca grain types lines differed significantly when grown under irrigated conditions of Porangatu-GO, region. The most productive lines in the two years experiments were BRS Supremo, CNFP 10214, CNFP 10800, CNFP 10806 and CNFP 10807 of the black grain type, and CNFC 10721, CNFC 10729, IPR Juriti, CNFC 10762, CNFC 10716, CNFC 10758, CNFC 10753, CNFC 10733, CNFC 10703, CNFC 10757 and BRS Pontal of the carioca grain type.

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GENETIC PARAMETERS IN CARIOCA TYPE BEANS IN THE EMBRAPA BREEDING PROGRAM - 2006 AND 2008

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Genetic parameters estimates allow inferences in genetic population structures being tested, enabling to evaluate its potential for breeding as well as to define strategies to evaluate segregating populations and to anticipate selecting gains. For common beans there are a reasonable number of estimates but insufficient, due to the existence of a huge diversity of cropping conditions and segregating populations used in breeding programs. The objective of this work was to estimate genetic parameters in populations of common bean lines commercial type carioca in the Embrapa Rice and Beans breeding program.

Trials were carried out at Ponta Grossa-PR in the wet season and in Santo Antônio de Goias-GO in the winter cropping season in 2006 (144 lines) and 2008 (100 lines). The experimental design was a triple square lattice 12x12 in 2006 and 10x10 in 2008 arranged in two four meter rows plot spaced 0.5 m and 15 seeds per meter.

The analysis of variance was applied on each experimental data and effective errors and adjusted means were calculated followed by the joint data analysis. Values of the experimental and genetic coefficients of variance and "b" coefficient were estimated as well as the broad sense heritability, according to Ramalho et al. (1993).

Regarding the genetic parameters estimate from the 2006 individual trials and 2008 the heritability estimates (0.67 and 0.87) and "b" coefficient (0.84 and 1.51) at Santo Antônio de Goiás were higher than those obtained in Ponta Grossa, suggesting that in those years the conditions for bean yield selection in Santo Antonio de Goiás were more favorable than those in Ponta Grossa. Genetic parameters estimates in the joint analyses were 0.72 and 0.86 for heritability, and 0.65 and 1.02 for "b" coefficient in 2006 and 2008 (Table 1). Those estimates were considered satisfactory in view of the complexity of the yield trait, a quantitative trait controlled by a number of genes of low individual effect and highly influenced by the environment with heritability estimates usually very low. The results obtained suggest the existence of adequate genetic variability in the Embrapa Rice and Beans carioca breeding program lines, enabling the obtainment of cultivars superior than those now in use.

Based on grain yield, disease resistance and agronomic traits evaluations, 77 superior lines were identified and selected in 2006 to make up the Lines Preliminary Trial (EPL) in 2007. Selected lines average yield was 358 kg ha^{-1}, higher than the general population average, enabling to estimate the general average of 4,076 kg ha^{-1}, consolidating a gain of 258 kg ha^{-1}, representing 6.75% of the original population average.

Based on grain yield, resistance to disease and agronomic traits evaluations, 40 superior lines were selected to make up the 2009 Preliminary Lines Trial (EPL). Selected population bean yield average was 131 kg ha^{-1}, higher than the general population average. Those lines made up a Preliminary

Trial with a general average of 2,142 kg ha^{-1}, representing a gain of 113 kg/ha and 5.23% of the original population average.

We can conclude that there is adequate genetic variation in the carioca grain type lines in the Embrapa Rice and Beans breeding program to obtain selection gains leading to the obtainment of cultivars superior than those in use.

Table 1: Summary of the joint analyses of variance and bean yield genetic parameters estimate in the carioca progeny test, in Ponta Grossa-PR wet season and Santo Antônio de Goiás-GO winter season, in 2006 (Q.M.1) and 2008 (Q.M.2).

F.V.	**G.L.(1/2)**	**Q.M. 1**	**Q.M. 2**
Treatment (T)	99/143	1628582.69**	462631.92**
Environment (A)	1/1	290872362.66**	2647899.80**
T x A	99/143	740655.59**	291425.08**
Effective Error Mean	342/506	453587.33	63676.69
GenotypeVariance		195832.56	66492.53
Phenotype Variance		271430.44	77105.32
Heritability (%)		72.14	86.23
Mean ($Kg.ha^{-1}$)		3818	1650
Experimental C.V. (CVe)		17.63	15.29
Genetic C.V. (CVg)		11.59	15.62
"b" - CVg/CVe		0.65	1.02

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

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GENETIC PARAMETERS IN YIELD RECURRENT SELECTION FAMILES IN CARIOCA TYPE BEANS

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Increases in common bean commercial type carioca yield potential have been gradual, besides the wide variability in the majority of its traits, especially grain yield. Therefore, when breeding programs work to improve one or more traits controlled by various genes, it is impossible to succeed in one selection cycle only. The only alternative is recurrent selection – a dynamic and cyclic system designed to gradually increase the desired allele frequency for a specific quantitative characteristic through repeated selection, evaluation and recombination cycles. The use of recurrent selection in autogamous plants enables the intercrossing of selected genotypes to form new genotype combinations. The objectives of this work were: to estimate genetic parameters; to evaluate families from recurrent selection breeding programs, comprising carioca type beans from Embrapa Rice and Beans Research Center, and to select superior families for the obtainment of lines to intercross to form new selecting populations.

In 2008 three carioca grain type recurrent selection trials with 78 $C_1S_{0:3}$ families and three controls were conducted: one in Santo Antonio de Goiás at the winter cropping season and two at the wet season in Ponta Grossa-PR and Sete Lagoas – MG. The experimental design was a 9x9 triple square lattice with two four meter rows (Santo Antônio de Goiás-GO and Ponta Grossa-PR) and two meters rows (Sete Lagoas-MG) spaced 0.5m and 15 seeds per meter. Disease responses (common bacterial blight and rust) were evaluates only in Ponta Grossa-PR; ranking from 1 (absence of symptoms) to 9 (maximum severity). At physiological maturity plant architecture and lodging were also evaluated, ranked from 1 (ideal phenotype) to 9 (totally undesirable). Visual assessment data were not included in the statistical analysis, but were considered as complementary information for yield. Yield was computed using the mass of each plot adjusted to 13% moisture. Data were subjected to the analysis of variance and the genetic parameters estimate was obtained using the Genes Program (CRUZ, 2001).

The genotypes evaluated showed genetic heterogeneity for grain yield in all trials and in the joint analysis, indicating the existence of genetic variability in all families tested. After the individual analyses of variance were performed, values of the experimental coefficient of variation, genetic coefficient of variation, 'b' coefficient and broad sense inheritability estimated. The individual experiments heritability varied from 50% to 70% and 66.7% was the estimative in the joint analysis (Table 1), considered satisfactory for bean yield, a quantitative trait controlled by many genes of low effect and highly influenced by the environment. This result corroborates the existence of enough genetic variability to obtain selection gains aiming the obtainment of cultivars superior to those now in use in those populations within the recurrent selection breeding program at Embrapa Rice and Beans Research Center. Although population variability were significant, the bellow 1 "b" estimate in all individual analyses and joint analysis (Table 1) indicated unfavorable selecting conditions, suggesting the inclusion of other selection sites to increase precision and selection consistence.

Line SRC-207102999 yielded most in the average, with (2,345 kg ha^{-1}), higher than all controls (BRS Pontal, BRS Estilo and BRS 9435 Cometa). 38 lines (48.7%) yielded higher than BRS Estilo (1,913 kg ha^{-1}), the control line that yielded the most. Regarding disease reaction, that line showed tolerance to rust and intermediary reaction to common bacterial blight. Regarding plant architecture it showed an intermediary behavior and no lodging.

Based on those results we could conclude that there is a wide variability within the base population of the recurrent selection breeding program for common beans carioca type grain yield at the Embrapa Rice and Beans Research Center. The large population and high intensive selection used within the recurrent selection programs indicate the possibility to select superior genotypes with a great number of alleles favorable to bean yield.

Table 1. Joint analysis of variance and genetic parameters estimate for bean yield of the recurrent selection program trials for carioca type grains at Santo Antônio de Goiás-GO in the winter cropping season; Ponta Grossa-PR and Sete Lagoas-MG at the wet cropping season, 2008.

F.V.	**G.L.**	**S.Q.**	**Q.M.**	**F**
Families (F)	80	22846250	285578	3.00**
Environment(A)	2	204986561	102493280	1077**
F x A	160	35952073	224700	2.36**
Average Effective Error	408	38802082	95103	
Genotype Variance			21164	
Phenotype Variance			31731	
Heritability (%)			66.70	
Mean ($kg.ha^{-1}$)			1926	
Experimental CV (CVe)			16.01	
Genetic CV (CVg)			7.55	
"b" - CVg/CVe			0.47	

** Significant at 1 % probability by F test.

ACKNOWLEDGEMENTS

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EVALUATION OF WHITE COMMON BEAN GENOTYPES IN BRAZIL

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"Carioca" commercial type is the most common bean produced in Brazil, followed by black beans (Del Peloso & Melo 2005); however other less consumed colored types such as purple, pink, red, brawn, jalo (kidney), striped and white are also produced. White grain beans have a great potential in the external market, since grains with that color and large size are well consumed in the United States and Europe. However, the number of cultivars with those characteristics at the farmer's disposal is scarce; therefore the Embrapa Rice and Beans breeding program has been working on the identification of genotypes with desirable characteristics to release new enhanced cultivars. The states of Paraná, Minas Gerais, Goiás and the Federal District of Brasilia are the largest producers of common beans, responsible for 55% of the total produced in the country with an output of 1,563.380 t on an area of 1,072.001 ha (FEIJÃO, 2010). The assessment of genotypes with potential to become a new cultivar is carried out in National Trials (VCU) in various environments representing the diverse conditions a new cultivar would be subjected. Thus, the search for genotypes with improved phenotype characteristics to be released as a new cultivar is our main goal.

In 2007, 2008 and 2009 years, 19 trials were carried out in the states of Paraná (nine at the dry and wet seasons); six in Goiás/Federal District and four in Minas Gerais (at the winter cropping season). The experimental design used was a completely randomized block design arranged in four plots with four meter rows and three replicates. Data were collected in the two central rows. In each trial ten white bean genotypes and two controls (Ouro Branco and BRS Radiante) were used (Table 1). The following characteristics were evaluated: 100 seed mass, plant architecture, lodging and disease resistance (anthracnose, common bacterial blight, angular leaf spot mildew and rust) using 1 (totally favorable) to 9 (totally unfavorable) raking scale. Yield data were submitted to the analysis of variance followed by joint analysis. For mean comparisons the Scott Knott test at 10% was used.

Data joint analysis showed good experimental precision (CV=17%) and significant differences ($P<0.01$) were detected among genotypes, environment as well as genotype x environment interaction. Regarding genotype performance, control BRS Radiante yielded the most (Table 1). This genotype has striped beans, released for planting in those states. Poroto, Alúbia and Branco Graúdo were the most productive when grouped with Ouro Branco; however Poroto and Alúbia showed inadequate plant architecture with tendency to lodging along with the highest grades for disease resistance, becoming inadequate for cultivar recommendation. Branco Graúdo genotype was graded highest for plant architecture and resistance to common bacterial blight, and intermediate for the other traits. Branco Graúdo genotype was ranked the best for plant architecture and bacterial common blight and intermediate for the other traits. The remaining genotypes were grouped in a third category through the means test. Among them, WAF 170 was ranked medium and low for disease resistance but ranked the best for bacteria common blight and anthracnose. WAF 75 showed good plant architecture, the best tolerance to lodging and larger beans. WAF 141 also presented good plant architecture and resistance to lodging but ranked low for disease resistance. Alubia

Argentina, besides being the worst ranked for plant architecture and lodging was also susceptible to all diseases tested, graded above 6; with 9 as maximum grade for all diseases.

Therefore, based on those observations, genotypes Branco Graúdo, WAF 170, WAF 75 and WAF 141 were selected as promising and will continue to be evaluated with a possible recommendation as a new white bean cultivar.

Table 1. Average yield (PROD) (kg ha^{-1}), average[(1)] and maximum[(2)] grades for plant architecture (ARQ), lodging (ACA), common bacterial blight (CBC), angular leaf spot (MA), mildew (OI), rust (FE), anthracnose (AN) and 100 seed mass (M100) of 12 genotypes of white type common beans, evaluated in 19 environments in 2007, 2008 e 2009 in Brazil.

GENOTYPE	PROD	ARQ	ACA	CBC	MA	OI	FE	AN	M100
BRS RADIANTE	2055 a	4[(1)]/5[(2)]	4/5	3/6	4/6	2/3	1/2	1/1	41
POROTO ALUBIA	1923 b	5/7	5/8	5/9	4/7	5/9	3/5	2/3	53
BRANCO GRAUDO	1865 b	3/5	4/5	4/7	2/5	5/8	2/8	1/1	49
OURO BRANCO	1798 b	3/4	3/6	4/7	2/3	6/8	1/3	2/4	50
ALUBIA ARGENTINA	1707 c	7/7	6/9	6/9	6/9	6/9	6/9	9/9	43
WAF 160	1698 c	4/5	4/8	5/8	3/4	7/9	1/3	1/1	48
WAF 130	1661 c	4/5	4/6	5/9	3/4	6/9	1/1	1/1	47
USWA 70	1647 c	3/5	3/4	5/8	1/1	7/9	1/1	1/1	65
WAF 170	1640 c	5/6	4/5	3/4	3/6	3/5	1/3	1/1	46
WAF 75	1639 c	3/6	2/4	4/9	3/6	5/8	1/3	2/5	57
WAF 157	1638 c	4/7	3/6	4/7	2/3	5/7	1/1	1/1	45
WAF 141	1587 c	4/5	3/5	3/4	1/2	4/7	1/1	1/1	45

[1]Means followed by the same letter do not differ among themselves (Scott Knott at 10% probability).

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EVALUATION OF EXPORT COMMON BEAN GENOTYPES IN BRAZIL

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Paraná, Minas Gerais, Goiás and the Federal District of Brasilia are the Brazilian leading common bean producer states, representing 55% of the total produced in the country, with a total out put of 1,563.380 ton on an area of 1,072.001 ha (FEIJÃO, 2010). The most consumed bean type is "carioca" (70%). Other types are: black, purple, pink, red, brown, jalo (kidney), mottled, and white (Del Peloso & Melo, 2005). Although Brazil is the largest beans producer, the amount exported is minimal due to high internal consumption, low acceptance and low market value abroad. An alternative to insert beans produced in Brazil in the international market would be to offer beans type Alubia (large white), Cranberry, Dark Red Kidney, Light Red Kidney, Pinto, and Navy (small White). However, the main obstacle for implementing that strategy is the availability of cultivars with that type of grain at farm level. To supply demand the Embrapa Rice and Beans research program is working on the identification of genotypes gathering desirable characteristics to indicate as new cultivars.

In 2007, 2008 and 2009 eighteen trials were carried out in the states of Parana (nine at the dry and wet seasons); six in Goiás/Federal District and four in Minas Gerais (at the winter cropping season). The experimental design used was a completely randomized block design with three replicates arranged in four plots with four meter rows and data collected on the two central rows. In each trial 15 genotypes of common beans were tested and from those 11 were promising lines (Cranberry, Light Red Kidney, Dark Red Kidney and Calima) and four controls: Jalo early harvest, BRS Radiante, Etna and Hooter (Table 1). Evaluations were carried out for the following characteristics: plant architecture, lodging and disease resistance (anthracnose, common bacterial blight, angular leaf spot, mildew and rust) using a 1 (totally favorable) to 9 (totally unfavorable) raking scale. 100 seed mass was also determined. Yield data were submitted to the analysis of variance followed by joint analysis. For mean comparison the Scott Knot test at 10% was used.

The joint analysis showed good experimental precision (CV=17%) and significant differences ($P<0,01$) were detected among genotypes, environment as well as genotype x environment interaction. Regarding genotype performance, control BRS Radiante yielded the most (Table 1). This genotype has striped seeds released for planting in those states. Genotypes Red Kanner, CAL-96, and BRS Embaixador yielded the same as BRS Radiante, being also resistant to anthracnose and rust, with 100 seed weight above 46 grams. BRS Embaixador presented the best grades for plant architecture and lodging. The two genotypes in the second average group along with controls Hooter and Etna were highly susceptible to anthracnose, with grades 7 (Poroto LRK-ARG) and 9 (Importado Notamil). In the third average group, four genotypes were grouped along with Jalo Precoce. Among those, Poroto DRK-ARG showed high susceptibility to anthracnose, Chennok and Light Red Kidney-ARG showed high susceptibility to common bacterial blight. Genotypes Montcalm and Poroto Bayo made up the fourth average group and were the least productive than all controls. Besides that, Poroto Bayo-ARG was the genotype with the worst performance for plant architecture, lodging, and disease resistance, besides yielding very small beans. Therefore, genotypes

Red Kidney, CAL 96, BRS Embaixador, Light Red Kidney-ARG, Chenook, and Montcalm were selected as promising, and will be evaluated to identify new bean cultivars for export.

Table 1. Average yield (PROD) (kg ha^{-1}), average(1) and maximun(2) grades for plant architecture (ARQ), lodging (ACA), reaction to common bacterial blight(CBC), angular leaf spot (MA), mildew (OI), rust (FE), anthracnose (AN) and 100 seed weight (M100) of 12 export type common bean genotypes, evaluated in 18 environments in the states of Goiás/Distrito Federal, Minas Gerais and Paraná (Brazil), in 2007, 2008 e 2009.

GENÓTIPO	PROD	ARQ	ACA	CBC	MA	OI	FE	AN	M100
RED KANNER	2027 a	4(1)/6(2)	4/7	4/7	3/5	4/7	1/1	1/1	46
CAL - 96	1988 a	4/6	3/4	2/3	2/3	5/8	1/1	1/1	54
BRS RADIANTE	1972 a	4/6	3/5	5/7	2/4	1/2	2/3	1/1	41
BRS EMBAIXADOR	1937 a	3/5	3/4	4/8	2/3	6/8	1/1	1/1	53
POROTO LRK-ARG	1850 b	4/5	3/4	4/8	1/2	3/7	2/3	3/7	51
HOOTER	1848 b	3/7	3/4	5/7	2/5	4/7	2/4	1/1	53
IMPORTADO NOTAMIL	1846 b	4/7	3/5	6/8	1/2	5/8	2/3	6/9	52
ETNA	1785 b	4/7	3/6	5/7	1/1	6/8	2/4	4/9	49
LIGHT RED KIDNEY-ARG	1742 c	6/8	5/8	5/9	3/5	3/5	1/2	1/1	53
JALO PRECOCE	1740 c	5/6	3/4	3/6	2/2	3/6	1/1	1/1	40
CHINOOK	1707 c	4/6	3/4	6/9	1/1	5/8	2/6	1/1	48
DIACOL CALIMA	1681 c	4/5	3/4	3/6	2/3	5/7	1/2	1/1	49
POROTO DRK-ARG	1632 c	6/7	5/8	5/8	2/2	5/8	3/6	4/9	39
MONTCALM	1540 d	4/6	4/7	5/7	2/3	6/8	1/2	1/1	50
POROTO BAYO-ARG	1443 d	7/9	6/9	5/8	5/9	5/8	6/8	9/9	39

[1]Means followed by the same letter do not differ among themselves (Scott Knott at 10% probability.

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FIELD EVALUATION OF PHENOLOGICAL TRAITS AND DISEASE RESPONSE OF MEXICAN BEAN LANDRACES

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INTRODUCTION. Collections of genetic resources are very important for agricultural research, because they could be the origin of genes to produce new genotypes for human benefit. It is necessary to identify outstanding traits of this germplasm, to be used in breeding programs. Frequently, native bean varieties have longer biological cycles and are susceptible to bacterial and virus diseases which present serious problems for bean growers, however it is a fact that natural variability could make it possible to find some adequate genotypes for these traits. The objective of this research was to characterize phenology and disease response of bean landraces from the State of Mexico, Mexico.

MATERIALS AND METHODS. During 2007, two hundred and five bean landraces from the State of Mexico were sown at the experimental station of Santa Lucía de Prías, in the State of México. The experimental plot was one 4 m-long row, alternating with one row without plants, to avoid contamination among genotypes. Data of flower color, days to flowering, days to ripeness, and disease incidence were registered, according to the standard scale of CIAT (1987). The scale has nine levels: 1 corresponds to very low incidence and 9 to very high incidence. In latter case, disease causes plant death. As reference check for growth habits four varieties were used: Flor de Durazno (type I), Jamapa (type II), Bayo Mecentral (type IIIa) and Flor de Mayo with long vines (type IVa).

Pesticides were not applied to the plot, however, mechanical control was used, through handpicking of some adults and larvae of Mexican bean beetle in order to avoid total destruction of foliage and pods in bean plants. All plants of each plot were harvested and grain production was estimated.

RESULTS AND DISCUSSION. Days to flowering vary from 41 to 120; and there were from 105 to 175 days to ripening; flowers were mostly white (52.9% of the genotypes), purple (37.9%), and pink (9.2%). Yield was from 10 g to 1540 g per plot. There was a negative correlation between disease incidence and yield (r= -0.36*). Ninety percent of genotypes showed growth habit type 3a and 4a.

Grain yield had high differences among landraces, the outstanding genotypes were 404, 412, 420, 1696, 408, 430, and 349 which produced from 1150 to 1540 g /row. Landraces 406 and 3076 exhibited the lowest grain production (Table 1). Anthracnose, rust, root rots, common blight, angular leaf spot, and virus were diseases detected in the nursery. The highest level of plant infection was caused by rust, root rots and common blight. The genotypes with the highest damage by root rots were 1662 and 3076 with score 5 and 6 (Table 1). For rust, the highest score was 5 for genotypes 7828 and 7840. While for common blight the highest score was 5 for the genotypes: 352, 353,386, 390, 3047, 3069, 7856, 7875, 7879 and 7888. Common blight and virus were the diseases with the highest number of infected genotypes including check varieties.

The outstanding genotype was 1706 which was not infected by common blight and other diseases, which suggests that it may have resistant genes with respect to them.

Table 1: Agronomic traits of some bean landraces of the State of Mexico, Santa Lucia de Prías, State of Mexico, Year 2007.

Genotype	Flower color	Flowering days	Growth habit	Maturity days	Yield (g/ 4 m of row)	Rust (1)	Root Rots (1)	Common blight (1)	Virus
404	Purple	61	41	174	1540	1	2	2	2
412	Purple	63	41	174	1440	2	1	2	1
420	Purple	58	31	138	1270	2	1	4	1
1696	Purple	65	41	175	1180	2	1	3	2
408	Purple	63	41	138	1175	4	1	2	1
430	Purple	58	31	138	1175	3	1	4	1
349	White	63	41	174	1150	1	1	2	2
3069	Purple	59	31	138	995	2	1	5	1
3069	Purple	59	31	138	995	2	1	5	1
Flor de mayo M-38	White	61	3a	122	805	1	1	3	1
7875	Purple/White	19	41	175	765	3	1	5	1
1706	**White**	**59**	**41**	**138**	**750**	**1**	**1**	**1**	**1**
7888	Purple	59	41	175	690	2	1	5	1
389	**White**	**75**	**41**	**174**	**520**	**1**	**1**	**1**	**4**
1662	White	59	3a	131	485	1	5	4	1
7856	White	84	41	175	475	1	1	5	1
7840	Purple	61	4a	175	425	5	1	4	1
Flor de Durazno	Pink	43	1	105	420	1	1	5	1
8003	Pink	43	10	105	420	1	1	5	1
413	**Purple**	**63**	**41**	**174**	**400**	**2**	**1**	**1**	**4**
390	Purple	61	31	138	355	2	1	5	1
353	White	61	31	138	350	2	1	5	3
7879	White/ Purple	61	41	175	345	1	1	5	3
7828	White/ Purple	83	41	175	325	5	1	3	2
352	White	61	31	138	295	3	1	5	4
352	White	61	31	138	295	3	1	5	4
3047	White	58	20	125	165	1	1	5	3
3047	White	58	20	125	165	1	1	5	3
386	Purple/White	41	1041	105	55	1	1	5	1
406	White	61	2	131	20	1	1	2	1
3076	Purple	65	2	122	10	1	6	3	1
Nursery average						1.77	1.22	3.2	2.31

(1) According classification standard scale of CIAT (1987).

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GRAIN QUALITY OF MEXICAN BEAN (*PHASEOLUS VULGARIS* L.) LANDRACES WITH DIFFERENT RESPONSE TO DISEASES

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It is necessary to know the genetic diversity that encompass agronomic adaptation and variability in grain quality traits of bean germoplasm to make it possible to use it as a source of genes that may improve both yield and grain quality traits that determines consumer´s preferences, in order to face the growing food demand in Mexico. It has been identified a wide variability in the characteristics of grain quality of landraces from different areas of Mexico (Jacinto *et al.*, 2002), which make it useful for bean breeding programs, however it is necessary to correlate quality and agronomic characteristics to ease the use of this genotypes. Important problems for bean production in Mexico are diseases, like anthracnose, rust, root rots, common blight, angular leaf spot, and virus. The objective of this study was to evaluate grain quality of bean landraces of the state of Mexico, whose susceptibility to the main diseases had been previously evaluated.

MATERIALS AND METHODS

During PV 2007, two hundred and five bean landraces from the state of Mexico were sown in Santa Lucía de Prías, México. The experimental plot was one row of 4 meters long; leaving one row with no plants, to prevent contamination among genotypes. As reference checks for growth habit the varieties Flor de Durazno (type I), Jamapa (type II), Bayo Mecentral (type IIIa) and Flor de Mayo with long vines (type IVa) were used. Incidence of the main diseases was previously scored according to the scale of CIAT (1987). All plants of each plot were hand threshed and grain production was estimated. Seed samples were kept at 5°C until the analyses were done. One hundred grain weight and volume, water absorption capacity, broken grains (granos abiertos) during cooking, solids in broth and protein content were determined in replicated samples. Cooking time was measured according to sensorial method in two samples of 25 grains previously soaked in water for 18 hours.

RESULTS AND DISCUSSION

Bean landraces exhibited highly significant differences among accessions grain weight and size (volume), water absorption capacity, as well as for cooking time, broken grains, thickness of broth, and protein content. Weight of 100 grains varied from 5.9 to 55 g in comparison with 9-58 g detected in landraces from different states of Mexico (Jacinto *et al*., 2002), in this study weight of grains was affected by diseases. The main diseases in the nursery were anthracnose, rust, root rots, common blight, angular leaf spot, and virus. For our purpose the summatory ($\sum$) of the incidence of all diseases in every accession was considered. The lowest score was 7 and the highest 16. The outstanding genotype was 1706 which was not infected by the common blight and others diseases, which suggest that may have a resistances genes for them.

Cooking time was among 48 and 147 minutes. The genotypes with lowest value were 406, 424, 379, 3073, 346, 358, 3080, 349, 383, 351 and 386 (between 48 and 55 minutes). The genotypes with the longest cooking time were 7897, 3045, 7895, 7898, 7886, 3074, 3054, 367, 3062 and 3083, (between

115 y 147 minutes). Genotypes with extreme cooking time or high or low protein content are presented in table 1. There was a negative correlation between cooking time and water absorption (r=-0.61**); accessions with higher water absorption capacity tend to be faster to cook. The amount of solids in broth was quite variable among landraces in comparison to the data of landraces from different states of Mexico whose solids in broth was from 0.33 to 0.60 % (Jacinto *et al*., 2000) the genotypes in this study exhibited a wider range showing thinner broths in some cases. There existed an association between grain weight and volume and the solids content in broth (r=0.59**) meaning that bigger grains tend to produce thicker broths, which are preferred by consumers.

Protein content varied between 19.2 and 34 %. The highest protein content (between 29 y 34%), was in the genotypes 7898, 1747, 3076, 7823, 7863 and 1679. The lowest protein content was detected in genotypes 429 and 427 (between 19.2 and 19.7 %) (Table 1). Higher protein content was associated with lower productivity (r=-0.56**), the four accessions with the highest protein content (>30 %) exhibited some of the lowest yield per row. The only statistical association between level of incidence of the diseases and quality traits was between the incidence of virus disease and protein content (r=0.35*). The only genotype which was not infected by the common blight and others diseases, landrace 1706, had low protein content (21.6 %) and long cooking time (>100 min). Results suggest that it is necessary to confirm this association because apparently grain quality and resistance to diseases are not closely linked traits and may be possible to use this variability in breeding programs.

Table 1. Grain quality of landraces from the state of Mexico, sown at Santa Lucia de Prías, state of Mexico during PV- 2007.

Genotype	Growth habit	diseases score (∑)	Yield of plot	100 grain wt (g)	Cooking time (min)	Protein (%)
7898	3a	11	21	15.5	117	34.0
1747	3a	15	45	25	62	32.7
3076	2	14	10	5.9	65	32.3
7823	4a	13	22	15.7	91	30.6
7863	4a	12	160	20.6	109	29.3
Jamapa	2	14	360	16.3	73	25.2
1706	4a	7	750	33.4	102	21.6
346	4a	13	920	29.9	53	24.6
Flor de Durazno	1	11	420	31.9	69	24.0
406	2	8	20	17.6	48	23.9
Flor mayo M-38	3a	9	805	29.4	71	23.6
424	3a	11	430	28.3	50	23.1
427	3a	10	675	22.1	99	19.7
429	3a	11	555	30.3	108	19.2

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COMMON BEAN LANDRACE POTENTIAL FOR CULTIVAR DEVELOPMENT AND DIRECT USE

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INTRODUCTION

The Convention on Biological Diversity (CBD) has as one of main goals the conservation, characterization, and sustainable use of biodiversity. The great amplitude of environmental conditions at which common bean (*Phaseolus vulgaris* L.) is cultivated in Brazil, results in multiple interactions between edaphic and climatic factors, that impose selection pressures favoring specific allelic combinations that lead to adaptation to these conditions and, as consequence, to an important genetic variability. The resulting populations from these processes are commonly known as landraces, and are usually highly valuable to breeding programs. Sources of resistance to fungal diseases and to low water availability have been detected from this germplasm (Antunes, 2008). Landraces resulting from the germplasm collection and characterization project underway at Embrapa Temperate Climate Research Center, are evaluated for the first time, along the cultivar development program, at the Internal Preliminary Trial I – EPI I, from where they might advance to the EPI Intermediary – EPI INT and from this, to the Internal Preliminary Trial II - EPI II. This article reports the performance of component landraces of EPI I in 2005/06, 2006/07 and 2008/09 cropping seasons

MATERIAL AND METHODS

Experiments have been conducted in 2005/06, 2006/07 and 2008/09 cropping seasons at Cascata Experimental Station, Embrapa Temperate Climate Research Center, Pelotas, Rio Grande do Sul State, Brazil, located at 31º37'49''S, 52º24'38''W. The experiments were constituted of one individual 3.0m long line of each of the landraces under test, with 0.5m between lines. Yield data was transformed to $kg.ha^{-1}$. The experimental design used was Federer's Augmented Blocks and checks were the cultivars BRS Expedito, black seeded, and Iraí, with white-dark red striped seeds. Each block comprised ten test lines, with the check cultivars at the beginning and at the end of the block. Test lines were evaluated with basis on the comparison with check lines through the graphic method, comprising the drawing of vertical lines in a graphic representing yield of individual test lines. Yields above the line connecting the mean yield of the check cultivars at each end of the block, were considered as promising lines. Landrace number under evaluation was 44, 58 and 205, in 2005/06, 2006/07 and 2008/09 cropping seasons, respectively. At sowing, the fertilization obeyed soil analysis requirements.

RESULTS AND DISCUSSION

EPI I structure and landrace performance can be found on Table 1. At 2005/06 cropping season 14 landraces reached superior yields, whereas 19 in 2006/07, and 66 in 2008/09, were considered as superior to the check cultivars. These data correspond to a mean percentage of 32.3 % of promising landraces by cropping season. Maximum yield detected was 3.996 $kg.ha^{-1}$ in 2008/09, surpassing in

111,28 % the mean yield of the checks at the respective block. An increase of 465% was observed in the number of tested landraces, from 2005/06 up to 2008/09 cropping season, an evidence of the efforts realized by EMBRAPA to collect, conserve and characterize the common bean landrace germplasm. Results have shown the existence of high yielding materials as well as innumerous sources of insect and disease resistance, and nutritional and functional outstanding lines, resulting in useful germplasm for cultivar development or even as cultivars, as such. It was also found great variability in seed coat color, being the percentage of black seeded entries 38.6, 32.7 and 20.9 in 2005/06, 2006/07 and 2008/09 trials, respectively.

The results confirm the high breeding value of the common bean landrace germplasm as source of favorable characteristics, as well as its high potential for direct use.

Table 1. Trial structure and landrace performance at the Internal Preliminary Trial I – EPI I in 2005/06, 2006/07 and 2008/09 cropping years at Embrapa Temperate Climate Research Center, Pelotas, RS, Brazil, 2010.

Cropping year	**2005/06**	**2006/07**	**2008/09**
Landrace number	44	58	205
Maximum yield ($kg.ha^{-1}$)	2447	1760	3996
Maximum above check cultivar mean yield percentage	34.4	102.6	111.3
Above check cultivar mean yield landrace number	14	19	66
Above check cultivar mean yield landrace percentage	31.8	32.7	32.2

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TECHNOLOGICAL QUALITY OF GRAINS IN THE RAINY CROP SEASON CONCERNING COMMON BEAN CULTIVARS

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INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is widely produced and consumed in Brazil, where the commercial type which pleases to the consumer the most is the one which has the "carioca" grain (cream in color and with brown strips), its being, then the most cultivated in the country. However, that concentration of production results into decreased price on the market and great demand in quality, not always reached by the farmer, who ends up commercializing his product with markdown. In this scenario, the entry of bean cultivars of special grain types on market can aggregate values to the final product values with the exploration of appropriate market niches. Some breeding programs have made new materials with these characteristics available, however, to make this use viable, it is needed to adequate not only the current production systems, but also to evaluate and make grain conservation suitable, since the characteristics of the new cultivars differ from those of the traditionally used cultivars. The objective of this work was evaluating the technological quality of freshly-gathered grains of different commercial groups of common bean.

MATERIAL AND METHODS

The cultivars were multiplied in the field in experimental area of the Agricultural Department of the Federal University of Lavras, in Lavras-MG, in the rainy crop season of 2008/2009, adopting the cropping practices usual to the crop in the region. Reaching the maturation date of each cultivar, the grains were gathered and threshed by hand and after natural drying to13% of moisture, submitted to the evaluations. The statistical design was the one of randomized blocks with five replicates and five treatments, cultivars BRS Radiante, BRS Ouro Vermelho, BRS MG Talismã, BRS Supremo and Bolinha (Table 1).

The percentage of imbibition before (PEANC) and after cooking (PEAPC) was determined according to **G**arcia-Vela & Stanley (1989) and Plhak et al. (1989), modified; **percentage of whole grains after cooking (PGI)** by means the evaluation of the grains utilized in Peapc, distinct and whole and split; average cooking time **(TMC)** by the Mattson cooker, according to Proctor & Watts (1987) and **volumetric expansion rate of grains after cooking (TEV),** according to Martin-Cabrejas et al. (1997). The analyses were conducted in the Plant Products Laboratory of the Food Science Department (DCA) of the UFLA. The data were submitted to the variance analysis and Scott-Knott test for mean clustering.

Table 1. Main characteristics of investigated cultivars.

Characteristic	Radiante[1]	Ouro Vermelho[1]	Talismã[1]	Supremo[2]	Bolinha[3]
Comm. group[4]	Others	Others	Carioca	Black	Others
Grain type	pinto	red	carioca	black	Yellow
Growth habit[1]	type I	type II/III	type III	type II	type II
Cycle	early	normal	medium	normal	-

[1]Vieira et al. (2006), [2]Melo et al. (2005), [3]Alves et al. (2009), [4]Classificação Ministério da Agricultura.

RESULTS AND DISCUSSION

The average values of the evaluated characteristics are presented in Table 2, where one can find that the cultivars did not differ as regards PEAPC, GIAC and TEV.

PEANC ranged from 88.6 to 94.2%, proving significantly influenced by the cultivars. As regards this characteristic, cv. BRS-Radiante stood out, while cv. Bolinha had its poorest behavior; the other cultivars showed intermediary percents.

As to the TMC of the grains, cultivars BRS-Supremo and BRS-Talismã stood out with faster cooking. The opposite occurred with cv. Bolinha, which also had already presented the lowest PEANC. Intermediary time was spent by the other cultivars (Table 2). The behavior of cv. Bolinha in relation to the PEANC and TMC is coincident with results of STANLEY & AGUILERA (1985) who related longer cooking time with lower water-holding capacity of grains, which can be due to the tegument impermeability, resulting into slow hydration during cooking. These authors and other point out, therefore, that the hydration capacity of grains before cooking can be a good indicative of cooking time (> hydration time, < amount of absorbed water, > cooking time). Other authors, such as DALLA CORTE (2003) and CARBONELL et al. (2003) disagree of that link, warranting poor correlation between these two traits. That situation seems to have occurred in the present work with cultivars BRS-Radiante (> PEANC and intermediary) and BRS-Supremo and BRS-Talismã (intermediary PEANC and < TMC).

Table 2 – Average values of PEANC, PEAPC, GIAC, TMC and TEV of grains concerning bean plant cultivars. Lavras- MG*.

Cultivars	PEANC (%)	PEAPC (%)	GIAC (%)	TMC (min)	TEV(g/mL)
Supremo	91.0 b	108.4	98.2	20.4a	0.8
Talismã	91.8 b	108.8	98.2	20.6a	1.0
Ouro Vermelho	91.8 b	110.0	98.8	23.0 b	1.0
Radiante	94.2a	100.4	98.2	23.2 b	1.0
Bolinha	88.6 c	96.8	99.0	25.8 c	1.0
General Mean	91.5	104.9	98.5	22.6	1.0

* The same letters in the column belong to the same group according to the Scott-Knott test at the level of 5% of probability.

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DRY BEAN SEED YIELD UNDER RAINFED CONDITIONS WITH TRADITIONAL AND IMPROVED TECHNOLOGY IN AGUASCALIENTES, MEXICO

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INTRODUCTION

Traditionally, producers of dry beans, under rainfed conditions in the semiarid North Central region of Mexico, sow bean in furrows 0.76 m width. Soil tillage is made with a pass of plowing disc and one or two passes of disking. Also, it is very common that the crop of bean under rainfed conditions is not fertilized, that fertilizers are applied in inappropriate quantities, or that fertilization is made in epochs where plants does not intake totally the fertilizer. Plant density oscillates between 85,000 and 90,000 plants per hectare, and there are few farmers who made rain harvest and soil conservation practices. With rain harvesting, crop will have more soil moisture that if this practice is not realized (Padilla *et al*., 2008), so that there will be more water for a higher plant density, but plants will need more nutrients so that additional fertilization will be needed.

MATERIALS AND METHODS

In 2009, a study was developed with bean under rainfed condition in Sandovales, Aguascalientes, Mexico. This site is located 2045 m.a.s.l. Recorded average rainfall during the growing season is 353.4 mm, average temperature is 16.3ºC, and length of growing season is 110 days. Soil has 0.45 m depth, 0.9% of organic matter, sandy loam texture, 1% slope, and pH 6.8. The aim of this work was to validate a strategy for an integral production of rainfed bean with a series of improved technological compounds applied during the growing season: a) Varieties: Pinto Saltillo, Flor de Mayo, and Azufrado Tapatío; b) sowing method in triple line on 1.52 width beds. Each line was separated 0.40 m and plant population was 170,000 plants per hectare; c) water harvesting practices: "in situ": Aqueel (The design of the Aqueel reservoirs ensures that far more of the water that lands on the field is retained for the benefit of the crop and vast reduction in run-off); d) leaf fertilization during the grain filling stage at a rate of 5.5 kg $ha^{-1}$and 4.2 l ha^{-1} of N-P_2O_5 (using urea as source of nitrogen and phosphoric acid for phosphorous); and e) minimum tillage method: vertical tillage (root cutter or Multiarado). This improved technology was compared to traditional technology used by farmers in the region such as plowing and disking, landrace cultivar, no use of rainfall harvesting practices and fertilizers, varieties not adapted to dry conditions, and plant density of 94,000 plants per hectare. Sowing was made on July 31. Straw and dry bean yield was obtained from the two central rows by three meter length in each treatment.

RESULTS AND DISCUSSION

Rainfall recorded from seedtime to harvest was 208.5 mm but uniformly distributed during the growing season. The obtained results show the efficiency of improved technical modifications to increase dry bean yields of evaluated varieties of beans, mainly because of a better distribution of moisture in the soil profile compared to that of traditional technology. In addition, soil erosion was

prevented by using the Aqueel, sow beds with three lines of plants due the reduction of water runoff and surface soil cover. Pinto Saltillo variety obtained the highest dry bean yield among the cultivars evaluated (Table 1).

TABLE 1. Dry bean yield with two types of tillage, three dry bean cultivars, and two methods of sowing in the Experimental Station Sandovales, Aguascalientes, México. 2009.

TILLAGE SYSTEMS	GENOTYPE	SEED YIELD (Kg ha^{-1})						Yield increase %
		CONVENTIONAL			TRADITIONAL			
		PP	SEED	STRAW	PP	SEED	STRAW	
P-TD	FMB	165,000	1,011.00	744.1	85,000	939.80	650.0	7.58
	AT	165,000	734.15	615.1	85,000	657.90	560.0	11.59
	PS	165,000	1,384.00	749.04	85,000	1,127.8	407.3	22.72
R-TD	FMB	165,000	466.29	456.40	85,000	282.00	317.2	65.35
	AT	165,000	406.80	515.90	85,000	360.30	352.4	12.91
	PS	165,000	902.80	545.70	85,000	571.70	407.3	57.91

P-TD – Plow-Tandem disk; Mu-Ra- Rootcutter-Tandem disk (minimum tillage); FMB – Flor de Mayo Bajío; AT – Azufrado Tapatío; PS – Pinto Saltillo; PP – Plant Population (thousands of planst ha^{-1})

Response of dry bean yield to tillage methods is showed in Table 1. A Yield reduction was observed with the rootcutter method compared to traditional tillage, contradicting a positive effect on dry bean yield reported by Padilla et al, 2008. A reason for this negative rootcuter effect on dry bean yield in this particular year was that the site was not cultivated in the last 20 years, so that soil compaction was the main soil factor which affected soil properties such as compaction, water infiltration, and soil moisture compared to that with traditional tillage. When a soil has not been cultivated for several years, it is recommended, in this area, to plow and disk the soil to break down its compaction and then, in the following years, uses minimum tillage methods. Theses results suggest that minimum tillage such as rootcutter, could have greater seed yield response when soil compaction is not a problem.

In general terms, it can be mentioned that production of dry bean under rainfed conditions with improved technology (tillage conservation, rain harvesting *in* situ, narrow furrows, high plant population, and adequate fertilization) can be more profitable than traditional technology in the semiarid North Central region of Mexico.

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GRAIN YIELD OF DRY BEAN CULTIVARS GROWN UNDER RAINFED AND IRRIGATED CONDITIONS AT TWO SOWING DATES

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INTRODUCTION

Common bean is the second most important crop in Mexico and this legume has played an important role in the diet of high percentage of people in our country. Nevertheless, average consumption has declined approximately by half compared to that consumed 30 years ago. The cultivated area with dry beans has also been reduced in more than 500 thousand hectares during last decade (1). This reduction in the cultivated area with dry bean is even more marked under rainfed conditions in the Mexican Highlands, mainly due to high production risks. On the other hand, dry bean cultivated under irrigated conditions represent less than 6% of the total cultivated area. Considering that predominate climate at the Highlands is semiarid, with an average precipitation of 300 to 350 mm during the growth cycle, drought stress is one of the main limiting factors affecting grain yield. Another constraint of dry bean production is the scarcity of irrigation water stored in dams or aquifers. Therefore, to guarantee enough dry bean production to satisfy consume demands in future years is important to develop both, new dry bean cultivars having better adaptation traits to water stress conditions, as well as crop management practices focused in reducing production risks and increase water use efficiency. In this study, grain yield of three dry bean cultivars grown under rainfed and irrigated conditions at two sowing dates was evaluated.

MATERIALS AND METHODS

The study was conducted at the Experimental Station of Pabellón (22º 09' North Latitude; 102º 17' West Longitude; and an altitude of 1912 masl) located in Aguascalientes state, during the summer of 2009. Climatic conditions in Aguascalientes are similar to those of the Highlands. Some soil characteristics of the experimental site are: Texture: sandy loam; pH: 7.5; Organic matter: less than 1.0%; Field Capacity: 20%; Permanent Wilting Point: 11%; Apparent Density: 1.4. Sowing dates were on May 27th and June 24th. The dry bean cultivars included were: Flor de Mayo Anita, Pinto Saltillo and Azufrado-26. These cultivars were obtained at the dry bean genetic improvement program of INIFAP and have been recently released. Cultivars were grown under rainfed (drought stress) and irrigated (non-stress) conditions. Fertilization was applied either to soil (40-40-00 at first cultivation) or foliar (Urea 2% + 1% Phosphoric acid at 60 days after sowing). An additional treatment without fertilization (check) was also evaluated. Experimental unit per sowing date consisted of 36 rows (twelve per cultivar) of 30 m long and 0.76 m apart for drought stress and non-stress treatments. Fertilization treatments were applied every four rows. Precipitation was recorded at daily bases from a near meteorological during the growing season. At the end of the growth cycle the following traits were registered from four samples of two central rows of 4.0 m long at each treatment: grain yield, drought intensity index [DII=1-(Xd/Xp)], where Xd is the mean yield under drought stress and Xp is the mean yield under non-stress (2) and harvest index (HI=grain yield/aerial biomass, excluding fallen leaves).

RESULTS AND DISCUSSION

Drought stress reduced drastically grain yield of all dry bean cultivars as compared to the non-stress treatment in both sowing dates. This grain yield reduction was clearly represented by the DII, with values from 0.67 to 0.96 (Table 1). These DII values are higher than that reported in an experiment with dry bean cultivars grown under rainout shelter (DII=0.50, Acosta-Diaz *et al*., 2009). These results are attributed to the low precipitation occurred especially during July and first half of August, since only about 90 mm were accumulated in a period of 50 days coinciding with flowering and pod filling stages, which are the most sensible stages to drought. This condition caused a severe drought stress, which in turn reduced the number of pods per plant and seeds per pod (data not shown). Among cultivars, Pinto Saltillo showed the highest reduction of grain yield, with a DII of 0.96 and 0.93 at the first and second sowing date, respectively. Flor de Mayo Anita could be a good alternative under rainfed conditions plus supplemental irrigation, since in the second sowing date only two irrigations were applied. Harvest index, which is trait directly related to grain yield, also showed a great reduction in the drought stress treatment. Regarding to the fertilization treatments, at the first sowing date soil fertilized plants under non-stress showed higher yields than foliar or non-fertilized treatments, but not at the drought stress treatment. Overall data suggests that foliar fertilization in dry beans may be a low-cost option.

Table 1. Grain yield (GY), drought intensity index (DII) and harvest index (HI) of three dry bean cultivars grown under non-stress (NS) and drought stress (DS) conditions at two sowing dates and soil or foliar fertilized. Pabellón, Ags., México.

Cultivar	Fertilization	First sowing date					Second sowing date				
		NS	DS		NS	DS	NS	DS		NS	DS
		GY (kg ha^{-1})		DII	HI (%)		GY (kg ha^{-1})		DII	HI (%)	
Flor de Mayo Anita	Soil	2525	326	0.87	49.5	20.9	2143	220	0.90	53.2	50.4
	Foliar	2414	416	0.83	48.1	22.5	1720	188	0.89	52.8	51.6
	Check	2026	216	0.89	50.3	13.2	2162	141	0.93	57.0	45.9
	Mean	**2322**	**319**	**0.86**	**49.3**	**19.0**	**2009**	**183**	**0.91**	**54.4**	**49.6**
Pinto Saltillo	Soil	1999	38	0.98	37.6	2.1	1617	112	0.93	51.0	33.8
	Foliar	1934	61	0.97	41.0	2.6	1827	104	0.94	50.0	33.9
	Check	1870	120	0.94	44.1	5.4	2148	171	0.92	52.5	42.1
	Mean	**1935**	**73**	**0.96**	**40.9**	**3.4**	**1864**	**129**	**0.93**	**51.2**	**37.1**
Azufrado -26	Soil	1558	342	0.78	41.9	24.3	183	61	0.67	26.9	30.2
	Foliar	1109	322	0.71	41.9	22.9	266	48	0.82	31.2	21.9
	Check	927	411	0.56	41.1	27.2	281	131	0.53	33.7	29.9
	Mean	**1199**	**358**	**0.70**	**41.6**	**24.9**	**243**[¥]	**80**	**0.67**	**30.9**	**24.7**

[¥] Low plant density

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NAMING AND RELEASE OF PHC-12 AND PHC-38, TWO RUNNER BEAN CULTIVARS WITH TOLERANCE TO SUB-OPTIMAL TEMPERATURE

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The Legumes Breeding Group at the Misión Biológica de Galicia (MBG), National Spanish Research Council (CSIC), announces the release of two runner bean (*Phaseolus coccineus* L.) cultivars named PHC-12 AND PHC-38.

The scarlet runner bean is a climbing perennial crop but it is often grown as an annual for dry seeds and immature green pods production in some parts of Europe. The runner bean cultivars are appreciated for their large seeds and culinary quality together with high yield.

As observed by the authors and local farmers, the runner bean generally requires moderate temperatures for good germination and growth and the optimum temperature ranges from 20ºC to 30ºC. Thus, temperature is a limiting factor for runner bean production and temperature under 10ºC at sowing delays both germination and plant emergence, lengthening the crop cycle and increasing production costs. Therefore, an alternative to make maximum utilization of the available growing period is to use cultivars tolerant to sub-optimal temperature at the germination and emergence stages.

The performance of cultivars under different growing conditions was evaluated in growing chamber at optimal (17ºC-day/15ºC-night) and sub-optimal (14ºC-day/8ºC-night) temperature on the basis of germination, earliness, ability to grow and vigor (Rodiño et al. 2007). Global culinary quality (GQ) of seeds was evaluated on a homogeneous sample of each runner bean cultivar by twelve independent observers according to Sanz and Atienza (2001) and Santalla et al. (2004) being scores above 40=bad quality; 39–30=acceptable; 29–20=very good, and scores lower than 19=excellent.

PHC-12 (previously tested as PHA-0311) has white large seeds (160 g 100 seeds^{-1}), very good culinary quality (GQ=25.1), earliness (46 days to first flower) and very high production under experimental conditions (50000 plants ha^{-1}), yielding 199 g plant^{-1}.

PHC-38 (previously tested as PHA-1025) has white large seeds (175 g seeds^{-1}), very good culinary quality (GQ=27.2), earliness (50 days to first flower) and high production under experimental conditions (50000 plants ha^{-1}), yielding 59 g plant^{-1}.

These cultivars are particularly recommended either for productions and breeding in temperate areas where spring season is humid and relatively cold. The cultivars are maintained and regenerated by hand pollination in nethouses at the MBG-CSIC and are released as a public nonexclusive germplasm. Small amounts of seeds are available from A. M. De Ron (amderon@mbg.cesga.es) and M. Santalla (msantalla@mbg.cesga.es), Legume Breeding Group, MBG-CSIC, P. O. Box 28, Pontevedra, Spain.

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2009 FINANCIAL STATEMENT

BEAN IMPROVEMENT COOPERATIVE

BALANCE AS OF January 1, 2009			**$ 14,832.30**
INCOME			
2009 Dues	$	3,449.00	
Extra CDs	$	190.00	
Extra Books	$	55.00	
Extra Articles for 2009 Report	$	75.00	
2008 Dues	$	26.00	
2010 Dues	$	129.00	
Back Issues	$	45.00	
Bank Interest	$	183.86	
TOTAL INCOME	$	4,152.86	
EXPENSES			
Labor Charges	$	1,341.00	
Student Travel Awards - 2009 BIC Meeting	$	5,000.00	
Postage, Copy Charges and Office Supplies	$	1,746.96	
Printing – Volume 52	$	1,230.38	
Google Checkout and PayPal Fees	$	75.96	
Bank Charges	$	92.00	
TOTAL EXPENSE	$	9,486.30	
BALANCE AS OF December 31, 2009			**$ 9,498.86**