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Cover: 'Patoles' (*Phaseolus coccineus*) beans planted in dry bean field in Flores Magon, Durango, Mexico. Photo courtesy of J. Kelly.

THE 51st ANNUAL REPORT OF THE BEAN IMPROVEMENT COOPERATIVE

The Bean Improvement Cooperative celebrated the Fiftieth anniversary at the 2007 Biennial Meeting in Madison, Wisconsin. The highly successful meeting had approximately 120 registered participants and featured 27 oral presentations and 38 poster presentations. The quality of both the oral and poster presentations was excellent. The meeting began with The Frazier-Zaumeyer Distinguished Lecture, entitled: '*From Ralph Corbett's Bean Field to Molecular Details of Broad Spectrum Potyvirus Resistance*' The stimulating lecture was presented by Dr. Molly Jahn, Dean of The College of Agriculture and Natural Resources at the University of Wisconsin, Madison. The meeting received generous support from the following organizations: Seminis Vegetable Seeds; Harris Moran Seed Company; Syngenta Seeds, Inc.; Del Monte Foods; Brotherton Seed Company; Central Bean Company; Crites Moscow Growers, Inc.; Hartung Brothers, Inc.; and the Michigan Bean Commission. The BIC wishes to recognize the financial support of these organizations that helped the meeting to succeed. On behalf of the BIC, I wish to acknowledge the very substantial assistance of the organizing committee, particularly Ken Kmiecik, Roxanne Mainz, Rob Gehin, Alyson Thornton, and Michell Sass and I wish to thank the sponsors and the participants for making the meeting a success.

At the Award Banquet five BIC members were recognized, Former BIC President Mike Dickson was honored with ASHS Vegetable Breeding Working Group Award of Excellence and two student awards were presented for the best oral and poster presentations at the BIC meeting.

The outstanding student oral presentation was entitled: 'Molecular diversity in the PvTFL1y sequence, a candidate gene for the determinacy (fin) locus' presented by Myounghai Kwak, University of California, Davis Paul Gepts, advisor [pg.10].

The outstanding poster presentation was entitled: 'Morphological and molecular characterization of Rhizoctonia solani isolates from western Nebraska dry beans' presented by Jorge Venegas, University of Nebraska, Lincoln Jim Steadman, advisor, [pg. 86].

On behalf of the BIC, I would like to recognize Soon Park for his years of dedicated service on the BIC Coordinating Committee and I wish to welcome Peter Pauls as the newest member of the coordinating committee. I wish to acknowledge leadership that Jim Beaver provided to BIC Genetics committee and welcome Kirstin Bett as a new member to the Genetics Committee.

A standardization of the nomenclature for common bean chromosomes and linkage groups has been proposed by Pedrosa-Harand et al [pg. 106]. The numbers will conform with those previously assigned to bean linkage groups, B1-B11. The proposal has the support of the BIC Genetics Committee [pg. iii-iv] and should simplify the reporting of bean chromosome numbers and linkage groups in future studies. The change has been made to the revised Bean Gene List posted on line.

The next BIC meeting is planned in Denver/Fort Collins in October, 2009. Details of the 2009 BIC meeting can be found at the BIC Web page (http://www.css.msu.edu/bic). Members are asked to check the web page periodically for upcoming events and deadlines related the BIC.

Dr. James D. Kelly, BIC President

BIC COMMITTEE MEMBERSHIP - 1957 to 2008

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

Awards Committee:

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

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).

REPORT OF THE BIC GENETICS COMMITTEE

(Minutes submitted by Carlos Urrea – abstracted)

The Genetic Committee met in Madison WI on October 31st, 2007 at 4:30pm. Decisions were made on six of the 10 topics discussed. A full version of the minutes can be found at www.css.msu.edu/bic

1. Topic: Regular cycle for review of the gene list and updating of the online list at the BIC website
Decision: The gene list will be reviewed and updated every six months. Each Annual Report of the BIC will include a section with new gene symbols. The complete list of gene symbols will continue to be posted on the BIC website and published every 5 years in the Annual Report of the BIC.

2. Topic: Review of published gene symbols or new gene symbols that have not gone through the genetics committee

A. *Phg* gene symbol for angular leaf spot resistance
James Beaver presented the proposed gene symbol for angular leaf spot (*Phg*) and indicated that allelism tests had already been completed (Mahuku et al., 2004). No Decision

B. Gene symbols for *Apion godmani* resistance
Several papers have been published using the *Agm* and *Agr* gene symbols for *Apion godmani* resistance, however these symbols have not been previously approved by the genetics committee. Matthew Blair presented the molecular mapping of *Apion godmani* resistance genes (Blair et al., 2006) and the previous assignment of gene symbols (Garza et al., 1996). No Decision

C. Gene symbols for Arcelin
Jim Myers presented the issue of the use of *Arc* or *Arl* gene symbols in the literature for Arcelin that confers resistance to bruchid insects. These gene symbols have previously been approved, but guidelines are needed for their application to molecular and genetic studies in tepary and common bean. No Decision

D. Anthracnose gene symbols
Celeste Gonclaves Vidigal presented results on the inheritance of anthracnose resistance in Mexico 222. No Decision

3. Topic: Nomenclature system
Paul Gepts suggested that the common bean community should use the linkage group nomenclature (Freyre et al., 1998) instead of the chromosome number nomenclature (Pedrosa et al., 2003).

Decision: The Genetics Committee will recommend the Freyre et al. (1998) nomenclature for linkage group nomenclature and orientation. Paul Gepts will contact the relevant groups concerning these new guidelines. These guidelines will then be posted on the BIC website. The prefix identification of each linkage group with the letter 'B' or 'Pv' is still under consideration. Either 'B' or 'Pv' will be selected.

4. Topic: QTL nomenclature
QTL nomenclature varies from study to study in common bean. There is a need for a common system for assigning QTL symbols.

Decision: Phil Milkas (convener), Matthew Blair, and Paul Gepts, will consult and decide on the best system for QTL nomenclature in common bean.

5. Topic: Conflicting complementation results with Widusa and PI 207262 using race 65 in two publications
There are conflicting complementation results from Widusa and PI 207262 from different groups that may be due to differences in the isolate of race 65 that is being employed. In addition, data from a number of different groups indicate that *Co-9* and *Co-3* are allelic.

Decision: Considering the allelism results presented, *Co-9* will be renamed *Co-3*^{3}. Jim Kelly will communicate this information to those scientists involved.

6. Topic: Posting of items regarding common bean genetics on the BIC website. Review process for posting of unpublished results on the BIC website.
Jim Kelly brought the issue of SSR data from different sources posted on the BIC website. There were several errors in some of the unpublished work on the website, thus the discussion involved whether or not unpublished work should be posted.

Decision: Post published information on the website. Post unpublished work with author names and a disclaimer so that the authors can be contacted in case questions arise.

7. Topic: Change in Membership of Genetics Committee
Decision: James Beaver stepped down from the Genetics Committee and Kirstin Bett will replace him.

8. Topic: Next Meeting
Recommendation to conduct a half-day meeting during the W1150 meeting in Puerto Rico in February, 2009 because of the lack of time to complete the Genetics Committee business during the short one hour meeting at the BIC.

ADDENDUM

Recent Decision in reference to "**Topic 3: Nomenclature system**" from the Genetics Committee meeting on 10/31/07 (above).

Decision: The revision of the linkage group nomenclature and orientation has been accomplished and approved by the Genetics Committee. It is available at: http://www.css.msu.edu/bic/PDF/Standardized%20Genetic%20&%20Physical%20Bean%20Map%202008.pdf. It has been agreed to drop the prefix B from linkage group names. When warranted, for example in comparative genomics, the prefix Pv (e.g., Pv1) can be added to distinguish linkage groups with the same number but from different species. A full discussion is provided in the paper by Pedrosa et al. in the current BIC report.

Questions or comments should be addressed to the chairman of the committee: **Dr. Tim Porch, USDA ARS SAA TARS, 2200 P.A. Campos Ave., Suite 201, Mayaguez PR 00680: ph. (787) 831-3435, ext. 254; fax. (787) 831-3386; and e-mail; maytp@ars-grin.gov**

Coordination of Genes and Gene Symbol Nomenclature - BIC Genetics Committee
The Genetics Committee is a sub-committee of the Bean Improvement Cooperative that organizes and coordinates activities that deal with *Phaseolus* genetics. The committee has served as a clearinghouse for the assignment and use of gene symbols. The committee also maintains the **Guidelines for Gene Nomenclature (last published in the Annual Report of the Bean Improvement Cooperative in 1988, 31:16-19 and supplemented in 1999, 42:vi).** The committee also evaluates materials submitted for inclusion in the Genetics Stocks Collection of the Plant Introduction System (for those rules see 1995 Annu. Rpt. Bean Improvement Coop. 38:iv-v).

2007 BIC AWARD RECIPIENTS

2007 FRAZIER - ZAUMEYER DISTINGUISHED LECTURESHIP AWARD

MOLLY JAHN

Dr. Molly Jahn was born in Michigan on June 4, 1959. When she was 11 years old, she began trialing vegetable varieties in her family garden and has continued her dedication to vegetable breeding ever since. With a family heritage of world-famous plant breeders, she was inspired to pursue her passion for biology and agriculture. Dr. Jahn received her bachelor's degree with distinction in biology from Swarthmore College in 1980. She completed her Masters degree in plant genetics at the Massachusetts Institute of Technology in 1983 and her doctorate on virus resistance in common bean with Dr. Mike Dickson and Dr. Henry Munger at Cornell University in 1988.

Dr. Jahn began her professional career at Cornell University in 1991 after completing a postdoc at UC Berkley and working as a research associate at the Boyce Thompson Institute. Her research has focused on plant gene discovery, the analysis of crop genome structure and function, and the application of these findings to crop improvement. Her research has successfully integrated research from fundamental studies on the relationship between model species and crops, to the study of breeding methods and the release of varieties. A few of the research accomplishments include, a detailed comparative genetic map of the Solanaceae, the broadest dicotyledonous comparative genetic system; the application of this comparative genetic map to the isolation of genes for an diversity of traits including disease resistance and product quality; the development of molecular markers for indirect selection; the assessment of naturally occurring genetic diversity for several plant traits; and the elucidation of the genes that control distinctive traits, such as pungency in *Capsicum*. In addition to these basic research successes, Dr. Jahn has 51 active commercial licenses in force for open-pollinated and hybrid varieties, and hybrid parents, and received the prestigious seed-industry award of All-America Selection (AAS) in 2002 for the squash variety 'Bush Delicata.' In addition to research on pepper and squash, Dr. Jahn has integrated worked with common bean, pumpkin, and melon, and has published her research in highly regarded journals. As a result of her significant contributions in plant research, she was inducted as an AAAS fellow in 2006.

Dr. Jahn has been successful in the development of a dynamic network for technology transfer and germplasm distribution in the US, successfully fostering collaboration between industry and the public sector. Dr. Jahn is the director of the Organic Seed Partnership and the Public Seed Initiative, both programs focus on extension using an alliance of the public, private, and non-profit sectors interested in improving the dissemination and utilization of public plant varieties and crop genetic diversity. Through formal and informal collaborations, Dr. Jahn has completed extensive research and extension activities worldwide. She is a member of the Board of Directors of the AVRDC (Asian Vegetable Research and Development Center) and she was also a member of the oversight committee of the collaborative crops research committee of the McKnight Foundation, both focused on agricultural development overseas.

Dr. Jahn has placed high value on successful teaching and training, and as a result, numerous graduate, post-doctoral, and visiting scientists have been trained through her program at Cornell. In August 2006, Dr. Jahn became the first female dean in the history of the UW-Madison College of Agriculture and Life Sciences. She now overseas a UW-CALS campus of 2,200 undergraduate students, 1,000 graduate students, and 270 faculty members, and a budget of more than $150 million. In January of this year she unveiled the new Wisconsin Bioenergy Initiative and outlined how the College is taking the lead in Wisconsin's bioenergy research. Dr. Jahn is an innovator, and a dedicated and skilled educator, who understands the importance of teamwork and the critical role of a land grant university in today's economy.

ROBERT L. GILBERTSON

Dr. Robert L. Gilbertson, Professor of Plant Pathology at the University of California-Davis, was born in Chicago and earned a B.S. degree in Wildlife Biology from the University of Massachusetts-Amherst in 1978. Bob earned a M.S. in Plant Pathology from the University of Massachusetts in 1980. He received his Ph.D. in Plant Pathology at Colorado State University in 1985. From 1986 to 1990, Dr. Gilbertson was a Research Associate and Assistant Scientist at the University of Wisconsin-Madison working on the USAID-funded Bean/Cowpea CRSP project.

For over 20 years, Dr. Gilbertson has conducted research on bacterial, fungal, and viral diseases of common bean. He has used molecular biology for pathogen detection and analysis of pathogen diversity and plant/pathogen genetics. Bob has worked on detection, characterization and molecular biology of geminiviruses. He and his team cloned and sequenced the complete genomes of six bean-infecting geminiviruses, and pioneered the development of DNA probe and PCR-based geminivirus detection methods. Dr. Gilbertson and his associates were the first to utilize particle bombardment to confirm the infectivity of cloned geminivirus DNA, in addition to developing agroinoculation techniques for *Bean golden yellow mosaic virus* (BGYMV) and *Bean dwarf mosaic virus* (BDMV). They inserted the green fluorescent protein gene into BDMV and followed the viral movement in susceptible and resistant common bean cultivars. This revealed that resistance involved a block in long-distance movement of the virus. The BDMV-GFP reporter was also used to clone a common bean gene involved in viral resistance. Dr. Gilbertson has also detected viruses infecting common bean in California, and helped incorporate resistance to BCMV and BCMNV into major market classes.

While at the University of Wisconsin-Madison, Dr. Gilbertson initiated studies on common bacterial blight caused by *Xanthomonas campestris* pv. *phaseoli* (*Xcp*) and *X. campestris* pv. *phaseoli* var. *fuscans* (*Xcpf*). He developed a dry-inoculum technique for infecting common bean in the field, and demonstrated that the bacteria could over-winter in common bean debris on the soil surface, but not in the soil. He established that common bean debris could be colonized by a diversity of non-pathogenic xanthomonds and used DNA probe and PCR detection methods to differentiate pathogenic from non-pathogenic xanthomonads. He used RFLP and repetitive element PCR analyses to elucidate the genetic diversity between and within *Xcp* and *Xcpf* populations. Moreover, he identified a genetically distinct *Xcp* population from East Africa with pathogenic specialization for the Andean bean.

Dr. Gilbertson and colleagues demonstrated the existence of genetically distinct pathogen populations of *Phaeoisariopsis griseola* (the cause of angular leaf spot) in different geographic regions of the world, and showed pathogenic specialization on the predominant common bean gene pool. This suggested co-evolution of the pathogen with the common bean, which had practical implications in terms of selecting appropriate pathogen isolates for resistance breeding for various production regions around the world. Dr. Gilbertson is an internationally renowned Plant Pathologist and an accomplished educator and leader. He teaches Introduction to Plant Pathology, Plant Bacteriology, Plant Virology, and Plant-Virus-Vector Interactions. He has mentored numerous Ph.D. students, visiting and postdoctoral scientists from around the world, and is actively involved in the Plant Pathology Graduate Program at UC Davis. He has served as an Editor for *Plant Cell Reports*, *Phytopathology*, *Plant Health Progress* and *Crop Protection*; and is currently the Editor-in-Chief for *Phytopathology*. Dr. Gilbertson has been an active member of the Western Regional W-1150 Bean Project and BIC. He also has been very active in international research and development since the mid 1980's. He currently has collaborative projects in the Caribbean, Central America, and West Africa. Bob has received numerous national and international awards including the Award from the Minister of Agriculture of the Dominican Republic for his research on the management of *Tomato yellow leaf curl virus* (1999). Dr. Gilbertson is also a Fellow of the American Association for the Advancement of Science.

WALTER EDWIN (ED) KEE Jr.

Walter Edwin (Ed) Kee Jr. was born on August 28, 1951 in Wilmington, Delaware. He earned a B.S. in Agriculture from University of Delaware in 1973. In 1975 he completed a M.S. degree in Plant Science, also from the University of Delaware, under the direction of Dr. Vernon Fisher. His thesis was titled "Evaluation and Implementation of Quality Measurement Techniques for Raw Lima Beans". Ed worked as the farm manager for Nassau Orchards, Nassau, DE from 1975 – 78. In 1978 Ed began his career with University of Delaware Cooperative Extension as the County Agriculture Agent for Kent County, Delaware. Since 1982 Ed has served as the State Extension Vegetable Crops Specialist, based at the Elbert N. & Ann V. Carvel Research and Education Center, Georgetown, Delaware.

As State Vegetable Specialist Ed advises farmers and conducts applied research on a variety of vegetable crops, including watermelon, cantaloupe, peas, sweet corn, and pickling cucumbers. It was Ed's work with lima beans, however, that motivated his involvement in the Bean Improvement Cooperative beginning in 1990. Since joining BIC Ed has hosted the BIC biennial meeting twice, first in Annapolis, MD in 1997 and then in Newark, DE in 2005.

Ed has been involved with research on all aspects of lima bean production: quality measurement, variety evaluation, flowering physiology, crop management, disease resistance, disease control, and mechanical harvest efficiency. When disease and other factors threatened the profitability of this crop in Delaware Ed brought together a diverse group of university faculty and extension staff to work on the problem. Ed has consistently encouraged faculty and graduate students to address the disease, production and mechanical harvest problems of lima beans. This research has kept Delaware lima bean growers and, in turn, regional vegetable processors competitive.

Ed's research and close relationship with the processing industry has resulted in new opportunities for Delaware vegetable growers. Ed was instrumental in attracting two new vegetable processing facilities to Delaware. One is a pickling cucumber grading facility. The other is a vegetable freezing facility which came to Delaware to procure lima beans but has since begun contracting with growers for peas and sweet corn as well. Ed is frequently in contact with individuals from all of the regional vegetable processors concerning potential areas of research.

Ed teaches an excellent class, "Issues in Agriculture", in which he takes students on day-long field trips to several farming operations and processing plants as well as brings in speakers on a range of topics, including: ag policy, environmental issues, land use, and international agriculture. Ed also teaches the University of Delaware's "Vegetable Science", and "Fruit Science" classes. His classes are hands-on and designed to give students a real taste of agriculture.

Ed is dedicated to supporting students. He enjoys interacting with students and has employed over 25 of them as summer workers in the vegetable research program and followed up by supporting them in their job search. In 2000 Ed and his wife Debbie established a scholarship to support students in the University of Delaware College of Agriculture and Natural Resources who are also athletes.

History is another of Ed's interests. In 1996 he earned a M.A. in Liberal Studies from the University of Delaware with a thesis project titled "Tense Times – Milford School Desegregation in 1954" and published in the journal Delaware History. In 2006 Ed published his first book, "Saving Our Harvest – The Story of the Mid-Atlantic Region's Canning and Freezing Industry." Both of these publications have led to numerous speaking engagements. Ed's most recent book, a collection of historical photographs entitled "Delaware Farming," was published in 2007.

HANS HENNING MUENDEL

Dr. Hans Henning Muendel was born on March 31, 1942 in Kosten Germany (now part of Poland) and moved to Oliver, British Columbia in 1951. Henning was awarded a B.SA. in Plant Science from the University of British Columbia in 1964. In 1966, Henning received an M.Sc. degree from the University of California at Davis, in Agronomy and Plant Breeding under the guidance of Dr. E.H. Stanford. The same year, Henning received a second M.Sc. degree in International Agricultural Development. During 1966-69, Henning was employed by CUSO to work at the Paniya tribal settlement in India as the Farm Manager for the Nilgiris Adivasi Welfare Association and later as a safflower breeder at the Nimkar Agricultural Research Institute. From 1972-74, Henning was employed as a wheat breeder in Kenya by CIDA. In 1973, Henning was awarded a Ph.D. in plant breeding from the University of Manitoba based on what breeding research under the guidance of Dr. Len Shebeski. In 1978, Dr. Henning Muendel began a long and distinguished career at the Lethbridge Research Centre of Agriculture and Agri-Food Canada. Initially, Henning was employed in the breeding and development of new crops. Henning made major contributions to cultivar development in safflower, hard red spring wheat and corn.

In 1987-88 and again in 1996 until his retirement in 2007, Henning was in charge of the dry bean breeding program at the Lethbridge Research Centre. In 1996, no dry bean cultivars grown in western Canada had been bred in this region. However over an 11 year period, Henning developed 14 dry bean cultivars (7 bean classes) and co-developed 1 new bean cultivar. Henning's bean breeding program concentrated on producing early maturing bean cultivars that are well suited to the short growing seasons of southern Alberta, Saskatchewan and Manitoba. Many of dry bean cultivars from Henning's breeding program also had improved resistance to anthracnose, white mould and bacterial wilt, while maintaining high yield and quality. Dry bean cultivars from Henning's breeding program now set the standard for small red, black and great northern cultivars in western Canada.

During his career, Henning also collaborated on a number of scientific studies to improve bean production in western Canada. Henning and Dr. H.C. Huang were the first to detect the presence of the three color variants of the bean bacterial wilt pathogen, *Curtobacterium flaccumfaciens* in western Canada. This led to the development of a new rapid method for screening dry beans for bacterial wilt resistance. Henning identified resistance to each of the variants of *C. flaccumfaciens* in dry bean cultivars and advanced breeding lines from western Canada. Together with his colleagues, Henning also launched a new study to investigate the inheritance of resistance to bacterial wilt in Canadian dry bean cultivars. Henning and his colleagues also were the first to identify *Erwinia rhapontici* as the pathogen responsible for the pink seed disease in dry beans. Henning participated in a national study to use a backcross program and marker-assisted selection to combine resistance to anthracnose, common bacterial blight and bean common mosaic virus in six different classes of dry bean. He identified physiologic resistance to *Sclerotinia sclerotiorum* and *Botrytis cinerea* in dry bean. In collaboration with Dr. R. Blackshaw, Henning also conducted agronomic studies on integrated cropping practices to improve weed control in dry beans.

The tremendous scope of Dr. Henning Muendel's research accomplishments is best exemplified by his extensive record of publications in scientific journals and his leadership role on many national and international research committees and projects. To date, Henning has published 90 scientific publications, 3 books, 2 book chapters and 210 miscellaneous publications. Henning also served as an editor of the refereed proceedings of three scientific conferences. Henning has served in various roles in the variety registration process (Prairie Registration Recommending Committee for Grain). He was a director of the Alberta Pulse Growers Commission and also served on the local arrangements committee for the BIC meeting held in Calgary in 1999 and for the Canadian Pulse Research Workshop held in Edmonton in 2002.

MATTHEW W. BLAIR

Dr. Matthew W. Blair, Germplasm Specialist and Andean Bean Breeder at the International Center for Tropical Agriculture (CIAT), graduated from Cornell University with his B.S. degree in 1987. He obtained a wide range of experience in plant breeding working as an undergraduate assistant for the potato breeding program at Cornell and as an assistant for the amaranth breeding program at the Rodale Research Center in Pennsylvania. He also served as an intern for an Asgrow melon breeding program in California and an Asgrow winter nursery in Puerto Rico. Dr. Blair's experience in international agriculture began in 1989 at the University of Puerto Rico where he refined his skills in Spanish and studied the inheritance of resistance to Bean Golden Yellow Mosaic (BGYM). A portion of his M.S. thesis research, which included first report the inheritance of the BGYM resistance gene *bgm*, was conducted in the Dominican Republic. During a semester at the University of Florida, Dr. Blair and collaborators made the first report of BGYM in southern Florida. He earned a Ph.D. in plant breeding from Cornell University in 1997. His dissertation research dealt with genetic fingerprinting of rice cultivars and the genomic location of the *xa5* gene for bacterial blight resistance in rice. Dr. Blair spent 17 months at Cornell University as a Post-doctoral Research Fellow where he conducted map-based cloning of a recessive gene for resistance to bacterial leaf blight of rice. He returned to bean research in 1999 as a Germplam Specialist / Andean bean breeder at CIAT in Cali, Colombia. His responsibilities at CIAT range from laboratory-based basic research using the latest molecular techniques to the improvement of large-seeded beans for small-scale farmers in Africa and South America.

Dr. Blair has established an international reputation for achievements in common bean breeding, genetics and genomics. He currently collaborates with bean research programs in the Americas, Africa and Europe, covering a wide array of topics. A few of Dr. Blair's research accomplishments documented in refereed journals include: the development of a microsatellite map for common bean; the mapping of genes for resistance to BGYM and the bean pod weevil; the QTL mapping of resistance to *Thrips palmi* Karny in common bean; QTL analysis of root traits related to adaptation to low soil fertility; studies of the phenotypic and genetic variability of common bean and tepary bean using microsatellite, AFLP and RAPD markers; the use of wild relatives to improve the seed yield potential and to broaden the genetic base of common bean; studies of the inheritance of climbing ability in common bean; and the development and release of improved climbing bean varieties and red mottled germplasm lines with resistance to BGYMV. Dr. Blair has been involved in an effort to generate and sequence 22,000 EST's for *Phaseolus vulgaris* through a Phaseomics consortium collaboration. These sequences have now been entered into GenBank. Dr. Blair has written or co-authored several review articles and book chapters dealing with the use of molecular markers and genomics to improve common bean. He is also a frequent contributor to the Annual Report of the Bean Improvement Cooperative.

Dr. Blair has provided numerous opportunities for post-doctorates and for graduate and undergraduate students from Colombia and many other countries to work in his laboratory. He currently serves on the Dissertation Committees or as the thesis advisor for many of these students. In addition, Dr. Blair has provided short to medium term training at CIAT in bean breeding and biotechnology to scientists from the Caribbean, South America and Africa.

Dr. Blair serves on the Steering Committee of Phaseomics and is a member of the Technical Committee of the Bean/Cowpea CRSP. He has served on the Genetics Committee of the Bean Improvement Committee and has participated in the review of grant proposals for the NSF Plant Genome Program. Dr. Blair serves as an external reviewer for several refereed journals.

IN MEMORY OF DONALD HAGEDORN

The bean community is saddened by the death of Donald J. Hagedorn who passed away on April 11, 2007. Don was born on May 18, 1919, and he received his undergraduate degree at the University of Idaho, and his Masters and PhD at the University of Wisconsin, Madison. Dr. Hagedorn was an internationally known plant pathologist and professor of plant pathology at the University of Wisconsin, Madison from 1948 until the time of his retirement in 1987. His dedication to field research led to the development of disease resistant peas and beans. A popular teacher and advisor, he provided a nurturing environment for the 48 graduate students who studied under his direction. Dr. Hagedorn published 320 scientific papers, many of which he presented at meetings and conferences in every area of the globe. His contributions to the field earned him many awards and recognitions, including the CIBA-Geigy Award in Plant Pathology, Meritorious Service Award from the Bean Improvement Cooperative in 1979, the National Pea Improvement Association, and the Forty-Niners Service Award for outstanding service to the canning industries, the APS Fellow Award from the American Phytopathological Society, and an Honorary Doctor of Science from his alma mater, the University of Idaho. In addition to his membership in several professional associations, Dr. Hagedorn was the co-organizer of both the International Working Group on Legume Viruses and the National Pea Improvement Association, and appointed to the US Plant Variety Protection Board in 1978 by the Secretary of Agriculture. Don was an avid fisherman, fishing the lakes and streams of Canada, Alaska, Idaho, and Montana. The Hagedorn Scholarship was established at the University of Wisconsin, and the University of Idaho in his memory.

THE 2007 FRAZIER-ZAUMEYER LECTURE

Bean Improvement Cooperative 50th Anniversary Meeting, Madison, WI

FROM RALPH CORBETT'S BEAN FIELD TO MOLECULAR DETAILS OF BROAD SPECTRUM POTYVIRUS RESISTANCE

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Genetic resistance to plant viruses has been used for at least eighty years to control agricultural losses to viral diseases. To date, hundreds of naturally occurring genes for resistance to plant viruses have been reported from studies of both monocot and dicot crops, their wild relatives and the plant model, Arabidopsis. While viruses are relatively simple genetic entities and much progress has occurred since the earliest studies, the mechanisms by which the many symptoms of disease are generated, and the mechanisms by which plants resist these effects remain largely unknown. In fact, the study of plant resistance genes raise many fundamental questions regarding the molecular, biochemical, cellular and physiological mechanisms involved in the plant-virus interaction and the evolution of these interactions in natural and agricultural ecosystems.

Of all the virus resistance genes that have been described in the last century of research, few, if any, have been as widely used in plant breeding as the *I* gene, named by Ali in 1950, but initially described by Pierce in 1934 based on a resistant selection made in the variety Stringless Green Refugee by Ralph Corbett of the Sioux City Seed Company. Because the *I* gene limits seed transmission of bean common mosaic virus (BCMV) and other legume potyviruses, this gene has been widely backcrossed into many bean varieties. There is considerable evidence that the dominant resistant allele, *I*, has arisen multiple times in *Phaseolus vulgaris*, in each case giving rise to a distinctive temperature-sensitive phenotype that typically involves a necrotic response to the virus under some conditions.

One other remarkable attribute of the *I* locus appears to be that mutations that confer this temperature-sensitive resistance to BCMV coincidentally confer resistance or lethal necrotic hypersensitivity to up to 8 additional potyviruses. In fact there are several well documented reports of co-segregation of resistance to plant viruses controlled by a simply inherited factor, e.g., Cook, 1961, Cockerham 1970, Schroeder and Provvidenti 1970, Provvidenti 1983. In each of these cases, pepper, potato, pea and bean, respectively, all the viruses involved are potyviruses, although some experienced plant breeders and a few short reports in the literature suggest that evidence for factors or crop genotypes with even broader resistance or tolerance beyond a single plant viral family. In the 1980s and 1990s, my advisors, students, collaborators and I published a number of papers that confirmed that the *I* locus controls resistance or a letha systemic necrotic reaction related to resistance to a large set of related potyviruses. More recently, we have shown that this resistance appears to have a dose-dependent quality, in that the inoculated phenotype of the heterozygote *Ii* could virtually always be distinguished easily from either homozygous parent, *II* or *ii*.

Inspired by these reports of co-segregation of resistance to multiple potyviral pathogens, I did the unthinkable for a bean breeder and began work in pepper in the early 1990s. Resistance initially reported in the 1950s in pepper for resistance to potato virus Y, pepper mottle virus and tobacco etch

virus showed monogenic recessive inheritance, in contrast to the dominant inheritance of the *I* gene. Based on our initial genetic studies in pepper confirming simple recessive inheritance and defining the breadth of the resistance spectrum of a series of alleles at a locus we now call pvr1 (pvr1 = potyvirus resistance locus), we showed that this locus encodes a factor (eIF4E) known to be essential for the initiation of genetic translation and highly conserved from pepper to yeast to mouse to human (Kang et al. 2004). Based on models of this protein, now known to account for virus resistance in many other plant species including pea, melon, Arabidopsis, tomato, we have identified specific amino acid changes that convert a plant from susceptible to resistant. Furthermore, we have shown that if we express mutant forms of eIF4E (pepper resistance alleles) under a strong promoter transgenically in a wildtype susceptible tomato plant, we can create a very strong dominant resistance to potyviruses (Yeam et al. 2007).

I predict that the famous *bc* loci defined by Drijfhout, known to limit necrosis when the *I* gene is overcome, may be alleles of *Phaseolus* eIF4E and its isoforms or proteins that physically interact with eIF4E and its isoforms.

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PIPE - PEST INFORMATION PLATFORM FOR EXTENSION AND EDUCATION

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The Legume PIPE consists of a network of 150 Sentinel plots in 30 states, provinces and districts of the U.S., Canada and Mexico; and is a spin-off from the Soybean PIPE; supported by various agencies within the USDA, legume commodities, and agribusinesses. Plots are monitored for pests such as soybean rust, soybean aphid and other insects, in addition to other regionally prevalent diseases such as white mold, common bacterial blight, and viruses such as *Alfalfa mosaic*, *Bean pod mottle*, *Bean common* and *yellow mosaics*, *Beet curly top*, *Cucumber mosaic*, and *Soybean mosaic*. PIPE enhances the role of IPM specialists by providing near real-time access to legume pest observations, model output, pest management information, as well as communication tools to support pest management decision making by growers during that growing season (Isard et al., 2006; Schwartz, 2007).

Legume PIPE – 2007 Activities and 2008 Plans: The goal of the sentinel plot component of the National Legume Risk Management Tool Development Project (PIPE) is to provide useful information for legume pest management through a national network of plots that are monitored for legume pests with an emphasis upon soybean rust (SBR). All states in the network monitored plots during the growing season, and some southern states also monitored "early sentinels" to determine overwintering success of SBR. Non-soybean hosts including other legumes (e.g., common bean, lima bean, lentil, chickpea, field pea) and kudzu were planted in sentinel plots. Each sentinel plot was monitored over the course of the growing season for approximately 12 weeks. The Legume PIPE consists of a network of 150 Sentinel plots in 30 states of the U.S., 3 Canadian provinces and one district in Mexico. Plots were monitored for pests such as soybean rust, soybean aphid and other insects, in addition to other regionally prevalent diseases such as white mold, common bacterial blight, and viruses such as *Alfalfa mosaic*, *Bean pod mottle*, *Bean common* and *yellow mosaics*, *Beet curly top*, *Cucumber mosaic*, and *Soybean mosaic*.

The State/Provincial Coordinator: (1) confirmed involvement of local cooperators and provided diagnostic training; (2) established linkage with the State Diagnostician (National Plant Diagnostic Network contact) to share primary pest information on Soybean Rust generated by the Sentinel Plot and/or other activities during the season; and (3) established linkage with the USDA/CSREES Soybean Rust Web Site and protocol to access resources and upload weekly survey data that was then made available to the public.

There were no suspicious samples of soybean rust detected in any Sentinel Plot or commercial field of any legume crop (other than soybean, jicama or kudzu) in the western or eastern region during 2007. Plans are underway to expand SBR (and other pest) monitoring on legume crops during 2008 with the addition of other priority legume diseases and pests such as white mold, common bacterial blight, Ascochyta leaf spot (of chickpea and lentil), viruses, Mexican bean beetle and aphids. There will be more outreach efforts by providing public access to the Legume PIPE during 2008, and access to an Image Gallery of priority pests of legumes. The public web site is available at: http://sbrusa.net. The threat to other legume crops such as common bean has been increasing

annually as more soybeans become infected earlier each year in the U.S., and now even in Canada and Mexico; and legume monitoring will be even more critical during 2008.

Soybean PIPE – SBR Summary for 2007: The Soybean Rust (SBR) web site reports that SBR has now been found in 331 counties in the U.S.; highest number of counties reporting the disease since it was first discovered in the continental U.S. in 2004. SBR has now been detected in one Province in Canada, in two states (3 municipalities) in Mexico, and in 19 States and 329 counties in the U.S including: 40 counties in Alabama (19 soybean), 33 counties in Arkansas (soybean), 24 counties in Florida (11 soybean), 48 counties in Georgia (14 soybean), four counties in Illinois (soybean), one county in Indiana (soybean), 14 counties in Iowa (soybean), nine counties in Kansas (soybean), three counties in Kentucky (soybean), 21 parishes in Louisiana (18 soybean), 26 counties in Mississippi (21 soybean), 37 counties in Missouri (soybean), four counties in Nebraska (soybean), six counties in North Carolina (soybean), 12 counties in Oklahoma (soybean), seven counties in South Carolina (soybean), seven counties in Tennessee (soybean), 26 counties in Texas (25 soybean), and nine counties in Virginia (soybean).

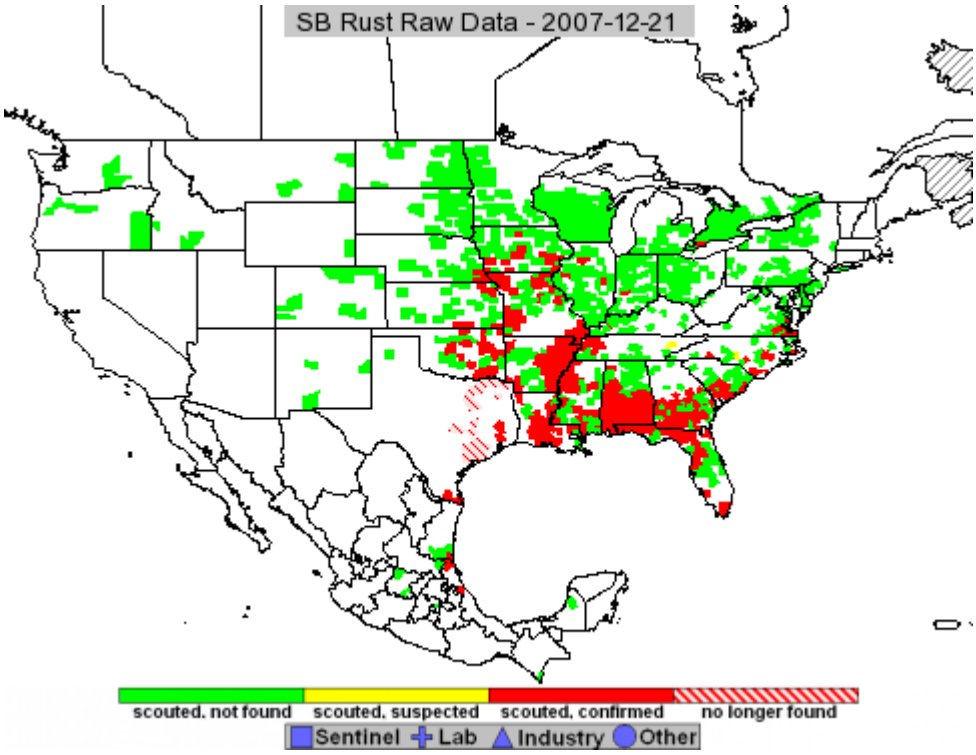

Figure 1. Soybean Rust (SBR) monitoring during 2007; darker spots indicate sites where SBR was confirmed on hosts including soybean (*Glycine max*), kudzu (*Pueraria lobata*) and jicama or Yam Bean (*Pachyrhizus erosus*) in Sentinel Plot or commercial fields [http://sbrusa.net].

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EFFECTS OF DRY BEANS ON THE DEVELOPMENT OF EXPERIMENTALLY INDUCED BREAST CANCER

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The social and economic burden caused by chronic diseases such as obesity, diabetes, heart disease, and cancer is enormous. To reduce the impact of these diseases, we must find ways to reduce their prevalence. Epidemiological studies have found links between the incidence of cancer and consumption of dry edible bean (*Phaseolus vulgaris* L.) in the human diet. Correa (1981) examined data from 41 countries and found a significant inverse relationship between bean consumption and morbidity due to breast, prostate, and colon cancer. Preclinical studies have also shown that bean consumption can reduce colon cancer (Hughes et al. 1997; Hangen and Bennink, 2003). Hughes et al. (1997) found that feeding pinto beans to laboratory animals reduced the incidence of colon cancer and the number of tumors compared to a diet without bean. Hangen and Bennink (2003) also reported that feeding black or navy beans to laboratory animals reduced both the incidence and number of colon tumors per animal by 50%.

At the outset of our work on "Defining the Health Benefits of Dry Beans", we decided to focus our initial investigations on ten market classes of dry beans representative of both Centers of Domestication (COD) and market classes that account for the majority of global dry bean production. The objectives of this research were to: *i*) determine if dry beans in the diet have cancer inhibitory activity in a preclinical model for breast cancer, and *ii*) determine if differences exist among dry bean market classes for anticancer activity.

METHODS

Beans evaluated in this study were represented by the commercial market classes small red, great northern (race Durango, Middle American COD), navy, black (race Mesoamerican, Middle American COD), dark red kidney, and white kidney (race Nueva Granada, Andean COD). All beans were obtained from Archer Daniels Midland Company, Decatur, IL, a bean seed processor that purchases commercially grown beans throughout the USA. Beans were shipped to Bush Brothers & Company, Knoxville, TN for canning using their standard commercial process, then sent to Van Drunen Farms where they were freeze dried and milled. The resulting powders were used to formulate diets with 60% bean meal in the diet of female Sprague Dawley rats. The control diet was an AIN-93 purified diet with no bean powder (Reeves et al., 1993). To induce mammary cancer female rats were given an intra-peritoneal injection of 1-methyl-1-nitrosourea (MNU) at 50 mg kg^{-1} body weight at 22 d of age according to published procedures (Thompson et al., 1995). The feeding trial was terminated 46 d post carcinogen administration and at necropsy, euthanized rats were skinned and mammary gland chains were examined at 5X magnification according to Thompson et al. (2000). All pathologies identified by visual inspection were excised and processed for histological classification to verify that they were malignant *vs* benign according to procedures described by Thompson et al. (2000). Cancer incidence (proportion of cancer bearing rats) and cancer multiplicity (average no of cancer tumors per rat) were based on histologically confirmed adenocarcinomas. All animal research was conducted under a permit and approved by the Colorado

State University Animal Care and Use Committee. The animal feeding trials were repeated using beans produced in the 2004 and 2005 crop year. Thirty animals per treatment per trial were used.

RESULTS AND DISCUSSION

Animal growth rate was unaffected by dry bean in the diet for both the 2004 and 2005 crop. We found that all dry bean market classes evaluated had an ability to inhibit experimentally-induced breast cancer. Beans in the diet of laboratory animals reduced the incidence of cancer from an average of 95% in the control group to 68% in animals fed beans ($P \leq 0.001$). Multiplicity of tumors was also reduced from an average of 3.24 tumors per animal in the control group to 1.45 ($P \leq 0.001$) in animals that were fed the bean diet. The main effects and interactions between crop year, dry bean market class, and the presence or absence of seed coat color were also evaluated. There were no main effects or interactions associated with crop year ($P = 0.95$) or seed coat color ($P = 0.49$) for cancer incidence or multiplicity; however, both variables differed among dry bean market class ($P = 0.024$). To determine if the genetic heritage of different market classes was associated with differences in carcinogenic response we conducted contrast comparison between beans representative of the Middle American and Andean COD. Cancer preventive activity was associated with the geographic origin of the bean market class based on COD. Dry beans of Middle American heritage (n= 4) had cancer multiplicity that was 40% higher than beans of Andean heritage (n=2). These results were reproducible based on tests conducted on beans from two consecutive harvest years. The differences for cancer inhibitory activity of dry beans based on COD will be used to gains insights about the mechanisms that account for this protective activity.

ACKNOWLEDGEMENT

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IMPROVED HARVEST INDEX IN DROUGHT RESISTANT COMMON BEANS AND POSSIBLE EFFECTS ON COMBINING ABILITY

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INTRODUCTION

Crop improvement has often resulted in enhanced harvest index, especially in the cereals. Poor harvest index is a characteristic of wild ancestors of crops, which often display survival mechanisms that are contrary to maximizing grain yield (Evans, 1993; Richards, 1997). Wild bean is a case in point. The wild bean ancestor of common bean germinates in a weedy environment among small trees and shrubs at the onset of seasonal rains, and must quickly develop a guide and climb to the top of the surrounding canopy to survive in a competitive environment. In this phase seed production must be suppressed until vegetative growth has assured access to light at the top of the canopy. Cultivated common bean may still have a delicate balance between vegetative and reproductive phases, and is prone to excessive vegetative growth and low harvest index, for example, in conditions of abundant soil moisture and adequate soil fertility. Thus, improving sink strength and harvest index may be a major challenge of genetic improvement of yield potential and abiotic stress tolerance of common bean.

MATERIALS AND METHODS

Drought resistant common bean lines in the small red (SER), small black (NCB), and carioca (SXB) classes were developed at the International Center for Tropical Agriculture (CIAT) in Cali, Colombia. Parental materials combined sources derived from race Durango, as well as small red seeded lines of race Mesoamerica. Several lines outyielded commercial checks of the same class and were identified as drought resistant for more intensive physiological study. Results of 20 genotypes (SER 47, SER 78, SER 109, SER 118, SER 119, SER 125, SER 128, SXB 405, SXB 409, SXB 412, SXB 418, NCB 226, NCB 280, RCB 273, Tio Canela 75, Carioca, SER 16, SEA 5, Pérola and DOR 390) are reported here. Tio Canela was included as a small red check, and DOR 390 as a black seeded check. Lines were planted in the July-September 2006 dry season under conditions of adequate soil moisture (irrigated) and terminal drought. A 4 x 5 partially balanced lattice design with 3 replicates was used. A number of plant attributes including canopy temperature, leaf area index, canopy dry weight, pod harvest index (dry wt of pods/dry wt of total biomass at mid-podfill x 100), grain yield and yield components were determined. Pod harvest index is used as a surrogate for harvest index (HI) because leaf fall at maturity makes HI difficult to measure accurately. Root growth and distribution (0-60 cm soil depth) were determined for four genotypes: SEA 5, Tio Canela 75, SER 16 and DOR 390.

SER 16 has expressed excellent combining ability, readily transmitting its characteristics of drought resistance, short bush habit, and productive branching. Therefore, SER 16 was employed in backcross-1 populations of (SER 16 x (SER 16 x *P. coccineus*)) for improving resistance to aluminum toxicity, using three different accessions of *P. coccineus* (runner bean). Individual plant selections were made within these populations and were progeny tested in the F_3 generation under aluminum toxic soil conditions.

RESULTS AND DISCUSSION

The data on pan evaporation together with rainfall distribution indicated that the crop suffered significant terminal drought stress during active growth and development. The mean yield under rainfed conditions was 1876 kg/ha compared with the mean irrigated yield of 2940 kg/ha. Among the 20 lines tested, three lines SXB 418, SER 109 and NCB 280 were outstanding in their adaptation to rainfed (water stress) conditions while Tio Canela 75, Pérola and Carioca were the most poorly adapted. Many lines including SER 16 showed improved pod harvest index. SER 109 was particularly outstanding in its ability to partition greater proportion of biomass to pods (Figure 1). The superior adaptation of SXB 418 to drought stress was associated with greater canopy biomass and lower pod harvest index. SER 78 had greater pod harvest index but average seed yield under drought due to greater proportion of pod wall biomass to grain yield. In contrast, results on root growth indicated that drought sensitive Tio Canela and DOR 390 were more deep rooted than the drought adapted SER 16 and SEA 5, suggesting that root growth occurred at the expense of photosynthate mobilization to seed under drought stress.

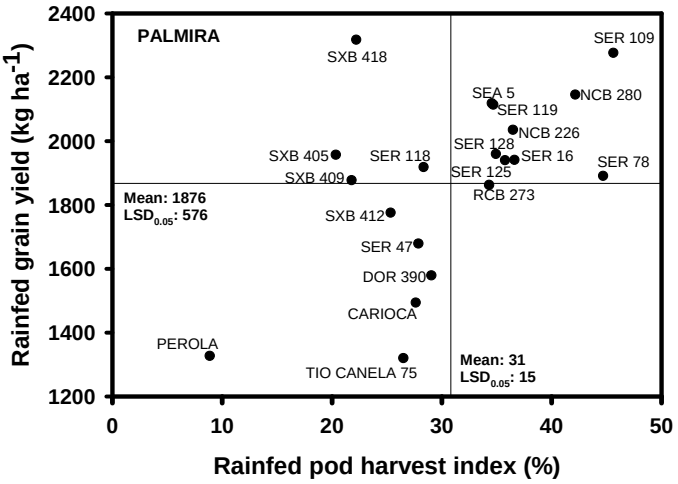

Figure 1. Identification of genotypes that combine superior seed yield with superior pod harvest index (upper right quadrant) under rainfed conditions in a Mollisol at Palmira.

Among the backcross-1 progeny of SER 16, productive plants were identified in all populations and progeny tested. Families from the cross with G 35346 were especially productive, expressing the positive traits of SER 16 (compact plant habit, productivity), but much improved resistance to aluminum toxicity over SER 16. We suggest that the combining ability of SER 16 is related to its ability to mobilize photosynthates to seed, resulting in enhanced HI. Furthermore, in the crosses with runner bean this trait may have contributed to improved quality of interspecific progeny, combining the typically large biomass of runner bean with improved harvest index of SER 16. Therefore, SER 16 may offer an option for introgressing traits from runner bean, leading to wider use of this genetic resource.

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MOLECULAR DIVERSITY IN THE *PVTFL1Y* SEQUENCE, A CANDIDATE GENE FOR THE DETERMINACY (*FIN*) LOCUS

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The determinacy trait has been selected during crop evolution to modify the partition of photosynthates between vegetative and reproductive growth, particularly to reduce overall plant vegetative biomass in favor of reproductive growth. Examples of this trait exist in several crop plants such as tomato, soybean, as well as common bean. Determinate common bean cultivars not only flower early but flower for a shorter time. Thus, in determinate bean cultivars, the resulting pod or seed yield is more uniform in size and developmental age and, therefore, easier to harvest. The determinate trait is controlled by a recessive gene, *fin*, which has been mapped on linkage group 1 (Koinange et al. 1996). The locus *fin* has pleiotropic effects on traits such as number of nodes on the main stem (NM), number of pods (NP), days to flowering (DF) and days to maturity (DM). In this study, we identify a potential candidate gene for determinacy and characterize molecular diversity for this candidate gene across the *P. vulgaris* germplasm

To build up a list of potential candidate genes for determinacy, we attempted to identify bean sequences that are homologous to flowering time genes in model species, mainly Arabidopsis. Sequences amplified with degenerate primers were confirmed through a BLAST search and were mapped onto the core genetic linkage map using the BAT93 × Jalo EEP 558 (Freyre et al. 1998) and Midas × G12873 (Koinange et al. 1996) recombinant inbred populations. Three *Terminal Flower 1* homologues (*PvTFL1x*, *PvTFL1y*, and *PvTFL1z*) mapped to the linkage groups 4, 1, and 7, respectively (Kwak et al. 2008). *PvTFL1y* co-segregated with the determinacy locus, *fin*, in the MG population, suggesting that *PvTFL1y* could be a candidate gene for the *fin* locus.

The determinate cultivar Midas has a 4.1 kb retrotransposon insertion at the end of 4^{th} exon in *PvTFL1y*. This insertion could lead inactivation of this gene, consistent with the recessive nature of the determinacy allele. A large-sized F_2 population (n=1472) was generated in the cross Midas x G12873. A PCR test was used to distinguish individuals with the determinate allele from Midas from those with the indeterminate allele from G12873. Concurrently, the determinacy phenotype of F_2 individuals was scored. In this large F_2 population, *PvTFL1y* co-segregated with the determinacy phenotype, suggesting that *PvTFL1y* is either the molecular version of the *fin* locus or very tightly linked to it.

The PCR test to identify individuals with a retrotransposon insertion into *PvTFL1y* was applied to 349 common bean wild and domesticated accessions from USA, Latin America, Europe, Africa and Asia. These samples include Andean and Mesoamerican samples as well as indeterminate and determinate accessions. One-hundred seventeen accessions with *PvTFL1y* alleles with retrotransposon insertion were identified; they were all determinate. No indeterminate line with such an insertion was found, confirming that *PvTFL1y* is a candidate gene for the *fin* locus. However, 42 determinate accessions without retrotransposon insertion in the *PvTFL1y* sequence were identified, suggesting that determinacy can originate from a different mutation at the *PvTFL1y* locus or that other loci can cause a determinacy phenotype.

The sequence of *PvTFL1y* in 44 representative accessions was determined, including the exons and introns of the gene, as well as a large stretch of sequence down stream from the gene. First, no indeterminate genotypes showed an allele with a retrotransposon insertion. Second, the only mutation found in indeterminate accessions were synonymous mutations that do not affect the protein product. Third, there were several mutations in the *PvTFL1y* locus of the determinate group that could to partial or complete lack of expression: in addition to the retrotransposon insertion already mentioned, a deletion of the entire coding region, 4 frame-shift mutations, a potential exon-splicing failure mutation, and 2 non-synonymous mutations. Lastly, no wild beans showed any inactivation haplotypes at the *PvTFL1y* locus, consistent with the absence of determinacy in wild beans. These observations again suggest that *PvTFL1y* is a candidate gene for determinacy but also that determinacy has multiple origins in common bean.

To analyze the lineage of the *PvTFL1y* haplotypes identified, a network tree of *PvTFL1y* sequences was generated. This analysis confirmed that the determinacy trait has multiple origins in the domesticated common bean germplasm as the determinacy phenotype was located in multiple branches in that tree. Haplotypes observed in wild beans were located in the center of the tree. Conversely, haplotypes associated with determinacy were located at the end of branches, suggesting that determinacy is a derived trait. *PvTFL1y* haplotypes with a retrotransposon insertion have a wide geographic distribution range that includes North and South America, as well as Europe, Africa, and Asia. Other haplotypes associated with determinacy have a more limited distribution within the Americas. The reason for the differential geographic distribution remains to be determined.

This study emphasizes the role of molecular approaches in identifying and conserving germplasm diversity. Also, this study suggests that different sources of the determinacy trait, which may facilitate the introduction of the trait into different genetic backgrounds. Experiments are under way to determine potential, more subtle differences in the determinate phenotype associated with the different *PvTFL1y* alleles, their expression, and their transmission across gene pools or ecogeographic races.

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MOLECULAR MAPPING OF GENES INVOLVED IN THE PHENYLPROPANOID PATHWAY IN BEAN (*PHASEOLUS VULGARIS* L.)

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INTRODUCTION

Previous genetic analyses identified 15 genes that control seed coat pattern and color in common bean (Prakken R, 1970). Some of these genes have been positioned on the common bean linkage map (McClean et al, 2002). It has been hypothesized that genes involved in the phenylpropanoid pathway correspond to some of the classical seed coat color genes in bean (Hosfield GL, 2005; Cotting and Hosfield, 2005).

In previous work, we aligned gene sequences available for phenylpropanoid pathway genes in other species, designed PCR primers for conserved regions and used the primers for RT-PCRs with bean seedling RNA. The sequences of the cloned cDNA segments were determined and confirmed by BLAST searches. Fragments of thirty four structural and regulatory phenylpropanoid pathway genes were identified.

The identification of the positions of these sequences in the bean genome would help to determine their roles in several important agronomic traits, including seed coat colour.

MATERIAL AND METHODS

To map the phenylpropanoid genes, their segregation patterns were determined in a recombinant inbred (RI) population derived from a cross between “Bat93” and “JaloEEP 558” (Nodari et al, 1993). This population is segregating for C, G, B and V seed coat color genes (Mcclean et al, 2002). DNA was extracted from fresh young leaves using CTAB DNA extraction method (Doyle and Doyle, 1990).

The phenylpropanoid genes were mapped using PCR (Polymerase Chain Reaction) and RFLP (Restriction Fragment Length Polymorphism) methods. The parental lines were tested for phenylpropanoid gene based polymorphisms by PCR using primers for the pathway genes designed for a previous study (Reinprecht et al, 2004). RI DNAs were screened with the primers and PCR conditions for those genes that gave polymorphic bands with the parental DNA. The polymorphism in the population was scored and the gene position on the bean linkage map was determined using JoinMap (Stam, 1993).

The RFLP method was used for genes that were monomorphic by PCR. For each sample 20ug of DNA was used. To identify the best restriction enzyme, the parental DNA was cleaved with six restriction enzymes (including: HindIII, EcoRI, BglII, DraI, BamHI and PstI), separated by agarose gel electrophoresis and blotted onto a positively charged membrane. The membranes were probed with fragments of the phenylpropanoid pathway genes. The probes were prepared by PCR amplification of clones using gene specific primers and DIG labeled dNTPs, hybridized to the membranes and visualized on X-ray film according to manufacturer instructions (Roche DIG

labeling kit). The polymorphisms were scored and a segregation analysis was performed with JoinMap.

RESULTS

So far we have been able to map nineteen genes from phenylpropanoid pathway in bean and put them on bean linkage map. Genes that have been mapped are as follows: Flavonoid 3'-hydroxylase (F3'H-1), laccase (LAC), anthocyanidin reductase (LAR), KAP2 transcription factor (KAP2), chalcone synthase (CHS), Myb4 transcription factor (Myb4-1), isoflavone reductase (IFR), isoflavone synthase (IFS), caffeate O-methyltransferase (COMT), isoflavone *O*-methyltransferase (IOMT), phenylalanine amonia-lyase (PAL1 and PAL2) and homeodomain protein regulatory factor (HD), vacuolar transporter (VT), Myb15 transcription factor (Myb15), ferulate 5-hydroxylase (F5H), cinnamyl alcohol dehydrogenase (CAD1), rhamnosyl transferase (RT1), glutathione S-transferase (AS1).

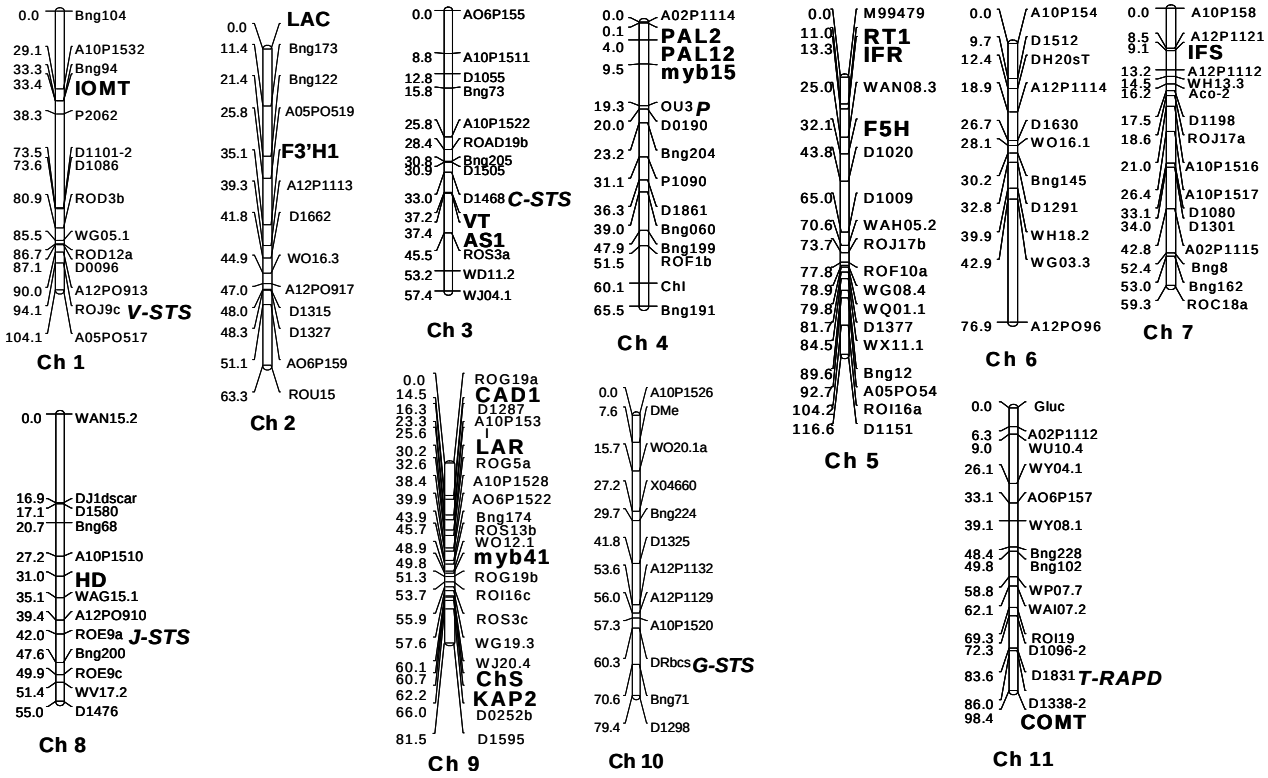

Fig.1. Position of the phenylpropanoid pathway genes (**Bold**) and markers for the classical color genes (***Italic***).

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UTILIZATION OF MICROSATELLITE MARKERS IN DIVERSITY ASSESSMENTS FOR COMMON BEAN

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INTRODUCTION

Microsatellite markers have been selected as the marker system of choice because of their low cost, high efficiency, whole genome coverage, robustness and minimum DNA requirements. In addition, they are highly polymorphic, co-dominant, PCR based and easily detectable. In common bean there are a range of microsatellite markers of which several sets of gene-based and genomic SSRs have been developed and mapped at CIAT. The aims of the CIAT program have been 1) to evaluate a large set of mapped SSRs for their diversity value (D), polymorphism information content (PIC) and heterozygosity level on a representative set of common bean genotypes and 2) to use the microsatellites in diversity assessment. This effort has been justified based on the need to select the most polymorphic markers for future analysis and apply them for germplasm characterization especially in the CIAT genebank and with National program partners.

MATERIALS AND METHODS

Plant material: A total of 44 genotypes were used in this study, representing wild accessions (3), landraces (17), breeding lines and cultivars (23) along with one tepary bean as an outgroup. All of the genotypes were of interest for biotic and abiotic resistance and/or grain quality traits. The genotypes were grouped in 3 parental surveys that were carried out separately with common controls, namely DOR364, a Mesoamerican genotype; and G19833, an Andean genotype, included in each survey.

Microsatellite analysis: The genotypes were evaluated for allelic diversity at 130 microsatellite (57 gene-associated and 73 genomic) loci. Amplification used genomic DNA template that had been extracted based on the miniprep procedure from Afanador et al. (1993). Microsatellite amplification and detection conditions were as reported in Blair et al. (2003). Markers that did not amplify were not considered further. To resolve allelic diversity as fully as possible, the PCR products for each survey were separated by electrophoresis for 1.5 hours at 120 constant volts on silver-stained 4% polyacrylamide gels. Microsatellite alleles for the control genotypes (DOR364 and G19833) were sized by comparison to the 10 and 25 bp molecular weight standards (Promega). Alleles of the remaining genotypes were compared to the control bands for each microsatellite so that molecular weights (in nucleotides) could be determined across parental surveys. Null alleles were not used in diversity assessment.

Data Analysis: The microsatellite alleles were coded into a binary data matrix that was analyzed by multiple correspondence analysis (MCA), using the CORRESP procedure of SAS (SAS Institute, 1989). Total diversity (H_t), intra population diversity (H_s) and inter population diversity (H_{si}) were also determined.

RESULTS AND DISCUSSION

Results of the multiple correspondence analysis and analysis of intra-population diversity and geneflow among the two principal clusters of genotypes corresponding to the gene pools and the subclusters corresponding to races is shown in Table 1. Intra population diversity (Hsi) was higher within the Andean genepool than within the Mesoamerican genepool and this pattern was observed for both gene-based and genomic microsatellites. Furthermore, intra-population diversity within the Andean races (0.356 on average) was higher than within the Mesoamerican races (0.302). Within the Andean gene pool, race Peru had higher diversity compared to race Nueva Granada, while within the Mesoamerican gene pool, the races Durango, Guatemala and Jalisco had comparable levels of diversity which were below that of race Mesoamerica. The divergence of the larger number of races in the Mesoamerican gene pool (Durango, Guatemala, Jalisco and Mesoamerica) was low compared to the races in the Andean gene pool (Nueva Granada and Peru). The distinction between races within each genepool has been further analyzed in two additional studies focusing on population structure in the Mesoamerican genepool (Diaz and Blair, 2006) and the Andean genepool (Blair et al., 2007). In conclusion, microsatellite markers have been useful for distinguishing races within the two major gene pools, and therefore supplement information collected from previous studies.

Table 1. Observed intra (H_s) and inter population (H_{si}) diversity for genotypes belonging to wild and cultivated common beans, to Andean and Mesoamerican gene pools and to races within each gene pool.

Category	N	Observed Heterogeneity			Value
		Gene-based (57)	Genomic (73)	Total	
Total	44	0.444	0.593	0.527	H_t
Species/Status[1]	44	0.429	0.575	0.511	H_s
Cultivated *P. vulgaris*	40	0.432	0.583	0.516	H_{si}
Wild *P. vulgaris*	3	0.388	0.477	0.437	H_{si}
Tepary Bean *P. acutifolius*	1	0.000	0.000	0.000	H_{si}
Gene pools	40	0.343	0.486	0.422	H_s
Mesoamerican	30	0.319	0.481	0.410	H_{si}
Andean	10	0.412	0.500	0.461	H_{si}
Races	40	0.253	0.363	0.314	H_s
Nueva Granada	4	0.215	0.352	0.292	H_{si}
Peru	5	0.397	0.436	0.419	H_{si}
Introgressed	1	0.000	0.000	0.000	H_{si}
Durango	4	0.154	0.325	0.249	H_{si}
Guatemala	2	0.246	0.292	0.271	H_{si}
Jalisco	3	0.257	0.367	0.319	H_{si}
Mesoamerica	21	0.289	0.430	0.368	H_{si}

[1]Status distinguishes wild versus cultivated *Phaseolus vulgaris*.

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APA LOCUS PROTEINS FROM TEPARY ACCESSION G40199 CONFERS RESISTANCE TO ACANTHOSCELIDES OBTECTUS IN COMMON BEAN INTERSPECIFIC BACKCROSS LINES

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INTRODUCTION

Bean bruchids are major seed storage pests of common bean. They have a cosmopolitan distribution, but are especially common in the tropics and subtropics of Africa and Latin America. Of the two major species, *A. obtectus* is adapted to more temperate environments than *Zabrotes subfasciatus*. *A. obtectus* is regarded as the more important pest because not only is it more broadly adapted, but it will also infest in field whereas *Z. subfasciatus* infests only in storage (both continue to multiply in storage). In East Africa, bruchids contribute to seasonal fluctuation of bean market prices with prices highest just prior to the main harvest. From 5-20% seed weight loss has been measured with 7 – 40% loss of marketable beans. Seed are of poor quality and have reduced germination.

Several *Phaseolus* seed storage proteins have been identified based on differences in electrophoretic patterns of total seed storage proteins. In addition to phaseolin, there is the arcelin-phytohemagglutinin-α-amylase inhibitor (APA) complex locus. This locus contains lectins/ phytohemagglutinins (PHA) that bind to glycans in intestinal epithelium of mammals and insects, the α-amylase inhibitors (α-AI) that bind to animal & insect α-amylases, and the arcelins (ARL) that are thought to be poorly digested and may alter insect gut structure. α-AI and ARL apparently evolved through gene duplication and divergence from PHA (Kami et al., 2006). Two classes of α-AI are known: α-AI-1 with specificity to animal α-amylases, and α-AI-2 that is specific to insect α-amylases. Seven alleles (*Arl-1 – 7*) have been described for the arcelins that have differences in insecticidal activity and species specificity. Several arcelins have strong resistance to *Z. subfasciatus*, but none possess strong resistance to *A. obtectus*.

P. acutifolius (tepary bean) possesses high levels of bruchid resistance, and is known to have lectin and lectin-like proteins (mainly α-AIs and arcelin-like proteins). Some wild and cultivated tepary beans exhibit bruchid resistance, and G40199 wild tepary was identified by CIAT as extremely resistant to both bruchid species. G40199 possesses a unique 33 kDa seed storage protein band compared to other common and tepary bean accessions, and this protein band appears to be associated with an arcelin (Kusolwa, 2007). The objective of our research is to develop African adapted dry bean cultivars with high levels of bruchid resistance. In this report we describe the effect of the APA locus when transferred to common bean on *A. obtectus* feeding and reproduction.

MATERIAL AND METHODS

Ninety-three $BC_2F_{2:3}$ families for ICA Pijao/G40199 and 33 $BC_1F_{2:3}$ families from ICA Pijao/G40199 BC_2//Rojo were used in this study (Kusolwa & Myers, 2005; 2006; Kusolwa, 2007). The families were characterized using SDS PAGE for the allelic state of the 33 kDa protein by evaluating 11 seeds per line. Insect feeding trials were conducted using a colony of *A. obtectus* that appeared spontaneously in the OSU breeding program materials. Vials containing 30 seeds for the ICA Pijao interspecific introgression population or 20 seeds for the Rojo interspecific backcross

population were infested with 15 *A. obtectus* adults and were kept undisturbed for 12 days at 25 ± 3°C. At that time, eggs were counted and the adults were removed. Seeds were observed daily for 72 days to record hatching adults. In the case of G40199 and some backcross lines, counts were extended to 100 days. Insect feeding parameters measured in this study included days after emergence for 50% F1 adults, number of adults emerged and rate of emergence, percent perforated seeds, percent seed weight loss, susceptibility index, severity of damage (number of seeds with ≥5 holes/seed, and weight of emerged adults (10 adults/sample).

RESULTS AND DISCUSSION

Significant differences were observed among families homozygous or heterozygous with, or homozygous without the G40199 APA locus. F1 adult emergence was progressively delayed as additional tepary alleles were added (Table 1.). Number of emerging adults was significantly reduced and seed damage was reduced when the tepary APA locus was present. Heterozygotes and homozygotes for the tepary APA locus were not significantly different from each other in terms of seed parameters (except for susceptibility index), but both classes were significantly lower than the class without the tepary APA locus. While introgression lines showed resistance, they were not as resistant as the original G40199 parent (Table 1). This suggests that other factors such as seed size and ratio of seed storage proteins to carbohydrates may influence amount of resistance. In addition to a reduction in emergence and seed damage, the size of bruchid adults emerging from seeds containing the tepary APA locus was significantly reduced, and this reduction in fitness carried over into the next generation even when adults were raised on seeds lacking the tepary APA locus.

Table 1. Means for bruchid damage parameters for an *A. obtectus* infestation trial of ICA Pijao introgression lines fixed or segregating for the tepary G40199 APA locus.

	Emergence		Seeds			
Genotype	50% (days)	No.	Perforated (%)	>5 holes (No)	SI[z]	Weight loss (%)
ICA-Pijao	44c	57a	52a	7.0a	3.9a	23a
arl/arl	46 c	64 a	48 a	6.5 a	3.7 a	18 a
ARL/arl	53 b	36 b	29 b	2.7 b	2.7 b	8 b
ARL/ARL	63 a	18 c	26 b	1.6 b	1.6 c	8 b
G40199	∞	0	0	0	0	0

[z]Susceptibility index

Overall, there appear to be economically significant levels of resistance in this material to *A. obtectus*. Several lines showed resistance very similar to G40199. Further trials are needed to determine field performance as well as cooking trials to evaluate palatability of lines carrying the tepary APA locus.

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MUTAGENESIS OF BAT 93 FOR TILLING IN COMMON BEAN

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In the absence of an effective transformation protocol, TILLING (targeted induced local lesions in genomes) of mutant populations allows for reverse genetics in common bean. EMS (ethyl methanesulfonate) creates a large number of point mutations and the mutation frequency seems to be independent of genome size, thus making it a widely used mutagen in plants for the development of large mutant populations. Therefore, using relatively small populations, it is possible to saturate the genome with mutations. The highest mutation frequency that yields adequate plant survival is selected in order to achieve optimal efficiency. Previous work in common bean used EMS concentrations ranging from 10-80 mM (Davis et al., 1988; Gautam et al., 1998). The efficiency of mutagenesis in common bean has been found to be affected by the concentration of EMS, the length of the treatment, and the ambient temperature (Pankhurst et al., 2004).

In this research, the mutagenesis population is being established using the genotype BAT 93, which was developed for high yield in tropical conditions at CIAT (Colombia). It is one of the parents of the core genetic common bean map, it has been used in the generation of a large BAC library, and it is amenable for production in temperate and tropical environments and under growth chamber, greenhouse or field conditions. BAT 93 also possesses a number of desirable characteristics, including disease resistance. Six EMS concentrations, including 0, 20, 30, 40, 50, and 60 mM, were used to treat seeds of BAT 93. After the 15 hour EMS treatment, the treated seeds were rinsed 20 times in water, and then planted in seedling trays and transferred to the greenhouse. A randomized complete block design was used with three replicates of each treatment. The experimental unit was a seedling tray containing 72 seeds. At 7 and 14 days after planting, the germination data was collected, and plant height data was collected on day 14. Ten randomly selected surviving seedlings were selected from each replicate and were transplanted to 15 cm round pots, watered regularly to avoid drought stress, treated with pesticides as needed, and fertilized. Seed yield component data was collected at harvest. The experiment was repeated twice.

The reduction in germination of BAT 93 in response to EMS concentration at 7 and 14 days followed a linear trend (Table 1). In addition, EMS treatment delayed germination as compared to untreated seeds. Thus, the evaluation of the seedlings 14 days after germination was found to more accurately reflect actual germination following EMS treatment. Significant differences in germination at 14 days were found between 0 mM EMS and EMS concentrations of 40 mM or higher. Although EMS concentrations of 60 mM resulted in germination rates of less than 10%, 50 mM EMS still yielded workable germination rates. Plant height at 14 days was severely affected by EMS and there was little variation in plant height at EMS concentrations above 40 mM. Seed yield components and other indices, including pod number, seed number, and harvest index, showed a similar response to EMS concentration. Significant reductions in pod number occurred with 30 mM EMS, and in seed yield and harvest index with 20 mM EMS. There were no significant differences in these yield traits at EMS concentrations above 30 mM. Above-ground vegetative biomass followed an opposite trend to seed yield from 0-30 mM EMS, as would be expected with yield compensation. At higher EMS concentrations (40-60 mM) however, vegetative biomass was increasingly affected by the EMS treatment. EMS treated seedlings that survived germination did not necessarily produce seed. Seedlings that survived EMS concentrations of 50 and 60 mM showed less

than 50% transplant survival. Overall survival showed a declining linear response with increasing EMS concentration. Large reductions in overall survival occurred with concentrations of 30 mM or higher EMS. Concentrations of 50 and 60 mM EMS resulted in overall survival rates of less than 10%.

Based on overall survival, phenotypic mutation rate, plant development, and yield of treated seed, 40 mM EMS is an appropriate concentration for the generation of a mutant population suitable for TILLING in genotype BAT 93. Using this concentration, a maximum number of mutations can be generated with a minimum population size and with relatively high efficiency. Higher concentrations of EMS resulted in overall survival rates of less than 10%, which are considered inadequate for efficient mutagenesis and population development, while lower concentrations (< 35 mM) resulted in a reduction in the frequency of phenotypic mutants. Overall survival is a better criterion than germination to evaluate EMS mutagen concentration, since EMS treated seedlings that survive germination, often do not produce seed.

Table 1. Effects of EMS mutagen concentration on BAT 93 survival and yield†.

			Survival		Height		Yield Components		
EMS conc.	Germination‡		Transplant survival	Overall survival	Plant height	Pod	Seed weight	Veg. weight	Harvest index
mM	7 days (%)	14 days (%)	%	%	14 days (cm)	#	grams	grams	
0	100.00	100.00	98.33	98.33	28.60	28.65	15.63	9.13	0.63
20	83.05	92.51	95.00	87.88	18.89	18.48	6.32	17.22	0.26
30	50.59	71.22	76.67	54.60	9.92	9.98	2.87	16.97	0.13
40	19.38	48.97	66.67	32.65	6.74	6.72	1.94	13.80	0.10
50	4.18	21.12	40.00	8.45	5.18	5.13	1.62	7.94	0.07
60	2.52	7.20	20.00	1.44	6.04	3.95	1.60	4.09	0.06

† Means of replicates shown.
‡ 0 mM EMS germination corrected to 100% for comparison to other treatments.

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RESISTANCE TO THE SOYBEAN RUST PATHOGEN (*PHAKOPSORA PACHYRHIZI*) IN COMMON BEAN CULTIVAR CNC

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Soybean rust (SBR), caused by *Phakopsora pachyrhizi*, is a new disease in the Americas that has moved swiftly from one country to another. *P. pachyrhizi* was first reported on soybeans in Paraguay (2001) and soon after in Brazil and Argentina (2002), Bolivia (2003), Uruguay and the USA, (2004), and Mexico (2005). Because *P. pachyrhizi* is a very aggressive pathogen and all commercial soybean cultivars are susceptible to SBR, severe yield losses in soybeans caused by SBR have been reported in Brazil, Argentina and other countries in South America and Southern Africa. Moreover, the SBR pathogen has a very broad host range of leguminous crops including common bean. SBR has been reported on common beans in South Africa (2005), the USA (2006), and Argentina and Brazil (2007). In a recent publication, 16 common bean cultivars were inoculated with six isolates of SBR from Asia, Africa, and South America (Miles et al., 2007). The common bean cultivar Compuesto Negro Chimaltenengo (CNC) was among the most resistant to all six isolates while Mexico 309 (Mx. 309) was susceptible. Here we report the inheritance of SBR resistance in CNC.

MATERIALS AND METHOD

Inheritance of SBR resistance in CNC was studied by crossing Mx. 309 (female) and CNC (male). F_1 seedlings were inoculated with the an isolate of the dry bean rust pathogen (*Uromyces appendiculatus*) to which Mexico 309 was susceptible and CNC was resistant to insure that they were the product of a cross. Because SBR inoculated seedlings are destroyed, this population was not inoculated with SBR in order to get F_2 seed. The F_2 seed was produced in a greenhouse in Beltsville, MD. Soybean check cultivars, parental lines (CNC and Mx. 309), and 241 F_2 plants were inoculated at the USDA-ARS Foreign Disease-Weed Science Research Unit Biosafety Level 3 Plant Pathogen Containment Facility at Ft. Detrick, MD, with a *P. pachyrhizi* isolate from Brazilian (BZ01-1). Disease severity was evaluated on the first trifoliate leaf using a 1 - 5 scale as described in table 1.

RESULTS AND DISCUSSION

CNC had significantly lower severity and sporualation than Mexico 309 (Table 1). A total of 140 F_2 plants from the cross Mexico 309 x CNC were resistant and 101 susceptible (Table 2). Based on severity, the segregation for SBR resistance on the F_2 population only fit a 9 resistant: 6 susceptible ratio with a Chi squared value of 0.26 and *p* value = 0.60 (Table 3). These results support the hypothesis that resistance in CNC to SBR is controlled by the interaction of two genes with complete dominance at both gene pairs; one dominant allele of each two genes is necessary to produce the resistant phenotype (A_B-) but either recessive homozygote is epistatic to the effects of the other gene.

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Table 1. Soybean rust mean severity and sporulation ratings of CNC and Mexico 309 *Phaseolus vulgaris* genotypes inoculated with six different isolates of *Phakopsora pachyrhizi*

Soybean rust isolate	Mean Severity[y]		Mean Sporulation[z]	
	CNC	Mexico 309	CNC	Mexico 309
BZ0-1	2.1	3.7	1.5	3.4
PG01-2	2.3	3.7	1.5	2.5
TH01-1	2.5	3.0	2.1	2.5
TW72-1	2.6	3.4	1.3	1.8
TW80-2	2.4	3.4	1.3	3.1
ZM01-1	2.6	3.4	1.8	2.6

[y]Severity: Based on lesion density, where 1 = visible symptoms, 2 = few scattered lesions present, 3 and 4 = moderate and abundant number of lesions, 5 = prolific lesion development over most of the leaf.
[z]Sporulation: evaluated on 1-5 scale, where 1 = no sporulation, 3 = sporulation present and equal to 26 to 50 % of a fully sporulating lesion, 5 = sporulation similar to a s fully sporulating lesion.

Table 2. Reaction of F_2 plants from the cross of Mexico 309 (S) x CNC (R) *Phaseolus vulgaris* genotypes to a Brazilian isolate (BZ01-1) of *Phakopsora pachyrhizi*

	First Batch	Second Batch	Total (1^{st} +2^{nd} batches)
Severity Reaction	Number of F_2 plants	Number of F_2 plants	Number of F_2 plants
1 (Resistant)	0	0	0
2 (Resistant)	6	10	16
3 (Resistant)	63	61	124
4 (Susceptible)	37	45	82
5 (Susceptible)	11	8	19
Total	117	124	241

Table 3. Resistant and susceptible severity reactions of an F_2 population derived from the cross CNC (R) x Mexico 309 (R) and inoculated with Brazilian isolate (BZ01-1) of *Phakopsora pachyrhizi*

	Number of Plants		Expected Ratio	Chi Square and *p* values	
	R[y]	S[z]	R:S	χ^2	*P*
F_2 (Mx.309xCNC)	**140**	**101**	**9:7**	**0.26**	**0.60**
F_2 (Mx.309xCNC)	140	101	13:3	45.90	0.0
F_2 (Mx.309xCNC)	140	101	15:1	517.02	0.0
F_2 (Mx.309xCNC)	140	101	3:1	35.84	0.0

[y]Resistant = Severity reactions 2 and 3; [z]Susceptible = Severity reactions 4 and 5.

COEVOLUTION OF THE BEAN RUST PATHOGEN *UROMYCES APPENDICULATUS* WITH ITS WILD, WEEDY AND DOMESTICATED HOSTS *(PHASEOLUS* SPP.) AT A CENTER OF DIVERSITY

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INTRODUCTION

Bean rust caused by the fungus *Uromyces appendiculatus* is a major constraint for bean production around the World. Although bean rust occurs worldwide, is most common in humid tropical and subtropical areas (Stavely, 2005). Rust diversity studies have shown high virulence and pathogenic diversity in Honduras (Araya et al., 2004, Sandlin et al., 1999 and Jochua, 2004). The high virulence diversity of *U. appendiculatus* in Honduras supports the hypothesis that this area is a diversity center for the fungal pathogen. Also, the occurrence of commercial varieties, landraces and wild beans growing in close proximity make this an appropriate region to study the host–pathogen interactions. The objectives of this study were: 1) to characterize and compare the virulence diversity of *U. appendiculatus* in wild, weedy and domesticated populations of *Phaseolus* spp. in Honduras using the virulence patterns obtained on the new standard 12 bean rust differentials 2) to assess the importance of the composition of domesticated (cultivated), weedy and wild *Phaseolus* species populations in *U. appendiculatus* virulence diversity throughout regions of Honduras.

MATERIALS AND METHODS

In November and December of 2002 to 2005 rust infected leaves and seeds were collected from wild, weedy (intermediate between wild and domesticated, introgression) and domesticated bean populations in Honduras. A total of 385 *U. appendiculatus* isolates derived from single pustules, were characterized on the 12 bean rust differentials. A mean disease score (MDS) was calculated for each isolate using the quantitative disease score. The MDS of an isolate is the average disease score across the 12 differentials. To identify the variables that contributed to variation among isolates, an analysis of variance was performed on the MDS of the isolates. To further describe the pathogen diversity in each location, descriptive population parameters such as number of pathotypes, pathotype frequency, virulence pattern and complexity, population richness and evenness were calculated.

RESULTS AND DISCUSSION

The MDS for each pathotype was not significantly different among populations (P= 0.5819). However, there was a significant difference for the MDS between species within populations (P= 0.0085). Moreover, when locations were compared on the basis of pathotype occurrence and frequency, differences among locations were evident (Fig.1). Cluster analysis based on MDS separated the 385 isolates into 91 pathotypes according to their virulence or avirulence on each of the 12 differentials. The virulence complexity of the 91 pathotypes varied from 3 virulent reactions to 11 virulent reactions on the 12 differentials comparing all isolates, 67% were virulent on 8 or

more differentials. The predominant pathotype, No. 43, comprised 22.9% of the isolates. Pathotype No. 43 was virulent on 10 of the 12 differentials and was present in 20 of the 28 host populations. The frequency of this pathotype in the 28 populations varied from 0% to 75%. The 10 most frequent pathotypes represented 69% of all isolates. None of the pathotypes were found in every population, while 15% of all isolates, were represented by only one isolate. The three diversity indexes calculated were similar among the majority of the populations in that most of the populations had values that overlap with each other. However, there were differences between some populations.

Long term coevolution of the bean rust pathogen in host populations composed of diverse genotypes, presumably with different genes for disease resistance, has favored highly virulent pathotypes. These pathotypes can cause disease when encountering most resistance genes utilized in bean breeding programs around the world, creating in Honduras a center for virulence complexity of the pathogen. Thus, the existence and proximity of wild, weedy and domesticated *Phaseolus species* in Honduras has played an important role in the virulence diversity found in *U. appendiculatus* populations.

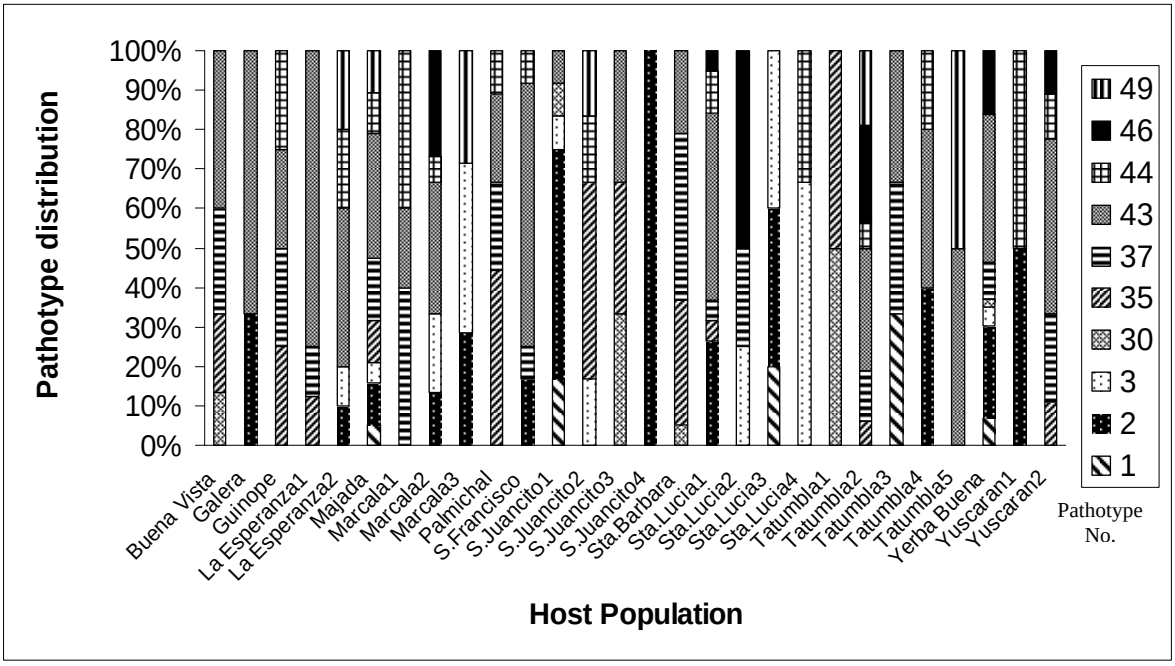

Fig. 1 Distribution of the ten most frequent *Uromyces appendiculatus* pathotypes collected in 28 *Phaseolus* spp. populations in Honduras.

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GENETIC IMPROVEMENT OF BEANS (*PHASEOLUS VULGARIS* L.) FOR GEMINIVIRUS DISEASE RESISTANCE

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INTRODUCTION

In Arizona, biolistic inoculation of hypocotyls from germinated seed was tested as an alternative to seeding and inoculating first trifoliolates for resistance screening of begomoviruses, and the former approach was found to be more efficient. This approach, together with scarification, resolved some of the problems associated with uneven seedling height (gene gun chamber space is limited) due to asynchronous germination in some lines. Additional seed was requested from various collaborators to replace lots that did not germinate well or at all.

METHODS

Candidate cultivars of selected *Phaselous vulgaris* L. in a core collection (Americas) were screened for geminivirus cross-resistance by inoculating seedlings with candidate viruses from phylogenetically divergent *Begomovirus* and *Curtovirus* genera: *Bean golden yellow mosaic virus* (Puerto Rico) *Bean calico mosaic virus*, *Squash leaf curl virus*, *Cotton leaf crumple virus* (CLCrV), and *Beet curly top virus* (BCTV)-Worland. All assays included 'Topcrop' and 'Red Kidney' as susceptible or tolerant/resistant controls from different centers of bean diversification. Field trials under natural BCTV pressure indicated Hystyle, Cardinal, G122, Othello, Zacatecano, Porrillo Sintetico, Moncayo, and Royal Red were highly resistant.

RESULTS AND CONCLUSIONS

The results of inoculations to date are shown below in Table 1. Nine of 15 lines experimentally inoculated with BGYMV, BCaMV, CLCrV, and SLCV exhibited a resistance response. Of those, Cardinal, Moncayo, Othello, Royal Red, DOR, T39 and UI-114 also were resistant to BCTV, indicating some extent of cross-resistance between virus genera and species in certain lines. In addition 'Red Kidney' is tolerant to *Squash leaf curl virus* (SLCV) (S) infection (perhaps also for Mesoamerican lines). Topcrop (Andean) is very susceptible to SLCV and *Squash mild leaf curl virus* (SMLCV), and this result also has been confirmed by PCR. SLCV and SMLCV are viruses from the Sonoran Desert; they and closest relatives are distributed throughout Mesoamerica and are not known to occur in South America.

Table 1. Results of geminivirus screening using biolistic inoculation for selected lines and varieties.

Line	Source	Type	Origin	Gene(s)	Geminivirus					
					BGYMV	BGMV	BCaMV	CTV	BDMV	TYLCV
A429	Beaver/Miklas	Dry	MA	bgm-1	R (PR)[1]					
Amarillo 154	AZ (Brown)[2]	Dry			S (GT)		S			
BAT1215 Baja tropico	CIAT	Dry	MA	Bgp-1?	R (PR)					
Benton	Syngenta	Snap	A				S	S		S
Bolon Rojo	AZ (Brown)	Dry			S (GT)		S			
Burros Argentinos	AZ (Brown)	Dry			S (GT)		S			
Caballero	AZ (Brown)	Dry			S (GT)		S			
Cardinal	Miklas	Dry	A	Bct?			R			
Carioca	AZ (Brown)	Dry			S (GT)		S			
Contender	?	Snap	A	?						R
Coscorron Corriente	AZ (Brown)	Dry			S (GT)		S			
DOR-364	Beaver/Miklas	Dry	MA	W12-QTL, Bgp-1?	R (PR)					
DOR-482	AZ (Brown)	Dry			R (GT)		R			
DOR-482 or 476	Beaver/Miklas/ CIAT	Dry	MA	bgm-1, W12, Bgp-	R (RP)					
Espada	Harris Moran	Snap	A				S	S		
Frijola	AZ (Brown)	Dry			S (GT)		S			
G122 (Jatu Rong)	Miklas	Dry	A	?	R (PR)			R		
G19833	CIAT/Miklas	Dry	A		S (PR)					
Genuine	Shamrock/Talo	Snap	A/MA	bgm-1	R (PR)					
Hilea	?	Snap	A							S
Hystyle	Harris Moran	Snap	A				S	S		
IAPAR 72(MD 820)	?	Dry	MA	?		R (BR)				
Monca 40	AZ (Brown)	Dry			S (GT)		S			
Moncayo	Syngenta	Snap	A	Bct parent			R	R	R	
Montcalm	Miklas	Dry	A					S (Mild)		
NY6020-4	Miklas	Snap	A	?				R [3]		
OjoCabraSantaRita	AZ (Brown)	Dry			S (GT)		S			
Othello	Miklas	Dry	MA	Bdm				R	R	
Porrillo Sintetico	Beaver/Miklas	Dry	MA	W12-QTL	R (PR)					
Porrillo Sintetico	AZ (Brown)	Dry			T (GT)		T			
PR9443-4	Beaver/Miklas	Dry	A	bgm-2	R (PR)					
PR9771-3-2	Beaver	Dry	MA	bgm-3, Bgp-2	R (PR)					
Primo	AZ (Brown)	Dry		Bct parent	S (GT)				S	
Primo	Syngenta	Snap					S	S		
Radical San Gil	AZ (Brown)	Dry			S (GT)					
Royal Red	Miklas	Dry	A	?	R			R	R	
SEL1309	CIAT/Miklas	Dry	MA		S (PR)					
T39	Miklas	Dry	MA	Bdm?					R	
Taylor Horticulture	Miklas	Dry	A					S		
Top Crop	?	Snap	A		S (PR)	S (BR)		S	S	
UI-114	Miklas	Dry	MA	?		S (BR)				
UI-44	Miklas	Dry	MA	Bdm?				R	R	
Venture	?	Snap	A	?						R
VR romano	AZ (Brown)	Dry			S (GT)		S			
XAN 176	Miklas	Dry	MA		S (PR)					
Zacatecano	?	Dry	MA	bgm-1?	R (PR)					

IS *FUSARIUM SOLANI* F. SP. *PHASEOLI* OR *F. LATERITIUM* CAUSING DRY ROOT-ROT IN COMMON BEAN?

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The common bean is cultivated in extended areas at diverse regions in Mexico. At the main producing areas in the highlands several *Fusarium* species have been frequently isolated from damaged bean roots. Most frequent and widely distributed are *F. oxysporum, F. solani*, *F. lateritium* and *F. reticulatum* (Montiel *et al*., 2005).

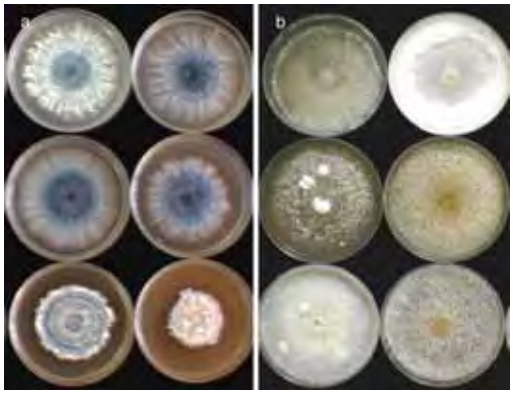
The cv. Montcalm, reported as susceptible to *F. solani* f. sp. *phaseoli* (Schneider and Kelly, 2000), was inoculated with more than 50 isolates of *F. solani* (Rodríguez, 2005; Guerrero, 2007) and none caused symptoms similar to those reported for this pathogen. This suggests that the isolates of *F. solani* obtained from bean roots at eight different states in the highlands of Mexico do not match the characteristics ascribed to f. sp. *phaseoli*, or that the symptoms ascribed to this f. sp. are not caused by *F. solani*, but by a different *Fusarium* species that has been misclassified.

Our aim is to present the evidence we have gathered which suggests that *F. lateritium* and not *F. solani* neither *F. solani* f. sp. *phaseoli*, is responsible for the dry root-rot of common bean in the highlands of Mexico. The first pieces of evidence are the symptoms caused by both species. Isolates of *F. solani* obtained in Mexico only causes a root discoloration, but never develop a reddish–brown coloration on the hypocotyls and primary root of the bean plant, as that described by several authors (Davet and Sardy, 1972; Bolkan, 1980; Schneider and Kelly, 2000); whereas isolates of *F. lateritium* produce the symptoms described for *F. solani* f. sp. *phaseoli* (Sánchez-Garcia *et al*., data no published).

The second piece of evidence implies the traits of both *Fusarium* species, which are the basis for its classification. Isolates from both species were identified following Nelson *et al*. (1983) and recently reconfirmed with the aid of The *Fusarium* Laboratory Manual of Leslie and Summerell (2006). Both species differ in their growth rate, after 10 days of growth the *F. lateritium* colony does not reach the edge of a 100 x 15 mm Petri dish, while isolates of *F. solani* regularly do. Hall (1981) did show images of *F. solani* f. sp. *phaseoli* in PDA media similar to our isolates identified as *F. lateritium* and with a growth rate that correspond to this last species. In addition, microconidia and chlamydiospores are normally scarce after 15 days growth on SNA and CLA media in *F. lateritium*, but abundant and conspicuous in *F. solani* f. sp. *phaseoli*.

The third piece of evidence consists in genetic differences observed between both species with the aid of molecular AFLP markers. Isolates identified as belonging to *F. lateritium* display a different pattern and reduced level of variation in comparison to those of *F. solani*. Our analysis in relation to symptoms and growth characteristics suggests that confusion may also exist in the identification of *Fusarium* species associated with the sudden death syndrome in soybean. Genetic analysis of *Fusarium* isolates from soybean and common bean from different geographic regions would allow

us to establish with greater confidence the identity and relationship between the *Fusarium* species causing these diseases.



(a) (b)

Figure 1. Growth of different isolates of *Fusarium lateritium* (a) and *F. solani* (b) from 21 to 28 days after being platted on PDA media. See the limited growth on the species in (a).

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FREQUENCY OF OCCURRENCE OF ROOT ROT PATHOGENS ON BEANS IN ECUADOR

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INTRODUCTION

Root diseases are known to be an important factor in the production of dry beans in all the major production regions in Ecuador and other Latin American countries; impacting both the quantity and quality of seed yield (Abawi and Pastor-Corrales, 1990). However, the incidence and severity of root diseases as well as their causal agent(s) vary greatly within and among production regions in each country. Thus, there is a need for accurate diagnosis of the causal pathogen(s) and the characterization of their pathogenic and genetic variation in order to develop locally adapted resistant cultivars and/or to design effective and sustainable management programs. Accordingly, an extensive survey of the root rot pathogens infecting beans in the Northern and Southern production regions in Ecuador was conducted during 2006 and 2007.

MATERIALS AND METHODS

Symptomatic and asymptomatic plants from 25 and 20 randomly selected bean fields in the northern and southern production regions, respectively were examined for the incidence and severity of infections by root rot pathogens. Large numbers of plants (>20) were carefully dug-up from several areas within each field and examined for the characteristic symptoms of pathogen infection on roots and lower stem tissues. In the laboratory, isolations were also made from surface sterilized tissues of selected plants collected from each field on acidified potato-dextrose-agar (PDA) plates to confirm the field diagnosis. Representative isolates of the recovered pathogens were purified by several hyphal-tip transfers on PDA plates and stored for characterization of their pathogenic and genetic variability.

RESULTS AND DISCUSSION

Five soilborne pathogens were confirmed to cause root diseases and serious yield losses on beans in Ecuador. The pathogens identified were *Rhizoctonia solani* (Rs, causes Rhizoctonia root and stem rots), *Fusarium solani* f. sp. *phaseoli* (Fsp, causes cortical root and stem rots), *Sclerotium rolfsii* (Sr, causes southern blight), *F. oxysporum* f. sp. *phaseoli* (Fop, causes wilt), and *Meloidogyne* spp. [root-knot nematodes (RKN), causes root-galling]. However, the prevalence of these pathogens and the damage of their resultant root diseases varied among the fields within a production region as well as between the two regions. For example, the frequency of infection symptoms caused by Fsp, Rs, Sr, Fop, and RKN were 84, 96, 40, 16, and 24% in the Northern region, respectively; whereas their observed frequencies in the Southern region were 70, 50, 10, 40, and 20%, respectively (Figure 1). Solid plantings of bush-type beans in rotation with vegetables are common in the Northern production areas, whereas climbing beans in association with corn predominate in the Southern areas. The latter might be the reason for the observed differences in the prevalence and severity of root diseases documented between the two regions. In addition, considerable variability in the occurrence of one or several root pathogens was evident among the fields examined within each

production region (Table 1). Furthermore, symptoms of insect damage (primarily stem borers) were observed at frequencies of 53 and 60% in the Northern and Southern regions, respectively. Root disease incidence and severity is generally higher in combination with insect activities, thus the need to manage them more effectively. Interestingly, both Rs and Fsp are the most predominate root pathogens in both production regions, thus they should receive the highest priority in the deployment of resistance genes into adapted bean germplasm. Currently, initial screening of promising parental bean lines for resistance to root pathogens is being conducted at the Tumbaco Experiment Station, where both Rs and Fsp predominate (Falconi et al., 2007). The bean lines that have been identified as promising are currently being evaluated against Fop under greenhouse conditions.

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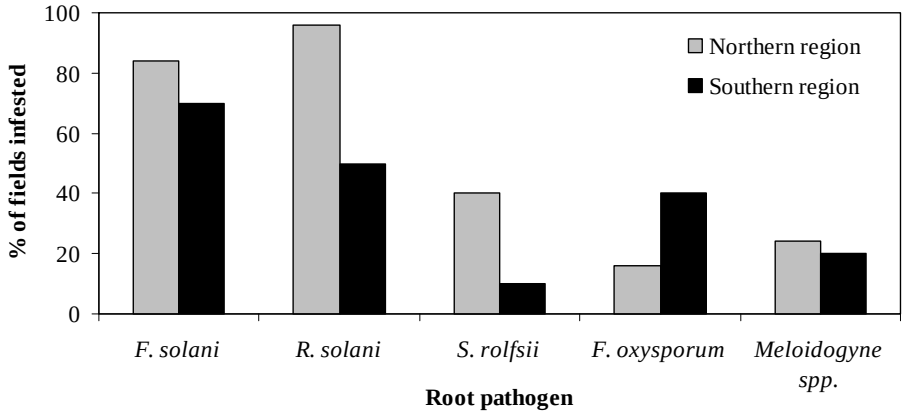

Figure 1. Recovery of selected pathogens from the roots of beans grown in the Northern and Southern production regions in Ecuador in November 2006 and April 2007.

Table 1. Variability in the incidence and severity of root pathogens isolated and identified in bean fields in Ecuador in November 2006.

Field	*F. solani*	*Rhizoctonia*	*Sclerotium*	*F. oxysporum*	RKN	Insects
1	-	+++	-	-	-	-
15	+	+	+++	-	+	-
16	+	+	-	-	-	+++
17	+	+	++	-	+	++
18	+	+	-	+++	-	-
19	-	+++	+++	-	-	+
20	+	++	+++	-	-	+
21	-	+	+++	-	-	-
24	+	+	-	++	-	++
25	+	+	-	-	-	-
27	-	-	-	+	-	++

BREEDING BEANS FOR RESISTANCE TO WEB BLIGHT

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Web blight, caused by *Rhizoctonia solani* Kühn can reduce seed yield and quality of common bean (*Phaseolus vulgaris* L.) produced in the hot and humid zones of Central and South America, the Caribbean and Africa. The bean breeding programs at the University of Puerto Rico and Zamorano have employed different strategies to develop bean lines with greater levels of resistance to this disease. Two cycles of selection for web blight resistance have been completed. The base population included diverse sources of resistance to the disease. This strategy has produced breeding lines with greater levels of web blight resistance than the black bean cultivar 'Talamanca' and greater seed yield and quality than the check cultivars 'Tio Canela 75' and 'Morales' (Table 1). In addition to web blight resistance, the pink bean lines PR0401-257 and PR0401-259 and the small red bean breeding line PR0401-277 have good seed yield potential, the *bgm* gene for resistance to Bean Golden Yellow Mosaic (BGYM) and the *I* gene for resistance to Bean Common Mosaic (BCM). The pedigrees of these lines include VAX 6, PR0607-29, BAT 93 and EAP9503-32A. Godoy-Lutz found these lines to have smaller lesion size than Pinto 114 when detached-leaves were inoculated with a virulent strain (AG-1-1B) of the pathogen (Beaver et al., 2002). Another strategy utilized the wild bean germplasm line PI 417622 as a new source of resistance to web blight. Some lines derived from crosses with PI 417622 have low web blight scores ($\leq$ 4.0), mean seed yields > 2,000 kg/ha and high quality seed in field trials inoculated with the pathogen (Table 2). It should be noted VAX 6 and BAT 93 are also progenitors of the most promising breeding lines from crosses with PI 417622. A few of these breeding lines had low web blight scores in field trials conducted in Honduras and Puerto Rico. During 2007, the white bean cultivar 'Verano' which is derived from a cross with VAX 6, also had a mean web blight score < 4.0. A longer term goal is the use of interspecific crosses between common bean and scarlet runner bean (*Phaseolus coccineus* L.) to broaden the genetic base of resistance to web blight. Field and greenhouse screening in Puerto Rico identified a few scarlet runner bean germplasm accessions from CIAT with resistance to web blight (Takegami and Beaver, 2000; Martinez et al., 2005). During the 2006 growing season, $F_{4:5}$ lines from the interspecific cross 'ICA Pijao / G 35006 // 5-593' were screened for reaction to web blight; individual plants were selected from rows having web blight scores $\leq$ 4. The $F_{5:6}$ lines were screened for web blight reaction during the 2007 growing season. We have also collaborated with CIAT in the development and evaluation of another interspecific (Pv x Pc) population from the cross 'ICA Pijao x G35163'. We plan to evaluate $F_{2:3}$ lines using the detached- leaf inoculation technique and detached leaves. During 2001, F_2 plants from the 'ICA Pijao / G 35006 // 5-593' interspecific population were crossed at Zamorano with 'Tio Canela 75' and 'Amadeus 77'. During 2003, F_3 plants from the crosses made at Zamorano were evaluated in the field in Jamastran, Honduras. Several lines in the trial had mean web blight scores $\leq$ 3.0. The most web blight resistant breeding lines have been distributed to cooperators in Nicaragua and Costa Rica. Results from these trials will help to determine if bean lines can be selected that have web blight resistance throughout Central America and the Caribbean.

Table 1. Mean web blight scores, seed yield and % damaged seed of advanced lines planted at Isabela, Puerto Rico in August 2006 and September 2007.

Line	First web blight score[1]		Second web blight score[1]		Seed yield (kg/ha)		% damaged seed	
	2006	2007	2006	2007	2006	2007	2006	2007
PR0401-257	3.4	3.7	4.0	4.8	2005	2566	3.9	3.7
PR0401-259	3.0	3.0	3.8	4.0	2103	3217	4.2	3.3
PR0401-277	3.0	4.0	4.4	4.8	1983	2397	6.1	6.8
Talamanca	6.2	5.5	7.8	5.5	1594	2071	5.4	5.3
Morales	5.6	5.0	6.4	5.7	1804	1533	17.7	15.1
Amadeus 77	4.6	5.5	7.0	5.5	1389	3295	8.6	6.0
LSD(0.05)	1.2	1.2	1.6	0.8	591	641	5.4	5.7
CV(%)	22.8	25.3	24.3	13.2	23.1	23.0	50.9	66.8

[1]Rated on a 1-9 scale where 1 = no symptoms and 9 = very severe symptoms (Van Schoon3.3hoven and Pastor Corrales,1987).

Table 2. Mean seed yield, % damaged seed and web blight scores from Puerto Rico and Honduras of F_7 lines derived from crosses with wild bean accession PI 417622.

Line	Mean seed yield in Puerto Rico (kg/ha)		Mean web blight score in Puerto Rico[1]		Mean web blight score in Honduras[1]
	2006	2007	2006	2007	2006
PR0650-27	2350	2160	3.8	5.0	4.3
PR0650-31	2603	2333	3.8	2.0	2.0
PR0650-32	2296	2779	3.3	2.5	3.0
PR0650-34	2240	1993	4.0	2.5	2.0
PR0650-41	2226	1473	4.0	4.0	2.3
Talamanca	1943	2206	4.8	6.5	
Morales	1360		6.8		
Verano		2686		3.5	
Tío Canela 75	1566		5.8		
Amadeus 77		2107		4.5	
LSD(0.05)	424	N.S.	1.8	1.8	
CV(%)	23.9	25.3	25.6	30.1	

[1]Rated on a 1-9 scale where 1 = no symptoms and 9 = very severe symptoms (Van Schoonhoven and Pastor Corrales, 1987).

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DEVELOPMENT OF A DIFFERENTIAL SET OF COMMON BEAN LINES TO SCREEN FOR WEB BLIGHT PATHOGEN VIRULENCE

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INTRODUCTION

Web blight, caused by the fungal pathogen *Rhizoctonia solani* (Rs), is an important disease of common bean in tropical areas. The pathogen spreads by airborne basidiospores, mycelial bridges between plants, rain-splashed sclerotia, infested soil debris, and infected seeds (Gálvez et al., 1989; Godoy-Lutz et al., 1994). *R. solani* is a complex species composed of subgroups within Anastomosis Groups (AG). Anastomosis group refers to hyphal fusion that occurs only between isolates of the same group (Gálvez et al., 1989). At least six subgroups of Rs cause symptoms of web blight (Godoy-Lutz et al., 2008). Field screening cannot separate disease avoidance due to architectural traits and physiological resistance to web blight (Takegami and Beaver, 2000). Therefore a fast, easy and reliable method of screening for pathogen virulence should include a set of differential bean lines. At present, no set of lines useful for screening virulence of different *R. solani* isolates in differential bean lines/cultivars is available.

MATERIALS AND METHODS

Twenty-six lines that were observed to have resistance or tolerance to web blight in field nurseries in Puerto Rico and Honduras were chosen to be screened with four isolates of *R. solani* from different anastomosis groups and subgroups in order to develop a set of differential lines. The isolates used were from AG 1 and AG 2 from different countries including Dominican Republic (AG 1-1F), Puerto Rico (AG 1-1E), Honduras (AG 2-2WB) and Cuba (AG 1-1A). The lines were evaluated using the detached leaf technique. The first trifoliate leaves of four plants of each line were inoculated with one of the four isolates and readings were taken at 24, 48 and 72 hours after inoculation. The leaves were scored according to the degree of penetration of the pathogen into the leaf tissue using the CIAT 1-9 scale (1=no penetration, superficial growth of mycelium; 9=leaf necrosis) (Schoonhoven and Pastor-Corrales, 1991). Another reading was taken by measuring the size of the lesion using the Scion Image Beta 4.02 Win software.

A second group of fourteen bean lines was also evaluated for reaction to web blight. This time the isolate from subgroup AG 2-2WB was not used because we were not able to produce the basidiospores that are needed for infection in the lab. Once again the detached leaf technique was used and two readings were taken: degree of penetration of the pathogen and area of lesion.

RESULTS AND DISCUSSION

Results obtained from both experiments (Tables 1 and 2) confirmed that there were significant differences in virulence between the isolates tested. Since these isolates represent different subgroups, this confirms previous results by Godoy-Lutz et al. (2008) that show that at least six different subgroups of Rs cause web blight symptoms in common bean. However, there were no significant differences among the lines for web blight reaction at 24, 48 and 72 hours after

inoculation, including the resistant and susceptible checks in both experiments. One hypothesis is that the resistance reactions of all these lines demonstrated in the field is attributed more to architectural traits than to physiological resistance of the plant. Also, it was shown that it is very important to take two types of readings, degree of penetration and lesion area, in order to determine resistance or susceptibility of a bean line to the pathogen. These two values do not appear to be correlated so both must be taken into account when screening for resistance to web blight.

A second hypothesis is that temperature differences (higher (99ºF/75ºF day/night) in Puerto Rico and Honduras, lower (80ºF/74ºF) in Lincoln) between previous tests showing differential response to field isolates or detached leaf tests with one of the same isolates can account for the different results. Further work will be conducted with the same lines under higher temperature conditions and with other bean lines also at >80ºF (26.7ºC) to test for significant differences between differential candidate bean lines.

Table 1. Mean lesion area (% of leaf area) and degree of penetration (1-9 score) for the first group of 26 lines tested.

	24 hrs		48 hrs		72 hrs	
Isolate	Lesion area	Degree of penetration	Lesion area	Degree of penetration	Lesion area	Degree of penetration
AG 1-1F	1.5	1.1	7	3.2	33.4	7
AG 1-1E	2.5	1.5	22.5	4.7	68.1	8.8
AG 2-2WB	0	1	0.35	1.6	1.2	4.6
AG 1-1A	2.3	1.3	18	3.9	61.6	8.5

Table 2. Mean lesion area (% of leaf area) and degree of penetration (1-9 score) for the second group of 14 lines tested.

	24 hrs		48 hrs		72 hrs	
Isolate	Lesion area	Degree of penetration	Lesion area	Degree of penetration	Lesion area	Degree of penetration
AG 1-1F	3.3	2.2	8.2	4.4	33.2	6.7
AG 1-1E	5.2	2.3	21.7	6.9	70.9	8.7
AG 1-1A	3.9	2.5	21.9	6.3	67.1	8.7

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TOWARDS THE IDENTIFICATION OF COMMON BACTERIAL BLIGHT RESISTANCE GENES IN *PHASEOLUS 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).

CBB resistance has been studied for a number of years, and has led to the development of several lines which have demonstrated resistance to *X. axonopodis* pv. Phaseoli. Recently, a CBB-resistant cultivar, OAC-Rex (registration no. 5491), tested as OAC 95-4, was derived from a cross between HR20-728 and MBE 7 made in 1988. Another CBB-resistant line, HR67, was produced by a series of crosses between Centralia, HR13-621, OAC Rico and XAN159 (Yu *et al*. 2000).

To aid in the identification of lines possessing CBB-resistance related genes, a number of molecular markers have been identified for various lines, including HR67 (Yu *et al*. 2000, Yu *et al*. 2004) and OAC-Rex (Tar'an *et al*. 2001). Although these markers represent a useful tool for breeding CBB-resistant lines, the actual genes involved in resistance are not yet known. The objectives of this project are to develop BAC libraries for two important CBB-resistant *P. vulgaris* lines (HR67 and OAC-Rex) and to identify genes associated with CBB resistance.

MATERIALS AND METHODS

DNA Isolation and Library Construction - High molecular weight (HMW) DNA from the fully expanded leaves of OAC-Rex and HR67, was extracted and encapsulated according to an established protocol (Zhang *et al.*, 1995). The encapsulated DNA was partially digested with 5 units of *Bam*HI (Roche) for 15 min. and electrophoresed through a 1% (w/v) low melting point agarose gel using a pulse field gel electrophoresis unit (BioRad). The DNA fragments between 100-400 Kbp were excised from the gel, and the DNA released through an enzymatic digestion with Gelase (EpiBio) according to the manufacturers protocol.

The BiBac2 vector was prepared according to the protocol of Hamilton *et al.* (1996). The ligation reactions contained 50ng of insert DNA and 1ug of prepared vector with 5U of T4 DNA ligase (Invitrogen). The reaction mixtures were incubated overnight at 4°C, and 2µl were used to transform DH10 *Escherichia coli* (Invitrogen) cells according to the manufacturers' instructions. Colonies were selected using a GeneTAK G3 automated workstation and transferred to 96-well plates containing LB media with 50 mg L^{-1} kanamycin. The plates were incubated overnight at 37°C.

Library Screening - The OAC-Rex and HR67 libraries were 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; Tao *et al.* 2001; Zhang and Wu 2000). The bands generated for each clone were used to assemble contigs using the FingerPrint Contig analysis software (AGCol) to align the fragments.

RESULTS AND DISCUSSION

OAC-Rex and HR67 libraries with 31,776 clones and 22,560 clones, respectively, were constructed. The OAC-Rex library has an average insert size of 150 Kbp, providing a library depth of 5.6. The HR67 library has an average insert size of 300 Kbp, with a depth of 8.1. Initial screens of the OAC-Rex library with the pv-ctt001 marker-derived probe identified 23 positive clones. These results were confirmed by PCR using primers for the pv-ctt001 marker (data not shown).

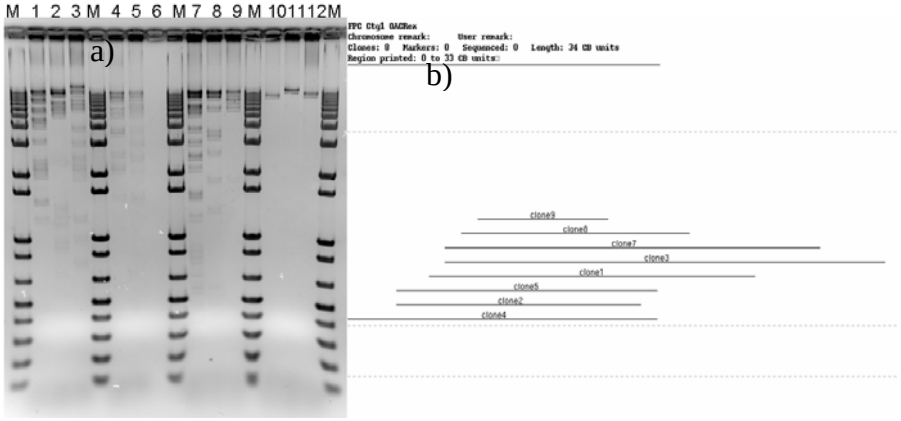

Figure 1. Contig construction from the OAC-Rex PV-ctt001 SSR marker. A) Clones identified from the membrane hybridizations were digested with *Hind*III, and separated according to size. B) The band patterns were analyzed with FPC Contig (Sanger) software and aligned into a single contig.

After separation by electrophoresis (Figure 1a) the bands from each clone were analyzed and they were aligned into a contig (Figure 1b). The library will be re-probed with fragments at the extremes of the contig (Clone 3 and Clone 4) to begin walking down the chromosome and expanding the size of the contig.

To identify clones containing CBB-resistance genes, selected clones will be transformed into a susceptible *P. vulgaris* line (OAC-Seaforth) and infected with *X. axonopodis* pv. phaseoli. The size of the resulting lesions will be measured, and clones resulting in reduced lesion size will be sub-cloned and sequenced. The genes identified in this study will facilitate the development of future CBB-resistant lines.

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EPISTATIC INTERACTION BETWEEN TWO MAJOR QTL CONDITIONING RESISTANCE TO COMMON BACTERIAL BLIGHT IN COMMON BEAN

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Resistance to common bacterial blight in common bean is a complex trait that is quantitatively inherited. Combining QTL is the current strategy for improving resistance (Singh and Munoz, 1999; Miklas et al., 2006), but interactions among different QTL are unknown. We examined the interaction between two independent QTL present in dry bean breeding line XAN 159. The QTL were studied in a near isogenic population consisting of 120 BC_6:F_2 plants. Each BC_6:F_2 plant was evaluated for disease reaction at several time points after pathogen inoculation and the dominant SCAR markers linked with QTL on linkage groups B6 (BC420~QTL) (Yu et al., 2000) and B8 (SU91~QTL) (Pedraza et al., 1997) were interpreted as codominant markers using real time PCR assays (Vandemark and Miklas, 2005). This enabled assignment of BC_6F_2 plants to all nine possible genotypes.

Briefly, the nucleotide sequences of BC420 (895 bp; GENBANK Accession EF553635) and SU91 (669 bp; GENBANK Accession EF553635) were used to identify sequences for real-time PCR primers and probes. The nucleotide sequences of the primers and the fluorochrome-labeled probes used in this study are as follows:

forward primer BC420F20, 5´-d-TGGCTCAGGTGGTTTGCAA-3´;
reverse primer BC420R111, 5´-d- GCGCCTGGGAACGATTT-3´; and
probe BC420T59, 5´-d-CCCCATTCGCAGCGTCGCA-3´;

forward primer SU91F2, 5´-d-CACATCGGTTAACATGAGTGATTTC-3´;
reverse primer SU91R86, 5´-d-CACACAAAGGAGGGATAAAAGAGATAA-3´; and
probe SU9134, 5´-d-CATATATCATCGCCTATTGTGT-3´.

In separate reactions for each plant sample, the 92 bp amplicon from the SCAR marker BC420 was amplified with the primer/probe set BC420F20- BC420T59- BC420R111, while the primer/probe set SU91F2- SU9134- SU91R86 was used to amplify the 85 bp fragment from the SCAR marker SU91. PCR was done in 50 µl reactions containing 100 ng genomic DNA, 450 nM forward primer , 450 nM reverse primer, 250 nM fluorochrome-labeled probe and 25 µl of 2X TaqMan™ Universal PCR Master Mix (Applied Biosystems). PCR and detection of fluorescence were performed using the GeneAmp 7000 Sequence Detection System (Applied Biosystems). All PCR was conducted using a thermocycling profile consisting of an initial cycle of 2 min at 50^{o} C, followed by a single cycle of 10 min at 95^{o} C, and then 40 cycles of 15 s at 95^{o} C and 1 min at 60^{o} C.

Reaction to CBB in BC_6:F_2 plants was characterized by an epistatic interaction between BC420 and SU91 such that: i) the expression of BC420 was epistatically suppressed by a homozygous recessive su91//su91 genotype; ii) SU91//SU91 and SU91//su91 genotypes conditioned an intermediate disease reaction when homozygous recessive for bc420//bc420; and iii) the highest level of disease resistance was conferred by genotypes with at least a single copy of both QTL (BC420//-; SU91//-). Segregation for resistance among BC_6F_3 plants derived from BC_6F_2 plants that were heterozygous for

both QTL did not deviate significantly from expected ratios of 9 resistant: 3 moderately resistant: 4 susceptible (Table 2). This is consistent with a recessive epistatic model of inheritance between two loci. These results indicate breeders will realize greatest gains in resistance to CBB by selecting breeding materials that are fixed for both QTL. This is a first report of a qualitative digenic model of inheritance discerning an interaction between two QTL conditioning disease resistance in common bean.

Table 1. Means separation of CBB response among F_6F_2 plants genotyped for BC420 (QTL) and SU91 (QTL) based on real-time PCR. Score 1 to 9 where 1 = resistant and 9 = susceptible.

	Plants	**14 DAI**
Genotype	**No.**	**1-9 score**
bbss	**9**	**8.33 a**
Bbss	**14**	**8.14 a**
BBss	**4**	**8.00 a**
bbSs	**28**	**3.16 b**
bbSS	**14**	**3.32 b**
BbSs	**29**	**1.64 c**
BbSS	**8**	**1.63 c**
BBSs	**9**	**1.17 c**
BBSS	**5**	**1.10 c**
Teebus (bbss)	**10**	**8.20**
XAN 159 (BBSS)	**10**	**1.05**

Table 2. Segregation for common bacterial blight reaction at 14 DAI in BC_6F_3 progeny of different BC_6F_2 genotypes for BC420 QTL and SU91 QTL.

	Segregation for Reaction to CBB in BC_6F_3 Progeny						
	Expected			**Observed**			
Genotype[b] (BC_6F_2)	**Resistant**	**Moderate Resistance**	**Suscept.**	**Resistant**	**Moderate Resistance**	**Suscept.**	**χ^2 / Prob**
BbSs	**47.25**	**15.75**	**21**	**41**	**22**	**21**	**3.31 / 0.19**

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GENETIC DIVERSITY IN *XANTHOMONAS CAMPESTRIS PV. PHASEOLI* AND *X. C.* PV. *PHASEOLI* VAR. *FUSCANS* AND THE INTERACTION WITH RESISTANT COMMON BEAN GENOTYPES

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Xanthomonas campestris pv. *phaseoli* (*Xcp*) and *X. c.* pv. *phaseoli* var. *fuscans* (*Xcpf*) cause common bacterial blight (CBB), a seed borne disease of common bean (*Phaseolus vulgaris* L.). Genetic diversity of the pathogen has previously been reported between and within strains of *Xcp* and *Xcpf*. Detailing the population structure of the pathogen in a localized growing region can identify the most prevalent strains and their relative pathogenicity, allowing for the identification of appropriate strains to be used in the direct selection for CBB resistance in a breeding program. Thus, the objectives of this study were to: 1) determine the genetic diversity of *Xcp* and *Xcpf* within a defined geographic region growing a single predominant type of bean (large-seeded dark red kidney beans of the Andean gene pool) and 2) determine the pathogenic variation of *Xcp* and *Xcpf* strains on a diverse set of common bean germplasm reported to possess CBB resistance.

During the summers of 2005 and 2006, a total of twenty-five dark red kidney bean fields were sampled for CBB in a Midwest U.S. production region. Leaf samples with CBB-like symptoms were collected and isolations from leaf lesions were performed on MXP medium. A collection of 369 xanthomonad-like strains (starch hydrolysis-positive) were analyzed for colony morphology on 523 media, pathogenicity on common bean, and genomic DNA fingerprint. The 369 strains were inoculated onto the cultivar Top Crop to test the pathogenicity of the strains. A total of 76 strains were non-pathogenic; had flat and nonmucoid colony morphologies; a repetitive element PCR (rep-PCR) fingerprint distinct from typical CBB bacteria; and were not positive with the *Xcp*/*Xcpf* specific X4e and X4c PCR primer pair. The remaining 293 pathogenic strains were positive with the *Xcp*/*Xcpf* specific X4e and X4c PCR primer pair.

Three types of colony morphologies were observed for the 293 pathogenic strains. The first was the yellow mucoid non-pigmented type, characteristic of the 'common' *Xcp* phenotype (168 P strains). The second type was yellow, mucoid and produced a strong brown pigment, characteristic of *Xcpf* (6 FH strains). The third type had a similar colony morphology to the *Xcpf* strains, but with noticeably less brown pigment production (119 Px strains).

Rep-PCR was used to examine the genotypes of these three phenotypes of CBB bacteria. The fingerprint of the yellow mucoid non-pigmented type was similar to that of known 'common' *Xcp* strains. The fingerprint of the 'intermediate' Px phenotype was very similar to the common *Xcp* fingerprint, but it could be distinguished based on fingerprints generated with REP and ERIC primers. This new fingerprint (Px) was associated with all 119 strains with intermediate brown-pigment production. A second new CBB genomic fingerprint was found for the 6 *Xcpf* strains isolated in this study. The rep-PCR fingerprint for these strains, generated with the ERIC primers, was a hybrid of fingerprints previously described for *Xcpf* strains from the New World (e.g., Puerto Rico) and the Old World (e.g., East Africa), containing both bands that differentiate these previously described *Xcpf* strains. Thus, we identified two new genotypes of CBB bacteria from our collection of Midwestern strains.

A representative strain for each CBB genotype from the Midwestern U.S., together with other previously identified genotypes of CBB bacteria, were selected to determine their pathogenic variation on a diverse collection of host genotypes previously reported to have CBB resistance. Each host genotype (30) was inoculated with each of these eight *Xcp/Xcpf* strains, producing 240 treatments that were organized in a randomized complete block design with four replicates. This experiment was repeated twice during the summer of 2007. The first trifoliolate leaf was inoculated by the razor blade technique with a bacterial concentration of $1x10^8$ cfu/ml and rated 21 days post inoculation on a 1-9 disease severity index (DSI).

The common *Xcp* strain from the Midwestern U.S. (Xcp25) was most pathogenic when all 30 host genotypes were analyzed together (DSI = 5.2). However, Xcp25 was not significantly more pathogenic than the Midwestern U.S. *Xcpf* (FH61) (DSI = 4.8), and all three *Xcpf* strains were not significantly different from each other [DSIs of 4.3 (East-African *Xcpf*), 4.5 (Puerto Rican *Xcpf*) and 4.8 (Midwestern *Xcpf*)]. The two genotypically distinct East African *Xcp* strains were less pathogenic (DSIs of 2.6 and 3.3) than all other *Xcp* and *Xcpf* strains. Thus, there was greater pathogenic variation within the *Xcp* strains than in the *Xcpf* strains. Pathogenic variation was even found among *Xcp* strains with the same genomic fingerprint (DSIs of 3.7-5.2).

When the 8 strains of CBB bacteria were considered together, VAX 3 (DSI = 2.2), VAX 4 (DSI = 2.3), VAX 5 (DSI = 2.4), and VAX 6 (DSI = 2.5) had the highest levels of CBB resistance. Montcalm and Top Crop were used as susceptible checks and had mean DSI scores of 6.2 and 7.1, respectively. Host genotypes with resistance derived from *P. acutifolius* (DSI = 3.3) or pyramided from various resistance sources (DSI = 3.2) were consistently more resistant than were landraces (DSI = 5.0), cultivars (DSI = 6.2) or breeding lines (DSI = 4.6) examined in this study. When the host genotypes were separated based on CBB resistance markers, genotypes with SU91 (DSI = 3.2), SAP6 + SU91 (DSI = 3.2), SU91 + LG5 (DSI = 3.0) or all three SCAR markers (DSI = 2.7) had higher levels of resistance than genotypes with no markers (DSI = 5.6) or SAP6 alone (DSI = 4.7).

In this study, no highly pathogenic strain of CBB bacteria was identified that could overcome the highest known levels of CBB resistance in common bean. Thus, even though 3 distinct CBB-causing bacterial genotypes (common *Xcp*, intermediate *Xcp*, and the hybrid *Xcpf*) were found in the Midwestern U.S., there was relatively little pathogenic variation among strains of these three genotypes. There was a significant quantitative host/pathogen interaction, in addition to a significant marker/strain interaction. However, no evidence of a qualitative gene-for-gene interaction was found in this host/pathogen system.

EVOLUTION OF SCREENING METHODS FOR DETECTION OF PHYSIOLOGICAL RESISTANCE TO WHITE MOLD IN COMMON BEAN

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Screening methods (SC) for the detection of physiological resistance to white mold [WM, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary] in the common bean (*Phaseolus vulgaris* L.) and related *Phaseolus* species have varied considerably over the past several decades. Availability of an effective, easy, reliable, repeatable, and economical screening method with resolving power that permits identification of large and small physiological resistance to WM will facilitate breeding, genetics, and pathology studies. Such SC also will facilitate formulation of effective complementary management practices to combat WM in the field and germplasm screening, enhancement, and cultivar development. Our objective was to briefly review some of the SC used in the past and report on the effectiveness of the most recent SC used in the greenhouse at the University of Idaho, Kimberly Research and Extension Center.

There is indirect and direct SC for physiological resistance to WM. The indirect SC includes (1) measurement of pathogen filtrate, (2) determining phenylanine ammonia-lyase activity, (3) oxalate test, and (4) use of molecular markers linked with white mold resistance quantitative trait loci (QTL). Also, real time PCR could be used to quantify the fungal DNA in susceptible and resistant genotypes. However, these indirect methods are not routinely used in germplasm screening, enhancement, and cultivar development. Most breeders and geneticist still heavily rely upon direct SC.

The effectiveness of SC for physiological resistance may depend upon (1) plant age and organ inoculated, (2) pathogen isolate aggressivity, (3) ascospore versus mycelial inoculant, (4) method of inoculation, (5) variation in inoculum load or density, (6) duration of inoculant contact with the plant, (7) rate of growth of pathogen on the plant, (8) measurement of lesion length versus the number of internodes infected, (9) measurement of incidence versus severity, and (10) time between inoculation and disease rating. Abawi et al. (1978) used ascospores to spray flowering plants derived from the common and scarlet runner bean crosses to determine inheritance of resistance. Schwartz et al. (1978) used colonized flowers inoculated with ascospores to screen for WM resistance. Hunter et al. (1981) sprayed ascospores on excised flowers and autoclaved snap bean pods.

Excised leaves, stems, and cotyledons were inoculated with the mycelial cultures to screen for white mold resistance. For the intact plant inoculations, Adams et al. (1973) used colonized oat seeds for stem inoculation and Hunter et al. (1981) used colonized celery stem for limited term inoculation. The straw-test or cut-stem inoculation method of Petzoldt and Dickson (1996) over time has become the method of choice. Despite the fact that the main stem is cut at desired internode of four to five weeks old plants and yield of resistant plants also are considerably reduced, the method is non-destructive. Thus, the straw-test is especially suitable for breeding and selection studies, germplasm enhancement, and cultivar development.

Over the past few years some modifications have been made in the straw-test to expedite germplasm screening, minimize escapes, and detect higher levels of physiological resistance. For example, instead of using pieces of cut-straw most researchers are using eppendorf pipette tips. This is easier and time saving. Also, changes in the number of inoculations per plant, number of mycelial plugs used per inoculation, and time between inoculation and disease rating are made according to challenges faced in germplasm screening. Initially, in our greenhouse test, we inoculated each four to five weeks old plant only one time using only one mycelial plug and rated at 28 hr, 72 hr, and at 7 days post inoculation. However, gradually it became difficult to differentiate among interspecific breeding lines derived from crosses of 'ICA Pijao' and *Phaseolus* species of the secondary gene pool, namely *P. coccineus*, *P. costaricensis*, and *P. polyanthus* that had gone through repeated cycles of the field and greenhouse screening. From the spring of 2007 we began inoculating each plant 2 to 4 times at a weekly interval as needed, each time using three mycelial plugs, and delayed disease rating until 27 days post inoculation and verified the reaction at harvest maturity. Also, Terán et al. (2006) modified the Petzoldt and Dickson (1996) rating scale to facilitate better differentiation among resistant, intermediate, and susceptible genotypes. The results of single, double, and triple inoculations at weekly intervals for few selected genotypes of the national Bean White Mold Nursery in 2007 are summarized in Table 1.

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Table 1. Response of some common bean genotypes of the National Bean White Mold Nursery to multiple inoculations using the modified cut-stem method in the greenhouse at University of Idaho, Kimberly, Idaho in 2007.

Genotype	Number of susceptible (≥7) plants (and white mold score)		
	July 13	July 20	July 27
Beryl	2 (6.6)	3 (8.2)	6 (8.5)
ICA Bunsi	2 (5.7)	4 (6.7)	4 (6.7)
BO 5055	1 (4.8)	4 (7.2)	5 (7.3)
Cornell 605	0 (4.0)	0 (4.3)	3 (5.8)
G 122	1 (5.8)	2 (6.0)	2 (6.0)

Thus, to identify common bean genotypes with high levels of white mold resistance use of two or more inoculations and delaying evaluation until 27 days or longer would be required. Use of a single inoculation and evaluations in 21 days (or less) post inoculation may permit identification of low levels of resistance and increase chances for escapes.

ONE CYCLE OF RECURRENT SELECTION FOR PHYSIOLOGICAL RESISTANCE TO WHITE MOLD IN DRY BEAN

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INTRODUCTION

White mold [caused by *Sclerotinia sclerotiorum* (Lib.) de Bary] is without doubt, the most important dry bean disease in North America. On average it can reduce seed yield by 40%, but in some cases losses may exceed 90%. Only low to moderate levels of resistance occur in a few Middle American (e.g., ICA Bunsi) and Andean (e.g., G 122, PC50, MO 162, and A 195) dry bean (1, 2, 3, 4). The low heritability, unreliable screening methods, large environmental influence, and use of inadequate breeding methods have hindered the success in breeding for white mold resistance. The backcross and pedigree methods (6, 7, 8) often used for white mold resistance breeding have been inadequate because they often used only one germplasm with low or moderate level of resistance. Recurrent selection method has been effective in improvement of quantitative traits such as seed yield. However, it has not been used in breeding for white mold resistance with one exception (9).

OBJECTIVE

Our main objective was to introgressing and pyramiding resistance to white mold in dry bean, using recurrent selection (RS) involving eight parental germplasm in three multiple parent populations.

MATERIALS AND METHODS

Eight parents with different level of white mold resistance (Table 1) were used to develop three multiple-parent populations, between 2005 and 2006. The population I (Pob I) had the pedigree USPT-WM-1/CORN 601//USPT-CBB-1/92BG-7; the population II (Pob II) had pedigree Chase/I 9365-25//ABL 15/A 195, and the population III (Pob III) was derived by combining the two double crosses thus had pedigree USPT-WM-1/CORN 601//USPT-CBB-1/92BG-7 /// Chase/I 9365-25//ABL 15/A 195.

Greenhouse Screening

The RS cycle Zero was initiated in the fall of 2006; 848 F_1 plants of each of Pob I and Pob II were screened in the greenhouse for white mold reaction. The cut-stem method was used approximately 25 days after germination. Using eppendorf tips the inoculum was put in contact with the cut stem. Multiple sequential inoculations were used. Reaction to white mold was rated 27 days after inoculation using a modified scale (5) where 1 = no sign of stem infection adjacent to inoculated mycelial plug, 3 = lesion was greater than one inch but not reached the first node, 4 = lesion reached the first node, but no further, and 7 = lesion reached the second node, but no further. Only highly resistant plants between and within populations were selected for intermating. Sixty-six resistant plants from Pob I, 80 plants from Pob II, and 60 of both populations were paired pollinated to generate families for cycle one. Those families were screened in the greenhouse at Kimberly in 2007.

Preliminary Results **-** Significant differences in the mean white mold score among selected plants from cycle zero and parents were observed. In Pob I, selected plants from cycle Zero, show a mean of 4.1 compared with 6.1 of their parents (Table 2). In Pob II, selected plants had a mean of 4.2 compared with 5.3 of their parents (Table 2). The selected plants from Pob III exhibited a mean of 4.2 compared with 5.7 of their corresponding parents (Table 2). Once again, in the cycle one selected families had a significantly low white mold score of 4.0 compared with unselected families in all three populations which had 5.9, 5.7, and 5.9 for population I, II, and III, respectively (Table 2). These preliminary results show that the mean white mold score of selected families from cycle Zero and cycle one was significantly lower than the mean for the parents; however, between cycles the gain was insignificant.

Table 1. Mean white mold score (WM) for eight breeding lines belonging to different market classes (MC), used to develop three multiple-parent populations.

Genotype	Origin[†]	MC	WM	Genotype	Origin	MC	WM
USPT-WM-1	M	PT	5.7	Chase	M	PT	5.0
CORN 501	A	PK	7.0	I 9365-25	I	PK	6.2
USPT-CBB-1	M	PT	7.3	ABL 15	M	PT	5.5
92BG-7	I	BL	4.3	A 195	A	CR	4.5

[†] M= Middle American, A= Andean, and I= *P. vulgaris / P. coccineus* interspecific breeding line.

Table 2. Mean white mold score of selected families from one cycle of recurrent selection for three multiple-parent populations evaluated in the greenhouse at Kimberly, Idaho during 2006 and 2007.

Population	Parents	Cycle 0			Cycle 1		
		Unselec.	Selected.	LSD (0.05)	Unselec.	Selected.	LSD (0.05)
Pob I	6.1	5.8	4.1	0.7	5.9	4.0	1.1
Pob II	5.3	6.1	4.2	0.8	5.7	4.0	1.0
Pob III	5.7	6.0	4.2	0.7	5.9	4.0	1.0

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IMPROVEMENT IN SCREENING FOR RESISTANCE TO *SCLEROTINIA SCLEROTIORUM* IN COMMON BEAN THROUGH CHARACTERIZATION OF THE PATHOGEN AND UTILIZATION OF MULTI-STATE NUSERIES

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Data and isolate collections from C. Kurowski (CA), R. Mainz (MN), J. Kelly (MI), P. Griffiths (NY), K. Grafton (ND), J. Myers (OR), P. Miklas (WA), H. Schwartz (CO), S. Singh (ID), K. Kmiecik (WI), R. Felix (Mexico), and A. Oppelaar (France)

Sclerotinia sclerotiorum, cause of white mold in common bean, has over 400 host species, and resistance is rare among its many hosts. There is no complete resistance to *S. sclerotiorum* in common bean. A lack of adapted resistance sources inhibits progress in breeding cultivars with white mold resistance. Repeatability of resistance expression has been a consistent problem in screening putative sources of white mold resistance. Screening difficulties can be reduced by using multiple location screening sites and understanding the role of pathogen variation in the screening system.

In major bean production areas in the United States, the bean breeder or plant pathologist at each different location uses their own standard screening isolate for greenhouse/laboratory tests. Often, these isolates have not been selected, but rather they are collected from cull piles (bean debris including sclerotia discarded from harvested fields). The genetic variation or variation in aggressiveness in these isolates from site to site has never been addressed. Nine isolates used in greenhouse screening and control isolate 1980 (the isolate of *S. sclerotiorum* that has been sequenced) were subjected to the mycelial compatibility groupings (MCGs) and aggressiveness tests. Also, isolates of *S. sclerotiorum* were collected from white mold field screening nurseries across nine states/countries; isolates were collected from G122 (more resistant), Bunsi (intermediate), and Beryl (susceptible) in each of three reps at the nine different screening sites from 2003-2005.

MCGs were used to test the isolates for clonality. If the different isolates grew together and formed a continuous mycelial mat on DS medium, the isolates were compatible and considered clonal. If the isolates formed a barrage line of dead cells where the hyphae met, the isolates were incompatible, and each isolate was considered unique. The ten greenhouse screening isolates formed six MCGs. MCG A was composed of clonal screening isolates from Nebraska, Oregon, and the control isolate. MCG B was formed by the Wisconsin and New York greenhouse screening isolates. MCG C contained the Colorado and North Dakota screening isolates. MCG D, MCG E, and MCG F were formed by the single screening isolates from Michigan, Idaho, and Washington, respectively. When the ten greenhouse isolates and the 146 field screening isolates were tested by the MCG assay, high variation was found within and between field locations. Sixty-four MCGs were identified when the 156 total isolates were tested; 36 of those MCGs (over half) were composed of a single isolate; and 6 of the 64 MCGs were formed by screening isolates from more than one location, i.e. an MCG was formed by greenhouse screening isolates, Minnesota field isolates, and Washington field isolates. MCG A and MCG B, two greenhouse screening MCGs, were clonal with isolates from field screening sites from different nursery locations. Greenhouse screening isolates MCG C, MCG D, and MCG F were clonal with at least one field screening isolate from the same location, i.e. the Michigan greenhouse screening isolate was clonal with at least one Michigan field screening isolate.

MCG E, formed by the Idaho screening isolate, was completely unique, and was not compatible with any of the other 155 isolates.

Genetic variation found by testing isolates using the MCGs varied by location. California and Washington had high within field variation. On the other hand, all eleven Minnesota isolates fit into two clonal groups, thus there was lower genetic variability at this location.

Unless genetic variation reflects the ability of the pathogen to cause disease (affects aggressiveness or virulence), this information is not likely to impact bean breeders and pathologists that screen for white mold resistance. Aggressiveness was tested in the isolates of *S. sclerotiorum* by the straw test (Petzoldt and Dickson, 1996). The stem of the bean plant was cut approx. 1" above the 4th node, and a straw filled with inoculum was placed over the cut end of the plant. The spread of the pathogen down the stem is measured after 8 days using a rating scale (Teran et al, 2005), 1=least aggressive and 9=most aggressive.

First, the ten greenhouse isolates were tested on cultivar G122. Then, the ten greenhouse isolates were tested on ten cultivars of different seed classes (different white mold resistance backgrounds). Finally, the 156 total screening isolates were also tested on cultivar G122. In all tests, significant differences were found between the isolates. However, when the isolate aggressiveness was compared to the MCGs the isolates came from, the MCGs were significantly different, but the isolates within MCGs did not differ in aggressiveness. This data supports the hypothesis that the differences in aggressiveness can be attributed to the MCG that the screening isolates form. The most aggressive MCG caused a straw test rating of 7.50 (susceptible reaction), and the least aggressive MCG caused a straw test rating of 2.92 (resistant reaction). Thus, the differences in aggressiveness caused by genetic variation in the isolates were important to consider when screening for resistance.

Pathogen variability exists in both greenhouse and field screening isolates. Use of the multi-site can provide more convincing evidence for putative resistance in bean lines. We recommend that a common greenhouse isolate(s) be selected for screening across locations.

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IDENTIFICATION OF SSR MARKERS LINKED TO RUST RESISTANCE IN ANDEAN COMMON BEAN PI 260418

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INTRODUCTION

Bean rust is a major disease of common bean that reduces yields and increase costs of dry and snap bean production in many parts of the world. Disease resistant cultivars offer the most effective, economical, and environmentally-friendly method of controlling bean rust. However, this strategy is complicated by the high virulence diversity of the rust pathogen *Uromyces appendiculatus*. Many races of this pathogen have been identified and reported in all bean production areas of the world. Conversely, several rust resistance genes have been identified and characterized in common bean. All of these genes so far identified and published are dominant. Some of these genes, such as *Ur-3*, *Ur-5*, *Ur-7*, and Ur-*11*, as well as unnamed genes *Ur-Dorado 108*, *Ur-Ouro Negro*, *Ur-BAC 6*, and *Ur-Dorado 53*, are from Mesoamerican bean genotypes, while other genes, such as *Ur-4*, *Ur-6*, *Ur-9*, *Ur-12*, and *Ur-13*; as well as unnamed genes *Ur-US#3* (*Ur-8*), *Ur-Resisto* (*Ur-10*) are from bean genotypes of the Andean gene pool. Invariably, rust resistance genes from the Mesoamerican gene pool have a broader spectrum of resistance than genes from the Andean gene pool. For instance Mesoamerican genes *Ur-3*, *Ur-5*, and *Ur-11* are resistant to 44, 70, and 89 races respectively, while *Ur-4* and *Ur-6* are resistant to 30 and 22 races respectively, of 90 races of the rust pathogen maintained at the USDA-ARS Bean Project in Beltsville, MD (Stavely, 2000). PI 260418 (collected in Bolivia) is the first Andean common bean with resistance to all but one of the same 90 races of the same bean rust pathogen mentioned above (Table 1). PI 260418 is susceptible only to Andean race 84 which was collected from an Andean bean in Colorado. The reaction of PI 260418 to the 90 races of the rust pathogen resembles the reaction of PI 181996 (*Ur-11*) which is also resistant to all but one race (Mesoamerican race 108) of the same 90 races mentioned above. We have studied the inheritance of rust resistance in PI 260418 and now we endeavor to find molecular markers linked to this resistance for use in marker-assisted selection. Microsatellite markers are PCR-based markers that have been developed for a wide variety of plant species including commercial crops. Microsatellites detect length polymorphisms at genetic loci that have simple sequence repeats (SSR). The objective of this study was to identify molecular markers linked to the rust resistance gene or genes present in PI 260418. These markers will be very useful in the introgression of this new rust resistance into dry and snap bean cultivars.

MATERIALS AND METHODS

An F_2 population (2-3773, 120 plants) from the cross Pinto 114 x PI 260418 was inoculated with four races of the rust pathogen; two Andean races (98 and 99) and two Mesoamerican races (63 and 85). Pinto 114 was susceptible and PI 260428 was resistant, respectively to all four races. DNA was extracted from 94 F_2 plants. Many primers (more 1300) were evaluated to identify those that were polymorphic between the two parents. Primers evaluated included published (146) and unpublished (278) bean primers, ESTs (174), soybean (715) and *Medicago truncutula* (4) primers.

RESULTS AND DISCUSSION

A total of 158 primers were polymorphic between Pinto 114 and PI 260418. Of the 715 soybean primers evaluated, only 90 produced amplification products but none were polymorphic between the two parents. Bean SSR primer pairs derived from bean genomic DNA sequences were more likely to produce amplification products (72%) than primers pairs from EST sequences (59%). About 28% of the SSR primers derived from genomic sequences detected polymorphisms between the two parents. We identified two single sequence repeats (SSR) markers closely linked to the region that confers resistance to the rust isolates used in this study. These markers were linked at a genetic distance of 20 cM (Figure 1). In addition these two SSR markers were closely linked to two other rust resistance loci.

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Table 1. Reaction of Andean bean PI 260418 and other Andean and Mesoamerican cultivars to selected Andean and Mesoamerican races of pathogen *Uromyces appendiculatus*

Andean (yellow) and Mesoamerican (blue) bean Cultivars	Resist. Gene	Reaction to Andean and Mesoamerican races of the rust pathogen													
		Mesoamerican							Andean						
		41	44	47	49	53	73	108	38	72	84	89	98	102	105
PI 260418		R	R	R	R	R	R	R	R	R	S	R	R	R	R
Early Gallatin	*Ur-4*	S	R	S	R	S	R	R	S	S	S	S	S	S	S
Redlands Pioneer		S	S	S	S	S	S	R	R	R	R	R	R	R	R
Pompadour Checa 50	*Ur-9*	S	R	S	R	S	R	R	R	R	R	R	R	S	S
Golden Gate Wax	*Ur-6*	R	R	R	S	S	R	S	R	S	R	S	S	S	S
Great Northern 1140	*Ur-7*	S	R	S	R	S	S	R	R	R	S	R	R	R	R
Aurora	*Ur-3*	R	S	S	S	R	S	R	R	R	R	R	R	R	R
Mexico 235	*Ur-3+*	R	S	R	R	R	S	R	R	R	R	R	R	R	R
Mexico 309	*Ur-5*	R	R	R	S	R	S	S	R	R	R	R	R	R	R
PI 181996	*Ur-11*	R	R	R	R	R	R	S	R	R	R	R	R	R	R

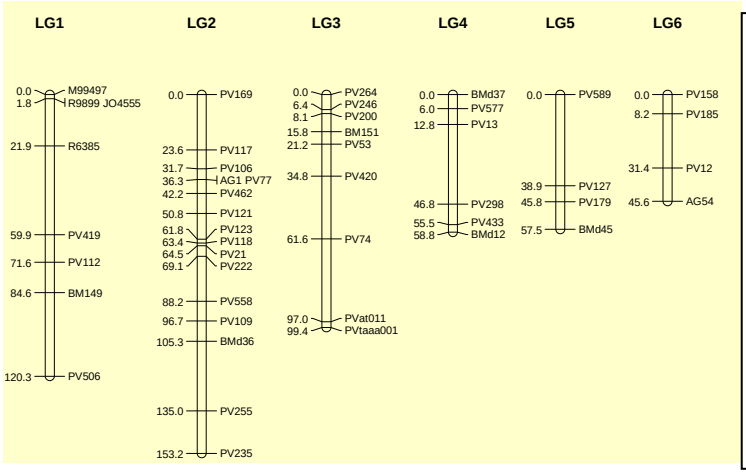

Figure 1. We identified two single sequence repeats (SSR) markers in linkage group (LG) 1 closely linked to the region that confers resistance in Andean bean PI 260418 to the rust isolates used in this study. These two SSR markers were linked at a genetic distance of 20 cM (Figure 1). In addition these two SSR markers were closely linked to two other rust resistance loci.

IDENTIFICATION AND INHERITANCE OF A NEW SOURCE OF HALO BLIGHT RESISTANCE IN COMMON BEAN

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Pseudomonas syringae pv. *phaseolicola* (*Psp*) is the casual agent of halo blight (HB), a bacterial disease of common bean (*Phaseolus vulgaris* L.) with worldwide importance. Halo blight is a seed-borne disease that attacks the foliage and pods. Nine races of *Psp* have previously been described with a set of eight differential *Phaseolus* genotypes. The *Psp* Race 6 is pathogenic on all of these differentials, and there is no known source of resistance for this race. The objectives of this research were to 1) identify common bean germplasm with resistance to *Psp* Race 6 and 2) determine how the resistance is inherited.

A set of common bean germplasm was obtained and screened with *Psp* Race 6 in a greenhouse in Davis, CA in 2007. Primary leaves were inoculated seven days post planting with the multiple needle method of inoculation and a concentration of $1x10^8$ cfu/ml of *Psp* Race 6. Disease symptoms were rated seven days post inoculation using a disease severity index (DSI) with a 1 – 9 rating scale [1-3 = resistant (R), 4-6 = intermediate (I), 7-9 = susceptible (S)]. In the screening of these potential HB resistant sources, it was noted that pinto US 14 showed a variable response to *Psp* Race 6 (Table 1). Plants showing this resistant reaction were selected and advanced using a plant-to-progeny test until a uniform resistant score of 2-3 was obtained.

To determine the genetics of this new source of resistance, a single cross was made between US 14 and the highly susceptible 92BG-7 (HB score 8-9). The F_1 was inoculated with *Psp* Race 6 as described above, and backcrossed to both the parents. The two parents, F_1, F_2 and both BC_1F_1 were assessed for HB reaction in a randomized complete block design with four replicates in the greenhouse. An F_3 progeny test was conducted with four replicates and this was repeated twice.

All 159 F_1 plants of the US 14 / 92BG-7 cross were susceptible, with a HB DSI range of 6-9 and a mean HB DSI of 7.1 (Table 2). These results suggest that the resistance in pinto US 14 is recessive. The F_2 segregation fit a 15S:1R ratio, suggesting that the resistance is controlled by two independent recessive genes having dominant alleles that confer susceptibility. Results of both backcrosses support this hypothesis, with the progenies of the backcross to US 14 (R) approaching a 3S:1R ratio and those of the susceptible backcross (92BG-7) being all susceptible (Table 2). However, the F_3 did not fit the expected ratio, due to a lower than expected number of resistant plants.

Following closer examination of the F_2, it was noted that 75 plants exhibited an intermediate (I) DSI of 4-6, a phenotype not observed in the parents. Thus, the F_2 distribution fit the ratio of **1**(9):**4**(8):**6**(7):**4**(4-6):**1**(1-3), with the numbers in parenthesis representing the HB DSI (Table 3). Thus, it appears that each dominant allele contributed to an increase in susceptibility. The backcross to susceptible 92BG-7 fits this model when the HB scores of each susceptible genotype are considered **1**(9):**2**(8):**1**(7). However, the resistant BC_1F_1 did not fit the expected ratio of **1**(7):**2**(4-6):**1**(1-3), because of a deficiency in the intermediate class. This inconsistency may be a function of the rating scale, or it is possible that a dominant allele at a given locus may not always confer susceptibility. In the F_3 progeny test, the resistant F_2 class (1-3) (e.g., aabb or aaBb) fit the expected

1I:1R ratio for this model. Thus, these results support the hypothesis that a dominant allele at one locus may not always confer susceptibility.

In conclusion, we report the first source of resistance for *Psp* Race 6 in common bean. Because the original US 14 was variable for resistance, the selected resistant pinto US 14 will be released later this year. The resistance in pinto US 14 is controlled by two independent recessive genes, with dominant alleles having a dosage effect. This information will be useful in transferring *Psp* Race 6 resistance to other market classes and for the further understanding of this complex host/pathogen interaction.

Table 1. The range and mean disease severity index (DSI) of selected common bean germplasm to halo blight caused by *Psp* Race 6.

Identification	**Mean**	**Range**
92BG-7	8.4	8-9
US 14	5.1	2-9
A 52 (ZAA 54)	9.0	9
A 53 (ZAA 55)	9.0	9
A 43 (ZAA 12)	9.0	9
Canadian Wonder	9.0	9
Chase	7.4	7-8
Edmund	7.0	7
Guatemala 196-B	9.0	9
Red Mexican UI 3	9.0	9
Tendergreen	9.0	9

Table 2. Reaction of common bean genotypes US 14 and 92BG-7, and their F_1, F_2, F_3 and backcrosses to halo blight caused by *Psp* Race 6.

Genotype	**Mean**	**Ratio**	X^2	***P* value†**
US14	2.3	…	…	…
92BG-7	8.4	…	…	…
F_1	7.1	…	…	…
F_2	6.5	237**S**:16**R**	0	0.05
BC_1F_1 US14	5.0	40**S**:43**I**:41**R**	3.88	NS
BC_1F_1 92BG-7	8.0	116**S**:0**R**	0	0.05
F_3	5.9	309**S**:26**R**	10.40	NS

†NS=non-significant or *P*>0.05.

Table 3. Dosage effect of the US 14 *Psp* Race 6 resistance in the F_2, F_3 and backcrosses.

Genotype	**Ratio**	X^2	***P* value†**
F_2	13(9):58(8):91(7):75(4-6):16(1-3)	3.27	0.05
BC_1F_1 US 14	40(7):43(4-6):41(1-3)	11.66	NS
BC_1F_1 92BG-7	24(9):69(8):20(7)	5.39	0.05
Resistant F_3	31**I**:17**R**	3.52	0.05

†NS=non-significant or *P*>0.05.

CHARACTERIZATION OF *PHASEOLUS VULGARIS* L. EMS MUTANTFAILING IN SEED DEVELOPMENT

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INTRODUCTION

Interspecific hybridizations between *P. vulgaris* and the two donor species, *P. coccineus* and *P. polyanthus*, are carried out to introgress desired traits into the recurrent species *P.vulgaris*. Those crosses lead to the abortion of immature embryos, particularly when the donor parents are used as female (Baudoin *et al.*, 2004). In order to isolate genes which can cause *Phaseolus* embryo abortion, plants from an ethyl methane sulphonate (EMS) mutagenized seeds of common bean were screened to isolate plants which failed in seed development. The suppressive subtractive hybridization technique was then used to identify transcripts that are differentially expressed in the mutant embryos.

MATERIAL & METHODS

Plants from an EMS mutagenized seeds of *P. vulgaris*, line BAT93 were screened to isolate plants deficient in seed development (Pankhurst *et al.*, 2004; Silue *et al.*, 2006). Seeds (40g) were treated with 200ml of 30mM EMS. The suppressive subtractive hybridization adapted from Diatchenko *et al.* (1996) was performed using degenerated seeds from the selected plants as tester and the normal seeds from the wild type as driver. Seeds were harvested at different stages of development. Fragments revealed after 2nd round PCR were sequenced and submitted to BLAST sequence homology analyses. Reverse transcription PCR was applied in order to study the relative expression of 3 transcripts during seed development 7 and 12 days after anthesis in mutant and wild-type samples.

RESULTS AND DISCUSSION

Among M2, M3 and M4 generations, 416 plants derived from sixty families were screened. Seven plants from family 522 (M 522) showed desired traits on a total of twenty-nine plants observed in this family. All the seeds produced from these plants aborted and embryos inside degenerated seeds failed to grow at different stages of development and showed abnormalities mainly in suspensors and cotyledons.

The suppressive subtractive hybridization allowed us to isolate eight cDNAs fragments. These cDNAs were cloned and sequenced. BLAST sequence homology analyses leaded to ten groups of proteins encoded by the cDNAs isolated: cytochrome P450 protein, cell wall-associated hydrolase, putative senescence-associated protein, myo-inositol 1-phosphate synthase (MIPS), Sucrose synthase (SUS), voltage-dependent anion channel (VDAC), peroxidase (PEROX), leucine rich protein, IMP dehydrogenase/GMP reductase and serine rich protein. Figure 1 shows the alignment of peroxidase

nucleotide sequence obtained in this study (SSH_PvE6, EF660341) with mRNA sequences in database sharing high identities.

On the basis of their score goal and their homology to gene sequences, five clones which correspond to cytochrome P450, MIPS, PEROX, VDAC and SUS have the best homology results. All of these five genes are expressed in plant seeds and are important for cell survival.

Reverse transcription PCR was applied in order to study the relative expression of VDAC, PEROX and MIPS transcripts during embryo development 7 and 12 days after anthesis in mutant and wild-type samples, with 18S rRNA as internal control (Figure 2). The expression levels of the transcripts differ between mutant and wild-type samples for all the transcripts, with a highest signal for the 7 days old wild-type sample.

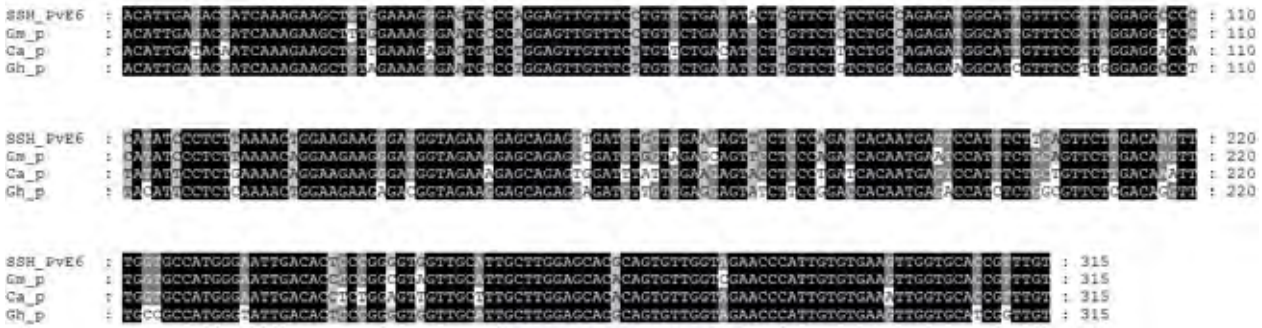

Figure 1. Clustal alignment of SSH_PvE6 (EF660341) nucleotide sequence with *Glycine max*, *Cicer arietinum* and *Gossypium hirsutum* peroxidase mRNA sequences. The sequences identities are 94% (Gm_p, AF039027) for *G. m.*, 87% (Ca_p, AJ271660) for *C. a.*, 82% (Gh_p, L08199) for *G. h.*

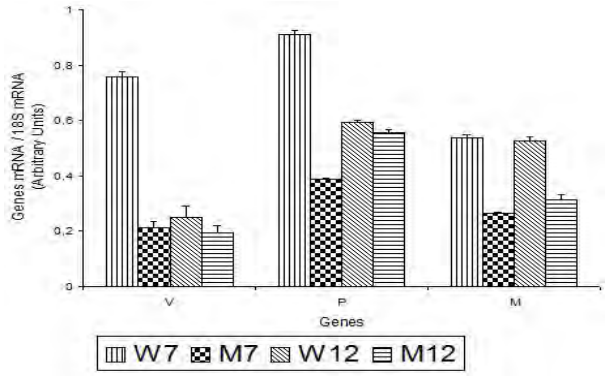

Figure 2. VDAC (V), PEROX (P) and MIPS (M) expression determined by RT-PCR in mutant (M) and wild-type (W) developing seeds at 7 and 12 days after anthesis. Expression of the genes corresponds to the ratio of the quantity of each gene divided by the quantity of the constitutive control 18S rRNA.

PROSPECTS

Crosses between the mutant described here and the wild type were carried out to estimate the genetic transmission of the mutation by analyzing the mutant plants ratio in F2 progenies. This study will be completed by histological comparison between the wild type and the mutant embryos during seed development.

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IMPROVEMENT OF THE SYMBIOTIC INTERACTION BEAN-RHIZOBIA

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INTRODUCTION

Crop legumes as the common bean (*Phaseolus vulgaris* L.) have been used extensively in agriculture over the past century, mainly for maintaining soil fertility. The low fertility has a negative impact on the legume-rhizobia symbiotic relationship reducing the ability of rhizobia to form nodules with optimal N_2-fixing capacity. The symbiotic fixation of nitrogen (SNF) provides an ecologically acceptable alternative to the high applications of nitrogenous fertilizers, and an economic alternative to the limited access to these fertilizers of the developing countries. Inoculation is required to increase yield through N_2 fixation and to reduce the external inputs. There is an important genotypic variability associated with SNF potential and amount of N_2 fixed, that emphasizes the need to explore the potential of indigenous rhizobial strains for improving the symbiotic performance of the common bean. The objective of this work was to identify common bean germplasm useful to improve SNF potential.

MATERIAL AND METHODS

Thirty six common bean populations and breeding lines from the MBG-CSIC collection were screened for their nodulation in glasshouse hydroponic-culture and in field to isolate the rhizobial strains. The reference rhizobial for the hydroponic culture was *Rhizobium tropici* CIAT 899. Plants were harvested at the flowering stage, between 42 and 47 days after sowing. Shoot, nodule and root components were separated and dried at 70 ºC for 2 days to constant weight and each fraction were weighted. The evaluation in field were carried out in two years and four locations, Pontevedra (42º 25'N, 8º 38'W 20 masl), Xinzo (42º 5'N, 7º 43'W, 620 masl), Ponteceso (43º 16'N, 8º 44'W, 400 masl) and Lalin (42º 40'N, 08º 07'W, 552 masl) arranged as a randomized complete block design with two replications. At flowering stage, five plants were collected and for each individual plant, the shoot was separated from the root and the number of nodules and the nodule and shoot dry weight were measured after drying at 80 ºC. At maturity, yield components were measured and rhizobial strains were isolated from root nodules.

RESULTS AND DISCUSSION

There was a wide variation associated with SNF potential and there were differences in the interaction nodulation-bean in different environments. In the hydroponic screening the landraces PHA-0704, PHA-0125, PHA-0227, PHA-0019, PMB-0127, PHA-0194, PHA-0191, PHA-0623 and PHA-0593 constituted a group with a significantly high growth with mean values of 10.49 ± 2.44 g shoot dry weight plant^{-1}. The highest nodule biomass was found in PHA-0704 (> 300 mg nodule dry weight plant^{-1}), PHA-0019, and PMB-0121 (> 100 mg nodule dry weight plant^{-1}). The variation in nodule mass per plants resulted from large variation in nodule number per plant. The field data indicate that the landraces PHA-0704, PHA-0019, and PHA-0155 and PHA-0704, PHA-0719, PHA-0593, PHA-0623 and PMB-0121 had a good shoot dry weight and nodule dry weight respectively. The accessions with high nodule biomass in field displayed the highest yield values also. PHA-0719, PHA-0593, PHA-0623 and PHA-0157 had a high yield in different soil environments. This variability emphasizes the need to explore the potential of indigenous rhizobial strains for improving

the symbiotic performance of *P. vulgaris*. The existence of genetic variation in SNF among bean landraces opens a real possibility for enhancing N_2 fixation through selection and breeding. These results indicate that the accessions PHA-0704, PHA-0719, PHA-0593, PHA-0623 and PMB-0121 could be incorporated into programs of genetic improvement, having an important role in the future of the agriculture. In addition, the genetic analysis of the different rhizobia isolates of the different soils environments has been starting.

Table 1. Characteristics of the common bean accessions studied in hydroponic culture and field trials.

Accession	Hydroponic culture		Field trials		
	Shoot biomass (g plant^{-1})	Nodule biomass (g plant^{-1})	Shoot biomass (g plant^{-1})	Nodule biomass (g plant^{-1})	Yield (g plant^{-1})
ALMONGA PMB-0222	6.71	0.097	18.13	0.103	7.82
ALUB.ENFESTA PMB-0127	9.84	0.116	13.90	0.084	9.03
ANDECHA PHA-0704	16.75	0.318	25.00	0.400	16.14
BELUGA PMB-0190	4.76	0.013	12.42	0.076	8.55
BOLITA PMB-0225	5.50	0.052	17.82	0.117	10.62
BORLOTTO PHA-0719	6.86	0.011	14.15	0.688	26.54
CERRILLOS PHA-0929	6.56	0.128	14.85	0.142	6.28
GANXET PHA-0593	8.92	0.085	15.50	0.563	16.57
GANXET PHA-0623	9.10	0.050	16.00	0.613	27.89
PEREGRINA PMB-0121	7.90	0.188	12.60	0.550	35.97
LINEX PMB-0244	4.03	0.016	14.07	0.047	9.46
MATHERHORN PMB-0220	2.59	0.037	15.05	0.049	8.39
MONTCALM PMB-0214	5.63	0.015	14.63	0.039	6.20
PALOMA PHA-0930	4.17	0.085	14.20	0.080	13.01
PHA-0006	4.52	0.030	13.33	0.063	10.84
PHA-0019	9.99	0.207	25.23	0.122	10.21
PHA-0118	7.04	0.126	14.83	0.058	11.26
PHA-0122	2.96	0.015	7.84	0.029	3.52
PHA-0125	10.98	0.075	14.77	0.024	9.13
PHA-0126	8.25	0.131	12.51	0.015	13.14
PHA-0148	4.56	0.037	16.43	0.020	8.54
PHA-0152	4.65	0.002	9.60	0.020	14.69
PHA-0155	7.64	0.037	21.03	0.007	7.23
PHA-0157	5.17	0.002	10.24	0.020	33.76
PHA-0179	8.26	0.132	13.98	0.031	17.16
PHA-0180	4.42	0.013	8.83	0.100	11.67
PHA-0190	5.53	0.010	19.50	0.048	6.76
PHA-0191	9.18	0.064	11.28	0.034	6.80
PHA-0194	9.40	0.019	18.28	0.028	14.29
PHA-0200	5.47	0.001	9.34	0.020	13.38
PHA-0203	7.81	0.129	20.34	0.177	7.47
PHA-0208	5.71	0.039	10.50	0.100	10.44
PHA-0220	8.20	0.019	13.47	0.200	13.57
PHA-0222	3.10	0.020	7.68	0.320	16.65
PHA-0227	10.27	0.121	12.41	0.047	5.55
PHA-0246	7.34	0.006	12.70	0.127	10.39

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LIMA BEAN BREEDING AND GENETICS RESEARCH AT THE UNIVERSITY OF DELAWARE

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INTRODUCTION

Green baby lima beans are the keystone crop for Delaware processing vegetable growers. In 2004 and 2005 Delaware planted over half of the US green baby lima bean acreage. The availability of green baby limas in Delaware has kept regional processors interested in Delaware growers and has attracted new processors to the state. Regional processors are increasing baby lima acreage in 2008.

Fordhook lima beans for processing are currently produced almost exclusively in Ventura County, CA. Delaware growers and regional processors are interested in producing Fordhooks in the East; however, the currently available Fordhook varieties produce poorly in Delaware.

Another class of limas of interest in the state is the large seeded pole limas. Pole limas are grown commercially by numerous fresh market growers in Delaware and bring premium prices at farm markets.

Baby Lima Bean Breeding

Breeding of baby lima beans in Delaware was initiated in 2004. In 2008 about a dozen baby lima lines from the Delaware breeding program will be included in the replicated lima variety trial. A project is also underway to develop baby lima lines that are homozygous resistant to lima bean downy mildew (*Phytophthora phaseoli*) races E and F. Resistance to each race is conferred by a single dominant gene.

Fordhook Lima Bean Breeding

Breeding of Fordhook lima beans in Delaware was initiated in 2005. Some promising F_4 lines and F_2 plants were selected in the field in 2007. The F_6 and F_4 generations from these selections will be grown in the field in 2008, as well as additional F_2 Fordhook crosses.

Hybrid Pole Limas

Pole limas are typically planted on a trellis with four to six feet between plants. It is not uncommon for growers to transplant, rather than direct seed, pole limas. In 2006 some pole lima crosses were made and the F_1 hybrid plants were grown in the greenhouse. We experimented with propagating these hybrid plants by taking cuttings. A few F_1 hybrid plants, successfully propagated from cuttings, were grown in two different locations in summer 2007. More pole lima F_1 hybrids are being generated for testing in summer 2008.

Lima Bean Mapping Populations

Three lima bean RIL populations are under development for future genetic mapping. The F_4 generations of these populations were grown in the greenhouse in fall 2007.

Table 1. Parents of the three lima bean mapping populations.

Cultivar	Growth Habit	Seed Size	Seedcoat Color/Pattern	Origin	Downy Mildew Resistance
Bridgeton	Determinate	Small/Flat	Green/Self	Mesoamerican	Resistant to races A, B, D & E
Dr. Martin	Indeterminate	Large/Flat	Greenish-white/Self	Andean	Reaction unknown
Fordhook 242	Determinate	Medium/Thick	Greenish-white/Self	Andean	Susceptible
Jackson Wonder	Determinate	Small/Flat	Buff & dark purple/Speckle	Mesoamerican	Susceptible

Segregation for observable phenotypic traits is apparent in all three populations. The Dr. Martin x Bridgeton population segregates for seed size, seedcoat color, and plant habit, including determinate/indeterminate growth habit. Indeterminate growth habit is conferred by a single dominant gene in this population, based on the segregation ratio in the F_2 generation (χ^2 p-value =0.53 for a 3:1 ratio). This inheritance pattern in lima beans was reported previously by Erickson (1992). The Bridgeton x Fordhook 242 population segregates for seed size, seed thickness, seedcoat color and plant habit.

The Bridgeton x Jackson Wonder population segregates for seedcoat color, seedcoat pattern and plant habit. Self-colored seedcoat is conferred by a single recessive gene in this population based on the segregation ratio in the F_2 generation (χ^2 p-value =0.21 for a 3:1 ratio) and the fact that plants with self-colored did not segregate for seedcoat pattern in subsequent generations. One of the parents of this cross, presumably Jackson Wonder, carries genes conferring red and light purple seedcoat colors. It also seems that the red/purple seedcoat color genes are tightly linked to the gene for the speckled seedcoat pattern, as there are no self-colored red, dark purple or light purple seeded plants in the hybrid population. The dark purple and buff seedcoat colors appear to be dominant to the other seedcoat colors.

Plants with crinkled leaves, stunted growth or sterile racemes were observed in the two populations generated from Andean x Mesoamerican crosses (Dr. Martin x Bridgeton and Bridgeton x Fordhook 242). Plants with crinkled leaves and stunted growth were also reported by Erickson (1992) in populations derived from crosses between U.S. baby lima cultivars and indeterminate accessions from Brazil. Some of the stunted plants in our lima mapping populations recovered and produced seed after treatment with napthaleneacetic acid, similar to the method used for *P. vulgaris* dwarf lethal plants described by Beaver (1993).

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EVALUATION OF NAVY AND BLACK BEAN GENOTYPES FOR RESISTANCE TO BACTERIAL WILT

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INTRODUCTION

Bacterial wilt, a seed-borne disease of dry bean, is caused by yellow, orange or purple variants of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (Cff). Symptoms include stunted growth and wilting of bean plants, and discolouration of bean seeds, with colour depending on the pathogen variant. Breeding for disease resistance is considered the most efficient way of controlling bacterial wilt of bean. Hsieh *et al.* (2005) identified dry bean lines primarily in the pinto, great northern and pink bean market classes with resistance to yellow and/or orange variants of bacterial wilt. With the exception of two black bean lines, all small seeded (black and navy) bean lines were susceptible to bacterial wilt. The objective of this study was to evaluate bean germplasm from navy and black bean market classes for resistance to the yellow, orange and purple variants of the bacterial wilt pathogen.

MATERIALS AND METHODS

The 16 navy and black bean genotypes included in this study were cultivars from Canadian dry bean breeding programs and germplasm lines from AAFC-Lethbridge. The isolates of Cff used were YSB-2 (yellow variant), OSB-3 (orange variant) and V254 (purple variant). Bean seeds were inoculated with each variant using the hilum injury/seed inoculation method of Hsieh *et al*. (2003). Beans were tested in groups of 8 genotypes. Group I was composed of navy bean genotypes, and group II was composed of black bean genotypes. Each genotype had 3 replicates of 20 seeds in a completely randomized design. The experiment was run twice for each variant.

Fourteen days after inoculation, each seedling was rated for severity of bacterial wilt on a scale of 0 to 5, according to the following scale: 0, no wilt symptoms; 1, wilt on one of the primary leaves; 2, wilt on both primary leaves; 3, wilt on first trifoliate; 4, death of seedling after development of primary leaves; 5, unemerged seedling or death before development of primary leaves. A disease severity index (DSI) was calculated for each replicate, using the formula: $DSI = \Sigma (nw)/T$, where n = no. of seedlings, w = wilt rating (0, no visible symptoms to 5, plant dead), and T = total no. of seedlings. DSI data for each group were analyzed separately using ANOVA, and Fisher's LSD was used to identify genotypes that had significantly ($P<0.05$) lower DSI.

RESULTS

Significant ($P<0.05$) differences in DSI were found among the tested genotypes in both navy and black bean market classes (Table 1). DSIs ranged from 1.95 to 3.71 for genotypes in the navy bean market class, and from 0.44 to 4.23 for genotypes in the black bean market class. The 'resistant' black bean genotypes L02F132, L02F130 and L02F140 and the 'less susceptible' navy bean genotypes CDC Whitecap and T9903 responded in similar fashion to all three variants of Cff.

Although the navy bean genotypes CDC Whitecap and T9903 had significantly reduced DSIs, it would be desirable to identify other navy bean genotypes with higher levels of resistance. The high levels of resistance observed in certain black bean genotypes such as L02F132 may be due to presence of phenolic compounds such as anthocyanins, but further investigation is required.

CONCLUSION

This study identified bacterial wilt-resistant germplasm in the black bean market class. Resistance was observed for all three (yellow, orange and purple) variants of the pathogen. Bacterial wilt-resistant black bean germplasm lines L02F132, L02F130 and L02F140 may be used as parents in the development of wilt-resistant cultivars. Further efforts are needed to identify and/or introgress higher levels of resistance to bacterial wilt in dry bean especially in the navy bean market class.

Table 1. Resistance reaction of navy and black bean genotypes to three variants of the bacterial wilt pathogen *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*.

		Disease Severity Index (0-5)		
Genotype	Market Class	Yellow Variant	Orange Variant	Purple Variant
Group I				
CDC Whitecap	Navy	**1.99**[1]	**1.98**	**1.95**
T9903	Navy	**2.05**	**2.18**	**2.25**
AC Skipper	Navy	2.49	2.89	2.99
Envoy	Navy	2.58	2.35	2.90
Cargo	Navy	2.69	3.12	2.94
Cirrus	Navy	3.22	3.71	2.98
Regent	Navy	3.44	3.39	3.24
Morden003	Navy	3.56	3.59	3.02
AC Cruiser	Navy	3.68	3.57	3.00
Group II				
L02F132	Black	**0.44**[1]	**0.59**	**0.69**
L02F130	Black	**0.67**	**0.82**	**0.91**
L02F140	Black	**0.63**	**0.91**	**1.01**
CDC Jet	Black	1.13	1.49	1.83
Black Violet	Black	1.13	1.84	1.78
AC Harblack	Black	1.23	1.72	2.13
AC Black Diamond	Black	1.33	1.59	1.40
CDC Expresso	Black	3.45	4.23	3.49

[1]Boldfaced number in each group indicates the genotype had significantly lower DSI (Fisher's LSD, $P<0.05$).

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ACKNOWLEDGEMENTS

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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). During 2007, the disease was detected in more than 300 fields in Nebraska, Colorado, and Wyoming. Affected fields were planted with dry bean 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 (EPPO/CABI, 1997). 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. The objective of this study is to screen the US dry core collection for bacterial wilt resistance.

MATERIALS AND METHODS

A total of 424 accessions from the National Plant Germplasm System (NPGS) collection of dry beans and the most current great northern cultivars grown in western Nebraska are being screened for bacterial wilt resistance in the Panhandle Research and Extension Center dry bean greenhouse facilities. Orion and Emerson are used as susceptible and resistant checks, respectively. The accessions are planted in an augmented block design. Each block consists of 23 entries plus 2 checks. Two seeds per accession are planted in each individual pot. The accessions have been planted three times. Ambient temperature is maintained at 27.8 ^{0}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 are inoculated at the V2 stage of development. One plant is 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 consist 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 third inoculation are: The great northern, Emerson, did not show symptoms in any of the three inoculations, suggesting a good level of bacterial wilt resistance. The current cultivars Marquis, Orion, Beryl, 99-131, and 99-136 were susceptible in all three evaluations. Three-hundred and ninety-two accessions (92.5%) were susceptible across the three tests. Thirty-two accessions (7.5 %) showed variability across the three evaluations. Maybe, there is some segregation within those lines to bacterial wilt. A fourth evaluation is in progress. Accessions with some variable responses will be tested a fifth time. The International Center for Tropical Agriculture (CIAT) bean core collection, consisting of a total of 1,586 accessions is being requested and will be tested for bacterial wilt resistance next year. One hundred and sixty-six wild beans are also included in that collection.

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ACKNOWLEDGEMENTS

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LEGUME PIPE—A NEW TOOL FOR DISEASE MANAGEMENT IN LEGUMES

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The Integrated Pest Management Pest Information Platform for Extension and Education (IPM PIPE) began as a dynamic, integrated national warning system to protect soybeans from soybean rust (SBR)(*Phakopsora pachyrhizi*) and to promote efficient and coordinated IPM decision systems (1). The IPM PIPE has succeeded through federal, state, university and industry cooperation in the day-to-day and long-term management of its programs. This concept of a national system that coordinates data from agricultural experts throughout the affected area and utilizes this data in a web-based system to monitor and analyze disease development and spread has been highly effective with SBR. In 2006, the IPM PIPE was expanded to include monitoring of soybean aphids (SA) (*Aphis glycines* Matsamura) and dry beans. Subsequently, the Risk Management Agency approached the IPM PIPE with questions about the disease and insect losses listed in fresh and dry beans, peas, chickpeas, lentils, and other marketable legumes (This group of crops will be designated by the term legumes for this poster). In 2007, the Legume PIPE has been organized and is starting to address these questions. The purpose of this poster presentation is to introduce the Legume PIPE and to inform others of its purpose and goals.

ORGANIZATION

Legume Plots. Twenty-seven states with insured legume acreages were requested to collaborate in the Legume IPM PIPE by establishing legume sentinel plots for monitoring and assay of disease in legumes. Legume sentential plots were established to parallel the size and profile of SBR plots. A total of 158 legume sentinel plots have been established in 2007. States were divided into Eastern and Western regions with Howard Schwartz coordinating the Western region and Marie Langham coordinating the Eastern region. Additionally, in order to expand monitoring for viral diseases into soybeans, 29 states with already established SBR sentinel plots were requested to complete virus assays in two of their SBR plots.

Pathogen Selection. Priority pathogens infecting legumes were identified during discussions with research and extension personnel.

Viral Diseases. Gail Wisler convened a nationwide group of legume virologists to identify important viral pathogens of legumes, estimate their regional priorities, compare sampling methods, and recommend assay methods. In discussion with regional specialists, *Bean pod mottle virus* (BPMV) (Genus: *Comovirus*; Family: *Comoviridae*) and *Soybean mosaic virus* (SMV) (Genus: *Potyvirus*; Family: *Potyviridiae*) were selected for testing in SBR plots during 2007. *Bean yellow*

mosaic virus (BYMV) (Genus: *Potyvirus*; Family: *Potyviridiae*), *Cucumber mosaic virus* (CMV) (Genus: *Cucumovirus*; Family: *Bromoviridae*), *Bean common mosaic virus* (BCMV) (Genus: *Potyvirus*; Family: *Potyviridiae*), *Alfalfa mosaic virus* (AMV) (Genus: *Alfalfamovirus* Family: *Bromoviridae)*, and *Beet curly top virus* (BCTV) (Genus: *Curtovirus* Family: *Geminiviridae*) were selected for testing in legume plots.

Fungal and Bacterial Diseases. Regional specialists were convened for discussions on important legume diseases in their areas. Regional and national priorities were considered, and these were compiled to form a list of pathogens by legume. From these lists, focus pathogens for 2007 were selected, sampling protocols established, and pest grids for each legume were developed. The pest grid compiles the pest, sampling time, number of samples, and identification method for high accessibility.

Tissue Blot Immunoassay. To provide a virus assay method for the Legume PIPE, Sue Tolin and Chet Sutula were awarded a USDA Critical Issues Program Grant for modification of tissue blot immunoassays (TBIA) from research scale assays to high through-put assays for the Legume PIPE. TBIA assays are being performed by National Plant Diagnostic Network (NPDN) diagnosticians. Collaboration with the NPDN is vital for success of this endeavor.

OUTCOMES

The Legume PIPE has organized 158 legume sentinel plots in 27 states, extended the monitoring plots into Canada and Mexico through collaborations with scientists from these countries, identified priority diseases for monitoring, established fungal and bacterial disease monitoring in these plots, developed a high output assay for virus sampling, established virus assays for BYMV, CMV, BCMV, and AMV for legumes, extended virus monitoring for BPMV and SMV in 58 SBR plots in 29 states, began conducting a limited assay trial for *Beet curly top virus* (BCTV), extended SBR monitoring to the Western US by the use of legumes, and established communication between scientists specializing in legumes across the US. Data is currently being collected for future reporting and modeling. The ultimate goal of the Legume PIPE remains to identify causes of loss in legumes and to assist producers in minimizing these losses. The 2007 growing season has begun our progress toward this goal.

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PHYSICOCHEMICAL CHARACTERISTICS OF COMMON BEANS RELATED TO QUALITY

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Different bean cultivars from a commercial group respond differently in terms of physicochemical parameters related to quality, and these responses can be decisive for cultivar selection. In this work some technological and nutritional traits of five dry bean (*Phaseolus vulgaris* L.) cultivars from commercial groups carioca (BRS Horizonte - A1; BRS Pontal - A2; and BRS Requinte - A3) and black (Grafite - A4; and BRS Supremo - A5) were evaluated. These cultivars were developed at Embrapa Rice and Beans Research Center, by the bean breeding program and were harvested in October 2006. Soluble solid contents were determined as well as grain color of both cooked and uncooked beans. Broth rheological measurements were performed at 24C, using Brookfield viscosimeter with concentrical cylinders, and electrical conductivity data were obtained at 25C.

Data presented in Figure 1 indicate that soluble solid content can be used as a quality parameter to determine dry bean shelf life status, considering that it quantifies the released solid amounts after the cooking process. Fresh harvested beans of both commercial groups carioca and black presented average values of soluble solids of 7.5% and 7.95%, respectively. Below these amounts beans can be classified as old. Bean soluble solid contents are affected by the cultivar itself, and can be used as a quality parameter to differentiate cultivar acceptability by consumers.

BRS Requinte and BRS Supremo, respectively from carioca and black groups, presented the highest contents of soluble solids. Soluble solid content data were not proportional to broth viscosity due to the cooking thermal treatment used. This has probably influenced protein solubility causing protein degradation and formation of aggregates followed by fast sedimentation, which generated incorrect viscosity results. To avoid such event, it may be necessary to try an alternative method, such as the Oswald viscosimeter. Broth viscosity has a Newtonian behavior which is independent of the solid contents in the broth (Fig 2) as well as of the variety tested. As far as tegument color is concerned, there is a correlation between uncooked and cooked beans, since gloss intensity (L) of the carioca commercial group is higher in uncooked grains. In contrast, tegument gloss of black beans is intensified after cooking (Table 1). In relation to “a” and “b” parameters, carioca uncooked grains presented less red and yellow colors as compared to cooked grains in which both parameters were intensified. Carioca grains presented the same behavior although less intense due to discoloration during the cooking process (Table 2). Broth electrical conductivity was also affected by variety (Figure 3) but may be used to determine quality of fresh harvested grains of both carioca and black commercial groups. Values above 3,87mS/cm and 3,95mS/cm, respectively for each commercial group, indicate that grains should be considered old.

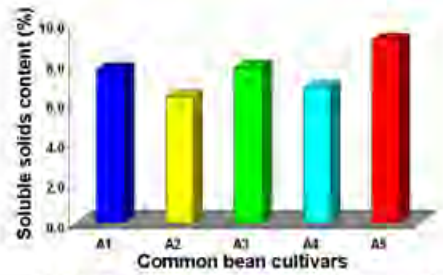

Figure 1. Soluble solids content of broth from common bean cultivars of the commercial groups carioca (A1=BRS Horizonte, A2=BRS Pontal and A3=BRS Requinte) and black (A4=BRS Grafite and A5=BRS Supremo).

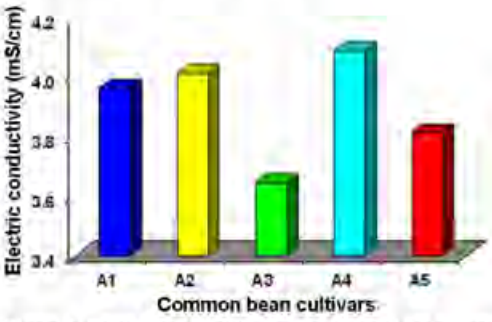

Figure 3. Electric conductivity values (mS/cm) for different analyzed common bean broth.

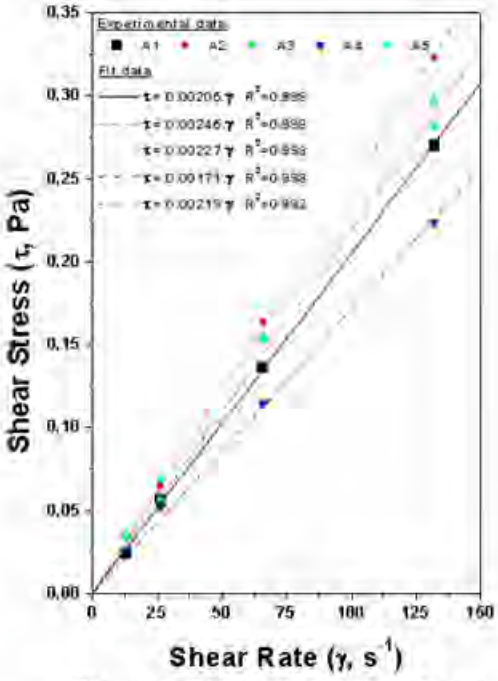

Figure 2. Shear stress (τ, Pa) as a function of Shear rate (γ, s-1).

TABLE 1 – Skin color results of different analyzed raw common beans.*

Samples	Color of raw skin		
	L	a	b
A1	57.255^{a}	2.555^{a}	10.475^{a}
A2	56.275^{a}	2.755^{a}	10.020^{a}
A3	53.300^{b}	2.577^{a}	8.855^{b}
A4	30.587^{A}	-0.495^{B}	-0.613^{B}
A5	30.633^{A}	1.473^{A}	0.295^{A}

* Means followed by identical lower case letters and capital letters in the same column are not different by Tukey test (P<0.05).

TABLE 2 – Skin color results of different analyzed cooked common beans of carioca and black commercial groups.*

Samples	Color of cooked skin		
	L	a	b
A1	41.863^{a}	5.295^{b}	13.240^{a}
A2	42.335^{a}	6.157^{a}	13.625^{a}
A3	42.877^{a}	4.965^{b}	12.005^{b}
A4	31.950^{B}	4.805^{B}	4.172^{B}
A5	33.447^{A}	9.992^{A}	6.050^{A}

* Means followed by identical lower case letters in the same raw are not different by Tukey test (P<0.05).

IDENTIFICATION OF ANTHRACNOSE RESISTANCE GENES IN COMMON BEAN CULTIVARS FROM PARANÁ STATE, BRAZIL

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INTRODUCTION

The anthracnose caused by *Colletotrichum lindemuthianum* is one of the main diseases that occurs in common bean crop. The use of resistant cultivars is characterized as one of the most efficient and economic alternatives in the control of this disease (Mahuku et al., 2002). For this matter, the utilization of assisted selection by molecular markers intimately linked to alleles of interest has shown efficiency on identifying host genotypes of alleles that confer resistance to anthracnose (Kelly and Vallejo, 2004). The allele *Co-4*^{2} can be identified by the molecular marker SCAR $SAS13_{950}$ (Young et al., 1998). The present work had the objective to identify SCARs molecular markers linked to *Co-10* and *Co-4*^{2} genes in common bean accessions and lines derived from the backcross Pérola x G 2333.

MATERIAL AND METHODS

Forty common bean accessions from Germplasm Bank of Núcleo de Pesquisa Aplicada à Agricultura (Nupagri) and 233 lines F_2RC_3 derived from backcross Pérola x G 2333 were analyzed to verify the presence of SCARs $SF10_{1072}$ markers linked to *Co-10* resistant gene and $SAS13_{950}$, linked to *Co-4*^{2} resistant allele. The DNA extracted from the 40 accessions was used as template for amplification reactions according to a methodology proposed by Edwards et al. (1991). The amplification reactions used the SCAR $SAS13_{950}$ marker. Trust analysis was carried out using data from resistant plants with presence of marker (R+), resistant plants without the marker (R-), susceptible plants with absence of marker (S-), and finally susceptible plants with marker (S+). Through these data trust marker, (T) was calculated for identification of resistance in the accesses utilizing the formula: T = (R++R-)/ (R++R-+S++S-).

RESULTS AND DISCUSSION

The combined genotype analysis estimated by markers and race reactions showed that most cultivars possess at least one resistance gene from Mesomerican cultivars (*Co-2*, *Co-3* and *Co-11* or *Co-4*^{2}). Marker $OPAS13_{950}$ was present in three cultivars resistant to races 73 and 2047. The magnitude of the trust analysis was 62 and 59%, respectively for SCARs $SF10_{1072}$ and $SAS13_{950}$ (Figure 1). The accessions Carioca Claro, Preto III, BGF12 and BGF13 exhibited the presence of the molecular markers $SF10_{1072}$ and $SAS13_{950}$ (Table 1). The marker linked to *Co-4*^{2} allele was observed in accessions Carioca I, Carioca IV, Jalo Pardo, BGF3, BGF4, BGF6, BGF8, BGF11, BGF14, BGF16, BGF17, BGF19 and BGF20. However, Carioca V, Carioca VI, Carioca Pintado I, Preto I and Preto II showed the presence of marker linked to *Co-10* gene. Out of the 233 F_2RC_3 lines analyzed by molecular markers, 80 of them revealed the presence of $SAS13_{950}$ linked to *Co-4*^{2} allele.

Table 1 - Amplification standard of the SCARs $SF10_{1072}$ and $SAS13_{950}$ and resistance to races 73 and 2047 in thirty-seven common bean accessions

Accessions	Pathotype of *C. lindemuthianum*		Presence of molecular marker	
	73	2047	$SF10_{1072}$	$SAS13_{950}$
BGF 1	S	R	-	+
BGF 2	S	R	-	-
BGF 3	S	R	-	+
BGF 4	R	S	-	-
BGF 5	S	R	-	-
BGF 6	R	R	-	+
BGF 9	S	R	-	+
BGF 11	R	R	-	+
BGF 12	R	S	+	+
BGF 13	R	S	+	+
BGF 14	S	S	-	+
BGF 16	R	S	-	+
BGF 17	R	S	-	+
BGF 18	S	R	-	-
BGF 19	R	S	-	+
BGF 20	R	R	-	+
Carioca I	S	S	-	+
Carioca II	S	S	-	-
Carioca III	S	S	-	-
Carioca IV	S	S	-	+
Carioca V	S	S	+	-
Carioca VI	S	S	+	-
Carioca Claro	S	S	-	+
Carioca Pintado I	S	S	-	-
Carioca Pintado II	R	S	-	-
Carioca Pitoko	S	S	-	-
Iapar 31	S	S	-	-
Preto I	S	S	+	-
Preto II	S	S	+	-
Preto III	S	S	+	+
Preto IV	S	S	-	-
Rosinha	S	R	-	-
Jalo Listras Pretas	S	S	+	-
Jalo Pardo	S	S	-	+
Jalo Mulato	S	S	-	-
Jalo Pintado I	S	R	-	+
Bolinha	S	S	-	-

(+) Presence of resistance mark; (-) Absence of resistance mark.

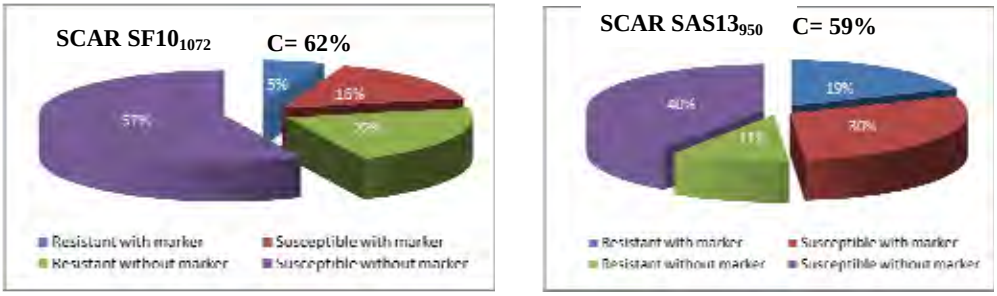

Figure 1. Results of the molecular analyses with the SCAR markers $SF10_{1072}$ and $SAS13_{950}$ and reaction to races 73 and 2047 of *C. lindemuthianum* in 40 accesses of common bean.

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We would like to thank CNPq, Capes and Fundação Araucária for the financial support.

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PARASEXUAL CYCLE AND GENETIC VARIABILITY OF *COLLETOTRICHUM LINDEMUTHIANUM*

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INTRODUCTION

Colletotrichum lindemuthianum is the causal agent of anthracnose, one of the main diseases in common bean (*Phaseolus vulgaris* L.). It presents high genetic variability that interferes on cultivars resistance durability (Kimati and Galli, 1970; Mahuku and Riascos, 2004). Although the sexual phase of *C. lindemuthianum* has already been reported in the laboratory, sexual reproduction in field conditions is not common. The present study had as objective to obtain *nit* mutants from *C. lindemuthianum* races and to use these auxotrophic mutants to obtain hyphae anastomoses and heterokaryons.

MATERIAL AND METHODS

The mutants that were unable to use nitrate as a nitrogen source (*nit*) were obtained in media + NaNO3 (0.2%) + KClO3 (3.0%). After that, mycelium plugs (5 mm) of each *nit* mutant were paired equidistantly apart (approximately 1.0 cm) on petri dishes containing BM + NaNO3 for vegetative complementation tests. Dishes were incubated at 22°C for 12 to 21 days and then examined for prototrophic heterokaryotic growth. Nitrate non-utilizing mutants (*nit*), derived from CL2047, CL73, CL65 and CL23 races were paired in all possible combinations to obtain heterokaryons.

RESULTS AND DISCUSSION

Prototrophic heterokaryons were obtained from pairings CL2047/CL65, CL2047/CL23, CL65/CL23, CL2047/CL2047, CL65/CL65, CL73/CL73, and CL23/CL23. The races CL2047, CL23 and CL65 were allocated in the vegetative compatibility group I (VCG I). Figure 1 shows that the pairings CL2047/CL73, CL73/CL65, and CL73/CL23 exhibited complete vegetative incompatibility by this method, and CL73 race was allocated in another vegetative compatibility group (VCG II). On the other hand, mutants *NitM* of CL2047, CL65 and CL23 races showed vegetative complementation among themselves and formed a dense line of growth in the contact area between the colonies (Figure 1).

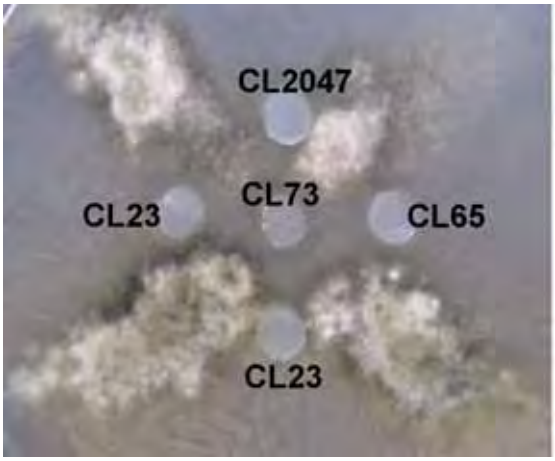

Figure 1. Vegetative compatibility among *C. lindemuthianum* isolates (CL2047, CL65 and CL23 – VCG I). The CL73 isolate (VCG II) does not form heterokaryons with isolates from VCG I.

Complementary *nit* mutants from the isolates were also paired in all possible combinations to determine their capacity for anastomosis. Isolates CL2047, CL65 and CL23 were found to carry out anastomosis with themselves (Figure 2) but none of them formed anastomosis with the CL73 isolate (results not shown).

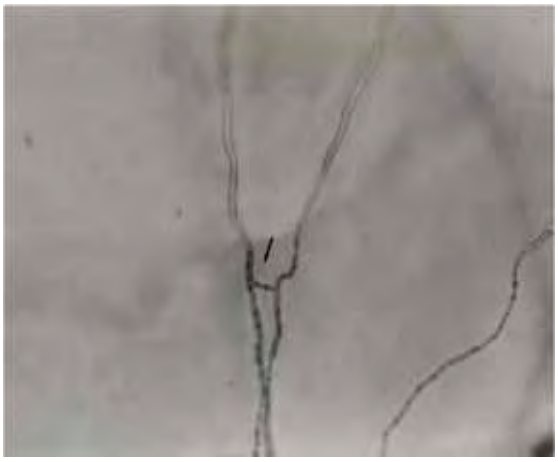

Figure 2. Anastomosis (arrow) between nit mutants CL 23 and CL2047.

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MOLECULAR CHARACTERIZATION OF *COLLETOTRICHUM LINDEMUTHIANUM* HAPLOIDS AND DIPLOIDS

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INTRODUCTION

The *Colletotrichum lindemuthianum* (Sacc. et Magnus) Lams.-Scrib., causal agent of anthracnose in common bean (*Phaseolus vulgaris* L) presents a wide genetic variability and it has motivated researchers to develop studies directed to the comprehension of the molecular interactions emphasizing this pathological system. The formation of conidial anastomosis tubes between conidia within acervuli has been observed during *C. lindemuthianum* conidiogenesis and described as a mechanism that favors exchange of nuclear material and organelles between incompatible strains. Conidial anastomosis tubes were detected in pairs or in multiple associations of conidia (Roca et al., 2003). Therefore, the present work had as objective to study the parasexual recombination in *Colletotrichum lindemuthianum* through analysis of auxotrophic mutants of races CL2047 and CL23 (haploids) using RAPD molecular marker.

MATERIAL AND METHODS

The experiments were carried out to obtain heterokaryons among the genetic complementary mutants. Mycelium plugs (5 mm) of each *nit* mutant were paired equidistantly apart (approximately 1.0 cm) on Petri dishes containing BM + NaNO3 for vegetative complementation tests. Those dishes were incubated at 22°C for 12 to 21 days and then examined for prototrophic heterokaryotic growth. The diploids CL2047-4//CL23-19 and CL23-14//CL23-15 were haploidized, which permitted the isolation of two recombinant parasexuals, haploids, CLrec6 and CLrec4. The DNA extraction was conducted according to the methodology proposed by Raeder and Broda (1987). The extracted DNA was submitted to PCR reactions using the primers RAPD OPC8 and OPF5.

RESULTS AND DISCUSSION

The recombinant CLrec4 presented an 800bp band which CL2047-4, when analyzed with the primer OPF5 (Figure 1). On the other hand, the analyses conducted with the primer OPC8 showed the presence of a 1,300bp band in the diploid CL23-14//CL23-15, which is not found in race CL23 (Figure 2). However, the recombinant CLrec6 presented two bands with 350bp and 800bp, being polymorphic in relation to the diploid CL2047-4//CL23-19. The recombinant CLrec4 demonstrated to be polymorphic in relation to the diploid CL23-14//CL23-15 by presenting a band with 1,000bp. The results showed the occurrence of a parasexual cycle in *C. lindemuthianum*, confirming the importance of this process in the variability generation in this pathogen.

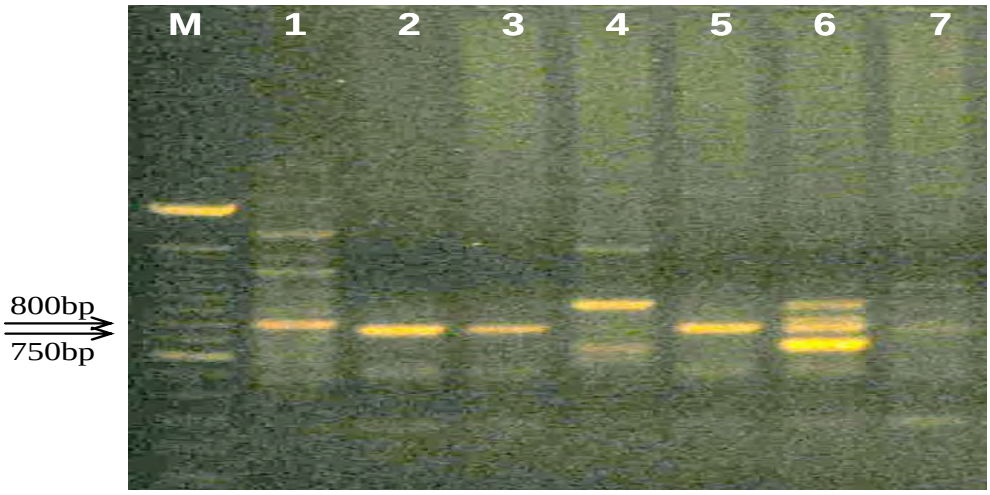

Figure 1 - Amplification of genomic DNA using OPF5 RAPD marker. Lanes are as follows: M, molecular weight marker (100bp ladder); 1, CL23; 2, CL2047; 3, CL2047; 4, CL23-14//CL23-15; 5, CL2047-4//CL23-19; 6, CLrec4; 7, CLrec6.

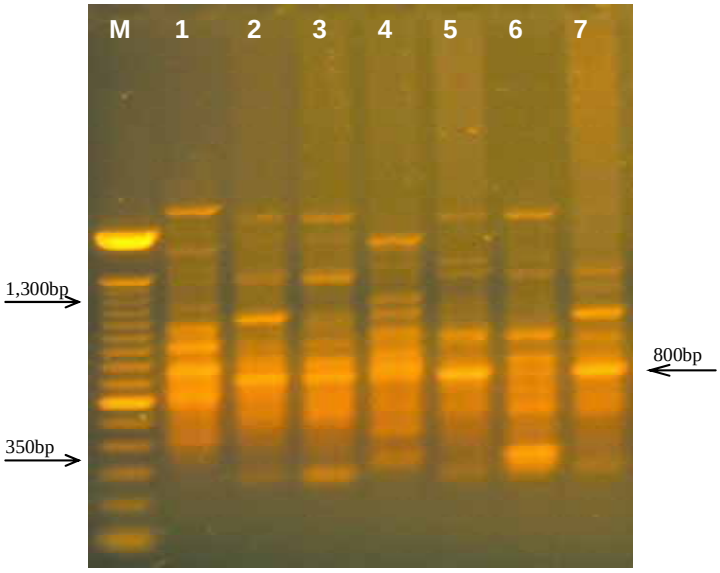

Figure 2 - Amplification of genomic DNA using OPC8 RAPD marker. Lanes are as follows: M, molecular weight marker (100bp ladder); 1, CL23; 2, CL2047; 3, CL2047; 4, CL23-14//CLR23-15; 5, CL2047-4//CL23-19; 6, CLrec4; 7, CLrec6.

ACKNOWLEDGEMENTS

This work was supported by CNPq, Capes and Fundação Araucária.

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GENETIC VARIABILITY WITHIN *COLLETOTRICHUM LINDEMUTHIANUM* RACE 65 ASSESSED BY RAPD MARKERS

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INTRODUCTION

Colletotrichum lindemuthianum (Sacc. et Magn.) Scrib. fungus, causal agent of common bean anthracnose represents wide pathogenic variability. Such fact consists mainly in limiting factor for resistant cultivars development, which represents as most practical method to control this disease. The present work had as objective to estimate the genetic variability in *C. lindemuthianum* isolates of race 65 from Paraná, Santa Catarina and Minas Gerais states, Brazil.

MATERIAL AND METHODS

Fourteen isolates of *C. lindemuthianum* race 65 from Paraná, Santa Catarina and Minas Gerais state were used in this study. The micelial DNA from the 14 isolates was obtained by the method of Raeder and Broda (1987) and amplified with 12 RAPD primers. RAPD bands were scored as present (1) or absent (0) for each isolate. The binary data generated a matrix obtained with the use of the Jaccard arithmetical complement index. The isolates were clustered by UPGMA method.

RESULTS AND DISCUSSION

The DNA amplification products of the *C. lindemuthianum* isolates reproduced a total of 63 polymorphic bands. Figure 1 shows the amplification by the OPC8. The isolates most dissimilar were CL65-1 and CL65-408, with 95% of dissimilarity, one from Paraná and another from Minas Gerais (Figure 2A). On the other hand, the isolates CL65-14 and CL65-21 from Santa Catarina were the most similar (Figure 2B). The cluster analysis grouped the isolates into five groups by UPGMA method with 0.70 similarity, showing molecular variability within the race CL65 (Figure 3). Similar results were obtained by Talamini et al. (2006), who demonstrated presence of molecular variability among isolates from race CL65.

The molecular analysis detected 93.0% of genetic variability within the race CL65 of *C. lindemuthianum* from Paraná, Santa Catarina and Minas Gerais states. The RAPD assays corroborated the broad genetic diversity of the pathogen and the results have been useful in breeding for resistance anthracnose.

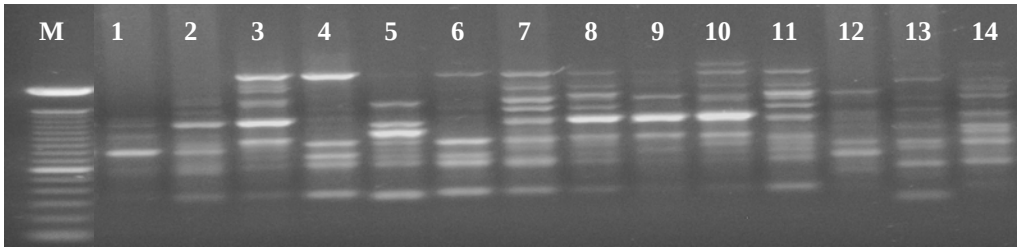

Figure 1 – Electrophoretic analysis of amplification products obtained with OPC8 RAPD marker. Lanes are as follows: M, molecular weight marker (100bp ladder); 1, CL65-1; 2, CL65-400; 3, CL65-5; 4, CL65-6; 5, CL65-9; 6, CL65-10; 7, CL65-14; 8, CL65-21; 9, CL65-22; 10, CL65-26; 11, CL65-29; 12, CL65-11; 13, CL65-408; 14, CL65-315.

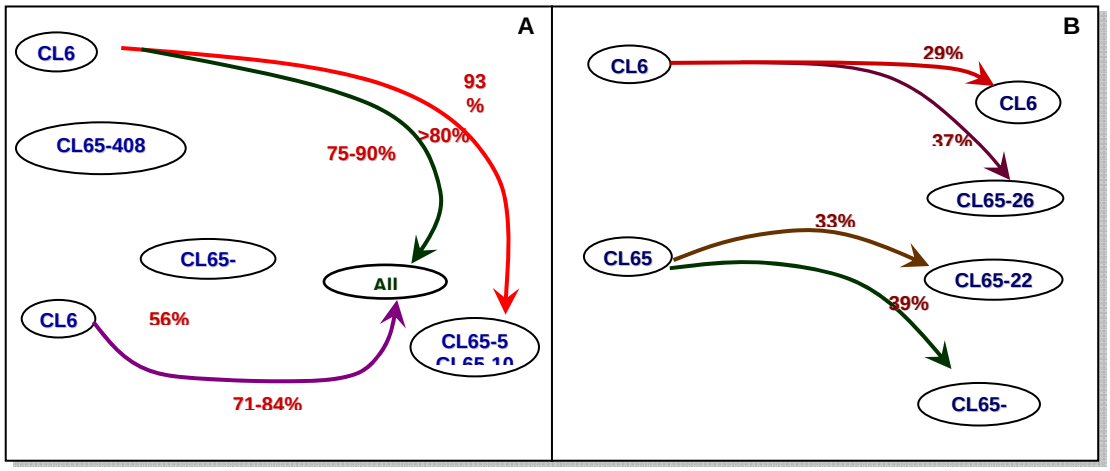

Figure 2 - The most dissimilar isolates (A) and the most similar isolates (B).

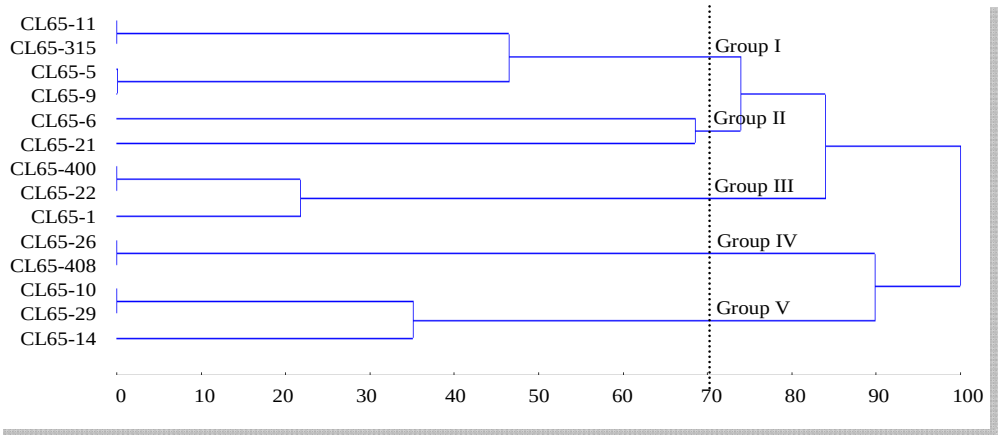

Figure 3 - Cluster analyses of 14 isolates of *Colletotrichum lindemuthianum* from race 65 based on UPGMA method obtained from 63 polymorphic fragments using the Arithmetic Complement of the Jaccard Index.

ACKNOWLEDGEMENTS

We would like to thank CNPq, Capes and Fundação Araucária for the financial support. P. S. Vidigal Filho and M.C. Gonçalves-Vidigal were supported by grants from CNPq, G.F. Lacanallo is recipient of fellowship from Capes.

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SCREENING OF EXOTIC DRY BEAN DROUGHT TOLERANT GERMPLASM IN WESTERN NEBRASKA

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INTRODUCTION

Intermittent water stress is the single most important abiotic stress affecting yield and seed quality of dry beans. Declining ground water and diminished surface water supplies during drought are not new problems for dry bean production areas, however, the drought across the inter-mountain west and Great Plains over the past six years has magnified yield losses. Water allocations are already in place in several Nebraska, Kansas and Colorado areas for both ground and surface water. The objective of this research was to screen and identify elite exotic dry bean germplasm for drought tolerance.

MATERIALS AND METHODS

Ten lines (seven drought tolerant lines from the International Center for Tropical Agriculture (CIAT), and three reference checks, Matterhorn, Bill-Z, and Beryl), identified from 110 lines tested in 2005, were planted in 2006 in replicated trials located near Scottsbluff and Mitchell, NE in non-stressed and stressed plots adjacent to each other as described by Terán and Singh (2002). Supplemental water was provided early to both the stressed and non-stressed plots to ensure successful stand establishment. The stressed and non-stressed plots were irrigated alike until flowering, at which time irrigation of the stressed plots ceased. A neutron probe access tube was placed in plots containing Matterhorn, Bill-Z and SEN 21, and soil water content was measured to a depth of 1.2 m. Drought intensity index (DII) (Fischer and Maurer, 1978), drought susceptibility index (S) (Fischer and Maurer, 1978), and geometric mean (GM) (Schneider et al., 1997) were calculated.

RESULTS AND DISCUSSION

The only rainfall received was 16.5 mm on 8/26 and 56.1 mm on 8/26 and 8/27 at Scottsbluff and Mitchell stations, respectively. However, plants were already physiologically mature. The DII value of 0.76 across both locations indicated severe drought conditions during the experiment. Beryl, Bill Z, SEN 3, and SER 22 had the lowest S and the largest GM suggesting some drought tolerance (Table 1). SER 26 had the lowest common bacterial blight severity in both environments (data not shown). On average, yield was reduced 76.2% across both locations (Table1), plants matured 6 d earlier (data not shown), and 100 seed weight was reduced 22.3 % (data not shown) in the stressed plots.

Table 1. Drought Tolerance Study parameters across two Nebraska locations in 2006.

Ent No.	CODE	Non-stressed Yield	Stressed Yield	Yield Reduction	Drought Susceptibility Index†	Geometry Mean‡
		lbs/A		%		
6	BERYL	2642.4	795.1	69.9	0.92	1449.5
5	MATTERHORN	2630.7	633.4	75.9	1.00	1290.8
7	BILL Z	2538.6	785.8	69.0	0.91	1412.4
2	SEN 3	2520.6	707.1	71.9	0.94	1335.0
1	SER 26	2504.1	554.9	77.8	1.02	1178.8
9	SER 22	2445.8	723.5	70.4	0.92	1330.2
8	SER 10	2332.6	498.5	78.6	1.03	1078.3
3	SEN 20	2031.5	383.4	81.1	1.06	882.5
4	SEN 21	1917.6	334.3	82.6	1.08	800.7
10	SEC 10	1852.4	161.6	91.3	1.20	547.1
	Grand Mean	2341.6	557.8	76.2		
	LSD (P=0.05)	360.9	247.9			
	CV%	17.4	61.7			

ACKNOWLEDGEMENTS

Financial support of the Nebraska Dry Bean Commission and the Burlington Northern Endowment Grant is appreciated. Germplasm provided by CIAT (S. Beebe) is also appreciated.

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GAMETE SELECTION FOR IMPROVING PHYSIOLOGICAL RESISTANCE TO WHITE MOLD IN DRY BEAN

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INTRODUCTION

In dry and green bean, white mold is the most devastating and widely distributed disease in the U.S. and other cool humid regions of the world. Only low to moderate levels of resistance occur in a few Middle American (e.g., ICA Bunsi) and Andean (e.g., G 122, PC50, MO 162, and A 195) dry bean (8, 3, 4, 6). The resistance to white mold in dry bean is quantitatively inherited (2, 5). Also a single dominant allele controlling resistance to white mold in *P. vulgaris/P. coccineus* populations was reported (1, 7). The availability of only a few dry bean germplasm with low levels of resistance, low heritability, unreliable screening methods, large environmental influence, and use of inadequate breeding methods have hindered the success in breeding for white mold resistance. The effect of gamete selection for introgression of white mold resistance using multiple-parent populations is not known.

OBJECTIVE

Our main objective was to introgress and pyramid physiological resistance to white mold in pinto bean, using gamete selection in two inter-gene pool populations.

Population Development

Based on evaluations made under field conditions at Parma Research and Extension Center and in the greenhouse at Kimberly in 2004 and 2005 eight breeding lines of diverse origin with different levels of white mold resistance were used to develop two multiple-parent populations. Population I (Pob I) had the pedigree USPT-WM-1/CORN 601//USPT-CBB-1/92BG-7, and population II (Pob II) was Chase/I 9365-25//ABL 15/A 195. First four single-crosses were made in the spring of 2006; then, doubles-crosses in summer of 2006. For each single cross, an average of 50 F_1 seeds were produced. For double-crosses, 1810 and 1049 F_1 seeds were produced for Pob. I and Pob. II, respectively.

Greenhouse Screening

In The fall of 2006, 848 F_1 plants of each population were screened in the greenhouse for white mold reaction. The cut-stem method was used approximately 25 days after germination. Using eppendorf tips the inoculum was put in contact with the cut stem. Multiple sequential inoculations were used. Reaction to white mold was rated 27 days after inoculation using a modified scale (9) where 1 = no sign of stem infection adjacent to inoculated mycelial plug, 3 = lesion was greater than one inch but not reached the first node, 4 = lesion reached the first node, but no further, and 7 = lesion reached the second node, but no further. In spring of 2007, 405 and 279 $F_{1:2}$ families belonging to Pob. I and Pob. II, respectively, were evaluated as described above. In summer of 2007, 37 and 16 $F_{1:3}$ families of Pob. I and Pob. II were again evaluated following the same methodology.

Preliminary Results

In $F_{1,}$ 405 families belonging to Pob. I were selected with an average white mold score of 4.0 while the overall population mean was 5.8. The 279 families from Pob. II showed intermediate and

resistant reaction to white mold, with an average of 4.0 compared with 6.1 for the population (Table 1). The mean white mold score of $F_{1:2}$ families was 7.6 and 7.7 for Pob. I and Pob. II, respectively, but the mean score of selected families in the same generation was 3.1 and 4.4 (Table 1). The overall mean white mold score of surviving $F_{1:3}$ families was 4.8 for Pob. I, and 4.5 for Pob. II; however, selected families in this generation showed a significant reduction in the incidence of white mold with average scores of 3.6 and 3.5, respectively (Table 1). A drastic reduction in the number of resistant families was observed simultaneously with the increase in resistance to white mold. From F_1 to F_3, Pob. I showed a 95.7% reduction while the increase in resistance was 62%. Similar tendency occurred in Pob. II with 98.1% of reduction in the number of families and 57% of increasing in white mold resistance. These preliminary results show the effectiveness of gamete selection in multiple-parent populations.

Table 1. Mean white mold score of families for two multiple parent populations evaluated during three generations in the greenhouse at Kimberly, Idaho during 2006 and 2007.

Population	F_1		F_2		F_3	
	Evaluated	Selected	Evaluated	Selected	Evaluated	Selected
Pob. I	848	405	405	37	37	36
WM Score	5.8	4.0	7.6	3.1	4.8	3.6
Pob. II	848	279	279	16	16	16
WM Score	6.1	4.0	7.7	4.4	4.5	3.5

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COOKING TIME IN SLOW VS. REGULAR DARKENING PINTO BEANS

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BACKGROUND

1533-15 is a slow darkening (SD) pinto bean developed at the University of Saskatchewan. It cooks faster than its close relative, the regular darkening (RD) CDC Pintium, whether it is stored under ideal (cold and dark) or typical (room temperature and light) conditions (Fig. 1). A population of RILs derived from a cross between CDC Pintium and 1533-15 has been developed and SD is controlled by a single recessive gene (Junk-Knievel *et al*. 2008). To establish whether the difference in cooking time between 1533-15 and CDC Pintium is related to the SD trait or a different factor, a set of SD and RD RILs were tested for cooking time following harvest and after storage.

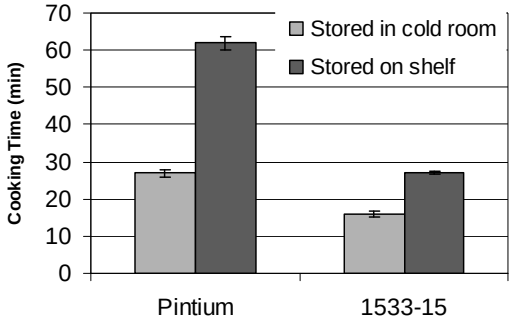

Figure 1. Cooking times of CDC Pintium (RD) and 1533-15 (SD) stored for 8 months in a darkened cold room or on a shelf and exposed to light.

MATERIAL AND METHODS

Plants were grown in a two rep test near Saskatoon in the summer of 2006. Harvested seed samples were cleaned and placed in a humidity-controlled chamber for several days to equilibrate the moisture content of the samples. Samples of each RIL were divided into three groups: one for immediate cooking (fresh), one for storage in the freezer (freezer) and one for storage on the shelf at room temperature (shelf). RILs were assessed for darkening phenotype using the Hunter Lab colorimeter L-values measured after exposing the samples to UV light for 48h (Junk-Knievel *et al*., 2007). The 9 lightest RILs and the 10 darkest RILs were selected for cooking tests.

Seeds were weighed then soaked in tap water for 4 hours. Soaked seeds were strained and re-weighed to determine hydration coefficient (soaked weight/dry weight; HC). Twenty-five randomly chosen seeds were nicked and placed in a Mattson cooker filled with boiling water. Cooking time was recorded when 20/25 seeds were punctured by the probes of the cooker. HCs were calculated for stored seeds 10 months after harvest to simulate 'old crop'.

RESULTS

The HC for fresh seeds was highly negatively correlated with cooking time (-0.94). 1533-15 had a higher HC and a corresponding faster cooking time than CDC Pintium (Fig. 2). While there

were large differences amongst the RILs for HC and cooking time following harvest, it could not be attributed to the darkening phenotype (RD vs SD) (Fig. 2). Interestingly, segregation of the RIL population for shiny and matte seed coats, independent of the darkening phenotype, led to the finding that shiny seed coat was correlated with lower HC and longer cooking time (Fig. 3).

No significant difference between SD and RD RILs for HC following storage under either condition was observed (p = 0.35 and 0.96 for freezer and shelf, respectively). Shiny RILs always had significantly lower HC than matte RILs (Fig. 3). Storage did not appear to affect HC for any phenotype (Fig. 3).

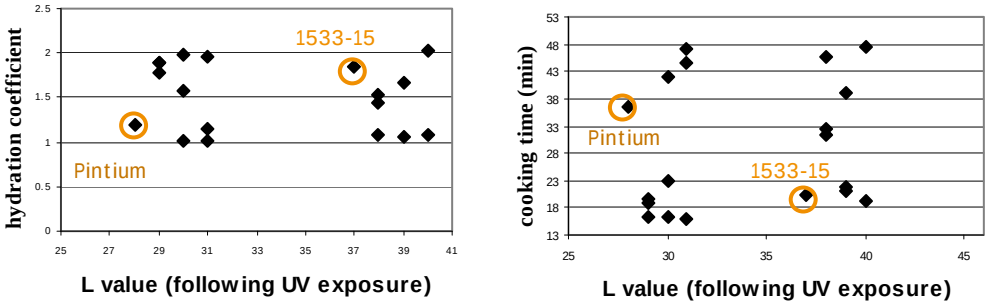

Figure 2. Hydration coefficient (left) and cooking time (right) for SD (high L-value) and RD (low L-value) RILs and parents following harvest. Average of two reps.

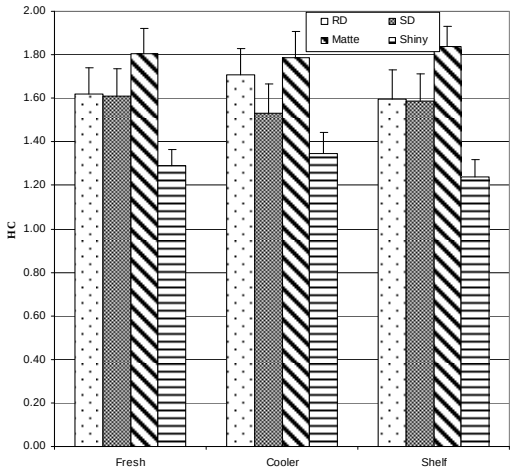

Figure 3. Average HC for RILs classified according to their seed coat darkening phenotype (RD or SD) or luster (shiny or matte). Data from one rep only.

CONCLUSIONS

Based on data from one year, the SD phenotype had no significant effect on cooking time, but seed coat luster had a significant effect with shiny beans having longer cooking times.
Seed coat luster and darkening phenotype are not correlated. A second year of testing is underway to confirm these results.

ACKNOWLEDGMENTS

Funding for this research kindly provided by the Saskatchewan Pulse Growers

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PHYLOGENETIC RELATIONSHIP OF LECTIN-LIKE PROTEINS EXPRESSED IN TEPARY AND COMMON BEAN

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Pulses contain seed storage proteins that deter insect predation of the grain. In particular, lectin family phytohaemagglutinins (PHA), α-amylase inhibitors (α-AI), trypsin inhibitors, and arcelins (ARL) are involved. PHAs and α-AIs show mammalian toxicity whereas ARLs and to some extent, α-AIs, seem specific to insect predation, mainly seed weevils or bruchids. Arcelin belongs to the APA (ARL-PHA-α-AI) complex locus. Seven alleles for arcelin have been discovered in wild accessions of common bean, and some of these have been incorporated into cultivated material. Some alleles provide high levels of resistance to the bean bruchid *Zabrotes. subfasciatus*, but none provide more than moderate levels of resistance to *Acanthoscelides obtectus*. Until recently, it was not known whether arcelins occur in *Phaseolus* species other than *P. vulgaris*, but the presence of these compounds in tepary bean (*P. acutifolius*) was suspected. Researchers at CIAT identified G40199, a wild tepary accession that is highly resistant to both bruchid species. The objective of our research was to transfer resistance to common bean and determine if arcelin was involved.

MATERIALS AND METHODS

Germplasm consisted of G40199, 'Brown Tepary', a cultivated tepary maintained at OSU, 'ICA Pijao', a small seeded tropical black that is cross compatible with tepary bean, and 'Rojo', a large red-seeded cultivar developed by Sokoine University of Agriculture in Morogoro, Tanzania. Interspecific hybrids were obtained from crosses between G40199 and ICA Pijao as described previously (Kusolwa & Myers, 2005). A candidate gene approach was used to identify sequences of the lectin-like proteins in G40199 and derivatives. Primers were designed from APA locus sequences of *P. acutifolius* deposited in Genbank and used to amplify PCR fragments from genomic and cDNA in the target genotypes. Bands of interest were excised from gels and sequenced. DNA sequence was translated into coding amino acid sequence for phylogeny studies. Sequences were compared to existing common bean and tepary sequences obtained from NCBI and EMBL databases. Neighbor joining algorithm in PAUP with 1000 bootstrap repetitions was implemented to obtain a phylogenetic tree. Sequences were aligned to further examine evolutionary relationships.

RESULTS AND DISCUSSION

G40199 was initially examined to determine its seed storage protein profile. A novel 33 kDa band was observed on SDS-PAGE protein gels in G40199, but not in cultivated tepary bean or common bean. The observed protein band was similar in size to that expected for arcelin of common bean and cosegregated with arcelin as revealed in genomic DNA. Using primers designed from previously published arcelin-like gene sequences from *P. acutifolius*, we demonstrated that both G40199 and Brown Tepary had putative arcelin proteins. Protein peptides sequencing (Kusolwa, 2007) confirmed that these proteins are functional arcelins.

Particularly surprising was the observation that G40199 possesses two separate genes for arcelin. These, as part of the complete APA locus found in this accession, may be the reason that it possesses such strong resistance against both major bruchid species of beans. We do not know how the two arcelin genes are physically arranged, but based on the work of Kami et al. (2006), would predict

that they lie in tandem with the other genes of the APA locus. The two genes are quite divergent, with one showing greatest similarity to the arcelin found in cultivated tepary, whereas the other appears unique to the wild accession.

We conducted a phylogenetic analysis of the novel lectin-like proteins observed in the tepary bean accessions used in the present study. Our neighbor joining tree for *P. vulgaris* was very similar to that presented by Lioli et al. (2003). The phylogeny fits well with the hypothesis that PHA is ancestral to α-AI and ARL, and that the latter two were derived independently from PHA. Our data suggests that arcelins and α-AI were both derived prior to the separation of *P. vulgaris* and *P. acutifolius* as species. Also, α-AI alleles from the two species appear to have diverged less than the arcelins. It is not possible to determine whether the rate of change in α-AI has been slower than in the arcelins, or whether arcelins are more ancient than α-AIs.

When sequences are aligned, three gaps were observed that characterize the phylogenetic relationships among the seed proteins (Mirkov et al., 1994). GAP 1 is associated with α-AIs of both species. GAP 2 occurs in all *P. acutifolius* arcelin sequences and *P. vulgaris* α-AI-2 (the latter of different size and probably being derived independently). GAP 3 separates PHA from the lectin-like proteins. Temporally, GAP 3 probably occurred first, GAP 1 next, with GAP 2 being the most recent event.

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PROGRESS IN CHARACTERIZATION AND TRANSFER OF WHITE MOLD RESISTANCE FROM RUNNER TO COMMON BEAN

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White mold (*Sclerotinia sclerotiorum*) is a serious disease of common bean (*Phaseolus vulgaris*). In snap beans, not only does it reduce yield and quality, but harvest lots with greater than 3% incidence of moldy pods are rejected at the cannery. One of the best sources of resistance to white mold is *P. coccineus* (runner bean). We first screened the *P. coccineus* USDA-NPGS plant introduction collection to identify accessions with highest levels of resistance (Gilmore et al., 2002). The resistant accession PI255956 was crossed to susceptible 'Wolven Pole' to create an F_2 mapping population. We characterized the population phenotypically for white mold resistance using the straw test, and mapped molecular markers and QTL associated with resistance. Subsequently, we crossed PI255956 to Oregon 91G bush blue lake green bean. The F_1 was backcrossed twice to 91G using the backcross-inbred (BCIB) method to create a $BC_2F_{4:6}$ population that was characterized phenotypically and mapped (Haggard & Myers, 2006). We summarize our findings from these mapping efforts.

Wolven Pole/PI255956 population: This population of 188 individuals was tested with an eight day and five week straw test. A five week reading of the straw test was implemented because of the resistance levels observed in *P. coccineus*. Random amplified polymorphic DNAs (RAPDs), simple sequence repeats (SSRs), and amplified fragment length polymorphisms (AFLPs) were used to create a map. Two-hundred fifteen markers were placed in thirteen linkage groups and spanned a total distance of 797 cM (LOD 4, 30 cM maximum distance between linked markers). We estimate that the *P. coccineus* map covers approximately 65% of the genome.

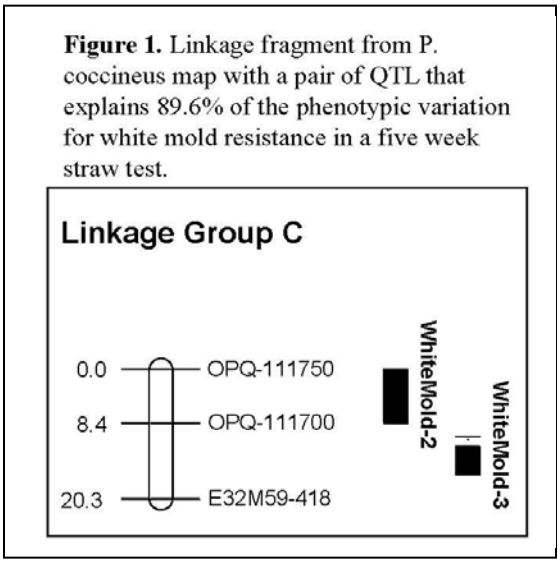

Figure 1. Linkage fragment from P. coccineus map with a pair of QTL that explains 89.6% of the phenotypic variation for white mold resistance in a five week straw test.

Single factor analysis revealed highly significant association with nine marker loci on four linkage groups. With composite interval mapping, four QTL were placed on this map. The two QTL related to the five week straw test explained 89.6% of the phenotypic variation (Fig. 1). Two other QTL, associated with the eight-day straw test results, explained 13.8% of the phenotypic variation.

Previously, we used interval mapping to place five QTL for white mold resistance on five separate linkage groups (Gilmore & Myers, 2004). The only QTL that matches the revised map is that placed on LG C. The previous map was based on ninety-four RAPD markers and 11 SSR markers mapped in 94 progeny, which may explain the difference in number of magnitude of QTL.

OR 91G/PI255956 BC-Inbred population: One-hundred fifteen BC_2F_4 lines were genotyped using AFLPs and SSR markers. Corresponding BC_2F_5 progeny were evaluated for resistance to white mold in a straw test repeated three times, and for oxalate tolerance in a laboratory test. BC_2F_6

lines were then tested for resistance under field conditions. Of 172 SSR primer pairs, 98 were polymorphic between parents. Of those, 77 were scorable in the progeny, and two revealed single introgressions. The remaining 21 SSRs were either monomorphic between progeny, or would not amplify in the progeny and were discarded. The single pair of AFLP primers amplified 56 scorable segregating fragments. The linkage map consisted of 11 linkage groups that correspond to 9 of the 11 core map linkage groups based on known SSR marker locations, and a single LG with no anchoring loci. The 11 LGs included 59 loci, covering a total genome length of 140 cM, or approximately 12% of the estimated length of the common bean genome.

Chi-square tests revealed significant divergence from the expected Mendelian segregation ratios at most loci. The only linked SSR marker that fit the expected ratio was BMd-52 on LG 09. Of the unlinked SSR markers, only PVag004 and PVat007 fit the expected ratio. While the homozygous recurrent parental marker class was represented at the expected rate over most loci, the heterozygotic marker class was overrepresented and the homozygous donor parental marker class underrepresented.

Single factor analysis of variance identified 29 marker loci contributing to response in at least one phenotypic test. One QTL conditioning 6% of the phenotypic variance for field resistance was identified by composite interval mapping on LG 09, anchored to the consensus linkage group b09 by SSR loci. Several SSR markers polymorphic between parents failed to segregate in the progeny, particularly those corresponding to bean core map linkage groups b01, b04, and b05.

Fourteen BCIB interspecific lines with snap bean characteristics (762/2-6, 811/43-4, 826/48-3, 828/48-5, 836/3-15, 840/4-6, 853/6-9, 856/7-2, 861/13-14, 880/11-1, 891/15-2, 897/18-1, 903/20-2, and 904/20-3) have shown white mold resistance similar to G122, NY6020, and Ex Rico over two field seasons.

CONCLUSIONS

Our initial mapping effort in *P. coccineus* led us to believe that several QTL with small effect conditioned white mold resistance in this species. As markers were added to the map, we discovered a pair of major QTL for resistance with additional minor QTL. The markers associated with this fragment did not map in the interspecific BCIB population, which suggests that this fragment was not transferred. Several entire linkage groups and many regions of other linkage groups were not represented in the BCIB population. This may represent an interspecies hybrid incompatibility barrier to recombination and transfer of genes from *P. coccineus* into *P. vulgaris*. We will test this hypothesis in another BCIB interspecific population that is currently under development.

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EVAPOTRANSPIRATION AND WATER USE EFFICIENCY FOR COMMON BEAN GENOTYPES UNDER NON-STRESS AND DROUGHT STRESS CONDITIONS

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Drought stress is the most important abiotic constraint in common bean production worldwide and results in significant yield reductions. A major effect of drought stress is the reduction in dry matter accumulation in common bean. The analysis of biomass production per unit crop area and per unit of water evaporated and transpired, provides an effective tool for the evaluation of genotypes under water-limiting conditions (e.g., Muñoz-Perea et al. 2007).

Two common bean genotypes were evaluated, including 'Morales', a small white cultivar (Beaver and Miklas, 1999), and a small red germplasm developed at CIAT (Colombia), SER 16. The field experiments were carried out at the Experimental Station of the University of Puerto Rico in Juana Diaz, PR, where the climate has been classified as semi-arid. Crop evapotranspiration was measured using 12 drainage type lysimeters and estimated using the general Penman-Monteith (P-M) method, based on 4 field-based weather stations (Allen et al. 1998) and variable aerodynamic (r_a) and surface resistance (r_s). Large replicated plots, measuring 9 m x 60 m, of each genotype under stress and non-stress conditions were used. Intermittent drought stress was applied in both years from the beginning of reproductive development (R1) to harvest. Water was withheld, allowing the soil to dry to 25% of total soil available water, at which point the irrigation was applied. In 2006, 18% less water was applied to the stress treatment (387.3 mm total) as compared to the non-stress (472.5 mm total) treatment, while in 2007, 30.3% less water was applied to the stress treatment (302.0 mm total) versus the non-stress (433.4 mm total) treatment. Water use efficiency (WUE) in this study was defined as the ratio of the field yield (kg) per unit of evapotranspiration (m^3).

Drought stress in the field was more severe in 2007 (Drought Intensity Index, DII=0.72) than in 2006 (DII=0.31), causing an average reduction in seed yield of 76% in Morales and 67% in SER 16, as compared to 33% for Morales and 29% for SER 16 in 2006. The lower stress during 2006 was associated with a larger number of rainfall events. The severity of drought stress in 2007 may have been due in part to windy and hot conditions during the pre-flowering and pod filling period, where the mean air temperature was 25.2°C in 2007 compared with 24.5°C in the same period in 2006. The cumulative evapotranspiration (ET) in the field was similar for Morales and SER 16 (Table 1). In 2006, cumulative ET during reproductive development (R1 to R8; DOY, Day Of Year, 66 to 97) was 118.2 mm for Morales and 108.7 mm for SER 16. During the same growth period in 2007, ET was 103.3 mm for Morales and 92.1 mm for SER 16. During seed maturity to harvest (DOY 98 to 104), the cumulative ET was 26.3 mm for Morales and 17.4 mm for SER 16 in 2006. For the same growth period in 2007, ET was 12.0 mm for Morales and 12.5 mm for SER 16. ET for Morales increased from 0.7 mm/day during vegetative development to 5.1 mm/day during pod filling in 2006, and from 0.6 mm/day to 4.6 mm/day in 2007 under non-stress conditions. For SER 16, ET increased from 0.4 mm/day during vegetative development to 5.1 mm/day during pod filling in 2006, and from 0.3 to 6.7 mm/day in 2007.

The low ET rates during vegetative development were associated with reduced surface area (smaller plants) and high surface resistance (r_s). Changes in r_s are associated directly with stomatal resistance (r_L) and leaf area index (LAI). The low levels of drought stress during 2006 did not generate significant changes in LAI and r_s; however larger differences in LAI, r_L and subsequently r_s were observed in 2007, which suggests that r_s is a sensitive parameter of ET during drought stress. The relatively low seasonal crop evapotranspiration values in this study are associated with short crop season (75 and 78 days in 2006 and 2007, respectively), relatively low plant density, climatic factors (low evaporative demand), and the irrigation system (drip) with low rates of evaporation. Published water requirements for dry bean for a 90 to 100-day season range from 350 to 500 mm depending upon the soil, climate and cultivar (Muñoz-Perea et al. 2007).

Table 1. Evapotranspiration and water use efficiency (WUE), for two common bean genotypes under field conditions, during 2006 and 2007.

Year	*2006*		*2007*		*2006*				*2007*			
Treatment	Non-stress	Stress	Non-stress	Stress	Non-stress		Stress		Non-stress		Stress	
	WUE (kg/m^3)†				Evapotranspiration (mm)‡							
					P-M	Lys.	P-M	Lys.	P-M	Lys.	P-M	Lys.
Genotype												
Morales	1.13	0.85	0.45	0.14	172.2	211.0	154.8	167.3	189.9	215.0	151.8	140.0
SER 16	1.37	0.92	0.42	0.16	147.2	142.0	157.6	100.0	166.3	152.5	137.1	107.2

† Water Use Efficiency (WUE) calculated as seed yield per unit of evapotranspiration.
‡ Penman-Monteith (P-M) method and lysimeters (Lys.) used to estimate evapotranspiration.

In this study, SER 16 showed a tendency toward higher WUE under drought stress conditions than Morales. Values for WUE in the field ranged from a low of 0.14 in 2007 under stress, to a high of 1.37 in 2006 under non-stress conditions. Muños-Perea et al. (2007) reported mean values of WUE in beans under strong stress of between 0.44 kg/m^3 and 0.11 kg/m^3, and under non-stress of 0.87 kg/m^3.

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INHERITANCE OF RESISTANCE TO *BEET CURLY TOP VIRUS* IN THE G122 COMMON BEAN LANDRACE

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The curtoviruses *Beet curly top virus* (BCTV), *Beet mild curly top virus*, and *Beet severe curly top virus*, commonly referred to as 'curly top virus' (CTV), are serious virus diseases of common bean in the semiarid regions of the western U.S. The three viruses are vectored in a persistent manner by the beet leafhopper *Circulifer tenellus*. The only effective control of CTV in bean is genetic resistance. We recently reported a SCAR marker (SAS8.1550) directly linked to the dominant *Bct* resistance gene that confers resistance to CTV (Larsen and Miklas, 2004). The marker is located on linkage group B7. During screening procedures to evaluate the robustness of SAS8.1550 for MAS purposes, the landrace Jatu Rong (G122) from India was the only Andean genotype with resistance to CTV that lacked the marker. The objective of this research was to determine whether G122 possesses novel resistance to the curly top viruses.

MATERIALS AND METHODS

Two populations of $F_{5:7}$ Recombinant Inbred Lines (RILs) were derived from separate F_1 seeds from a cross between G122 x Taylor Horticultural (CTV-susceptible). The populations were comprised of 98 RILs in total. Approximately 40 seed per RIL were planted in rows 3 meters in length in a randomized complete block design with three reps over two years at Prosser, WA in 2006 and 2007. Disease incidence based on the number of infected plants within a single-row-plot was used to measure phenotypic response. Because none of the plants expressed intermediate reactions to infection with CTV, individuals were rated either resistant or susceptible. Presence or absence of CTV in select plants was verified by ELISA or polymerase chain reaction (PCR).

The RIL population was also planted in the greenhouse for use in DNA extractions. DNAs bulked from six CTV-resistant and six CTV-susceptible RILs, respectively, were extracted from bean plants at the first trifoliate stage using FastDNA spin columns (Q-Biogene, Irvine, CA). After adjusting DNA concentrations to 0 ng/µl, random decamer primers (Operon Technologies, Inc. Alameda, CA) were screened for RAPD DNA markers detected as amplified fragments present in one bulk but absent in the other as viewed on agarose gels. RAPDs detected between resistant and susceptible bulk DNAs within populations and verified for cosegregation among individuals comprising the bulks were then assayed across the entire population of 98 RILs. QTL were identified by regression of a marker on disease incidence phenotype using single factor ANOVA (PROC GLM in SAS). A probability level of <0.05 was used as a significance level to declare presence of a QTL. JoinMap 4.0 was used to construct linkage maps.

RESULTS AND DISCUSSION

Infection pressure was uniform across both years as indicated by the high level of disease incidence for the susceptible parent Taylor Horticultural. The mean CTV incidence within 40 plants of the susceptible parent was 8.8 and 11.8 for 2006 and 2007, respectively. Population A (52 RILs) exhibited greater susceptibility ($\overline{X}$ = 4.3 and 4.5 in 2006 and 2007, respectively) than Population B (46 RILs) ($\overline{X}$ = 2.8 and 2.7 in 2006 and 2007, respectively), the reason for which is not immediately clear. G122 landrace may be heterogeneous for minor genes that cause slight differences in reaction

to CTV. We identified three dominant RAPD markers, Q14.925, R15.460, and S11.625, derived from G122 that were completely linked with each other. Genetic analysis of the 98 $F_{5:7}$ RILs revealed that the markers were associated with a major effect QTL that exhibited stable expression across both years and populations (Table 1). The phenotypic variation explained by the QTL in Population A (43.8%) was greater than in Population B (21.9%). Markers Q14.925 and R15.460 also were polymorphic in the BJ core mapping population enabling integration of the QTL to linkage group B6. The *bc-3* gene which conditions resistance to *Bean common mosaic virus* is also located on the B6 linkage group but is not closely linked with the QTL described here. Three additional linked RAPD markers I10.520, S11.580 and K9.925 detected a QTL with minor effect. Expression of this QTL was detected both years, but only in Population A (Table 1). The linkage group associated with this QTL has not yet been identified. Multiple regression analysis indicated these QTL had an additive effect. Additional analysis confirmed that SCAR SAS.1550 for the *Bct* gene located on linkage group B7 was not present in G122. An F_2 from a cross between G122 x Cardinal (which possesses *Bct*) segregated for susceptibility to CTV (data not shown). These findings, combined with the location of the major QTL on B6, indicate that G122 possesses novel resistance to CTV. This is the first report of a QTL for resistance to CTV.

Table 1. Identification (by one-way ANOVA) of two independent QTL conditioning resistance to BCTV as measured by disease incidence across two field trials and two populations, A=52 RILs and B=46 RILs, derived from separate F1 from G122/Taylor Horticulture cross.

RIL population	Linkage group	RAPD marker	Linkage distance (cM)	% phenotypic variation - explained disease incidence (R^2) 2006	2007
A	B6	Q14.950	0	40.3 (P<0.0001)	47.3 (P<0.0001)
		R15.460	0		
		S11.625	0		
	unknown	I10.520	0	10.2 (P<0.022)	7.8 (P<0.047)
		S11.580	1.9	14.6 (P<0.005)	11.8 (P<0.013)
		K9.925	5.2	11.3 (P<0.019)	8.2 (P<0.049)
B	B6	Q14.950	0	26.4 (P<0.0003)	17.3 (P<0.004)
		R15.460	0		
		S11.625	0		
	unknown	I10.520	0	3.9 (ns)	7.8 (ns)
		S11.580	1.9	5.8 (ns)	6.8 (ns)
		K9.925	5.2	ns	ns

Multiple regression of markers S11.625 + S11.580 was equal to 49% (P<0.006) and 32% (P<0.07) for populations A and B, respectively, for 2006 and 53% (P<0.015) and 24% (P<0.06) for populations A and B, respectively, for 2007.
ns - denotes non-significant values.

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MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF RHIZOCTONIA SOLANI ISOLATES FROM WESTERN NEBRASKA DRY BEANS

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Rhizoctonia root rot of beans, caused by *Rhizoctonia solani*, is common throughout the world and causes one of the most economically important root and hypocotyl diseases in dry beans (*Phaseolus vulgaris*). In the United States, losses of more than 10 percent have been observed in conventional tillage systems, while those in minimal or no-till systems have been documented at 20-30 percent. In western Nebraska bean production fields *R. solani* is one of the main pathogens that causes bean root and crown rot. However, there have not been reports for this region on the characterization of anastomosis groups (AGs) and subgroups involved in these diseases. The objectives of this study were to determine the AGs of western Nebraska *R. solani* isolates associated with bean root rot using specific DNA markers, to develop a phylogenetic relationship analysis based on polymorphic DNA markers, and to determine morphological characteristics of isolates.

Fifty-nine *R. solani* cultures isolated from dry bean collected in five counties of western Nebraska were grown on potato-dextrose agar to determine size of sclerotia and appearance of cultures. These isolates were grown on V8-liquid media for three days and then stained with DAPI (4',6-Diamidino-2-phenyl-indole) to determine number of nuclei in hyphal cells (nuclear condition). For the DNA analysis, isolates were grown on potato-dextrose agar for three days, and then DNA from mycelia was extracted using the UltraClean™ Plant DNA Isolation Kit adjusted to *R. solani* DNA conditions. PCR reactions were conducted by using the standard PCR master mix and amplification protocols described by Godoy-Lutz *et al.* (2008). AG analysis of the isolates was determined by presence or absence of twelve AG specific ITS rDNA markers. Polymorphic bands, obtained by two SSR (GACA, GTG) and two URP (URP-2R, URP-6R) markers, were scored using a binary system (1 and 0) for the molecular data and the simple matching co-efficient (SM) in NTSYS pc version 2.0 was used to generate a similarity matrix. A dendrogram was constructed using the unweighted pair grouping method by mathematical averaging (UPGMA) and the genetic distance was estimated by through Dice Coefficient.

Forty-eight isolates produced microsclerotia (<1 mm in diameter), two produced macrosclerotia (5 to 20 mm in diameter), and nine isolates did not produce sclerotia. Based on the nuclear condition, 7 binucleate and 52 multinucleate (> 2 nuclei per cell) isolates were described. Use of specific ITS rDNA markers assigned *R. solani* isolates to AG-1-ID, AG-2-2IIIB, AG-2-2IV, AG-2-2LP, and AG-4. No markers were found in nine isolates; however, using morphological traits and ITS 5.8s rDNA sequences, those isolates were assigned to AGs. The phylogenetic dendrogram, confirmed the AG clusters (Figure 1). Three main branches grouped AG-4, AG-2 and AG-1-ID separately. However, AG-4 isolates were divided in three distinct subgroups separated by significant genetic distance. Morphologically similar AG-4 isolates from those three phylogenetic groups were sequenced using ITS 5.8s rDNA. Sequence revealed three homogeneous groups (HGs) HG I, II and III described by Stevens Johnk and Jones (2001) from culture appearance and fatty acids, and by Kuninaga *et al.* (1997) from ITS sequences. The appearance of our HGs matched with those AG-4 HGs cultures

used by Johnk and Jones (2001). Most of the *R. solani* isolates associated with bean root rot in five counties in western Nebraska were AG-4 and AG-2-2IIIB, and phylogenetic AG clusters determined using specific DNA markers were associated with specific morphological features.

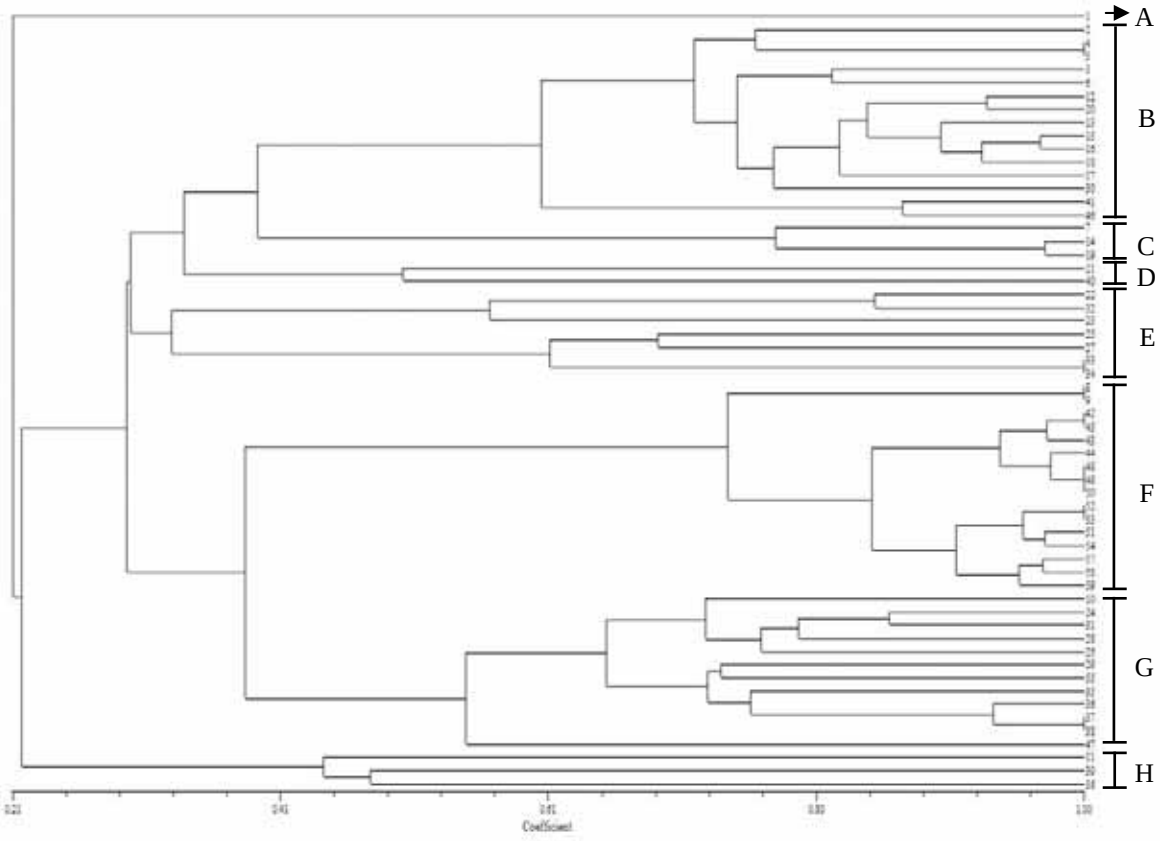

Figure 1 - Dendrogram of 59 *Rhizoctonia solani* isolates obtained from similarity coefficient after UPGMA clustering of band data generate by four different primers. A = AG-2-2IV; B = AG-4-HG II; C = AG-2-2IIIB/IV/LP; D = AG-4-HG I; E = Binucleate; F = AG-2-2IIIB/LP; G = AG-1-ID; H = AG-4-HG III.

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PROGRESS IN THE IDENTIFICATION OF GENETIC VARIATION FOR TOLERANCE TO *CUCUMBER MOSAIC VIRUS* IN *PHASEOLUS VULGARIS* L.

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INTRODUCTION

Wisconsin continues to be the primary producer of processing snap beans in the U.S., however, beginning in 2000, dramatic increases in aphid-transmitted viruses have adversely impacted late-season snap bean production in the state. Based on statewide survey data collected in Wisconsin year-to-year fluctuations in aphid and virus pressure and in the number of species present have occurred (1;2;3). Coincidently, the combination of c*ucumber mosaic virus* (CMV) and the soybean aphid (*Aphis glycines* Matsumura) vector has been detected each year in Wisconsin since 2000. Symptoms caused by CMV include leaf blistering, interveinal chlorosis, off-colored and twisted pods, plant stunting, and flower abortion. Snap bean cultivar evaluation trials have concluded that there are currently no commercial varieties available with resistance to CMV although some tolerance has been observed (4,5,6). We have screened germplasm for resistance to CMV and have identified Plant Introductions (PI) with tolerance to CMV.

A recombinant inbred line (RIL) population (H2-RIL) derived from a cross between a selection within PI 619437 (selection 2313.9.1000 – hereafter TL for tolerant line) and Hystyle (susceptible to CMV) has been developed and is currently being field evaluated to determine the inheritance of tolerance to CMV.

MATERIALS AND METHODS

Germplasm Evaluation & Parent Selection - An array of germplasm including PI accessions from the *Phaseolus vulgaris* L. core and reserve collections, several RIL populations as well as commercial snap bean cultivars were screened in replicated field trials from 2002-2007 (Table 1). Repeatable variation in symptomatology was observed in all years and locations with the exception of 2007. In 2002, individual plants selections were made within three PI accessions (PI 557487, PI 594325 and PI 619437) based on a symptomless phenotype and a negative CMV titer and screened repeatedly in the greenhouse in 2002 and 2003 to determine if the selections were resistant to CMV, tolerant or escapes. These selections were also field evaluated in 2004 and 2005 in replicated trials at Hancock Agricultural Research Station, Hancock, WI and in cooperation with Dr. Walt Stevenson, UW-Madison Dept. of Plant Pathology in three production field trials throughout Wisconsin with a previous history of high virus pressure (5,6). With the exception of 2007, TL remained symptomless over years and locations and was crossed with MV185, a commercial snap bean tolerant to CMV and to Hystyle to create two RIL populations. These populations (M2-RIL and H2-RIL) were screened in replicated trials in 2006 and 2007, respectively. Accession PI 309881 was planted each year as the resistant check (hereafter RL for resistant line).

Symptomatology & ELISA - Visual symptomatology ratings were taken twice each growing season. Composite leaf samples were harvested from each plot at approximately 60 days after planting and screened using Enzyme Linked Immunosorbant Assay (ELISA) for the presence of CMV and *alfalfa mosaic virus*.

RESULTS & CONCLUSIONS

Unlike previous years, both RL and TL had virus symptoms and a positive CMV titer in 2007. The conflicting results from previous years and 2007 must be studied further. Resistance and tolerance may have been defeated due to a new strain of CMV, excessive inoculum and aphid pressure or environmental conditions such as temperature. Nevertheless, breeding for tolerance to CMV in snap beans may be an acceptable strategy until resistant varieties can be developed.

Table 1. Summary of germplasm screened from 2002-2007 and corresponding results.

Year	Germplasm Evaluated	Results
2002	▪ *170 P. vulgaris PI accessions previously reported as having a degree of virus resistance to an array of viruses* ▪ 60 Eagle x Puebla 152 RIL (EP-RIL) ▪ 10 commercial cultivars	▪ *Within the 170 PI accessions, seed was harvested from 77 individual plant selections having a symptomless phenotype and a –CMV titer. These selections were screened in the greenhouse and narrowed to 32.* ▪ Repeatable variation in EP-RIL for aphid preference ▪ 10 commercial cultivars with a +CMV titer and virus symptoms
2003	▪ **423 PI accessions from *P. vulgaris* core collection & commercial checks** ▪ *32 symptomless selections from 2002* ▪ ***RL as resistant check***	▪ **16 of 423 accessions and MV185 with a symptomless phenotype and all 16 with a +CMV titer** ▪ *32 selections narrowed to 12 (TL symptomless and a +CMV titer)* ▪ ***RL symptomless and a –CMV titer***
2004	▪ **16 symptomless PI accessions from 2003 core collection & commercial checks** ▪ *12 symptomless selections from 2003* ▪ ***RL as resistant check***	▪ **All 16 PI accessions and MV185 symptomless and a +CMV titer** ▪ *4 of 12 selections symptomless and a +CMV titer at 4 WI locations (TL symptomless and a +CMV titer)* ▪ ***RL symptomless and a – CMV titer***
2005	▪ **200 random PI accessions from the *P. vulgaris* reserve collection** ▪ 32 pre-1950 commercial cultivars ▪ **16 symptomless core collection accessions from 2004 & commercial checks** ▪ *Of the 12 selections from 2004, TL with best symptomatology ratings to date* ▪ ***RL as resistant check***	▪ **All reserve collection accessions with a +CMV titer and visual symptoms** ▪ All pre-1950 cultivars with a +CMV titer and visual symptoms ▪ **2 of 16 PI core collection accessions with better than or the same virus symptomatology ratings as MV185 at 4 WI locations** ▪ *TL symptomless and a +CMV titer at 4 WI locations, chosen as parent of RIL populations* ▪ ***RL symptomless and a –CMV titer***
2006	▪ MV185 x 2313.9.1000 (M2-RIL) (Symptomless x Symptomless) 131 F3 families, parents and checks ▪ ***RL as resistant check***	▪ Repeatable variation in M2-RIL for virus symptomatology ▪ *Selection TL symptomless and a +CMV titer* ▪ ***RL symptomless and a –CMV titer***
2007	▪ Hystyle x 2313.9.1000 (H2-RIL) (Susceptible x Symptomless) – 90 F_3 families, parents and checks ▪ ***RL as resistant check***	▪ No repeatable variation in H2-RIL for symptomatology ▪ *Selection TL with symptoms and a +CMV titer* ▪ ***RL with symptoms and a +CMV titer***

ACKNOWLEDGEMENT

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A NEW APPLICATION FOR SCAR MARKER SAE19890

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INTRODUCTION

The dominant genes *Ur-3* and *Ur-11* are two of the most important genes conveying resistance to the common bean rust pathogen *Uromyces appendiculatus*. These genes, clustered towards the end of linkage group B11 (Miklas *et al.*, 2002) were originally linked in repulsion (Stavely, 1998). However, they were successfully linked in coupling at the USDA-ARS-Vegetable Lab, Beltsville, and subsequently introduced into breeding lines BelDakMi-RMR-14 to -23 and BelMiNeb-RMR-7 to –13 (Pastor-Corrales, 2003).

Molecular markers are becoming increasingly important in resistance breeding. A number of markers have been developed for both *Ur-3* and *Ur-11*, but have limited applicability. The SCAR marker $sAE19_{890}$ (de Queiroz *et al.*, 2004), derived from RAPD marker $OAE19_{890}$ is linked in repulsion to *Ur-11* (from PI 181996) and reported to be between 6.2±2.8 cM (Johnson *et al.*, 1995) and 1.0 cM (Oliveira *et al.*, 2002) from the gene. The marker was tested for applicability in local breeding material at the ARC- Grain Crops Institute. It was absent from all important local cultivars, but found to be present in some accessions containing the *Ur-3* gene, for example Nep 2 and Aurora. Nep 2 was the source of *Ur-3* for the pinto cultivar Kodiak (JD Kelly, personal communication), from which *Ur-3* was introduced into the *Ur-(3+11)* coupling (Pastor-Corrales, 2003). The purpose of the present study was to determine the usefulness of the SCAR marker $sAE19_{890}$ as a coupling marker for *Ur-(3+11)*.

METHODS

Using standard conditions and methods, important accessions (Table 1), as well as three F_2 populations segregating *for Ur-(3+11)*], were inoculated with *U. appendiculatus* race RSA-Ua1 (which overcomes *Ur-3*+ as in Nep 2, but not *Ur-11*) and with race TZ-Ua-11 (which overcomes *Ur-11* as in the "Bel" lines, but not *Ur-3*). Whatman® FTA technology was used to isolate DNA from the leaves. A micro punch was used to obtain single sample discs, 1.2 mm in diameter, from the FTA cards. Disks were washed with FTA reagent followed by isopropanol to remove chlorophyll and impurities, and air dried. Amplification reactions contained 12.5 µl of 2x Promega PCR Master mix, 1.0 µl of each sAE19 forward and reverse primers, 10.5 µl nuclease free water and the FTA disc. The PCR amplification protocol was as follows: an initial denaturation for 5 min at 94ºC, 40 cycles of denaturation for 15 sec at 94ºC, annealing temperature for 1 min at 45ºC, extention for 1.5 min at 72ºC, with a final elongation for 7 min at 72ºC. PCR products were separated on 3% agarose gels, stained with ethidium bromide.

RESULTS AND DISCUSSION

The reactions of the accessions to races RSA-Ua1 and TZ-Ua-11, as well as the presence or absence of the marker, are shown in Table 1. The marker was absent from all Bel lines with *Ur-11* only, and present in all resistant plants of Bel lines reported to have the *Ur-(3+11)* coupling. BelDakMi-RMR-22, which segregated for its reaction to races RSA-Ua1 and TZ-Ua-11, showed a parallel polymorphism for the marker (Table 1). For the populations segregating for the *Ur-(3+11)* coupling, although marker based selections would have been 78% correct and only 2% (shaded) of the resistant plants would have been discarded, 20% (shaded) were positive for the marker but susceptible to one or both races. There is, therefore, still an urgent need for further markers for these genes, in particular, markers that can, together with $sAE19_{890}$, serve as flanking markers for the *Ur-(3+11)* coupling. As *Ur-3* and *Ur-11* are both block genes (a series of tightly linked genes from which segments can be lost during crossing), flanking markers would also prevent the selection of lines exhibiting partial loss of

either gene. The implication of using the marker as a repulsion marker for *Ur-11* is that, where *Ur-3* is present, it is likely to be lost together with the marker. It is preferable to use the now available *Ur-(3+11)* coupling as resistance source. If the existence of plants that have lost the marker but not *Ur-(3+11)* can be confirmed, these could be used as a source of *Ur-(3+11)* linked in repulsion to the marker. Other important resistance sources, as well as segregating populations, are presently being screened for the presence of $sAE19_{890}$.

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Table 1. Reactions of accessions with and without *Ur-(3+11)* to *Uromyces appendiculatus* races RSA-Ua-1 and TZ-Ua-11 and SCAR marker $sAE19_{890}$

Accession	Rust reaction*		SCAR $sAE19_{890}$	No of plants
	Race 1 (overcomes *Ur-3* but not *Ur-11*)	Race 11 (overcomes *Ur-11* but not *Ur-3*)		
With *Ur-(3+11)*				
BelDakMi-RMR-14; -16; -18; -19; -20; -21; -23; BelMiNeb-RMR-7; 10; 12; 13	R	R	+	26
BelMiNeb-RMR-8	R; MR	R	+	6; 1
Segregating for *Ur-(3+11)*				
BelDakMi-RMR-22	R	R	+	4
	R	S	-	4
PC 3161 RR (segregating *for Ur-(3+11)* but not *Ur-11*	R	R	+	18
	R	S	-	7
	R; MR	S	+	9
PC 3164 RR (segregating for *Ur-(3+11)*	R	R	+	25
	R; MR; S	S	-	13
	R; MR	R	-	3 (one confirmed)
	R; S	S	+	7
PC 3875 BC2 RR (segregating for *Ur-(3+11)*	R; MR	R	+	3; 6
	S	R	-	1
	R	S	+	1
	MS	R	+	1
With Ur-3 *and parents*				
Nep 2; Aurora, Helderberg	S	R	+	bulks
51051; A 295 from GCI germplasm	R	R	+	bulk; 3
Rudá = CIAT line A 285 (DNA from Brazil)	nd	nd	+	bulk
Cornell 49242 (male parent of Aurora)	R	R	+	1
Black Turtle Soup-17-GDN (GCI Germplasm#178) (?female parent of Aurora) (segregating)	S	R	+	5
	S	R	-	1
	S	S	-	2
Mexico 235; Ecuador 299	S	R	-	bulks
BelDak-RR-2 (Ur-3 from Mexico 235); Teebus-RR-1 (Ur-3 from BelDak-RR-2)	R	R	-	3; 1
Rio Tibagi (male parent of A285)	S	R	-	1
With *Ur-11*; without *Ur-3*				
PI 181996; PI 310762	R	R	-	8; 1
BelDakMi-RR-1	R	S	-	1
BelMiDak-RR-3, -9; -11	R	S	-	6
BelMiNeb-RMR-3; -4; -5	R	S	-	7; 4; 8
Sederberg; 3 backcross lines from Kranskop-group	R	S	-	1 each
Without *Ur-3*; without *Ur-11*				
Mexico 309; BelNeb-RR-1	R	R	-	bulk;3
Jalo EEP 558 (G 09603) (Core Map parent); BAT 93 (Core Map Parent); Kranskop; Kranskop-HR-1; Jenny; Teebus; Brown Beauty; Bonus (SA)	S	S	-	bulks

- R = resistant, S = susceptible, MR = moderately resistant; MS = moderately susceptible

THE *Bct-1* LOCUS FOR RESISTANCE TO BEET CURLY TOP VIRUS IS ASSOCIATED WITH QUANTITATIVE RESISTANCE TO BEAN DWARF MOSAIC VIRUS IN COMMON BEAN

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Host resistance provides effective control of some diseases induced by geminiviruses in common bean (*Phaseolus vulgaris*). A recessive gene *bgm-1* conditions resistance to *Bean golden yellow mosaic virus* (BGYMV) and is located on linkage group B3 near the *bc-1*^{2} gene for resistance to *Bean common mosaic virus* (Blair et al., 2007). The dominant *Bct-1* gene conditions resistance to *Beet curly top virus* (BCTV) and is located on linkage group B7 (Larsen and Miklas, 2003) in the vicinity of a QTL for resistance to BGYMV (Miklas et al., 1996; 2000). These genomic associations of host resistances to different geminiviruses, and between a geminivirus and a potyvirus as in the first example, suggest presence of individual resistance genes with broad effect against multiple virus species and/or presence of resistance gene clusters formed from gene duplication events with subsequent divergence for specific resistance against different viruses. We sought to examine the effect of the *Bct* gene that conditions resistance to BCTV (curtovirus) against another distinct geminivirus, *Bean dwarf mosaic virus* (BDMV), a begomovirus.

Ninety-four $F_{5:7}$ RILs (Moncayo/Primo) were previously characterized for reaction to BCTV across three disease field nurseries (Larsen and Miklas, 2003). Sixty-seven RILs had *Bct-1* gene and resistance to BCTV and 27 RILs lacked the gene and were susceptible to BCTV. Reaction to BDMV was determined by sap-inoculation in a growth chamber. Sixteen plants of each RIL and the parents Primo and Moncayo were mechanically inoculated with BDMV. Primary leaves of 10 to 14-day-old plants were dusted with celite and rubbed with infected tissue ground by mortar and pestle in 0.1 M potassium phosphate buffer (pH 8.0). Symptoms were rated using a disease severity index (DSI) of 1 to 4 approximately 21 DAI, where 1 = no obvious symptoms, 2 = mild: only mosaic on young leaves and no stunted growth, 3 = moderate: leaf curling, mosaic and dwarfing beginning at the 2nd to 3rd trifoliolate stage and stunted plant growth, and 4 = severe: leaf curling, mosaic and dwarfing beginning at the 1st trifoliolate stage and severely stunted growth. Viral DNA was detected by PCR in plants rated 2, 3, and 4.

Uniform disease reaction for the parents was observed (Table 1). Moncayo with resistance to BCTV had complete resistance to BDMV, and Primo susceptible to BCTV was completely susceptible to BDMV. The F_1 population was resistant to BDMV indicating dominant inheritance. The RILs expressed a quantitative disease reaction to BDMV (Table 1). The RILs with *Bct-1* had plants with a DSI mostly rated 1 or 2 for BDMV reaction with a mean DSI of 1.42 (Table 2). RILs without *Bct-1* had plants mostly rated 3 and 4 for BDMV with a mean of 3.07. Generally, RILs scoring <2.0 for BDMV reaction also possessed BCTV resistance, and RILs scoring > 2.0 were susceptible to BCTV. Six RILs had a weak association between BDMV and BCTV reactions. *Bct-1* explains 69.6% of the phenotypic variation for reaction to BDMV based single factor ANOVA.

Seo et al. (2004) demonstrated that germplasm of Andean origin was susceptible to BDMV, whereas most Middle American (MA) germplasm was resistant. The pinto bean Othello (Race Durango) possessed a dominant gene *Bdm* which conditioned qualitative resistance to BDMV. Most snap

beans originate from the Andean gene pool, and many possess genes purposely introgressed from the MA gene pool. Moncayo is a snap bean with resistance to BCTV due to transfer of *Bct-1* from the MA gene pool. In summary, *Bct-1* or a closely linked gene confers quantitative resistance to BDMV. This quantitative resistance to BDMV is different from the qualitative resistance conferred by the *Bdm* gene. The genomic location of *Bdm* is unknown. Resistance to BCTV, BDMV, and BGYMV is associated on linkage group B7.

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Table 1. Quantitative BDMV reaction for a subset of RILs (Moncayo/Primo).

RIL	Plants inoculated	DSI=1	DSI=2	DSI=3	DSI=4	Mean DSI	BCTV rxn
	No.	Number of plants				1 to 4	
Sample of co-segregation between BDMV reaction and *Bct-1* gene observed for most RILs							
MP-1	16	7	8	1		1.6	R
MP-4	14		5	4	5	3.0	S
MP-5	12	7	4	1		1.5	R
MP-6	10			6	4	3.4	S
MP-11	15	14	1			1.1	R
Few RILs with weak association between BDMV reaction and *Bct-1* gene							
MP-19	14		11	3		2.2	R
MP-30	8	4		2	2	2.3	S
MP-75	14	5	5	4		1.9	R
MP-77	7	4		3		1.9	S

Table 2. Summary of BDMV disease reaction for RILs grouped based on R and S reaction to BCTV.

		Average BDMV reaction (on a per line basis)					
Group	Total RILs	Avg. Inoculated	DSI-1	DSI-2	DSI-3	DSI-4	Mean DSI rating
	No.	Number of plants					1 to 4
R to BCTV - *Bct*	67	13.4	8.7	3.9	0.6	0.2	1.4
S to BCTV – *bct*	27	12.4	0.8	1.3	5.9	4.4	3.1

SELECTION OF MARKERS FOR MAPPING AND CLONING DISEASE RESISTANCE IN COMMON BEAN

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INTRODUCTION

Infestation of diseases is a major constraint of subsistence production and economic yield of common bean. Development of cultivars with improved genetic resistance to pest and diseases is the primary goal of bean breeding programs throughout the world.

DNA-marker-based genetic linkage maps have been developed and exploited to identify, tag, and map disease resistance genes and QTL in common bean. The availability of DNA-based markers within the past 20 years has provided new opportunities and challenges to bean researchers. While numerous molecular markers are available, especially for resistance to bacterial, fungal, and viral diseases in common bean, proportionately a few are routinely used in breeding programs. The recent development of SSRs from coding and non-coding sequences (Blair et al. 2003) and tentative consensus (TC) sequences (McConnell et al. 2006) in common bean have created further opportunities for mapping and tagging genes in breeding program.

Several classes of resistance genes encode proteins containing leucine-rich repeats (LRR). Amino acid sequences for a number of these resistance sequences contain strong similarity to nucleotide binding sites (NBS). We have analyzed NBS-LRR type disease resistance gene sequences in common bean and generated 37 molecular markers. In this study, our goal was to screen these markers along with 34 SSRs (Blair et al. 2003) and 21 markers developed from TC sequences (McConnell et al. 2006) among parents of seven mapping populations segregating for different disease resistance traits.

MATERIALS AND METHODS

Plant Materials: DNA was extracted from green house grown common bean parents of seven mapping populations using the protocol supplied by dry bean breeding group, department of plant sciences, North Dakota State University, Fargo, ND.

Development of PCR Primer: Common bean NBS-LRR type disease resistance gene sequences were downloaded from NCBI GenBank and were aligned with multiple sequence alignment software CLUSTALX (1.81). Phylogenetic trees were depicted to evaluate the relationship among different sequences using Neighbor-Joining options of the software. Initially, four NBS-LRR complete coding gene sequences were chosen to design a series of primers based on sequence alignment. The large (~3kb) gene sequences were divided into small ordered overlapping fragments and a total of 37 primer pairs were designed to amplify genomic DNA of ~500 bp, using the web-based PCR-primer designing program 'Primer3'. The primer design also ensured that there is sufficient overlap of the fragments, in order to obtain the sequence of the primer sites and their flanking nucleotides. In addition to these primers, 34 SSR primer pairs previously developed from coding and non-coding sequences of common bean (Blair et al. 2003) and 21 primer pairs developed by analyzing common bean tentative consensus (TC) sequences (McConnell et al., 2006) were also used.

Amplification of common bean genome: The screening of the primers developed from NBS-LRR gene sequences was performed using a PCR program consisting of one cycle of 95ºC for 3 min; 40 cycles of 95ºC for 1 min, from 55 to 57ºC for 1 min, and 72ºC for 2 min; and one cycle of 72ºC for 10 min. The PCR program for SSR primers was consist one cycle of 95ºC for 3 min; 40 cycles of 95ºC for 1 min, from 55 to 57ºC for 1 min, and 72ºC for 2 min; and one cycle of 72ºC for 10 min. For primers developed from TC sequences, the PCR protocol was one cycle of 95ºC for 3 min. followed by 40 cycles of 95ºC for 20 sec, from 52 to 58ºC for 20 sec, and 72ºC for 2 minutes. The PCR program ended with a 7 minutes cycle of 72ºC for final extension. In all cases the amplified products were separated on 2% agarose gel with 60 volts and run for 200 minutes.

RESULTS

Forty-nine percent (49%) of these primers were polymorphic across different parental combination and markers developed from TCs were found to be highly polymorphic (76%) followed by SSRs (65%), and NBS-LRR types (27%). These primers showed varying degrees of polymorphism across different mapping populations. The highest number of primers (46) showed polymorphism in BAT93/JaloEEP558 mapping parent combination followed by A55/G122 (36) and Aztec/ND 88-104-04 (28) while the least polymorphism was observed in DOR364/XAN176 and Benton/NY6020-4 (each with 19 primers) (Fig. 1).

The map location of NBS-LRR type markers has yet to be determined. Sequencing of the amplified products from the NBS-LRR type primers across different parental lines is in progress. Population specific polymorphic markers selected here will be useful in mapping and tagging in different disease resistance traits.

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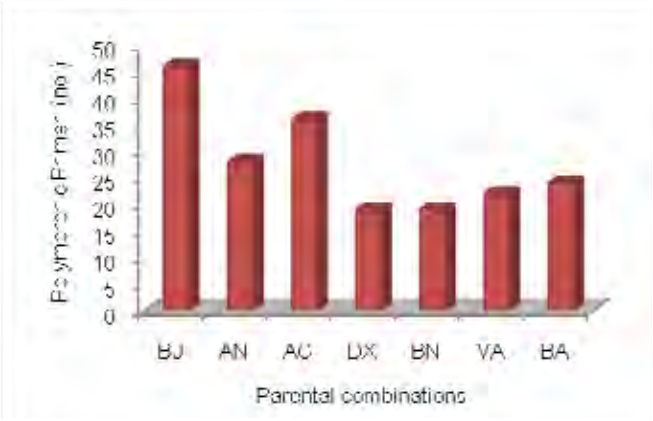

Fig. 1. Distribution of polymorphic primers among different parental combinations of mapping populations in common bean
BJ = BAT93/JaloEEP558, AN = Aztec/ ND88-104-04, AG = A55/G122, DX = DOR364/XAN176, BN = Benton/NY6020-4, VA = Voyager/Albion, and BA = BelNeb-RR-1/A55

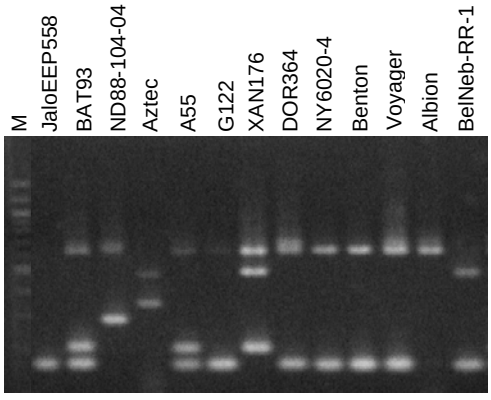

Fig. 2. Polymorphisms for NBS-LRR primer, Pv2356 among parental lines segregating for disease resistance traits

INHERITANCE AND ALLELIC RELATIONSHIPS OF ANTHRACNOSE RESISTANCE IN COMMON BEAN MEXICO 222

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INTRODUCTION

Anthracnose of common bean (*Phaseolus vulgaris* L.), caused by *Colletotrichum lindemuthianum* is one of the most economically important fungal disease responsible for extensive yield losses worldwide. The development of resistant cultivars is the most effective method of controlling anthracnose in common bean and new resistance sources must be constantly sought. 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., 2007b). However, it was reported the presence of two independent genes in Mexico 222 conferring resistance to races 7 and 8 (Kelly and Vallejo, 2004; Gonçalves-Vidigal et al., 2007a). Therefore, this study had as objective to determine anthracnose resistance inheritance in Mexico 222 to race 7, and also to verify resistance independence of the *Co-3* gene from the characterized *Co-4, Co-5, Co-7,* and *Co-9* genes through allelism tests.

MATERIAL AND METHODS

The resistance inheritance to anthracnose in Mexico 222 was evaluated in two segregating populations, where compatible races were chosen to produce an S x R reaction with the following cultivars: MDRK (susceptible to race 7) and Widusa (susceptible to race 23). Parents, F_1 and F_2 were inoculated with the respective race under environmentally controlled greenhouse conditions.

The allelism tests were conducted with F_2 populations from the crosses Mexico 222 x PI 207262, Mexico 222 x MSU 7-1, Mexico 222 x BAT 93 inoculated with race 7. Additionally, were carried out tests with the F_2 population derived from the cross MSU 7-1 x PI 207262 which was inoculated with race 64. Seedlings were evaluated for their disease reaction using a scale from 1 to 9 (Pastor-Corrales et al., 1995) 7d after inoculations.

RESULTS AND DISCUSSION

Evaluation of the parental genotypes, F_1 and F_2 populations revealed that the resistance to anthracnose in the cultivar Mexico 222 to race 7 is controlled by two dominant genes, whereas its resistance to race 23 is conferred by a single dominant gene (Figure 1).

Allelism tests revealed the lack of segregation among 369 F_2 individuals from the cross Mexico 222 x PI 207262; among 115 F_2 individuals from the cross Mexico 222 x BAT 93; and among 125R:0S F_2 individuals from the cross Mexico 222 x MSU 7-1, inoculated with race 7, supporting the assumption that the same locus provides resistance to race 7 in these cultivars (Figure 1). Additionally, a lack of segregation observed in the 158 F_2 individuals from the cross MSU 7-1 x PI 207262 inoculated with race 64, suggesting that the dominant resistant gene in this two cultivars are located at the same locus. Since Mexico 222 has a different resistance spectrum from BAT 93, these data would indicate that BAT 93 carries a new allele at the *Co-3* locus. Allelism tests showed that anthracnose resistance in Mexico 222, MSU 7-1, PI 207262 and BAT 93 to the race 7 is conditioned by the same resistance locus. According to Porch (2008) *Co-3*[3] was firstly described by Geffroy et al., 1999 and is located on linkage group 4 (Freyre et al., 1998). This gene has also shown

to be present in PI 207262 cultivar (Alzate-Marin et al., 2007). Therefore, once Mexico 222 carries two genes conferring resistance to race 7, we propose that the second anthracnose resistance gene in this cultivar should be designated *Co-15*.

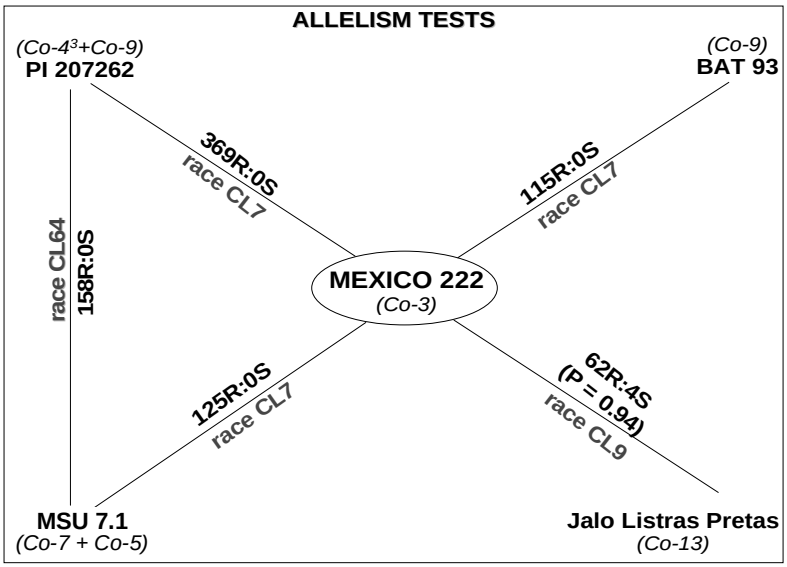

Figure 1. Allelic relationships of anthracnose resistance in Mexico 222.

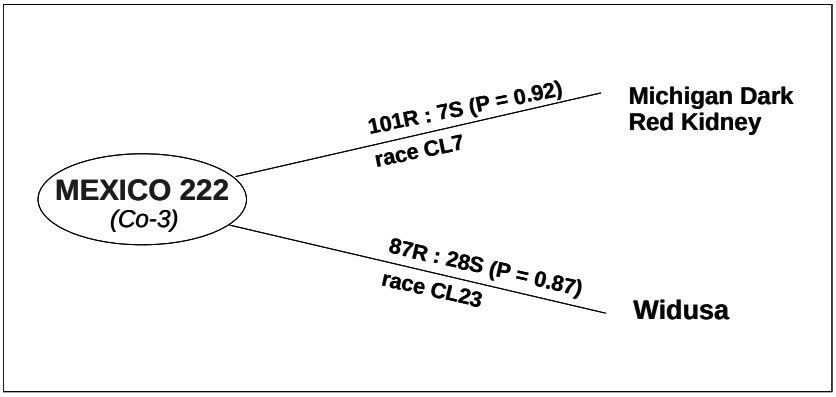

Figure 2. Inheritance of resistance to anthracnose in Mexico 222.

ACKNOWLEDGEMENTS

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AN IMPROVED *IN VITRO* REGENERATION OF COMMON BEAN (*PHASEOLUS VULGARIS* L.)

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INTRODUCTION

Common bean is a crop with important genetic resources which include germplasm with agronomic traits such as resistance to adverse climatic conditions, pest and disease resistance, and high nutritional quality (1); however, some genes that confer specific traits are either not present in bean germplasm or conventional breeding may result time consuming. The recalcitrant condition for in vitro regeneration of this crop has been overcome and we developed an efficient and stable protocol to obtain fully developed plants as a requirement for genetic transformation (2).

OBJECTIVE

To establish an *in vitro* regeneration protocol from embryonic axes for four common bean cultivars.

MATERIALS AND METHODS

Embryonic axes isolated from mature seeds of four common bean cultivars G13637 (G1), Ica palmar (G4523) (IP), Pinto Saltillo (PS) and Flor de Mayo Anita (FMA) were used as explants. Seeds were superficially sterilized with chlorine gas (commercial chlorine (Cloralex®) and 12N HCl, v/v 5:0.16) for 17 h. Seeds were then embedded in double distilled and sterile water for 24 h to ease embryo extraction.

Induction media consisted of GM B5 (3) or MS (4) amended with myo-inositol (100 mg/L), pyridoxine (1 mg/L), thiamine (10 mg/L), sucrose (2%) Phytagel (2.8 mg/L), benzyl aminopurine (10 mg/L) and adenine hemisulphate (0 or 20 mg/L). Media was adjusted to pH 5.8. Elongation-rooting medium consisted on the basal medium with no growth regulators.

Nine Petri dishes containing 10 embryos each, per treatment and per cultivar, were incubated for 5 days. After this period meristematic shoots and roots were removed and the embryonic axes were cultivated in the same media. Explants were transferred to fresh Induction Medium every two weeks until full differentiation. Shoots were then transferred to Elongation-Rooting Medium for complete development and rooting. Growth conditions consisted of 8 h darkness and 16 h light, with 45 mmol/m2/s light intensity and 25 °C. Each experiment was done with three replicates.

RESULTS

All treatments induced 1 to 2 meristematic bud clusters. A 0.5 mm wide bud clusters were observed at the internodal area, four days after they were excised (AE, After Excised). These results were consistent with previous reports (2).

Embryonic axes grew readily in GMA and GMO treatments (10 mm-20 mm) after 3 days in Induction Medium. Bud clusters increased in size eight days AE (1.5 mm wide and 1mm high) and

shoot differentiation was observed 25 days AE, forming true leaves from a 6 mm wide shoots. Shoots were separated from their original explants and transferred to Elongation-Rooting Medium for 30 days. Fifty five days AE, 5-7 cm plantlets with a 2-3 trifoliate leaves and differentiated stems and roots were obtained. From each bud cluster we obtained 2 to 3 plants.

Embryonic axes cultivated in MS0 y MSA treatments showed necrosis of the tissue after nine days in Induction Medium AE. However, still more than 90% of embryonic axes showed bud cluster formation after ten days of culture AE. Approximately 40 days AE the first differentiated buds were observed but more than 50% of the embryonic axes and bud clusters were completely necrotic decreasing the regeneration efficiency. Once shoot fully differentiated (47 days AE), they were transferred to Elongation-Rooting Medium for 25 days, where only 1% of the cultivated axes developed into a whole plant.

The regeneration efficiency for FMA was high in all 4 treatments compared to G1, IP and PS, suggesting that this is candidate genotype for genetic transformation experiments (Table 1). Contrary to previously reported (2), the addition of adenine did not influence the regeneration efficiency regardless of basal medium. GM0 treatment provided the highest efficiency for the 4 cultivars (96.7 to 100 %) (Table 1).

Table 1. Regeneration efficiency of four cultivars of common bean in different Induction Media.

	Regeneration Efficiency[1] (%)			
TREATMENT	**FLOR DE MAYO ANITA**	**G13637**	**ICA PALMAR (G4523)**	**PINTO SALTILLO**
GM0	)7.8[2] ± 4.4	100 ± 0	96.7 ± 5.0	96.7 ± 5.0
GMA	)5.6 ± 7.3	97.8 ± 4.4	90.0 ± 8.7	92.2 ± 8.3
MSO	3.3 ± 11.2	31.1 ± 12.7	28.9 ± 14.5	17.8 ± 8.3
MSA	1.1 ± 16.9	31.1 ± 16.2	46.7 ± 14.1	15.6 ± 7.3

[1] Numbers represent bud clusters/embryonic axes times 100.
[2] Values represent an average of three Petri dishes with 10 embryos each and three replicates

CONCLUSIONS

- A consistent organogenic regeneration protocol is reported for *Phaseolus vulgaris* from embryonic axes with levels above 97%.
- GM B5 medium induced organogenic regeneration in 37% more than MS medium.
- Addition of adenine did not impact regeneration efficiency.
- This protocol will be used in transformation experiments due to its regeneration efficiency.

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PHYTOCHEMICAL EQUIVALENCE OF BLACK BEAN CULTIVARS TO NEGRO 8025, AN OUTSTANDING CULTIVAR FOR REDUCING CHRONICALLY DEGENERATIVE DISEASES IN RATS

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Consumption of black and pinto seeded genotypes have shown a beneficial effect from a health point of view (1,2). Among 12 cultivars, Negro 8025 and Pinto Durango displayed the highest reduction of glucose and cholesterol in blood (3), and in the number and size of colon cancer tumors in rats (2). It has been hypothesized that different seed phytochemicals are responsible of such effects (4). Given the difficulty of assessing the biological effect of all black seeded cultivars that are consumed, we hypothesize that if the phytochemical profile of a black cultivar was similar to that of Negro 8025, then the same biological effect might be observed. Experimental black seeded lines and cultivars bought from local markets were analyzed along with a sample of T-39 produced in Celaya, Gto. in 2005. Total fiber was measured with the Dietary Fiber Assay kit (SIGMA, TDF-100A). Total phenolics and total tannins were determined following George *et al*. (5) and Desphande and Cheryan (6) methods, respectively. Anthocyanins were determined according to Abdel-Aal and Hucl (7) and oligosaccharides by HPLC using the method of Muzquiz *et al.* (8). Isoflavones (daidzein and genistein) were assessed by HPLC. With exception of lines (NSLB/8025/N203)-201 and (NSLB/8025/N203)-250 and cultivar Negro Zacatecas, the rest of lines and cultivars showed similar fiber content as Negro 8025 (Table 1). Negro Queretaro, a landrace cultivar, showed an outstanding content of 18.95 %, 50% higher than that of Negro 8025. It has been mentioned that fiber is one of the main components responsible for the prevention of colon cancer (4). Also, fiber is believed to be involved in the hypocholesterolemic effect of foods (9). All lines and cultivars showed higher total phenolics than Negro 8025, with the exception of line (NSLB /8025/N203)-250 that showed 22% less total phenolics (Table 1), other lines and cultivars showed from 11.6 % (NG0 99176) to 176 % (NG0 99038) higher total phenolics in comparison to Negro 8025. As for tannin and anthocyanins content NG0 99176 had a lower content than Negro 8025 (Table 1). Lines (NSLB /8025/N203)-201 and (NSLB/8025/N203)-250 also showed lower anthocyanins content as well as Michigan 1 and 2 market samples. These last two samples had the lowest tannin content. Plant phenolics participate in decreasing intestinal tumors in animal models of adenomatous polyps (11).

Table 1. Fiber content, total phenolics and anthocyanins content of cooked black bean seeded bred lines and cultivars compared to that of Negro 8025.

Line/cultivar	Fiber (%, dwb)	Total phenolics (mg/100 g, db)	Total tannins (mg/100 g, db)	Anthocyanins (mg/100 g, db)
(NgINIFAP/Ng 8025)-100	11.3 ± 1.0	402 ± 22	25.5 ± 2.0	1.7 ± 0.0
NG0 99176	6.4 ± 0.3	286 ± 11	13.3 ± 1.1	2.6 ± 0.0
NG0 99038	12.7 ± 1.2	711 ± 21	68.8 ± 3.6	54.8 ± 2.0
(NSLB/8025/N203)-201	12.7 ± 1.1	676 ± 46	32.1 ± 2.2	80.7 ± 3.1
(NSLB/8025/N203)-250	10.1 ± 1.6	211 ± 16	46.1 ± 3.9	14.9 ± 1.0
Negro Durango	15.9 ± 1.7	458 ± 19	132.4 ± 6.3	24.0 ± 1.3
Negro Jamapa	10.6 ± 1.6	352 ± 17	47.8 ± 1.3	15.9 ± 0.8
Negro Querétaro	19.0 ± 2.1	390 ± 16	47.8 ± 1.0	34.3 ± 3.2
Negro Otomí	13.7 ± 1.3	378 ± 10	59.6 ± 3.2	25.8 ± 1.5
Negro San Luis	15.9 ± 2.3	377 ± 18	44.3 ± 2.1	33.3 ± 2.9
Negro Tacana	13.0 ± 1.2	343 ± 15	36.1 ± 2.0	6.9 ± 0.9
Negro Zacatecas	9.7 ± 0.1	613 ± 31	53.7 ± 2.8	42.6 ± 2.9
Negro Michigan (1)[1]	13.3 ± 2.0	347 ± 21	6.7 ± 0.2	12.1 ± 1.1
Negro Michigan (2)	15.5 ± 1.4	487 ± 31	6.7 ± 0.2	17.5 ± 1.9
T-39	15.4 ± 1.8	378 ± 19	39.6 ± 2.0	43.8 ± 4.7
Negro 8025 (check)	**12.5 ±1.2**	**258 ± 11**	**38.4 ± 1.2**	**29.3 ± 2.2**

[1]samples were bought from the market at Irapuato, Gto. (1) and (2) Queretaro, Qro.

Oligosacharides and isoflavones. Only four cultivars showed similar (Michigan 2) or higher (Negro Durango, Negro Otomi, and Negro San Luis) raffinose content than Negro 8025 (Table 2). Also, all lines and cultivars showed higher stachyose content than Negro 8025 with exception of line NG0 99038. Non-verbascose was detected. Oligosaccharides along with resistant starch and insoluble fiber are partially responsible for the prevention of colon cancer. This type of fiber is fermented by bifydo bacteria in the colon producing short chain fatty acids which act as potential antiproliferative differentiation agents (11). On the other hand, lines (NSLB/8025/N203)-201 and (NSLB/8025/N203)-250 and Negro Zacatecas had higher daidzin content than Negro 8025; meanwhile all genotypes including Negro 8025 showed similar genistin content (Table 2). Seeds of Negro Durango, Negro Queretaro and T-39 contained more than 75% of all phytochemicals compared to Negro 8025; meanwhile, line NG0 99176 showed less than 75% of all phytochemicals with exception of total phenolics. Our hypothesis is that cultivars Negro Durango, Negro Queretaro and T-39 will show a similar effect on colon cancer and diabetes in rats as Negro 8025; meanwhile, line NG0 99176 will not shows the same beneficial effect. It will be of great interest to assess these cultivars in colon cancer or diabetes to confirm such hypothesis.

Table 2. Oligosaccharide (raffinose and stachyose) and isoflavone contents (mg/100g, db) of cooked black seeded bean bred lines and cultivars.

Line/cultivar	Raffinose	Stachyose	Daidzin	Genistin
(Ng INIFAP/Ng 8025)-100	1023 ± 22	500 ± 12	44.7	0,9
NG0 99176	907 ± 6	540 ± 29	61.8	0,9
NG0 99038	890 ± 12	225 ± 4	118.8	1,3
(Negro Bola/8025/N203)-201	893 ± 10	338 ± 6	155.8	1,1
(Negro Bola/8025/N203)-250	964 ± 28	535 ± 11	68.0	0,8
Negro Durango	2489 ± 40	277 ± 12	76.1	0,9
Negro Jamapa	1452 ± 35	555 ± 10	62.2	1,0
Negro Querétaro (landrace)	1783 ± 38	315 ± 12	88.0	0,9
Negro Otomí	3399 ± 21	379 ± 8	50.7	0,9
Negro San Luis (landrace)	2563 ± 19	319 ± 62	59.8	0,0
Negro Tacana	1305 ± 21	591 ± 11	54.8	0,8
Negro Zacatecas (landrace)	1025 ± 10	754 ± 45	107.5	1,1
Michigan (1)	1282 ± 12	570 ± 2	76.8	0,9
Michigan (2)	2129 ± 47	518 ± 78	79.1	0,9
T-39	1972 ± 13	427 ± 5	90.9	1,0
Negro 8025	2087 ± 18	238 ± 8	94.4	1,1

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ANALYSIS OF ELEMENTS IN COMMON BEAN (*PHASEOLUS VULGARIS* L.) THAT PROMOTE AND INHIBIT IRON ASSIMILATION

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INTRODUCTION

Common bean seeds contain antinutritional compounds, such as proteinase inhibitors, saponins, polyphenols and phytates that reduce the nutritional potential by diminishing digestibility or nutrients bioavailability. Phytates and some phytate degradation products as well as iron-binding polyphenols are well-known inhibitors for minerals absorption, particularly non-heme iron (2, 11). However, there are other compounds that can enhance iron absorption or reverse the effect of dietary inhibitors (2, 5) such as ascorbic acid (4, 5), EDTA (8) or by degradation or removal of phytic acid (7, 12). The analysis of elements that promote and inhibit iron assimilation in bean has been oriented mainly to seeds; however, understanding the levels of these elements in bean leaves considering the high levels of iron found in this plant structure, would provide valuable information to nutritional breeding programs about, not only the actual iron content, but also regarding other components that would make this microelement bioavailable. The objective of this study was to compare total phytate, tannins and ascorbic acid contents in leaf and seed in four bean cultivars and plant developmental stages, in order to establish those elements and levels that are critical in bean consumption.

MATERIALS AND METHODS

Leaves (L) and seeds (S) of 4 cultivars: Flor de Junio Marcela (FJM), Azufrado Higuera (AH), Negro Jamapa (NJ) and Pinto Villa (PV) were sampled in four different developmental stages; 50% flowering (EI), pod seed filling (EII), full pod filling (EIII) and physiological maturity (EIV). Sampling was done in four replicates; all tissues were lyophilized at -50 °C and $50x10^{-3}$ M BAR and ground in a stainless steel grinder. Condensed tannins were quantified according to the vanillin test (3), and detection was done by spectrophotometry (500 nm). Phytic acid extraction was done with thrichloride acetic acid (ATA 3%), and detection by spectrophotometry (480 nm) (14). Finally, ascorbic acid detection was performed by HPLC at 200-300 nm (13).

RESULTS AND DISCUSSION

Leaf tissue had 9 to 12-fold more iron than seeds regardless of cultivar (Table1). Iron content in leaves showed significant differences among cultivars ($p=0.001$) where PV had the highest iron content (643±31 ppm) followed by FJM, AH and NJ, but there was no difference among developmental stages ($p=0.527$). This suggests that leaves can be consumed for its iron content at any developmental stage due to its constant level across plant development.

Tannins showed similar levels in leaves of all four cultivars (372-436 mg/100g) ($p=0.542$). These values were higher than in seeds (NJ, FJM and AH, 313-344 mg/100g) except PV which had superior values (>500 mg/100g) (Figure 1a). In the analysis by developmental stage leaf and seed had similar tannins content ($p=0.569$).

Phytic acid content indicated differences between leaf and seeds. Seeds of NJ, FJM and AH showed up to 4 mg/g, this indicates a difference of up to twelve times above that of leaf values. On the other hand, differences among leaf developmental stages were observed, where EII-L had higher values (1.92 mg/g) than the other three stages. Leaves of FJM and NJ showed higher content of phytic acid, with a 6:1 and 4:1 relationship, respectively, in comparison to AH. However, seeds of AH showed higher phytic acid content than the other three cultivars. In general, seeds have higher levels of tannins (from 2 to 20 times more than leaf) (Figure 1b).

Table 1. Iron content in different plant organs and developmental stages of four cultivars of common bean (*Phaseolus vulgaris* L.).

CULTIVAR	LEAF	SEED	DEV. STAGE	LEAF	SEED
AH	490[1]±31	52±3	EI	489±31	-
FJM	516±31	53±3	EII	516±31	-
PV	643±31	51±3	EIII	510±31	-
NJ	439±31	52±3	EIV	573±31	52±3

[1]PPM

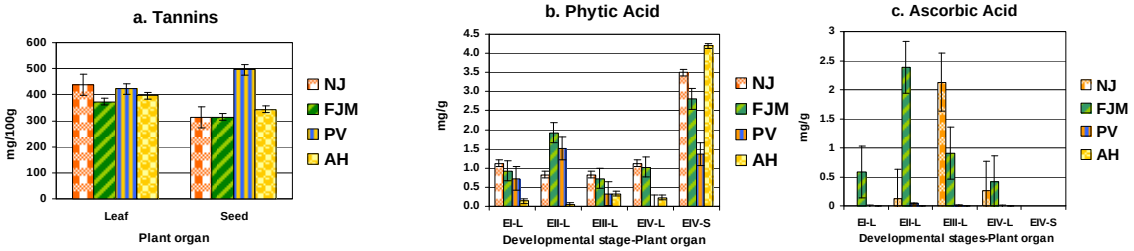

Figure 1. Analysis of elements that inhibit (a. tannins; b. phytic acid) and promote (c. ascorbic acid) iron assimilation in common bean at different developmental stages and cultivars. I-L, 50% flowering-Leaf; II-L, beginning of pod filling-Leaf; III-L, seed filling pod-Leaf; IV-L, physiological maturity-Leaf; IV-S, physiological maturity-Leaf; IV-S, physiological maturity-Seed.

Ascorbic acid was not detected in seeds of the four cultivars; however, leaves of FJM (EII L) (2.38 mg/g) and NJ (EIII L) (2.13 mg/g) showed modest levels. Ascorbic acid reverses the effect of iron assimilation inhibitors and is one of the most powerful and efficient well known promoters of non-heme iron absorption (8, 9, 10). The interaction observed in different investigations between ascorbic acid and phytates has wide nutritional implications. In diets with high phytates content, the desired levels in ascorbic acid should also be higher (2:1, ascorbic acid:phytates, respectively) (6, 10). In iron deficient populations an increased intake of foods with ascorbic acid and iron could have important public health implications, especially in populations subsisting mainly on plant food based diets.

CONCLUSIONS

-Bean leaf tissue had 9-12 times more iron content than seeds; this suggests that leaves can be consumed as an iron source.

-Tannins showed higher values in leaves than seeds. The highest phytic acid content was present in seeds. Both antinutritional compounds reduce the nutritional potential by diminishing digestibility or bioavailability of iron.

-Ascorbic acid reverses the effect of iron inhibitors and is one of the most powerful and efficient well known promoters of iron absorption. However, the levels observed are not enough to reverse the antinutritional effects of the other compounds.

-Leaf could be consumed as iron source with foods containing ascorbic acid.

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PHYTOCHEMICAL CONTENT OF BLACK SEEDED BEAN CULTIVARS AFTER COOKING AND FRYING

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Seed of different bean cultivars have been included in diets and tested in rats with induced diabetes and colon cancer to determine the effects of their consumption on the control of these diseases. Outstanding cultivars were Pinto Zapata and black seeded Negro 8025 (1). Phytochemical characterization is essential to study the effect of bean consumption on colon cancer and diabetes. Such information could guide us on identifying which seed component(s) are involved in the biological effect. In Mexico, all the commercial classes of dry beans that are produced are consumed cooked or refried; therefore, the objective of this study was to determine the effect of such processes on the phytochemical profile of black bean cultivars. Black seeded beans constitute the largest consumed commercial class in Mexico, grown mostly in the central and southeast regions.

MATERIAL AND METHODS

After cooked, broth and seeds were lyophilized, samples were divided for biochemical determinations and for refrying; refried beans were then analyzed. Total phenolics were determined according to literature (2), as well as total tannins (3) and total anthocyanins (4). Tested bean genotypes included bred cultivars and lines as well as landraces Negro San Luis, Negro Zacatecas and Negro Queretaro; these three belong to the Jalisco race and are shiny seeded, as is cv. Negro Otomi. The rest of the genotypes are opaque seeded from the Mesoamerican race.

RESULTS

Phenolic compounds. As expected, the content of these compounds was high in row seed and without exception diminished after cooking and refrying (Table 1). The extent of reduction was different among genotypes, however. Negro Otomi and Negro San Luis (shiny seeded) and line NGO 99176 (opaque) displayed the lowest contents after refrying, whereas Negro Zacatecas (shiny) showed the highest. The variation found among genotypes was large (5) and could be utilized in breeding, however the stability of these compounds after cooking and refrying must be considered.

Table 1. Total phenolics (mg/100 g whole seed) in row, cook and refried grain of black seeded bean genotypes.

Genotype	Raw bean	Cooked bean	Refried bean
(Ng INIFAP/8025)-100	503,2	402,9	306,7
NG0 99176	1020,5	286,4	117,0
NG0 99038	1118,0	711,4	262,4
(NSLB/8025/N203)-201	852,7	676,0	282,0
(NSLB/8025/N203)-250	979,6	210,9	301,1
Negro Michigan (1)	805,6	347,2	213,6
Negro Michigan (2)	803,2	486,8	320,6
T-39	682,8	377,7	380,3
Negro 8025	1089,4	257,9	401,9
Negro Durango	1059,3	457,5	371,9
Negro Jamapa	672,5	351,9	310,8
Negro Querétaro	713,4	390,3	383,7
Negro Otomí	688,0	377,6	126,6
Negro San Luis	601,5	376,6	171,8
Negro Tacana	572,6	342,9	356,9
Negro Zacatecas	1062,3	612,5	519,8

Anthocyanins content: Row beans displayed higher content, while refried beans the lower. Therefore, the processes of cooking and refrying caused a generalized reduction in content, although non-uniform across genotypes. NGO 99038 showed the highest content after being cooked followed by a sample of Negro Michigan purchased in Queretaro and T-39 harvested in Celaya. All the shiny seeded genotypes displayed relatively high contents. After being refried T-39 displayed the highest content followed by Negro Zacatecas (shiny), Negro 8025 (opaque) and Negro Jamapa (opaque). The five-bred lines displayed low content after refrying than the rest of the tested genotypes. It may be worth to conduct these determinations before releasing a cultivar or even just testing for the loss of pigments in soaking water.

Tannin content: Tannins were readily reduced with cooking and refrying, and even after conducting these processes a large variation was observed among genotypes. Similar variation was reported among black seeded bean genotypes (5, 6).

CONCLUSIONS

Cooking and refrying diminished the content of most phytochemicals, and regardless of preparation, there was large variation among bean genotypes; variation that can be utilized in breeding better cultivars.

Table 2. Anthocyanins and tannin contents (mg/100 g whole seed) in raw, cook and refried grain of 16 black seeded bean genotypes.

	Anthocyanins			Tannins		
Genotype	Raw bean	Cooked bean	Refried bean	Raw bean	Cooked bean	Refried bean
NI/8025)-100	76,8	1,7	0,3	1016,7	25,5	38,4
NG0 99176	84,1	2,6	0,1	1067,6	13,3	45,5
NG0 99038	102,7	54,8	0,4	1197,6	68,8	34,9
(NSLB/8025/N203)-201	62,5	8,7	0,8	1471,2	32,1	70,2
(NSLB/8025/N203)-250	105,7	14,9	0,2	1413,2	46,1	31,4
Negro Michigan 1	99,2	12,1	15,5	351,0	6,7	53,7
Negro Michigan 2	58,1	47,5	31,1	444,6	6,7	25,5
T-39	71,8	43,8	39,8	38,6	39,6	38,4
Negro 8025	54,1	29,3	29,8	881,2	38,4	74,9
Negro Durango	84,5	24,0	17,6	1170,0	132,4	31,4
Negro Jamapa	42,7	15,9	28,3	11,1	47,8	40,8
Negro Querétaro	62,7	34,3	22,8	408,4	47,8	31,4
Negro Otomí	42,8	25,8	14,2	770,5	59,5	23,1
Negro San Luis	81,7	33,3	11,2	155,0	44,3	1,96
Negro Tacana	48,7	6,9	9,5	14,0	36,0	1,96
Negro Zacatecas	64,8	42,6	32,9	1233,2	53,7	31,3

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STANDARD NOMENCLATURE FOR COMMON BEAN CHROMOSOMES AND LINKAGE GROUPS

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Several DNA-based linkage maps have been developed for common bean since 1992 (reviewed by Gepts *et al*. 2008). From these initial maps, a core common bean linkage map was developed in the BAT93 x Jalo EEP558 recombinant inbred line (RIL) population (Freyre *et al*. 1998). Subsequent studies have continued to map additional markers in this population, making the core map a reference map for the species (e.g., Blair et al. 2003; Papa et al. 2005).

The first common bean mitotic chromosome nomenclature was proposed by Moscone *et al*. (1999). Chromosomes were characterized with respect to size, morphology, heterochromatin content and distribution of rDNA genes by fluorescent *in situ* hybridization (FISH) and assigned numbers from 1 to 11, based on size from largest to smallest, using the European cultivar 'Wax' as a reference. But correlation to genetic linkage groups came only in 2003, when RFLP markers from the Florida map (Vallejos *et al*. 1992) were used to assign each linkage group to a chromosome pair by FISH (Pedrosa *et al*. 2003). Chromosomes were named following the nomenclature proposed by Moscone *et al*. (1999).

Due to the large variation in number and size of the 45S rDNA loci, however, chromosome size is highly variable within *Phaseolus vulgaris* (Pedrosa-Harand *et al*. 2006). Chromosome number, therefore, does not reflect chromosome size. The largest chromosome of Wax, named chromosome 1 by Moscone *et al*. (1999), for example, is the smallest chromosome in BAT93 and in many other accessions. The commonly used cytological criterion for naming chromosomes, namely size, is therefore not applicable for common bean as a whole.

Because the numbering of linkage groups according to Freyre *et al*. (1998), B1 to B11, has been widely used by the bean community, it was agreed during the Phaseomics III meeting in 2004 that chromosomes should be reassigned numbers based on the linkage group nomenclature. This new chromosome numbering scheme was applied in a recent publication (Pedrosa-Harand *et al*. 2006). We therefore present a modified version of Figure 4 from Pedrosa *et al*. (2003), in which chromosomes are re-named according to Freyre *et al*. (1998). Because chromosomes are always represented with the short arm on top and the long arm on the bottom, linkage groups B1, B2, B3, B4, B6, B9 and B10 were rotated top to bottom. Linkage groups B5, B7, B8 and B11 were kept in their original orientation (Figure 1). A new version of the core map, with the new orientation for some linkage groups, is now available on the BIC website (http://www.css.msu.edu/bic/PDF/Bean%20Core%20map%202007.pdf). The correspondence between the actual and previous chromosome nomenclature, as well as with linkage groups in the core and Florida maps is presented in Table 1. Henceforth, the chromosome/linkage group numbers will consist of arabic numerals only (dropping the B prefix). When the situation warrants it (e.g., comparative linkage mapping), a prefix Pv can be added (e.g., Pv1) to facilitate comparisons of linkage groups across species.

[*] Supported by CNPq, Brazil.

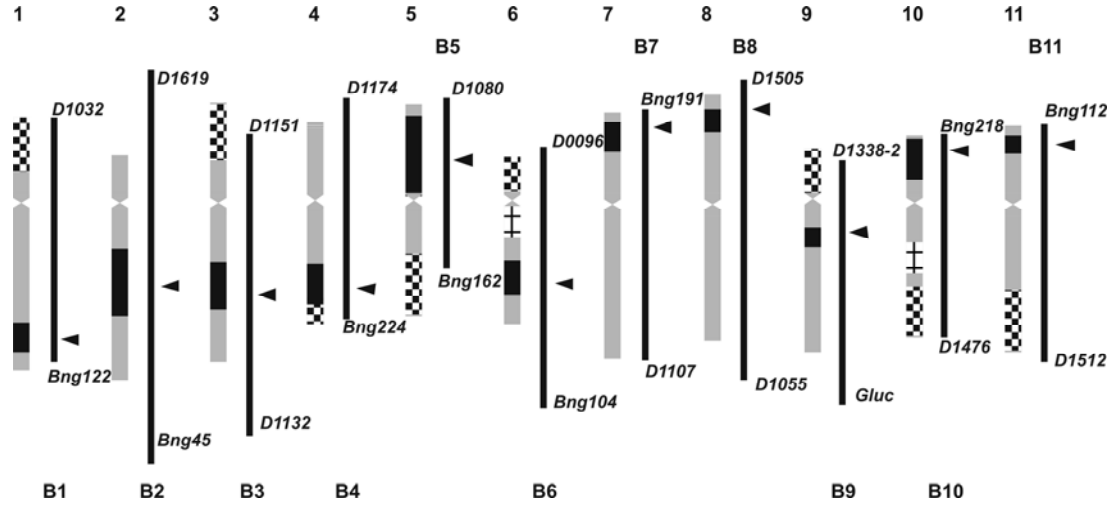

Fig. 1. Idiogram of *Phaseolus vulgaris* Calima chromosomes (modified from Pedrosa *et al*. 2003 and A. Pedrosa, unpubl. results) numbered according to the corresponding linkage groups of the core map (Freyre et al. 1998). Linkage group names are written on the bottom when linkage groups have been rotated to reorient them according to chromosome arm length. Position of RFLP clones used for the correlation of linkage groups (■), 45S rDNA (⛝) and 5S rDNA (⊞) and chromosomes are indicated. Approximate location on the core map of Bng clone pools used in *in situ* hybridization by Pedrosa et al. (2003) is indicated by arrowheads. Labels at the extremities of linkage groups represent distal RFLP markers according to Freyre et al. (1998).

Table 1. Correspondence between the new common bean chromosome nomenclature and previous linkage group and chromosome designations

New chromosome/linkage group nomenclature	1	2	3	4	5	6	7	8	9	10	11
Freyre *et al*. 1998	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11
Pedrosa *et al*. 2003	2	9	5	10	7	1	4	3	11	8	6
Vallejos *et al*. 1992	H	D	C	B	E	G	A	F	K	I	J

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DOES THE *FIN* GENE FOR DETERMINACY CONTROL MERISTEM FATE OR EARLY FLOWERING?

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The determinacy trait in *Phaseolus* beans is a component of the plant growth habit. It refers to the termination of the stem (whether main stem or side branches). In indeterminate plants, the terminal meristem continues producing the modular units that constitute a bean plant. Such modules consist of a subtending internode, a leaf (cotyledon, primary leaf, or trifoliolate leaf), and an inflorescence originating from the axil of the leaf (Tanaka & Fujita 1979; Debouck 1991). Although an inflorescence is produced at the nodes, the main activity of the terminal meristem in indeterminate plants is to develop the vegetative scaffold of the plant. In determinate plants, the terminal meristem, after producing modular units, is converted from a vegetative to a reproductive state. In its reproductive state, it produces a terminal inflorescence, effectively ending the vegetative development of the stem.

In most determinate plant types of the domesticated bean gene pool, the appearance of terminal inflorescences take place rather early, often around the fifth trifoliolate node. In these plant types, determinacy is an important component of the type I bush growth habit as defined by CIAT (Singh 1982). However, later appearances of terminal inflorescences have also been described, e.g., by Debouck (XX) in climbing beans from Argentina (more recently defined as type V growth habit: XX). Such later appearance can also be observed in segregating progenies, e.g., Midas (domesticated, determinate) x G12873 (wild, indeterminate), in which the full range from early to late determinates segregate. Thus, the correlation between determinacy (defined as a morphological trait) and earliness is not absolute. This observation raises the following question: what is the gene action of the *fin* locus, which controls determinacy? Is it a meristem fate locus? Or is it an early flowering locus? A meristem fate locus controls the outcome of meristematic activity, in this case, vegetative (module production) vs. reproductive (terminal inflorescence). An early flowering causes this transition from vegetative to reproductive state to occur earlier rather than later.

How can we distinguish between these two hypotheses? The meristem fate hypothesis posits that beans are originally indeterminate; these include wild beans and most domesticated beans. Determinate beans, regardless of their growth habit or flowering time, result from mutations at the *fin* or other loci, which change the organs produced by the meristem as explained in the previous paragraph. In contrast, the flowering time hypothesis states that all beans are determinate, including wild and domesticated types, whether climbing or bush types. Under normal circumstances, however, the determinacy trait is not expressed because it is pre-empted by the partitioning of photosynthates to pod and seed development instead of further vegetative growth that would eventually lead to the expression of determinacy. An alternative occurrence is abscission of stem ends, which takes place when flowering catches up with leaf deployment (Fig. 1) . Mutations in the *fin* gene, under this hypothesis, would not alter the basic function of the terminal meristem, but would only move up the development of a terminal inflorescence to an earlier vegetative stage when and where it can be observed.

The two hypotheses can be differentiated by a simple experiment. The basic goal is to prevent the transition to a full-fledged reproductive phase in an indeterminate cultivar. This can be achieved through precluding the transition of the flowering stage into the pod development and pod filling stages by systematically removing flowers in an indeterminate genotype. If one continues observing the production of modular units and plants remain indeterminate, then this observation would provide support for the meristem fate hypothesis as the indeterminate phenotype persists even when the life cycle of the plant is artificially extended. This indeterminate genotype does not carry a mutation for determinacy. In contrast, the observation of a terminal inflorescence provides support for the flowering time hypothesis. The determinate nature of all bean genotypes is hidden in the indeterminate genotypes by the lateness of expression of the determinacy gene.

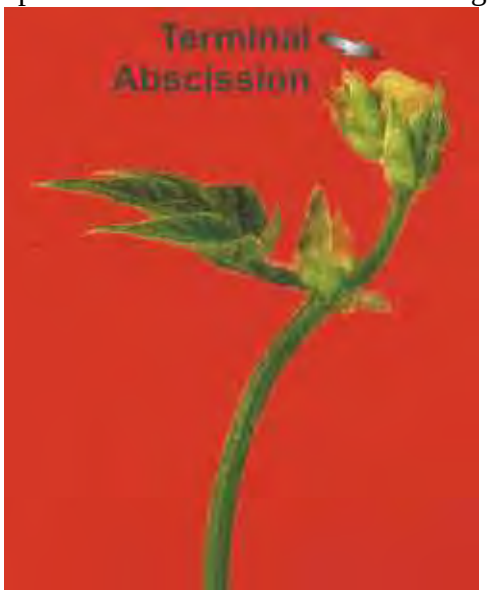

Figure 1. Terminal abscission in indeterminate genotype BAT41 as indicated by yellowing of stem organs.

This experiment was actually conducted with the cultivar Yolano, cultivated in a greenhouse in Davis, CA. Systematic removal of flowers led to an extension of the life cycle of Yolano plants and the eventual appearance of a terminal inflorescence. Thus, we tentatively conclude that *fin* is a flowering time gene rather than a meristem fate gene. Similar experiments with other indeterminate genotypes are needed to confirm this conclusion.

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LINKAGE OF THE *Rf* AND *V* GENES WITH ONE OF THE TWO PRINCIPAL GENES FOR LEAF VARIEGATION IN COMMON BEAN

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Coyne (1966) described a mutable gene system expressing variegated foliage in common bean. His genetic hypothesis included a mutator gene *mu* and an unstable gene *us*. The gene *us* mutates to *Us* in the presence of *mu*, expressing green areas, whereas the yellow leaf areas have the genotype *us us mu mu*. He crossed Nebraska #1 with numerous other bean varieties and PI lines, all with normal foliage. In the F_2 he observed segregation of 15 normal to 1 variegated plant for most of the cross combinations. Thus, both *mu* and *us* express as recessive genes. In summary, each F_2 plant expressing variegation has genotype *us us mu mu*, where each green leaf sector is produced by *mu* mutating *us* to *Us*. I retain Coyne's gene symbol *Mu* for the mutator locus, but change his *us* gene symbol to *Var* for the *variegated* locus. Thus, *Var* is the non-mutable allele and *var* is the mutable allele, where var^m is the symbol for the mutated gene.

MATERIALS AND METHODS

Florida breeding line 5-593 has normal foliage, black seed coats, and bishops violet flowers due to *V*. Over a period of 15 years, I have crossed 5-593 with numerous varieties and PI lines, observing in F_2 progenies a low frequency of plants with variegated foliage (data not shown). Those results agreed with those of Coyne (1966). Lamprecht's line M0169 (now PI 527858) has normal foliage, black seed coats, and salmon red flowers due to *Sal* V^{wf} (Bassett, 2003).

Table 1 experiment. The cross 5-593 x M0169 was made, and selections for plants with variegated foliage and bishops violet (*V V*) flowers were made in F_2. A genetic stock with reclining foliage (*rf*) was used to make the backcross *rf* BC_1 to 5-593 x (*Rf*) variegated F_3 plants (derived from field F_2 variegated selections). Eleven F_1 plants with normal foliage were observed from this cross. The backcross F_2 for the 11 F_1 progeny was planted in the field, and data were recorded on segregation for reclining foliage and leaf variegation. Data analysis and linkage calculation were performed.

Table 2 experiment. The original cross was 5-593 (*V*) x M0169 (V^{wf}), and selection was made for a single plant with normal foliage and bishops violet (*V*/-) flowers in the F_2. A 70-plant F_3 progeny was grown in the greenhouse, and selection was made for 13 plants with normal foliage (non-variegated) and bishops violet (*V*/-) flowers. Those 13 F_3 selections were progeny tested (F_4) in the field, and data were recorded on segregation for flower color and leaf variegation. Data analysis and linkage calculation were performed.

RESULTS AND DISCUSSION

Bassett (1991) reported linkage between the *V* and *Rf* genes of 10.17 ± 0.83 map units. The data in Table 1 permitted the calculation of repulsion phase linkage between the *Rf* gene and either *Mu* or *Var* of 22.79 ± 2.8 map units. The data in Table 2 permitted calculation of coupling phase linkage between the *V* gene and either *Mu* or *Var* of 13.8 ± 2.4 map units. Those three linkage estimates permit orientation of the three genes, *Rf*, *V*, and *Mu* or *Var* in linkage group B6 of the Davis, California core map.

Rf ---------- 10 ---------- *V* ---------- 14 ---------- *Mu* or *Var*

Arbitrarily choosing the *Mu* gene locus for illustration, one can arbitrarily assign the *Mu* gene to 5-593 and *mu* to M0169. In the Table 1 experiment, 5-593 had hypothetical genotype *Mu rf*, whereas M069 had *mu Rf*. Thus, the linkage of nearly 23 map units was in repulsion phase. In the Table 2 experiment, 5-593 had hypothetical genotype *Mu V*, whereas M0169 had *mu* V^{wf}. Thus, the linkage of nearly 14 map units was in coupling phase. The genes *Rf* and *V* are both linked to *Mu*, giving only two possible orientations: 1) *Rf* and *V* are on either side of *Mu* or 2) *Rf* and *V* are on the same side of *Mu*. If orientation 1 is correct, then there would be 37 map units between *Rf* and *V*. The data of Bassett (1991) demonstrated that about 10 map units separated the two genes. With orientation 2, the map distance between *Rf* and *V* is only about 1 map unit different from expected, viz., 23 -14 = 9 versus 10. Hence, orientation 2 is the better fit.

Table 1. Segregation for reclining foliage and variegated leaves in the F_2 from the cross *rf* BC_1 5-593 x F_3 plants selected for variegated leaves in F_2 and F_3 from the cross 5-593 x M0169.[z]

Non-reclining foliage *Rf* -		Reclining foliage *rf rf*			
Normal	Variegated	Normal	Variegated	χ^2 (9:3:3:1)[y]	*P* value
572	269	269	15	81.3	< 0.001

[z]The gene segregating for either normal or variegated foliage expression could be either *Mu* or *Var*. The data from the eleven progenies were combined after determining that the individual F_2 plots significantly deviated from the expected segregation ratio.
[y]The orthogonal contrasts were $\chi^2_{Rf} = 0.036$, $P = 0.85$; $\chi^2_{Mu\ or\ Var} = 0.036$, $P = 0.85$; $\chi^2_L = 81.2$, $P < 0.001$. The repulsion phase linkage between *Rf* and either *Mu* or *Var* was calculated to be 22.79 ± 2.8 map units.

Table 2. Segregation for flower color and variegated leaves in F_4 progenies from 13 F_3 parents with normal (non-variegated) foliage and bishops violet (*V*/-) flowers.[z]

Bishops violet flowers *V* -		White flowers V^{wf} V^{wf}			
Normal	Variegated	Normal	Variegated	χ^2 (9:3:3:1)[y]	*P* value
723	63	63	196	114.2	< 0.001

[z]The gene segregating for either normal or variegated foliage expression could be either *Mu* or *Var*. The data from the thirteen progenies were combined after determining that the individual F_4 plots significantly deviated from the expected segregation ratio.
[y]The orthogonal contrasts were $\chi^2_V = 0.165$, $P = 0.69$; $\chi^2_{Mu\ or\ Var} = 0.165$, $P = 0.69$; $\chi^2_L = 113.8$, $P < 0.001$. The coupling phase linkage between *V* and either *Mu* or *Var* was calculated to be 13.8 ± 2.4 map units.

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MORPHOLOGICAL CHARACTERIZATION OF THE EMBRYO IN COMMON BEAN SEED

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INTRODUCTION

Dry beans are grown in Mexico in many rain-fed areas, where drought often occurs early in the growing season. Early establishment of a seedling is important to obtain high yields in such areas. In many crops it has been suggested that seeds with a larger embryo should produce vigorous seedlings with larger roots and leaves that give to the crop a head start (Dharmalingam and Basu, 1987; López-Castañeda *et al*., 1996; Martinelli and Moreira de Carvalho, 1999) improving their chances to establish and produce a high yield. The objective of the present work was to determine the morphological characteristics of the bean embryo that may increase the bean seedling vigor.

MATERIALS AND METHODS

Five common bean (*Phaseolus vulgaris* L.) cultivars: two high-yielding (Flor de Junio Marcela and Flor de Mayo Bajío), drought-tolerant (Flor de Mayo Corregidora) and drought-susceptible Flor de Mayo RMC selected under field conditions, and a low-yielding landrace (Michoacán-128) were studied. Three groups (200, 260 and 320 ± 5 mg individual seed weight) of ten seeds were selected per cultivar. The seeds were weighed in an electronic precision balance and the cotyledons were excised from the embryo axis complex (radicle-hypocotyl axis plus two foliage leaves). The following embryo axis complex variables were determined: length of the radicle-hypocotyl axis (LRHA, mm), its width (WiRHA, mm), area of the two foliage leaves (ATFL, mm^2) were determined with the software Image Tool for Windows Program (Wilcox *et al*., 2002). Weight of: the radicle-hypocotyl axis (WeRHA, mg), the two foliage leaves (WeTFL, mg) and weight of the whole embryo axis complex (WeWC, mg) were determined with a precision balance. An individual datum was considered one replication. The data for each seed weight class (200, 260, 320 mg) in each one of the five cultivars were analyzed as a complete randomized design. On the other hand, the average of the data for each weight for each variety was calculated and analyzed also as a complete randomized design (SAS, 2000). Least Significant Differences of Tukey (LSD, $p \leq 0.05$) were calculated for mean comparison.

RESULTS AND DISCUSSION Differences in morphological characteristics of the embryo axis complex were significant for all the cultivars and for the different seed weights. Seeds of 320 mg had heavier embryos than those of 260 and 200 mg due to a higher WeTFL and WeWC. Embryos excised from seeds of 320 mg also had greater ATFL and LRHA than those excised from seeds of 260 mg. They also had higher ATFL, LRHA and WiRHA than embryo axis complex excised from seeds that weighed 200 mg each (Table 1). Studies on mungo bean (*Vigna radiata*) (Dharmalingam and Basu, 1987), maize (Martinelli and Moreira de Carvalho, 1999) and small-grain cereals (López-Castañeda *et al*., 1996) showed a close relationship between seed and embryo weight. Large single primary leaves and large root meristems in the embryo are traits of prime importance for the early leaf expansion and root growth of seedlings in temperate cereals (López-Castañeda *et al*., 1996). Cultivars Flor de Mayo RMC and Flor de Mayo Corregidora had higher WeWC than Flor de Junio Marcela, Flor de Mayo Bajio and Michoacan-128; the high WeWC of Flor de Mayo RMC was due to a high WeTFL, WeRHA, ATFL and WiRHA, while the high WeWC of Flor de Mayo Corregidora was due to a greater WiRHA and LRHA than the other genotypes (Table 2).

Comparison of the drought resistant genotype, Flor de Mayo Corregidora *vs.* the susceptible genotype Flor de Mayo RMC shows that although these genotypes differ in the WeWC, they had similar weight and length of the radicle-hypocotyl axis, yet the drought resistant genotype had a thinner radicle-hypocotyl axis than the drought susceptible genotype (Table 2). These morphological embryo axis complex characteristics may help to identify genotypes better suited to rain-fed environments with early drought; however, further studies on the embryo morphological characteristics, seedling root, shoot growth, and crop root system may help identify ways to improve drought resistance and seed yield of dry bean.

Table 1. Weight of the whole embryo axis complex (WeWC), weight of the two foliage leaves (WeTFL), weight of the radicle-hypocotyl axis (WeRHA), area of the two foliage leaves (ATFL), and length (LRHA) and width (WiRHA) of the radicle-hypocotyl axis for three different seed weights. The numbers represent the average for five bean cultivars.

Seed weight (mg)	WeWC (mg)	WeTFL (mg)	WeRHA (mg)	ATFL (mm^2)	LRHA (mm)	WiRHA (mm)
320	45.0	9.0	36.1	8.6	4.9	0.87
260	38.7	7.4	31.2	7.2	4.4	0.84
200	32.3	6.6	25.7	6.4	4.0	0.79
LSD ($p \leq 0.05$)	1.7	0.6	1.7	0.5	0.1	0.04

Table 2. Weight of the whole embryo axis complex (WeWC), weight of the two foliage leaves (WeTFL), weight of the radicle-hypocotyl axis (WeRHA), area of the foliage leaves (ATFL), and length (LRHA) and width (WiRHA) of the radicle-hypocotyl axis for five bean varieties. The table values represent the average of 30 data, ten for each seed weight.

Cultivar	WeWC (mg)	WeTFL (mg)	WeRHA (mg)	ATFL (mm^2)	LRHA (mm)	WiRHA (mm)
Flor de Mayo RMC (Drought susceptible)	45.7	9.5	36.2	8.9	4.6	0.95
Flor de Mayo Corregidora (Drought resistant)	41.3	6.8	34.5	6.6	4.7	0.84
Flor de Junio Marcela (High seed yield)	38.7	7.4	31.3	6.8	4.4	0.87
Flor de Mayo Bajío (High seed yield)	34.9	7.5	27.4	7.2	4.4	0.75
Michocan-128	32.8	7.2	25.6	6.8	4.1	0.75
General average	38.7	7.7	31.0	7.3	4.4	0.83
LSD ($p \leq 0.05$)	2.5	0.9	2.5	0.7	0.2	0.06

CONCLUSIONS

The seed weight determines the weight of the whole embryo axis complex; large seeds have heavy embryo axis complex, which in turn is determined by a greater weight of the radicle-hypocotyl axis and weight of the two foliage leaves.

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INTROGRESSION OF POPPING ABILITY INTO COMMON BEANS ADAPTED TO TEMPERATE REGIONS

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Nuña beans, or "popping beans", are grown in the high Andes of South America at 2 to 3,000 meters above sea level. They have also been found at pre-Inca archaeological sites and continue to be a popular staple in many areas of Ecuador, Peru, and Bolivia (http://naturalscience.com/ns/news/news38.html; validated 10 March, 2008). Nuña germplasm from this region has photoperiod sensitivity and Type IV growth habit; therefore, it is not adapted to production in temperate regions of North America (Zimmerer, 1992). Nuña beans are called popping beans because cotyledonary cells expand due to water vaporization when heated (Spaeth et al., 1989; National Research Council, 1989). Tohme et al. (1995) recognized that nuña beans are unsuitable for commercial production in temperate zones because they are photoperiod sensitive. They suggested that popping ability be combined with bush growth habit, early maturity, and photoperiod insensitivity. Nuña cultivars that possess these characteristics would be necessary for commercial production in temperate zones of the USA.

In a previous study, we used single crosses between nuña bean accessions and 'Sacramento' light red kidney to combine popping ability with photoperiod insensitivity, determinate growth habit, and adaptation to Colorado environments (Ogg et al., 1998). However, we did not recover lines with high popping ability, and selections were dominated by lines that did not pop (Figure 1). Furthermore, only two lines had popping frequencies greater than 40%. Kmiecik and Nienhuis (1997) used a backcross crossing protocol with the nuña parent as the recurrent parent to enhance popping ability among progeny. They identified BC_1 generation progeny that had popping frequencies greater than 70%. Their results suggested that introgression of the popping trait was possible with backcross breeding. Consequently, we selected two of the best lines derived from our single crosses to utilize as backcross parents (female) to nuña accessions (Table 1). The resultant BC_1F_1 plants were increased in the greenhouse and $BC_1F_{1:2}$ progeny rows were planted in the field at Fruita, CO. Ninety BC_1F_2 plants were selected for Type I growth habit and photoperiod insensitivity and planted to progeny rows the following season for additional selection. Thirty four of the most adapted progeny rows were identified and 10 to 15 plants were selected from each row based on early maturity and suitable agronomic traits. To evaluate popping ability in these selections, ten seeds from each plant were placed in a ceramic crucible and heated in a microwave oven at 50% power for 2 min. The number of popped seeds for each plant was expressed as a percentage.

Plants derived from the backcross scheme had improved popping percentage (Table 1; Figure 2). Mean popping percent ranged from 37 to 69% and all three BC populations had plants with 100% popping (Table 1). The portion of plants that had $\geq$ 80% popping ranged from 4 to 40% in the BC populations. These results indicate that backcrossing the single cross progeny to nuña increased mean popping proportion and allowed the development of plants that had 100% popping. We also recovered adapted, photoperiod insensitive, Type I growth habit lines from the inbred backcross populations (data not shown). These results demonstrate that popping ability can be introgressed into dry bean germplasm adapted to temperate zones using an inbred backcross breeding strategy.

Table 1. Pedigrees of backcross progeny and popping results of BC_1F_3 plants.

Pedigree of backcross populations	No. of $BC_1F_{2:3}$ plants selected	% Popping plant^{-1}		Percentage of plants with ≥ 80% popping
		Mean	Range	
Sacramento/ PI 293356//PI 316018	229	37	0 to 100	4
Sacramento/ PI 293356//Piemco	239	38	0 to 100	8
Sacramento/Piemco//PI 316023	20	69	20 to 100	40

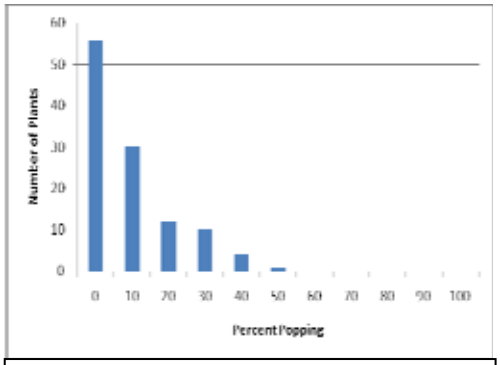

Figure 1. Frequency distribution among plants for popping percentage in the single cross progeny.

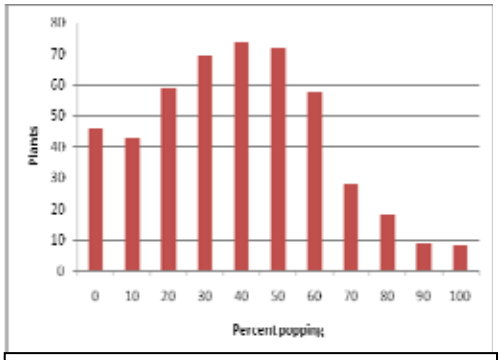

Figure 2. Frequency distribution among selected plants for popping selections in the BC_1F_3 progeny.

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MAPPING OF QTL INVOLVED IN THE GENETIC CONTROL OF SEED TRAITS IN COMMON BEAN

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INTRODUCTION

Several genetic maps based on molecular markers have been reported in common bean (*Phaseolus vulgaris* L.) although a limited number of quantitative trait loci (QTL) have been incorporated in these genetic maps, being necessary a continuous effort in that respect. The QTL mapping has been mainly focused on genetic resistances rather than morphological or agronomic traits. Seed traits such as seed dimensions or seed weight are important traits in common bean. In this work, several QTL involved in the genetic control of four seed traits are incorporated in a genetic map developed from a recombinant inbred line population.

MATERIAL AND METHODS

A mapping population formed by 104 $F_{2:7}$ recombinant inbreed lines (RILs) derived from the cross Xana x Cornell 49242 was developed using the single seed descent method. 'Xana' is a determinate line, obtained in the SERIDA, having very large white seeds (100 g/100 seeds). 'Cornell 49242' is an indeterminate line having very small black seeds (23 g/100 seeds).
The population was grown and evaluated in Villaviciosa, Asturias (Spain) for three consecutive years (2004, 2005, and 2006). Two repetitions per line were analyzed in each evaluation. A total of four morphological quantitative traits were evaluated according to standard descriptors (IBPGR, 1982): seed length (mm), seed width (mm), seed height (mm), and seed weight (g). Each trait was estimated as the average of 10 measurements per year and per plot.

A genetic map was previously developed by Pañeda (2005) in this RIL population using the software JoinMap V3 (van Ooijen and Voorrips, 2001). This map included 177 AFLP markers, 26 SSR markers, 33 ISSR markers, 28 SCAR markers, 13 seed protein loci and 3 morphological loci (*Fin, P* and *asp*) grouped in 11 linkage groups. The linkage groups were identified based on the positions of microsatellites used in a map previously published by Blair *et al.* (2003). Quantitative trait loci were located using QTL Cartographer V2.5 (Wang *et al.*, 2005). Significant QTL were found through composite interval mapping analysis (CIM) with restrictive conditions. The CIM was carried out using 2 cM for walk speed and 300 permutations with a 5% significance level. The criterion in the QTL identification were LOD > 3 and a proportion of variance explained, R^2 > 10 %.

RESULTS AND DISCUSSION

The four traits showed continuous distributions in the RIL population and they were normally distributed (Figure 1). A total of 16 QTL were identified, distributed among 7 linkage groups: B2, B3, B6, B7, B8, B9 and B10 (Table 1). Five QTL explaining73 % of variation were identified for seed length. One of them (SL_1) was associated to the marker SW13, a SCAR linked to gene *I/i*. Four QTL explaining 67 % of variation were identified for seed width and one of them (SWi_1) was associated to the marker ROC11, a SCAR linked to gene *Bc-3/bc-3*. Loci *I/i* and *Bc-3/bc-3* are implicated in the genetic resistance to potyvirus. Two QTL explaining a 26 % of variation were

identified for seed height, one of them (SH_2) being associated to locus *P/p*, involved in the genetic control of seed color. Finally, five QTL explaining 80 % of variation were identified for seed weight. The molecular markers or loci closely linked to the QTLs found in the present work could be used as an indirect selection tool in breeding programs involving seed dimensions.

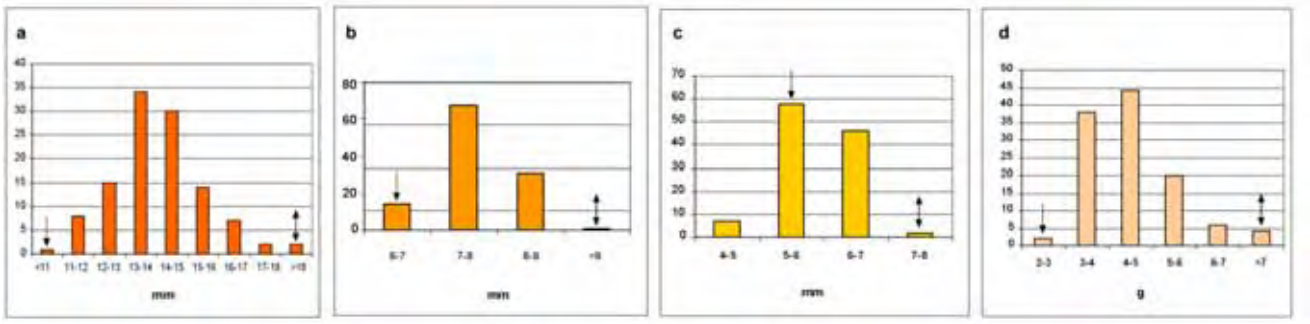

Figure 1. Frecuency distributions for seed traits: **a**: seed length, **b**: seed width, **c**: seed height, and **d**: seed weight of recombinant inbred lines derived from the cross Xana x Cornell 49242. *Arrows* indicate phenotyphic value of parents: ↓ Cornell 49242, ↕ Xana.

Table 1. Quantitative trait loci for four seed traits identified in a RIL population derived from the cross Xana/Cornell 49242. The proportion of variance explained (R^2) by each QTL and the LOD values for the associated marker are indicated. In addition, the phenotype (means) in the two possible genotypes in the RIL population is shown for the four traits. LG= linkage group. SE= standard error. P_1= Xana, P_2= Cornell 49 242.

							Phenotype population			
							Genotype P_1		Genotype P_2	
Trait	QTL	LG	Marker*	Marker type	LOD	R^2 (%)	Mean	SE	Mean	SE
Seed length	SL_1	B2	Sw13	SCAR	3,2	12	14,7	± 0,2	13,7	± 0,2
	SL_2	B3	$MCATETC^{220,95}$	AFLP	5,2	12	14,9	± 0,3	13,7	± 0,2
	SL_3	B6	ROC11	SCAR	4,8	15	14,8	± 0,2	13,5	± 0,2
	SL_4	B8	$MCTGEAT^{191,78}$	AFLP	3,4	20	13,9	± 0,2	14,6	± 0,3
	SL_5	B10	$MCATETC^{72,69}$	AFLP	3,3	14	14,7	± 0,2	13,8	± 0,2
Seed width	SWi_1	B6	ROC11	SCAR	7,8	22	7,9	± 0,1	7,4	± 0,1
	SWi_2	B8	$(ACTG)_4^{850}$	ISSR	4,1	25	7,5	± 0,1	7,8	± 0,1
	SWi_3	B9	$(AC)_8YG^{694}$	ISSR	3,6	10	7,6	± 0,1	7,7	± 0,1
	SWi_4	B10	$MCATETC^{240}$	AFLP	3,8	10	7,8	± 0,1	7,6	± 0,1
Seed height	SH_1	B3	$MCATEAG^{166}$	AFLP	4,5	14	6,2	± 0,1	5,6	± 0,1
	SH_2	B7	*P*	Morphological	3,5	12	6,0	± 0,1	5,7	± 0,1
Seed weight	SW_1	B6	$MCTGEAC^{115,62}$	AFLP	6,4	18	5,2	± 0,2	4,1	± 0,1
	SW_2	B7	$MCTGEAC^{276,38}$	AFLP	5,6	15	4,9	± 0,2	4,2	± 0,1
	SW_3	B7	$(AC)_8YT^{1029}$	ISSR	3,1	10	4,9	± 0,2	4,4	± 0,1
	SW_4	B8	$MCTGEAT^{191,78}$	AFLP	5,0	22	4,3	± 0,1	4,9	± 0,2
	SW_5	B8	$MCTGEAG^{167,36}$	AFLP	3,9	15	4,8	± 0,2	4,4	± 0,2

* Associated marker with the QTL

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VEGETABLE BEAN RECOMBINANT INBRED LINES (*PHASEOLUS VULGARIS* L.) REACTION TO BEAN WEEVIL (*ACANTHOSCELIDES OBTECTUS* SAY)

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INTRODUCTION

Bean weevil (*Acanthoscelides obtectus* Say) attacks bean seeds (*Phaseolus vulgaris* L.) and cause huge economic storage losses in Bulgaria. Pest control is difficult and includes applying of both chemical products and non-chemical measures. Breeding for genetic-based resistance is one of the environmental friendly and ecology saving methods, which was one of our bean breeding program priority trends. Recombinant inbred lines (RILs) with round or flat stringless pods, and pest infestation index below 10 % have bean released as a result of long term breeding program at *Marits*a Vegetable Crop Research Institute (Maritsa VCRI) in Plovdiv. (3, 4). Looking for the interaction and relationship between the pest and host plant we have bean studied various bean genotypes reaction towards *A. obtectus* on one hand, and host influence on pest biological indicators on the other hand (2, 4). Present study goal is to determine the resistance of vegetable bean RILs from Maritsa VCRI germplasm collection to bean weevil under non controlled field conditions and natural infestation. Further more these RILs will be valued and classified to enrich the local market with new high-grade vegetable bean cultivars.

MATERIALS AND METHODS

Three RILs and two control-varieties vegetable bean were taken in non controlled field experiment under natural been weevil infestation for the vegetation period of two years. Representative samples of 300 pods (100 pods from three replications) in physiological maturity were taken for each of the bean genotypes. After 50 days storage without chemical treatment, seeds were collected from the pods and valued as followed: 0 for non penetrated seeds; 1; 2; 3 and 4- for penetrated seeds with one, two, three, and four exit hole respectively. Percentage of seed infestation and infestation index (%) was estimated using collected values and Mc Kinney's formula.

RESULTS AND DISCUSSIONS

There is genotype differentiation among *Phaseolus vulgaris* L. susceptibility to *Acanthoscelides obtectus* Say (1, 5). Genotype's stability finds expression in reducing the number of penetrated seeds and emerged adults, manifested in current study through percentage of infested seeds and infestation index (%). It is based on adult females' less preference for laying their legs on the resistant genotypes' pods on one hand, and larvae's unwillingness to bore through the seedcoat on the other hand.

That genotype differentiation was proven to be true by our two years study and the results we have obtained (fig. 1a, b). It was revealed that the higher percentage of infested seeds (17.71 %) and average infestation index (11.62 %) showed control variety Starozagorki black, followed by second control variety Oreol (11.65 % and 7.16 % respectively). Quite different reaction was observed in RILs № 612, 614 and 620: RIL № 614 showed the lowest percentage of infested seeds (1.92 %) and average infestation index (1.21 %), followed by RIL № 620 (2.12 % and 1.27 % respectively) and RIL № 612 (2.89 % and 2.27 % respectively). These results suggest that the plants from the tested RILs are less favorable host for been weevil.

RIL № 614 possesses green flat pods and white, elliptic seeds and it is designed for both fresh market and processing. Its increased agronomic and gustatory trends, multiple disease resistance and bean weevil tolerance make it advisable to future utilization in Bulgarian vegetable cropping systems.

Cultivars *Tangra* and *Pagane*, recently released from RIL 620 and 612 respectively, in *Marits*a Vegetable Crop Research Institute in Plovdiv were developed to meet some requirements such as high yield, excellent taste and flavor, firm string less pod flesh, straight flat pods, disease resistance, early pod market maturity. These quality packed cultivars are showed from the present study to be enriched with extra tolerance to bean weevil.

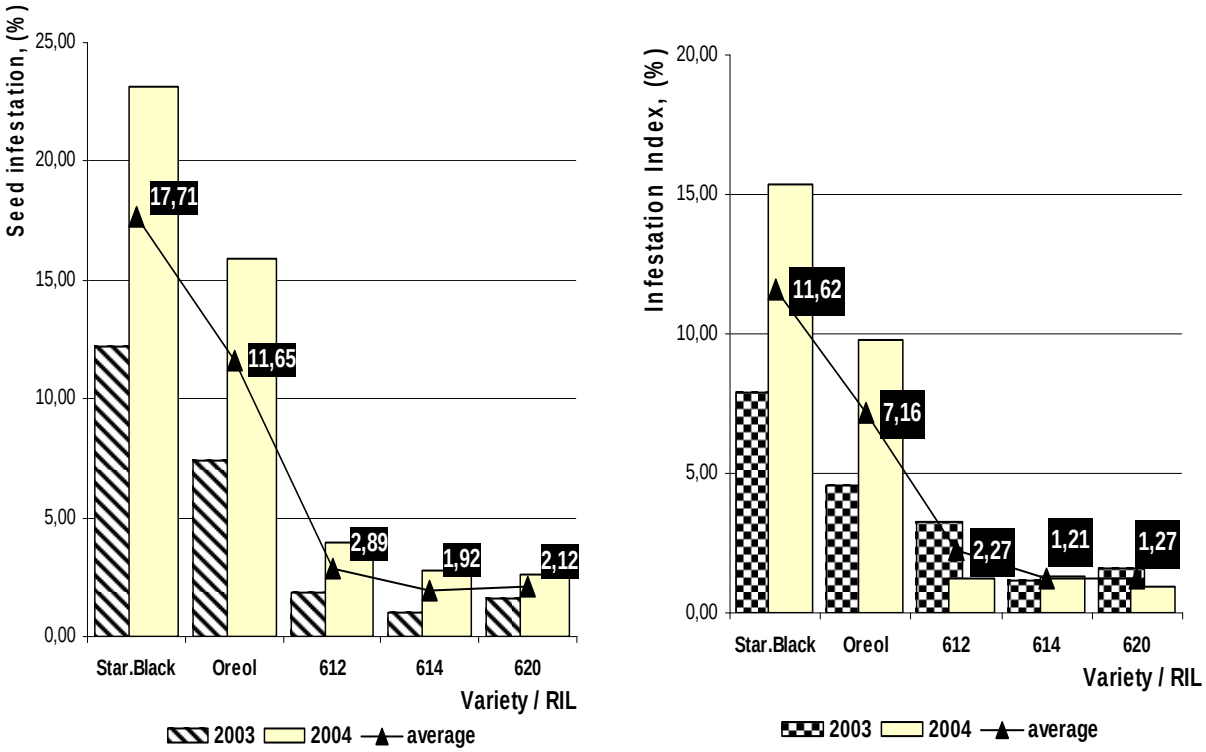

Figure 1 /a, b/. Bean weevil (*Acanthoscelides obtectus* Say) infestation on vegetable bean (*Phaseolus vulgaris* L.) lines.

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NEW POPULATIONS OF WILD COMMON BEAN DISCLOSED IN NICARAGUA

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The distribution of wild *Phaseolus vulgaris* L. in southern Central America is not yet fully documented. The species is present in Chiapas, Mexico (Acosta Gallegos et al. 1998), Guatemala (Azurdia et al. 1999; Toro et al. 1990), Honduras (Beebe et al. 1997), El Salvador (Freytag & Debouck 2002), Nicaragua (Delgado Salinas 2001; Freytag & Debouck 2002), Costa Rica (Araya Villalobos et al. 2001), but unknown from Panama (Freytag & Debouck 2002). Because of recent field work the survey is perhaps complete only for Costa Rica (González Torres et al. 2004). For Nicaragua, only three populations – as herbarium specimens - were known (Delgado Salinas 2001; Freytag & Debouck 2002) before this work. With view of confirming/ expanding the above information, and assessing the conservation status of existing populations *in situ*, we carried out an exploration in NW Nicaragua in December 2007 - January 2008, along a methodology described elsewhere (Debouck 1988).

RESULTS AND DISCUSSION

We have found 24 populations for four wild species (*P. leptostachyus, P. lunatus, P. oligospermus*, and *P. vulgaris*), with a total of 116 herbarium vouchers, collected in five departments of Nicaragua (Table 1). All seem new records about the presence of these species, namely that of *P. vulgaris*, in Nicaragua. Typically this species thrives in the tall understory of modified oak woodlands on fertile deep soils under good rains. The life zone is that of Lower montane humid forest (bh-MBS), according to Holdridge & Tosi (1971), which occupies a very small acreage in Nicaragua, on the slopes of the mountains protected from humid winds coming from the Caribbean sea of Nicaragua (Incer Barquero 2000). The rural inhabitants know it as 'fríjol de venado' o 'fríjol venado' [bean of the deer], and it seems that it has been consumed at some time. Populations #3202 and #3205 will disappear *in situ* unless small protected areas are established with a management plan with the participation of the rural communities. Populations #3216 and #3218 in contrast can easily be maintained *in situ* if there is an effective conservation plan of the Biological Reserve Quiabuc and its buffer zones close to the town of Estelí. This type of survey should be continued up to the full inventory of wild bean populations for Nicaragua.

ACKNOWLEDGEMENTS

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Table 1 – List of all materials found during this exploration.

Collection No	Species	Department	County	Date	Alt masl	Lat N	Long W
3196	*lunatus*	Managua	Sn Fco Libre	11-12	410	12.39.30	86.05
3197	*leptosta*	Estelí	Estelí	11-12	930	13.04	86.20
3198	*leptosta*	Nueva Segovia	Dipilto	12-12	940	13.47	86.35
3199	*lunatus*	Nueva Segovia	Dipilto	12-12	1030	13.47.30	86.35.30
3200	*lunatus*	Madríz	San Lucas	12-12	890	13.23	86.37.30
3201	*leptosta*	Madríz	San Lucas	12-12	980	13.23.30	86.38
3202	*vulgaris*	Madríz	Las Sabanas	12-12	1080	13.23	86.38
3203	*oligosper*	Madríz	Las Sabanas	12-12	1250	13.22	86.38.15
3204	*lunatus*	Madríz	Las Sabanas	12-12	1390	13.21.30	86.38.30
3205	*vulgaris*	Madríz	Las Sabanas	12-12	1370	13.21.15	86.38.30
3206	*oligosper*	Madríz	Las Sabanas	12-12	1400	13.20.45	86.38.15
3207	*lunatus*	Madríz	Palacagüina	13-12	490	13.23	86.25
3208	*oligosper*	Estelí	Condega	13-12	880	13.22.30	86.16
3209	*lunatus*	Estelí	Condega	13-12	880	13.22.30	86.16
3210	*lunatus*	Jinotega	Sn Sebastián Yali	13-12	1010	13.17.30	86.11.30
3211	*lunatus*	Jinotega	Sn Sebastián Yali	13-12	940	13.15.30	86.09
3212	*lunatus*	Jinotega	Sn Rafael Norte	13-12	1000	13.12	86.08
3213	*lunatus*	Estelí	Estelí	14-12	950	13.07	86.23.30
3214	*leptosta*	Estelí	Estelí	14-12	1250	13.05.45	86.26
3215	*oligosper*	Estelí	Estelí	14-12	1250	13.05.45	86.26
3216	*vulgaris*	Estelí	Estelí	14-12	1250	13.05.45	86.26
3217	*lunatus*	Estelí	Estelí	14-12	1510	13.06	86.26
3218	*vulgaris*	Estelí	Estelí	14-12	1350	13.06.30	86.26.30
3219	*leptosta*	Estelí	Estelí	14-12	1230	13.07	86.27

PHASEOLIN DIVERSITY OF NICARAGUAN COMMON BEAN GERMPLASM HELD AT CIAT

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INTRODUCTION

Tapia (1987) reported the presence of three species of the genus *Phaseolus*, namely *P. vulgaris*, *P. acutifolius* and *P. lunatus* in Nicaragua. Of these three species, *P. vulgaris* is the most important with a large number of landraces, which are distributed in the most important agroecological zones of the country. Numerous collection missions have been carried at different points in time (1952, 1960, 1980´s, 1990´s) and most of the landraces collected are stored in genebanks of different international and national institutions (Gómez et al. 2004). The purpose of this work was to disclose the phaseolin variability of the Nicaraguan collection held at CIAT as a contribution to their conservation and use. Additionally this information can help to plan new collections of Nicaraguan germplasm.

MATERIALS AND METHODS

Three hundred thirty five accessions of common bean (*Phaseolus vulgaris* L.) are available from the Nicaraguan collection held in CIAT, which include 329 landraces, 5 commercial varieties and one bred-line. To facilitate the characterization and later use of Nicaraguan germplasm, a sampling on the Nicaraguan collection was done. In total, 148 accessions of common bean were selected using three criteria to represent the total diversity of the species in this country. These criteria are: (1) All the cultivars selected are originally from Nicaragua and are representative of the geographic distribution of the species in this country, mainly in the most important regions of bean production. (2) the most frequent landraces. (3) The agromorphological, agroecological and passport data were utilized as indicators of genetic diversity to select these 148 accessions. The seed storage proteins were analyzed using the 1D-SDS-PAGE technique (Brown et al. 1981) and confirmed later in 2D-IEF-SDS-PAGE (O'Farrel, 1975).

Table 1. Geographic distribution of the types of phaseolin found in 148 accessions of *Phaseolus vulgaris* L. collected in Nicaragua.

Department/Types of Phaseolin	**CH**	**S**	**T**	**C**	**Total**
Boaco	4	6			10
Carazo	6	8			14
Chinandega	12	3			15
Chontales	8	2			10
Esteli	8	2			10
Granada	16	6			22
Jinotega	18	2		1	21
Madriz	8				8
Masaya	2	2			4
Matagalpa	6	4			10
Leon		2			2
Managua		6	2		8
Nueva Segovia		4			4
Rivas	6	4			10
Total	**94**	**51**	**2**	**1**	**148**

RESULTS AND DISCUSSION

Four electrophoretic types of phaseolin were found among the 148 Nicaraguan common bean accessions analyzed. The 'CH' phaseolin was present at the highest frequency (64%), followed by the 'S' type with 35%, and the 'T' and 'C' types with 1% (Table 1).

The diversity in phaseolin types possibly indicates that the bean cultivated in Nicaragua has not experienced an important genetic interchange between the Mesoamerican and Andean gene pools, as it has happened in other American regions (Paredes and Gepts, 1995; González et al. 2003; and Toro and Ocampo, 2004). The 'CH' phaseolin was present in 94 accessions, reporting an extensive distribution in the most important regions for Nicaraguan bean production, especially the regions near to the Nicaraguan lakes (Managua and Nicaragua) and the North region that borders with Honduras (Figure 1). The 'S' and 'CH' types are typical of the small and medium seeded Mesoamerican beans. These are of particular interest to breeders and agronomists since they may represent more than 65% of world bean production (CIAT data). Therefore, understanding genetic diversity within the Nicaraguan germplasm collection has important implications for genetic improvement of the common bean in the Mesoamerican lowlands.

Figure 1. Distribution geographic of the phaseolins present in 148 accessions of the Nicaraguan common bean collection held at CIAT.

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DEVELOPMENT AND TESTING OF MID-ELEVATION, COMMERCIAL-TYPE, ANDEAN CLIMBING BEANS

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INTRODUCTION

Climbing beans have been an important component of traditional societies for centuries most often grown in intercropping but also as monocultures. In pure stands and in the tropics, climbing beans have among the highest yield potential of any beans especially as compared to more commonly grown bush beans. Climbing beans have expanded in certain areas of Africa but contracted in certain areas of Latin America, while in both continents production has switched from intercropping to monoculture. Climate change and changes in agriculture pose risks to the production of climbing beans but also provides challenges. A principal challenge has been the production of new well-adapted, high-yielding varieties for higher temperature environment and for monocrop systems of staking or trellising. Most currently-available climbing beans come from high-altitude areas of Central and South America and do not grow well in lower elevations or hotter climates. Currently, there are also very few climbing bean varieties with the red mottled or red kidney seed types that are preferred in many areas of the Eastern Africa and South America. Therefore, an additional challenge for breeders is to develop climbing bean varieties that produce grain with the proper color and size. Our research has addressed this by developing and testing commercial type climbing bean varieties that are adapted to monocropping and to lower elevation (800 to 1800m) production systems in Latin America and Africa.

MATERIALS AND METHODS

We developed a set of 62 mid-altitude climbing beans from various climbing bean x bush bean crosses and by selection for heat tolerance over four generations of pedigree selection (F2 to F6). The selections were coded as MAC (mid-altitude climbing) bean lines in the F7 generation. Of the selections, 27 were red mottled seeded, 13 were large-red and 32 were cream mottled, all with average seed sizes around 50 g / 100 seed. Yield data was collected at trials across two sites in Colombia for 55 MAC lines (Darien at 1450 masl and Palmira at 1000 masl) and across two sites in Uganda for a set of 11 MAC lines (Namulonge at 1150 masl and Kachwekano at 1830 masl). In addition, several MAC lines have been entered into national yield trials in Kenya (MAC13, MAC34 and MAC64). Testing of these genotypes in Colombia and Uganda has been conducted during the rainy seasons with randomized complete block design experiments. G685, G2333, G2337, ICA Viboral and Calima Darien were used as checks in the experiments in Colombia while in Uganda three previously released climbing beans, NABE 8C, NABE 9C and NABE 10C, were used as checks. Experiments in Palmira were protected from insect damage (primarily Empoasca, Epinotia, Thrips and Mites), while the experiments in Darien had preventative fungicide treatment at planting and again at flowering. The climbing beans were supported on bamboo and wire trellises in Colombia and by staking material in Uganda. Data collected in Colombia included yield per plant (Y/P), pods per plant (P/P), grain per plant (G/P), days to flowering (DF), days to maturity (DM) and harvest index (HI) based on stem and pod weight. Agronomic adaptation (AA) and climbing ability (CA) were evaluated on 1 to 9 scale (where 1=good and 9 = poor). Plant height (PH), raceme length (RL), number of pods per raceme (NP), pod length (PL), number of vines per guide (NV) and internode length at a height of one meter above the ground (IL) were evaluated for two plants per row and averaged to produce plot values. Data collected in Uganda included yield in kg/ha, days to flowering and days to maturity.

RESULTS AND DISCUSSION

The analysis of variance for the experiments conducted in Colombia showed that genotype and location effects were significant for all the traits measured while GxE effects were observed for all traits

except for pods per plant (Table 1). This showed that climbing bean agronomic traits are sensitive to environmental conditions at the different altitudes represented by the sites in Colombia, something which has been frequently observed but rarely quantified. Among the control genotypes, high-elevation Andean climbers (ICA Viboral, Calima Darien) yielded almost nothing in Palmira and even suffered from poor adaptation at a mid-elevation site like Darien while Mesoamerican climbing bean check varieties, G685, G2333, G2337, had problems of adaptation in the hot seasons that occurred in Palmira. Meanwhile many of the advanced MAC lines outperformed these checks in Palmira, indicating a higher level of heat tolerance in these genotypes compared to G2333 and G685 which are standard varieties for climbing bean areas of Eastern Africa and to ICA Viboral a standard variety grown in Colombia. In Uganda, an AMMI biplot analysis of yields for the MAC lines shows the stability of several genotypes compared to previous climbing bean releases and their adaptation to the lowland site of Namulonge versus the highland site of Kachwekano (Figure 1). The majority of the climbing beans in both environments matured in 100 to 140 days from planting depending on the altitude of the test site with earliest maturation times in the lower elevation sites compared to the higher elevation sites.

In conclusion, these trials have given us a new appreciation of the multiple characteristics that make up a good climbing bean variety and the sensitivity of climbing beans to genotype x environment interaction. These elements are being factored into further breeding of mid-altitude climbing beans. In addition, disease resistance is being incorporated into MAC lines to counteract the greater prevalence and effect of virus infection at mid altitude sites. Several MAC lines have been or are planned to be release as varieties in Colombia, Kenya and Uganda. Rapid adoption of MAC13 through participatory varietal selection has been observed in Colombia and Kenya while yield increases and increased market opportunities for farmers growing MAC31 (released as NABE12C) has been observed in Uganda.

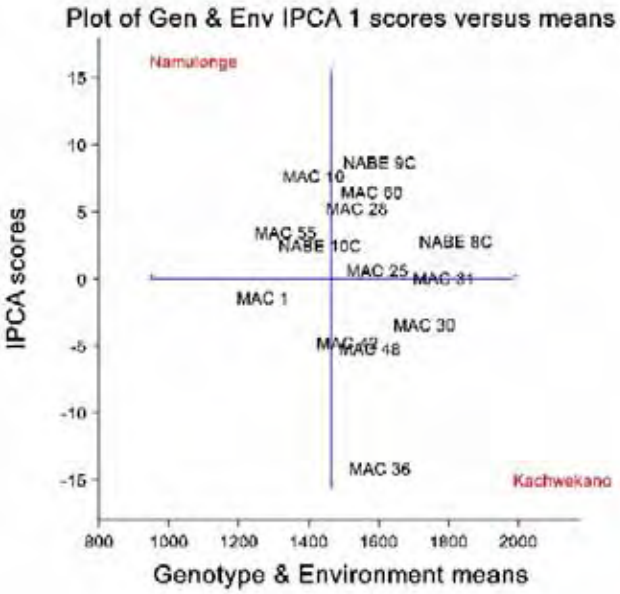

Figure 1. AMMI biplot of 11 MAC climbing bean lines evaluated for plot yield in two sites in Uganda.

Table 1. Significance (F statistic) of location, genotype and genotype x location effects in the trials for 55 MAC climbing bean lines in two sites in Colombia.

Source:	1.1.	**P/P**	**Y/P**	**AA**	**CA**	**PH**	**DM**	**IL**
Rep(loc)	2	0.7 ns	1.13 ns	0.25 ns	0.93 ns	0.64 ns	0.09 ns	0.24 ns
Loc	1	192.85 ***	469.14 ***	745.87 ***	86.98 ***	264.35 ***	5.41 *	36.49 ***
Trt	54	1.58 *	2.42 ***	8.79 ***	1.78 **	2.33 ***	3.17 ***	2.65 ***
Loc x Trt	54	1.33 ns	2.04 ***	5.9 ***	1.18 ns	1.5 *	1.62 *	1.15 ns

Significance at P=<.001 (***), .01 (**), .05 (*) or not significant (ns), indicated.

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YIELD AND ANALYSIS OF GROWTH IN CLIMBING SNAP BEAN (*PHASEOLUS VULGARIS* L.) BASED ON THE SOWING DATE

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INTRODUCTION

The agricultural practices are determining in the production of snap bean (*Phaseolus vulgaris* L.). Thus, with the sowing date that involves changes in solar radiation, temperature, etc., the crop is provided with the appropriate conditions, so it can express its maximum growth and yield. Hernandez (1995) found in snap bean that the yield and its components decrease with the delay in the sowing date in the photoperiod-sensitive varieties. On the other hand, Esquivel (1999) found in Pinto Villa and Tlaxcala, bean for grain varieties, sowed on 12 of June, the highest rates of growth in the area of Chapingo, México (temperate climate). The aim of the present study was to determine the effect of the sowing date on the yield and growth analysis of climbing snap bean Hav-14.

MATERIALS AND METHODS

The study was done under rainy field conditions in Montecillo, México (19° 29 ' N, 98° 53 ' O, to 2250 m of altitude), and in the less dry of the arid climates, with rains in summer, 14,6 °C as the annual average temperature and 558,5 mm of precipitation (BS1, García, 2005). The soil is clay (mólico Fluvisol, Flm), with 2 to 3% of organic matter and pH of 8 in the first 30 cm of depth. The sowing dates, of snap bean Hav-14, of indeterminate climbing habit of growth, were done since May 2nd until July 1st of 2005 with a 15 days frequency , and a 5.2 plants by m^{-2} density, with trellises . The experimental design was randomized complete blocks. Three harvest were done at the 35, 80 and 120 DAS (days after of sowing), per sowing date, to determine: leaf area, number of buttons and flowers, snap bean yield (g m^{-2}), the mean relative growth rate ($\overline{RGR}$) and the mean net assimilation rate ($\overline{NAR}$) (Escalante and Kohashi, 1993).

RESULTS AND DISCUSSION

The results suggest that the leaf area and the yield components showed significant changes due to sowing date, therefore with the July 1st, seeding time, snap bean has the smallest number of buttons (2) and flowers (5) per plant at 120 DDS.

Table 1. Leaf area and yield components based on sowing date in snap bean Hav-14. Summer 2005.

Treatment		Leaf area (dm^2) $plant^{-1}$			Buttons number $plant^{-1}$			Flowers number $plant^{-1}$		
		30 DAS	80 DAS	120 DAS	30 DAS	80 DAS	120 DAS	30 DAS	80 DAS	120 DAS
Sowing date	1	2 d	5 e	55 b	0.35 a	4.3 a	**7.7 a**	0 c	1.35 b	**27.5 a**
	2	3 c	10 d	**52 c**	0.35 a	2.6 b	1.8 c	0 c	0.35 b	11.3 b
	3	4 b	36 a	**76 a**	1 a	5 a	5.4 b	0.5 b	5.2 a	39.3 a
	4	3 c	26 c	49 e	0.6 a	4.7 a	5.2 b	0 c	4.9 a	9.2 b
	5	5 a	30 b	50 d	0.87 a	4.1 a	**1.6 c**	0.12 a	3.6 a	**4.6 b**

Averages with the same letter within columns are statistically equal (Tukey 0,05)

No. = Number, 1 = 2nd of May, 2 = 17 of May, 3 = 1st of June, 4 = 16 of June, 5 = 1st of July, DAS = Days after seedtime.

The table 2 indicates that the yield decreases when the sowing is delayed, so in the second of May seedtime, the highest yield is 1,18 kg m^{-2} and in the first of July seedtime the lowest is 0,55 kg m^{-2}. The yield has a close relation with the $\overline{RGR}$ and $\overline{NAR}$. These results indicate that the sowing date determines the growth rate and the yield of pole snap bean. In the area of Montecillo, Mexico, sowing date with the highest yield is the 2nd of May.

Table 2. Yield and growth rates of snap bean Hav-14 based on the date of sowing. Montecillo, Mex. Summer 2005.

DS	Yield (kg m^{-2})	$\overline{RGR}$ (g g^{-1} day^{-1})			$\overline{NAR}$ (g dm^2 day^{-1})	
		P	S	T	S	T
2 May	1.18 **a**	0.07 a	0.01 c	0.04 **a**	0.07 a	0.12 **a**
17 May	0.94 a	0.06 a	0.01 c	0.03 b	0.03 c	0.05 c
1 June	0.83 ab	0.08 a	0.02 b	0.03 b	0.05 b	0.08 b
16 June	0.82 ab	0.05 a	0.04 a	0.03 b	0.05 b	0.06 c
1 July	0.55 **b**	0.06 a	0.04 a	0.02 **c**	0.05 b	0.03 **d**

Averages with the same letter within columns are statistically equal (Tukey 0,05)

DS = date of sowing, $\overline{RGR}$ = average rate of relative growth, $\overline{NAR}$ = mean net assimilation rate,

DAS = days after of sowing. P=1- 30 DAS; S= 30-80 DAS; T = 80-120 DAS.

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RECONSTRUCTING PLANT ARCHITECTURE OF VEGETABLE BEAN (*PHASEOLUS VULGARIS* L.) FOR EFFICIENT AND COMPETITIVE PRODUCTION

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Strategies employed by bean breeders to improve yield include ideotype breeding, based on an ideal plant architecture that is expected to: (i) maximize yield trough enhanced morphological adaptation to specific cropping systems or environments; (ii) improve disease avoidance and (iii) adaptation to mechanical harvesting of bush beans in monoculture (1, 3). The wide range of variability for plant type in cultivated beans has been classified into four growth habits. Type I is the only determinate habit, whereas Types II, III and IV are indeterminate, differing in vine growth extension and climbing ability (2). Our bean breeding program earliest attempts of genetic modification of growth habit were successful in converting pole or climbing snap bean to determinate bush types. Study the architectural differences among 29 determinate bush types of garden bean suitable for mechanical harvest is the aim of the current investigation.

MATERIALS AND METHODS

There were used 29 genotypes from Maritsa Vegetable Crops Research Institute bean germplasm collection. Conventional equipment was used for planting bean accessions on the experimental plots of 3 m^2 of four replications. Samples of 12 plants in technical ripeness were evaluated for following architectural characters: plant height (PH), stem height (SH), number of branches per plant (NBP) and pods arrangement toward the stem (PA) both in vertical and horizontal line.

RESULTS AND DISCUSSION

A major focus of our bean breeding programs has been to (i) increase the space between the fresh pods to avoid disease infections and the ground and (ii) decrease pods concentration around the main plant stem in order to reduce losses from mechanical harvesting. Pods arrangement in vertical and horizontal direction depends on genotype. To demonstrate that we used architectural characters PH, SH, NBP and PA, and MS Excel program to build figures that correspond to three average plant habit models.

Habit model I (fig. 1). Bean plant is an upright 60 cm height bush. Vertical pods arrangement is between 10-15 and 50-55 cm in height with maximum concentration at about 20-35 cm. Horizontal pods arrangement is up to 20 cm sideward from the main stem, efficiently distributed. That habit model enables mechanical harvesting, monoculture growth of garden bean and may prove to be a valuable strategy in future diseases avoidance. It was possessed from 11 of all tested genotypes.

Habit model II (fig. 2). Bean plant is an upright 50 cm height bush. Vertical pods arrangement is between 5-10 and 40-45 cm in height with maximum concentration at about 15-25 cm. Pods distribution under 15 cm and over 25 cm is very unequal and asymmetrical. Horizontal pods arrangement is up to 10 cm sideward from the main stem with very high concentration. That plant habit model on one hand allows increasing crop density aiming to improve yield of fresh garden bean, but enables diseases epidemics and caused by them yield losses on the other hand. Highly concentrated pods amplify the risk of mechanical damage of the fresh pods. Ten of all tested genotypes possess that plant habit.

Habit model III (fig. 3). The plant is an upright 55-60 cm height bush. Vertical pods arrangement is between 5-10 and 50-55 cm in height with maximum concentration at about 25-35 cm. Pods

distribution along the whole length of the stem is very equal and symmetrical. Horizontal pods arrangement is up to 15 cm sideward from the main stem, non concentrated. Eight of all tested genotypes possess that plant habit, with pods that don't reach the ground. These are highly recommended for breeding garden bean varieties for mechanical harvesting in monoculture cropping.

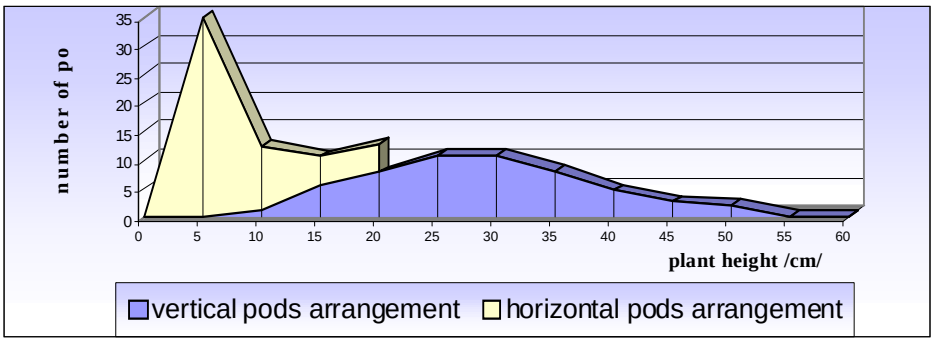

Figure 1. Plant habit model I

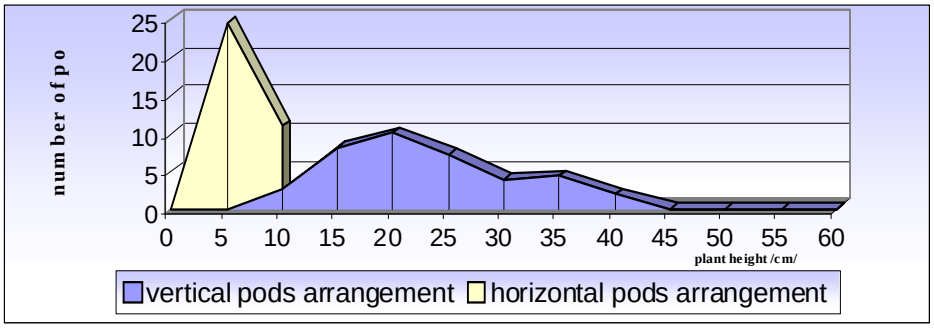

Figure 2. Plant habit model II

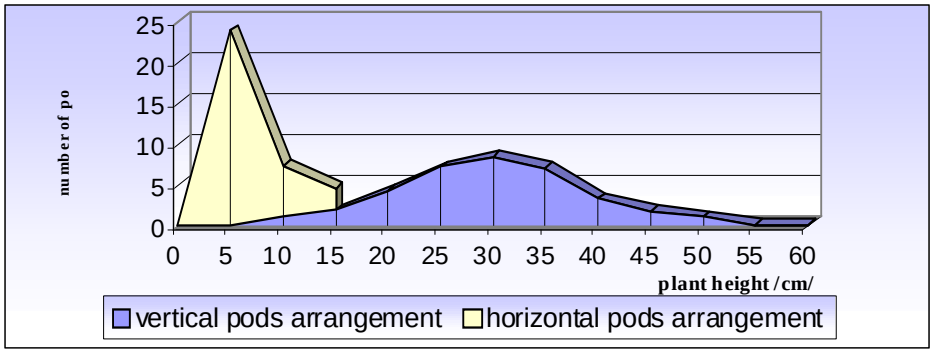

Figure 3. Plant habit model III

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EVALUATION OF CONDENSED TANNINS IN TEPARY BEAN GENOTYPES

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INTRODUCTION

Limited studies have shown tepary beans (*Phaseolus acutifolius L.*) to have higher levels of zinc and iron (Bhardwaj & Hamama 2004) and lower levels of tannins (Benitez et al. 1994) than common beans (*Phaseolus vulgaris L.*). Since tannins are antinutrients that prevent absorption of iron and iron concentration is a key nutritional trait, this would make tepary bean a good candidate for use in biofortification programs. However little is known about variability for tannin content in tepary bean. Therefore, the objective of this research was to evaluate condensed tannin content in seeds of 26 tepary bean genotypes from the CIAT collection through a butanol-HCl method, developed for common bean and determine diversity for this critical nutritional trait.

MATERIALS AND METHODS

Plant Material: We analyzed 18 cultivated and 7 wild tepary bean genotypes in this study plus a common bean control genotype, ICA Pijao. Samples of 10 seeds from each genotype were used for the analysis. For each of the cultivated accessions, the seed coat was separated by hand and ground for the extraction of tannins; while for each of the wild accessions, whole seed was used, as it was impractical to remove the seed coat from these smaller seeded genotypes.

Extraction procedures: Extraction was carried out with 70% acetone and diethyleter using 10mg of ground seed coat according to Blair et al. (2006). Spectrophotometric detection was via a butanol-HCL method (Porter et al 1986) which extracts most of the tannins present in a sample but which does not guarantee the complete exclusion of other compounds such as anthocyanins or other polyphenols. This occurs because in the reaction with butanol in an acid solution, proanthocyanidins (tannins) are converted to anthocyanidins, which absorb light at 550nm as do Anthocyanins and other related compounds; leading to some overestimation in the quantification. To avoid this, we used a blank to eliminate the matrix effect and at the same time the possible interference from these compounds. The blank is a sample similar to the samples for analysis and treated in the same conditions, but with the difference that in the butanol reaction, this blank is treated with butanol-water and not butanol-HCl.

Calibration curves: The calibration curves used for data transformation from absorbance to percentage of tannins were color curves for *Phaseolus vulgaris* (Blair et al., 2006). Given the predominant colors in the tepary beans that were analyzed the calibration curves for cream and black seeded genotypes were used for cultivated and wild accessions, respectively.

RESULTS AND DISCUSSION

This first attempt to evaluate tannin content in tepary bean was a useful screening of the effectiveness of the butanol-HCl method in this species, along with an initial exploration of the variability of this important nutritional trait. One notable observation was that variability for tepary bean seed coats was similar to previously observed variability in better-studied common beans. The butanol-HCl method was found to be a fast and good alternative to screen samples, however, techniques like HPLC are more sensitive and accurate and would allow a better understanding of qualitative and quantitative profiles of tannins in tepary beans. Our current results can be summarized as follows:

Cultivated tepary beans: We found variability for condensed tannin content among cultivated tepary bean (Table 1) from undetectable levels to 15 mg / g of seed coat for G40022. In comparison, the control common bean genotypes ICA Pijao had total condensed tannins of 16.3 mg / g of seed coat. Color variation for the genotypes used in the analysis ranged from white, yellow, cream, grey to black; with some mixed color or two-tone genotypes. Overall, the highest values for tannin content among the cultivated tepary beans were found in yellow seeded types, which were the predominant type in the

survey (white beans were for the most part excluded given that they have very low tannin content which was confirmed by the results presented here). White beans in this study had undetectable levels of condensed tannins. Meanwhile, the cream or brown mottled and black speckled seed types had intermediate values. The range in tannin content found for the 25 genotypes was within the normal range observed for *P. vulgaris* (House et al., 2002), although in general, there was a clear tendency toward low tannin content.

Wild tepary beans: Wild genotypes were complicated to analyze because of their size and in addition all had the same color (grey/black mottled). The total condensed tannin content on a total seed weight basis (rather than on a seed coat basis) was very similar ranging from 0.1 to 4.7 mg/g of seed coat. When assuming approximately 25% seed coat over total seed percentage these values ranged higher than for the cultivated accessions which might be expected due to the darker tones of wild versus cultivated genotypes. Given the similarity between genotypes and the difficulty in estimating an exact conversion percentage it is difficult to make firm conclusions about variation for tannin content in wild tepary beans.

Table 1. Condensed tannin content (mg tannin/g sample) in 25 tepary bean accessions and one common bean control (ICA-Pijao) measured through Butanol-HCl analysis.

Genotype	Status[1]	Color	Soluble Tannins[2]	Insoluble tannins	Total Tannins
G40001	Cultivated	White	0.0	0.0	0.0
G40007	Cultivated	White	0.0	0.0	0.0
G40006	Cultivated	Cream mottled	0.0	1.3	1.3
G40013	Cultivated	Black speckled	5.6	2.9	8.5
G40019	Cultivated	Black	5.1	2.5	7.7
G40021	Cultivated	White	0.0	0.1	0.1
G40022	Cultivated	Yellow	12.2	3.0	15.1
G40025	Cultivated	Yellow	8.1	3.4	11.5
G40033	Cultivated	Yellow	8.5	3.7	12.2
G40037	Cultivated	Yellow	7.0	3.7	10.8
G40066	Cultivated	Yellow	6.5	3.7	10.2
G40068	Cultivated	Yellow	7.4	3.4	10.7
G40084	Cultivated	Brown mottled	2.2	2.5	4.6
G40110	Cultivated	Black speckled	5.2	2.4	7.5
G40112	Cultivated	Yellow	6.3	3.7	10.0
G40161	Cultivated	Yellow	4.6	1.0	5.5
G40200	Cultivated	Brown mottled	7.9	3.0	10.9
G40237	Cultivated	Yellow	8.8	3.5	12.3
G40186	Wild	Grey/Black	0.9	0.6	1.4
G40106	Wild	Grey/Black	nd	0.6	0.6
G40240	Wild	Grey/Black	3.1	1.6	4.7
G40055	Wild	Grey/Black	nd	0.1	0.1
NI576	Wild	Brown/Black	nd	1.1	1.1
P.a 78	Wild	Grey/Black	nd	1.0	1.0
P.a 19	Wild	Grey/Black	nd	1.1	1.1
ICA Pijao	Common bean	Black	14.1	2.3	16.3

1/ Condensed tannins measured in seed coat samples for cultivated tepary and common beans and in whole seed samples for wild tepary beans.
2/ nd = none detected.

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FOLATE CONTENT IN SELECT DRY BEAN GENOTOYPES

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Dry edible beans are a good natural source of folate (½-cup serving of cooked beans provide 35% daily value of folate). Recognized healthful benefits of folate in the human diet include reduced birth defects (Czeizel and Dudás, 1994), decreased plasma homocysteine level which is a risk factor in cardiovascular disease (Boushey et al., 1995), reduced risk of several types of cancer, and decreased age-related memory loss as claimed in a recent report (http://www.worldhealth.net/p/randomized-trial-memory-of-adults-50-75.html).

Variable folate content reported among dry bean market classes (cranberry and dark red kidney, respectively, were reported to contain 183 and 65 mcg per ½-cup serving (http://www.northarvestbean.org/html/schoolbasics.cfm) indicates a potential for genetic manipulation of folate content using traditional plant breeding methods. Release of high folate bean cultivars would provide a value-added "nutritionally improved" food to consumers. Our objective was to determine folate content of dry bean genotypes harvested from the same field trial.

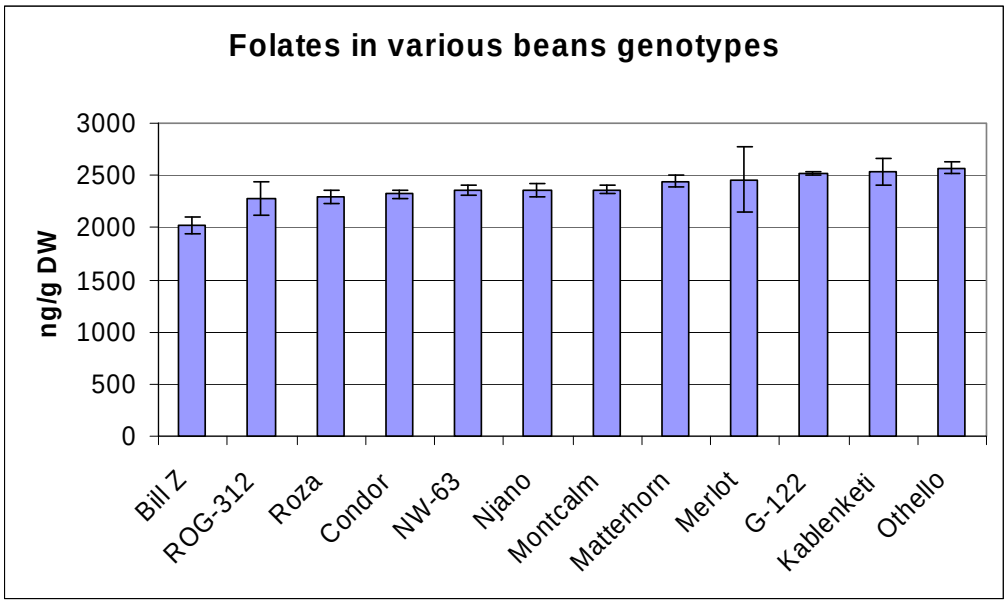
Twelve dry bean genotypes representing different U.S. market classes ('Othello' and 'Bill Z' pinto, 'ROG 312' and 'Roza' pink, 'NW-63' and 'Merlot' small red, 'Condor' black, 'Matterhorn' great northern, 'Montcalm' dark red kidney, G 122 cranberry-like landrace from India, and 'Kablanketi' purple-speckled and 'Njano' yellow landraces from Tanzania, were grown in a field trial in Othello, WA, in 2005. Seed from plots harvested Sep 2005 were dried 100°F for 48 h, cleaned, and stored at room temperature ~70°F until processing in June 2006.

Folates were extracted by a tri-enzyme treatment (Pfeiffer et al., 1997) with the following modifications. 200 mg of pulverized uncooked seeds was homogenized in 10 mL of extraction buffer (50 mM HEPES/50 mM CHES, pH 7.85, containing 2% (w/v) sodium ascorbate and 10 mM 2-mercaptoethanol, deoxygenated by flushing with nitrogen), boiled for 10 min, and cooled in ice. The homogenate was treated with protease (1 mL at 4 mg/mL; 4.5 units per mg) for 2 h at 37ºC, boiled for 5 min, and cooled in ice. The sample was then treated with α-amylase (1 mL at 20 mg/mL; 43 units per mg) and rat plasma conjugase in large excess for 4 h at 37ºC, boiled for 5 min, and cooled in ice. After centrifugation for 10 min at 3000 × *g*, the supernatant was transferred. The residue was resuspended in 5 mL of extraction buffer, and recentrifuged for 10 min. The combined supernatants were adjusted to a 20-mL final volume with extraction buffer, flushed with nitrogen, freezed in liquid nitrogen, and stored at –80ºC until analysis. Recoveries were estimated by adding a mixture of standards (5-formyl-THF, folic acid, 5-methyl-THF, 5,10-methenyl-THF, each in equal amounts) during the homogenization in Hepes/Ches buffer (300 ng of standards mixture was added per gram of dry sample).

Lactobacillus rhamnosus (ATCC 7469) was used to determine total folate content as described by Horne and Patterson (1988) with the following modifications. The *L. rhamnosus* inoculum was prepared by mixing 1 mL of cryoprotected cells with 4 mL of 9 g/L NaCl, followed by centrifugation for 10 min at 3000 × *g*, and resuspension of the bacterial pellet in 5 mL of 9 g/L NaCl. The folic acid casei medium (double strength) was prepared as recommended by the manufacturer, with the addition of 8 g/L sodium ascorbate, and sterilized by 0.2-μm filtration. Wells of a 96-well plate (Falcon Microtiter Plates) contained 150 μL of double-strength folic acid casei medium containing either 5-formyl-THF standard (from 5 to 60 fmol of the (6*S*)-isomer in a maximum volume of 11 μL of extraction buffer) or 5 μL of bean extract at various dilutions. Sterile water was added to adjust total volume to 300 μL. Ten microliters of bacteria were added to each

well. The plate was incubated at 37ºC for ~18 h. Bacterial growth was measured at 630 nm on a BioTek Instrument EL 311 SX microplate autoreader (BioTek Instrument, Winooski, VT) and analyzed with the KCJr EIA application software. Results were calculated by reference to a standard curve using 5-formyl-THF and were expressed as nanograms of 5-formyl-THF per gram of sample. Folate values were corrected for the endogenous folate contents in rat plasma conjugase, α-amylase and protease.

Folate content ranged from 2016 to 2569 ng g^{-1} DW (dry weight), with the largest difference representing a 27% increase between genotypes within the pinto bean market class (Fig. 1). Han and Tyler (2003) report a significant difference for folate content between pinto and navy market classes but not for cultivars within a market class. The range in folate content observed among genotypes in this study is quite small compared to the 182% difference reported between dark red kidney and cranberry bean (http://www.northarvestbean.org/html/schoolbasics). Differences in analytical methods, sample source, and sample preparation, likely contributed to the disparity between our results and those reported elsewhere. Our samples derive from a common field trial and were stored similarly which contributed to more uniform test material, whereas sample source and treatment in the previous study are unknown. The wide range in folate content observed in the previous study could arise from many factors including differences for environment and location of production, year of harvest, storage, and commercial processing, among the bean samples tested. Han and Tyler (2003) report significant location effects on folate content in lentil. Goyer and Navarre (2007) found about a 3-fold difference in folate content among 70 potato genotypes, and reported that location effects were less than genotype effects. Perhaps a larger survey of bean genotypes will reveal a wider difference in folate content amiable to manipulation by breeders than that observed among the 12 genotypes examined in this study.



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ULTRAVIOLET SPECTRAL FINGERPRINTS: A SIMPLE APPROACH FOR CLASSIFICATION OF BEAN CULTIVARS

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INTRODUCTION

Beans and legumes have been grown and eaten for thousands of years. Dating back to 2500 BC these healthy protein filled foods have been found in Egyptian tombs. Legumes play a critical role in human and animal nutrition since they provide rich sources of protein, calories, certain minerals, and vitamins (1). Dry beans (*Phaseolus vulgaris* L.) are important sources of proteins for many Asian, Latin American, and African countries. Total per capita consumption of dry beans intake has increased in United States during the last two decades (2). This increase in dry beans consumption has been attributed to multiple factors included increased immigration, wide-spread interest in ethnic foods, and recent interests in the beneficial health properties of phenolic compounds found in beans and other plant products (2,3).

In our previous communications, we had reported the determination of phenolic acids and polyphenols in the dry bean cultivars commonly consumed in the United States (4,5). These detailed analytical procedures are expensive, time consuming, and require expensive instrumentation. In continuation of our research on development of methods for beans analysis, we have evaluated the applicability of a simple inexpensive ultraviolet-visible spectral fingerprinting method for categorization of different bean cultivars commonly grown and used in United States.

MATERIALS AND METHODS

Bean samples. As reported earlier, all nine varieties of bean samples belonging to nine market classes, pinto (Maverick), black (Eclipse), navy (Norstar), dark red kidney (Red Hawk), light red kidney (California Early), small red (Red Merlot and UI-239), Alubia (Beluga), cranberry (Taylor Hort), and pink (UI 535) were grown in three different states (Maryland, Michigan, and Nebraska). Eight samples from different plants per variety were collected and analyzed from each growing site.

Chemicals. HPLC-grade MeOH was purchased from Fisher Chemicals (Fair Lawn, NJ). Deionized water (18.2 MΩ•cm) was obtained in-house using a Nanopure diamond analytical ultra pure water purification system (Model # D11901, Barnstead Intl., Dubuque, IA). Polyvinylidene difluoride (PVDF) syringe filters with pore size 0. 45 μm were procured from National Scientific Company (Duluth, GA).

Extraction. All bean samples were ground in a coffee grinder and stored under nitrogen at temperature < -60^{o}C until analyzed. Approximately 250 mg of powdered bean sample (particle size < 0.825 mm) was placed in a screw cap vial with 5 mL of MeOH:H_2O (60:40, % v/v). The mixture was sonicated in an ultrasonic bath (Branson 2510, Branson Ultrasonic Corporation, Danbury, CT) at 40 ^{o}C for 30 min. The mixture was centrifuged (Model GT2, West Chester, PA) at a low speed (5000 rpm) for 10 min. The supernate was transferred into a separate vial and the residue was

extracted for two more times with 2.5 mL of fresh MeOH:H_2O (60:40, % v/v). The volume of the combined extract was adjusted to 10 mL with MeOH:H_2O (60:40, % v/v). All extracts were stored in vials under nitrogen at temperature < -60 ^oC until analyzed. An appropriate aliquot of each extract was filtered using PVDF syringe filter (pore size 0. 45 μm) prior to ultraviolet spectral analysis.

Data acquisition and analysis. The Ultraviolet (UV) spectral fingerprints of bean extracts were recorded on a Lambda 25 spectrophotometer (Perkin-Elmer, Boston, MA). All spectral data were converted to the American Standard Code for Information Interchange (ASCII) files and exported for chemometrics analysis. Pre-processing of the data matrices was performed on Excel (Microsoft, Inc., Belleview, WA, USA) and PCA was performed using Pirouttte 3.1 (Infometrix, Inc., Bothell, WA, USA).

RESULTS AND DISCUSSION

This study has shown that chemometrics analysis of the ultraviolet-visible spectral fingerprints can provide useful information for categorization of different bean cultivars. The results indicate that analysis of variance-principal component analysis (ANOVA-PCA) provides easily interpreted visual plots and the data matrices can be further employed to calculate the variance contributed by the different experimental parameters. The variance among samples grown at different sites was around 2%. Over 80 percent of the variance was attributed to cultivar and approximately 10 % of the variance was due to plant variability. The analytical uncertainty was estimated to be around 7%. The ultraviolet-visible spectral fingerprinting in combination with ANOVA-PCA offers a simple inexpensive methodology for categorization of powdered dry bean cultivars. A similar approach was used for differentiating two broccoli cultivars grown under different experimental conditions (organic, conventional, and different selenium concentration).

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VOLATILE COMPOUNDS OF DRY BEAN SEED (*PHASEOLUS VULGARIS* L.)

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ABSTRACT

Volatile compounds of uncooked dry bean (*Phaseolus vulgaris* L.) cultivars representing 3 market classes (black, dark red kidney and pinto) grown in 2005 were isolated with headspace solid phase microextraction (HS-SPME), and analyzed with gas chromatography mass spectrometry (GC-MS). A total of 62 volatiles consisting of aromatic hydrocarbons, aldehydes, alkanes, alcohols and ketones represented on average 62, 38, 21, 12, and 9 x 10^6 total area counts, respectively. Bean cultivars differed in abundance and profile of volatiles. The combination of 18 compounds comprising a common profile explained 79% of the variance among cultivars based on principal component analysis (PCA). The SPME technique proved to be a rapid and effective method for routine evaluation of dry bean volatile profile.

INTRODUCTION

The low Canadian per capita dry bean consumption at 2.5 kg per annum in 2005 [1], may be due to the unsophisticated taste and flavor consumers generally associate with dry bean products despite their nutritional and health benefits. Flavor, an important factor in overall acceptability, and cooking time were two main characteristics used by consumers to select a given type of bean according to a survey conducted in Mexico [2, 3]. Flavor is most often ascertained on cooked or canned bean. Presently, flavor of canned bean is judged subjectively for flat, dull, bitterness, acid, sweet and off-flavor by a trained and experienced sensory evaluation team with scores ranging between 2.4 to 3.2 (2=fair, 3=good and 4=very good). Studies of flavor in dry bean [4, 5, 6] were preceded by identification of about 90 volatile components of canned whole bean [7].

SPME is a simple, sensitive, robust, reliable, low cost and very popular fast screening sampling technique based on analyte diffusion that combines the advantages of both static and dynamic head space for qualitative volatile analysis [8]. Information currently available on the volatile components of dry beans is deficient and full investigations of the uncooked beans are long overdue. In the present study, HS-SPME was applied as a solvent-free sample preparation method, with GC-MS analysis, to provide the initial investigation of the volatile profile of dry bean from Manitoba. This is the first step in unraveling and elucidating bean volatiles as a prerequisite in developing new cultivars for increased economic value and novel bean ingredients for health and functional food uses.

MATERIALS AND METHODS

Black bean cultivar AC Harblack, CDC Rio and Onyx; pinto bean cultivar AC Pintoba and Maverick; and dark red kidney cultivar ROG 802 and Red Hawk grown in 2005 in southern Manitoba were used in this study. The bean seeds were stored in a dry room (23°C, 15-20% relative humidity) prior to analysis.

Ten grams of freshly ground dry bean seed sample was used to extract volatiles using SPME. Grinding and collection of headspace volatiles were performed in triplicate from the same lot for each sample. After extraction, the analytes were thermally desorbed at 250°C for 2 min in the injection port of an Agilent 6890/5973 GC-MS and separated on a Supelcowax 10 polar column, 60 m x 0.25mm with a 0.50 μm film thickness. Data were collected with Agilent enhanced ChemStation

software (standard MSD version) and searched against the NIST (v. 02) and Wiley (v. 138) libraries (Palisade Corp., Newfield, NY). Compounds were identified by preliminarily library search, and identities were confirmed by comparison of their GC retention time with eight internal standard solutions of C_7-C_{22} n-alkanes and MS ion spectra. Levels of flavour components were determined from the average of three replicate chromatograms, calculated and expressed as the area units of their abundance (total area counts, TAC).

Data were subjected to analysis of variance by the general linear models (GLM) procedure, means comparison by Duncan's test, and Principal component analysis according to Statistical Analysis System [9].

RESULTS AND DISCUSSION

A total of 62 headspace volatile compounds isolated by SPME were tentatively identified in dry bean seeds by GC-MS. Cultivars differed in relative abundance of extracted volatiles with low (< 70 x 10^6 TAC) (black bean AC Harblack and dark red kidney ROG 802), intermediate (115 x 10^6) (Red Hawk) and high (> 190 x 10^6) (AC Pintoba, CDC Rio, Maverick and Onyx) abundance. The volatile profile of AC Harblack and ROG 802 consisted of only 32 and 25 compounds, respectively, and in addition had the lowest content of 13 compounds. The low volatile compounds detected in AC Harblack and ROG 802 probably indicates suppression of their lipoxygenase enzyme, particularly the lipoxygenase 3 enzyme involved in aroma biosynthesis [10].

The volatile classes included 14 alkanes (4.6 to 42.6 x 10^6 TAC for AC Harblack and AC Pintoba, respectively), 11 aldehydes (14.4 to 55.8 x 10^6) and 10 aromatic hydrocarbons (29.1 to 83.4 x 10^6 for AC Harblack and Onyx, respectively), 10 alcohols (3.6 to 19.3 x 10^6 for AC Harblack and AC Pintoba, respectively), 7 ketones (4.3 to 12.7 x 10^6 for ROG 802 and CDC Rio, respectively), 3 terpenes (0.8 to 14.7 x 10^6) and 2 furans (0.4 to 2.7 x 10^6 for AC Harblack and AC Pintoba, respectively). The aromatic hydrocarbons, aldehydes, alkanes, alcohols and ketones represented 62.2 ± 23.7, 37.8 ± 16.4, 21.1 ± 15.2, 11.5 ± 6.5 and 8.8 ± 3.6 x 10^6 TAC, respectively. The SPME method for profiling bean headspace volatiles may be acceptable as a first step for segregating bean types and later can be applied in genetic improvement of flavor in dry bean. Basic knowledge of volatile compounds constituting the unique dry bean flavor can facilitate better quality control of raw materials and also help product developers meet flavor-delivery challenges.

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COLOR LOSS IN TWO BLACK BEAN POPULATIONS

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INTRODUCTION

Black bean (*Phaseolus vulgaris* L.) is especially prone to loss of seed color during the thermal processing prior to canning (BIC, 2008). This color leaching results in a canned bean product that appears brown or washed-out, and visually unappealing to consumers. Prior work undertaken to better understand the physiology and genetics associated with this leaching suggests the value of a rapid screen of black bean breeding lines at an early generation with limited quantities of seed and supplies. One such method, the soak water color test, was recently published by Bushey and Hosfield (2007). Their method requires ten seeds per line, along with minimal lab facilities and time, to indirectly screen for color retention in black bean. The objectives of this current work were twofold. The first was to purify and re-establish the original populations used to develop this technique as a genetic resource to facilitate future study of black bean color retention. The second was to verify the reproducibility of this technique for future use in breeding for color retention.

MATERIALS AND METHODS

Seed of two populations previously established by G.L Hosfield was obtained from USDA-ARS. Population 1 ('Black Magic' x 'Shiny Crow') consisted of 93 recombinant inbred lines (RILs), while population 2 ('Black Magic' x 'Raven') consisted of 106 RILs. Several of the bulks in population 1 segregated for both shiny and dull seed within a line, so a single seed descent purification process in the MSU greenhouse was immediately undertaken for each line during spring 2007. At maturity, single plant rows were established at the Saginaw Valley Bean and Beet Research Farm near Saginaw, MI. Rows were harvested as bulks and data collected on each line included: total seed weight, seed coat (dull or shiny), 100-seed weight, and dry seed color (measured with HunterLab LabScanXE, Reston, VA.). Two samples of 10 seeds each were then taken from each line and tested using the soak water color test as described by Bushey and Hosfield (2007). Soak water color was determined both as an L-value using a HunterLab UltraScanXE and as a visual rating from 1=clear to 5=very dark.

In addition, eleven of the lines in population 1 that were segregating for shiny and dull seed coats were randomly chosen for use in creating a group of near isogenic lines (NILs). The only differences in procedure from that described above were three shiny and three dull seeds for each line were planted in the greenhouse and then bulked prior to planting in the field in 2007.

RESULTS AND DISCUSSION

The amount of seed obtained for each line within the populations varied from 53 to 963g, with most lines producing sufficient seed to facilitate future work in replicated field plots. The few lines that produced little seed were the result of plant rows containing very few plants. Seed size, measured as 100-seed weight, ranged from 15.7 to 27.0g. On average, population 1 had larger seed size, with a mean of 21.2g, while population 2 was slightly smaller with a mean of 19.7g (Table 1). As expected, population 1 segregated by line for seed coat luster; 39 lines had shiny seed coats, while 54 were dull. All lines in population 2 had dull seed coats, as expected.

Dry seed color was nearly the same between the two populations. However, differences between shiny versus dull seed coats became apparent in the soak water color test. In this test, a lower L-value for the soak water indicates more color loss from the bean, thus a higher L-value is more desirable. Population 1, where 39 lines had shiny seed coats, had an average L-value of 75.6 and visual rating of 3.1. In contrast, population 2, with all dull seed coats, had an average L-value of 61.3 and visual rating of 4.5. As shown in Table 1, both populations had similarly low L and visual

values, but the high values were much higher in population 1, reflecting the presence of lines with shiny seed coats that did not leach as much color into the soak water. Similar trends were observed when comparing the 11 NILs differing only in seed coat. Again, dry seed color was very similar between the two groups, while soak water color was much lighter in the shiny group that tended to leach less (Table 2).

Table 1. Trait means and ranges for five traits in two populations segregating for color retention based on the 'Soak Water Color Test' (Visual rating 1=clear 5=very dark).

	Population 1: Black Magic x Shiny Crow					**Population 2**	
	Overall	**Dull**		**Shiny**		**Black Magic x Raven**	
Trait	**Mean**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**
100-Seed Weight (g)	**21.2**	21.2	15.7-27.0	21.2	17.0-26.8	19.7	15.1-24.7
Dry Seed Color (L)	**19.3**	19.0	17.2-23.4	19.8	17.2-21.4	19	16.2-22.6
Soak Water Color (L)	**75.6**	68.2	46.5-90.8	85.9	53.9-97.0	61.3	43.1-75.5
Soak Water Color-Visual	**3.1**	3.9	1.5-5.0	2.0	1.0-5.0	4.5	2.5-5.0

Table 2. Trait means and ranges for five traits measured on 11 pairs of NILs selected from population 1.

	Population 1 Shiny		**Population 1 Dull**	
Trait	**Mean**	**Range**	**Mean**	**Range**
100-Seed Weight (g)	22.2	17.8-26.1	25.6	22.2-31.0
Dry Seed Color (L)	19.8	18.0-22.2	19.2	18.2-20.3
Soak Water Color (L)	85.5	73.8-98.5	61.9	51.8-78.7
Soak Water Color-Visual	1.9	1.0-3.5	4.5	3.0-5.0

CONCLUSIONS

The data demonstrate that much of the variation for color loss originally present in two populations was maintained throughout the process of purification and renewal. This genetic variation for black bean color retention presents a unique opportunity for continued study of this economically important trait. Individuals in population 1 that have a dull seed coat facilitating water uptake, but a high L-value for soak water color would be particularly interesting to breeders (Table 1). The occurrence of these lines suggests that crossing between dull and shiny black beans represents a viable approach to improved processing quality when coupled with an early generation selection for dull seed and enhanced color retention. In contrast, the L-values and visual scores in population 2 underscore the difficulty that breeders must confront in retaining processed seed color when crossing two lines with dull seed coats. In addition, the group of NILs developed represent a useful genetic tool for studying other changes associated with differences in seed coat luster. While it is evident that shiny or dull seed coats cause beans to take up water differently and therefore influence their color retention, future analysis at the molecular level is needed to elucidate additional genetic differences. Such studies will provide practical knowledge useful for breeding future black bean varieties with improved processing characteristics.

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QUANTIFYING THERMAL DEGRADATION OF ANTHOCYANINS IN BLACK BEAN FLOUR (*PHASEOLUS VULGARIS* L.)

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ABSTRACT

Black bean (*Phaseolus vulgaris L.)* flour is a natural source of anthocyanins, a beneficial antioxidant. Previous studies have examined and quantified the total anthocyanin content in black beans; however, the degradation during thermal processing has not been studied. This study examined the change in anthocyanin content during thermal processing using samples with a moisture content (wet basis) of 25%, similar to that found in commercial extrusion processing applications. The samples were sealed in 50 x 70 mm cans (inner diameter) which were heated at retort temperature of 265°F for 15, 30, and 60 min. Retort and can center temperatures were measured with thermocouples. These measured temperatures were compared to predicted temperatures generated by Comsol finite-element software. Thermal conductivity (k) and specific heat (C_p) as functions of temperature were estimated via nonlinear regression in Matlab simultaneously integrating with Comsol. These properties were used to generate internal can temperatures for each heating trial, based on measured retort temperatures. Total anthocyanin content was measured before and after treatment. Degradation rate constant (k110°C) and activation energy (E_a) were estimated via nonlinear regression in Matlab software. The thermophysical properties estimated at 100°C were k = 0.369, W/m◦C and C_p = 3059 J/kg◦C. The kinetic parameters were k110°C = 0.0442/min and E_a = 17.06 kJ/gmol. The results of this study can be used to design low-moisture cooking processes for bean flour that minimize anthocyanin degradation.

INTRODUCTION

Black bean flour is a natural source of anthocyanins, which provide both natural color and antioxidant capabilities. Anthocyanins have shown to be beneficial to human health (Stintzing & Carle, 2004), and have also shown the potential to destroy free radicals, which may lead to their use in the prevention of degenerative diseases, such as Alzheimer's disease (Choung et al, 2003).

Previous studies have examined and quantified the total anthocyanin content in many types of bean, such as black beans (Macz-Pop et al, 2004; Salinas-Moreno et al, 2005) and kidney bean seed coat (Choung et al, 2003).

However, these studies only examined the anthocyanin content in the unprocessed bean flour; anthocyanin degradation during thermal processing has not been studied. The objective of this study was to examine the change in anthocyanin concentration in black bean flour during low-moisture thermal processing.

MATERIALS & METHODS

Sample Preparation: Black bean samples were ground to produce black bean flour. Water was added to the flour, mixed in a KitchenAid mixer for 30 mins, and left to equilibrate overnight until a moisture content (wet basis) of 25% was achieved.

Sample Treatment: The samples were sealed in 50 x 70 mm cans (inner diameter) which were heated at retort temperatures of 265°F for 15, 30, and 60 min.

Anthocyanin Extraction & Quantification: Anthocyanin concentration and degradation was determined by using the method of Cash et al. (1976). 7 g samples of bean flour (25% MCwb) were mixed with 10 ml 0.025M citrate buffer and centrifuged for 60 minutes. Total anthocyanins were extracted by mixing 1 ml of the supernatant of the centrifuged sample with 9 ml of an ethanol: 1.5 N HCl (85:15 ratio) solution (Skalski & Sistrunk, 1973). These samples sat at room temperature for one hour. The absorbance was read at 535nm.

Black Bean Thermal Property Determination: Thermal conductivity (k) and specific heat (C_p) as functions of temperature were estimated via nonlinear regression in Matlab simultaneously integrating with Comsol. These properties were used to generate internal can temperatures for each heating trial, based on measured retort temperatures. Anthocyanin degradation rate constant (k110°C) and activation energy (E_a) were estimated via nonlinear regression in Matlab software.

RESULTS

The thermophysical properties estimated at 100°C were k = 0.369, W/m◦C and C_p = 3059 J/kg◦C. The kinetic parameters were k110°C = 0.0442/min and E_a = 17.06 kJ/gmol.

CONCLUSIONS

The values established for anthocyanin degradation during heating of black bean flour will enable better low-moisture cooking processes for bean flour that minimize anthocyanin degradation.

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EFFICIENCY IN SEED PROTEIN EXTRACTION FROM COMMON BEAN CULTIVARS GROWN IN MEXICAN NORTHERN HIGHLANDS

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is grown in 1.1 million of hectares in Mexican Northern Highlands. Significant grain surplus and marketing problems are observed during high production years. Due to women job involvement, significant reduction has been observed for common bean consumption among Mexican urban population (Sánchez *et al*., 2001). Market alternatives for common bean are needed to increase farmers' income and recover healthy tradition in Mexican food. Grain industrialization is an important alternative to use seed benefic elements as protein, fiber, starch, polyphenols, etc. Those elements present in common bean seed could be used as functional components in processed foods. The objective was to evaluate efficiency in protein extraction using two common bean cultivars widely grown in the Mexican Northern Highlands.

MATERIAL AND METHODS

Commercial grain samples for Pinto Saltillo and Negro San Luis (Negro Bola) cultivars were obtained in Los Llanos, the main common bean producing area in Durango State. Samples were milled and proximate analysis was performed in whole grain flour according to micro-Kjeldhal (AOAC, 1990) and Bradford (Bradford, 1976) methods. Bean protein was extracted in two separate stages such as aqueous extraction followed by saline extraction. For aqueous extraction common bean grain flour was suspended in water (1:8 w/v), shaken for 15 min at 5°C and centrifuged for 5 min at 4000 g. Water-insoluble solids was protein extracted using 0.5 N saline solution, shaken for 15 min at 5°C and centrifuged for 5 min at 4000 g. Supernatant in both aqueous and saline solutions were acidified (pH 4.5) for protein precipitation and an additional 4000 g centrifugation cycle was applied. Isolated protein was neutralized, quantified and spray dried to obtain protein powder. Protein quantification was performed in original flour, products obtained in each extraction and discarded residues in order to establish process efficiency. Protein determinations were performed in duplicate using Bradford protein assay.

RESULTS AND DISCUSSION

Protein content in original flour sample reached 21 % in Pinto Saltillo and 20 % in Negro San Luis, according to micro-Kjeldahl method. Using Bradford assay grain flour protein content reached 124.9 mg g^{-1} of grain flour in Pinto Saltillo and 87.0 mg g^{-1} of grain flour in Negro San Luis. Aqueous extraction recovered 47.3 % of total protein content in Pinto Saltillo and 52.4 % in Negro San Luis (Figure 1). Saline extraction yielded 26.3 % of total protein in Pinto Saltillo and 16.8 % in Negro San Luis. Efficiency in protein extraction reached 73.6 % in Pinto Saltillo and 66 % in Negro San

Luis. Pinto Saltillo showed 17.5 % for protein losses after aqueous extraction, 3.1 % after saline extraction and 6.2 % in solid rest. Losses registered in Negro San Luis reached 20.1 % after aqueous extraction, 3.5 % after saline extraction and 6.5 % in solid rest. Losses observed in supernatant obtained after aqueous extraction need to be reduced increasing acidifier solution dosage and centrifugation speed and time period. Genetic breeding increased grain protein content and extractable proportion in Pinto Saltillo compared to Negro San Luis. Protein extraction performed combining aqueous followed by saline extraction showed high efficiency and represents an industrial option in Mexican Northern Highlands where common bean is widely grown. Extracted and spray dried protein form a bland tasting white powder which could be used to fortificate processed foods.

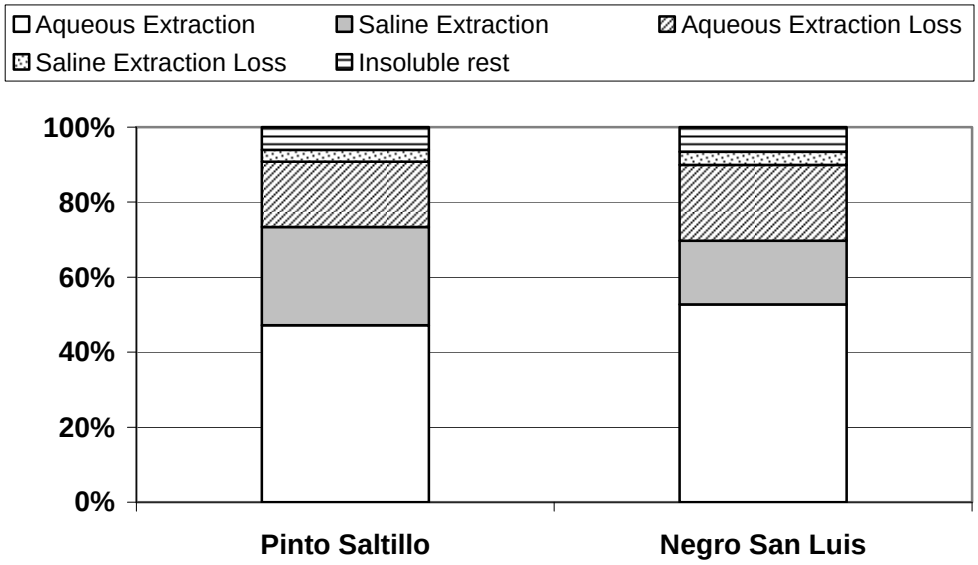

Figure 1. Proportions of protein recovery in different products obtained using aqueous and saline extraction in grain of two common bean cultivars.

ACKNOWLEDGEMENTS

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SENSORY EVALUATION OF EXTRUDED LIGHT RED KIDNEY BEANS (*PHASEOLUS VULGARIS* L.)

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INTRODUCTION

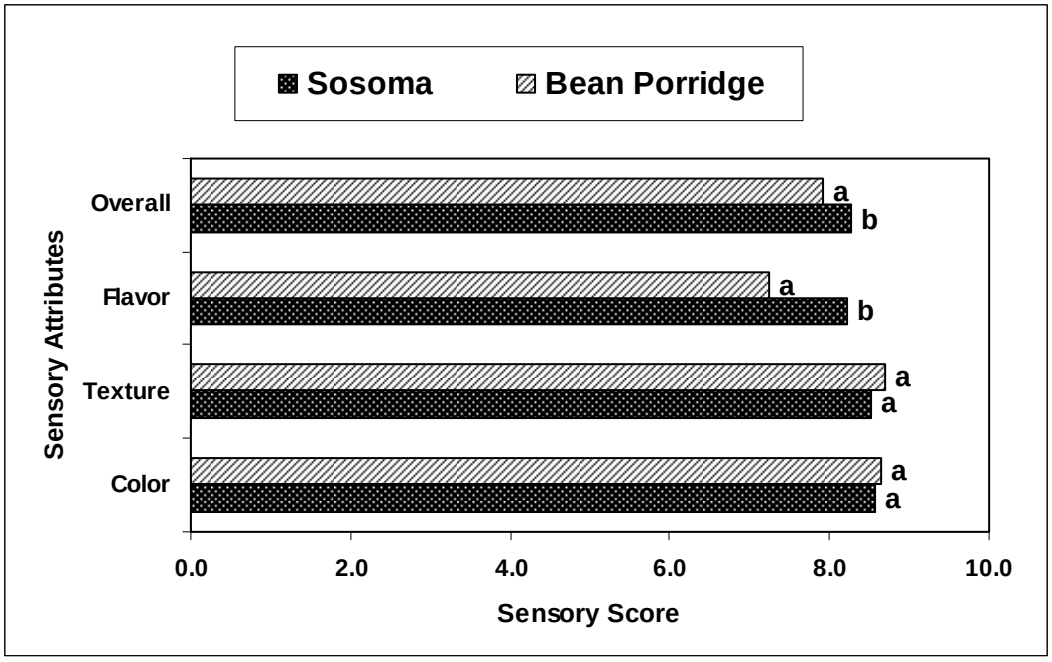
Dry beans (*Phaseolus vulgaris* L.) can be an extremely beneficial component of all diets because they are high in complex carbohydrates, protein and dietary fiber, and low in fat, calories and sodium, and are cholesterol free. In developing world, dry beans are an important source of protein and calories for middle and low-income families because animal protein is very expensive. In Rwanda, beans provide up to 60% of protein needs and 25% of calories in the diet (MINIPLAN, 1988). Large quantities of wood and charcoal used to cook the beans 3-4 hrs leads to deforestation and erosion. Low-cost extruders are seen as one of the best alternatives for dry bean cooking, especially in countries with low income, because the typical existing extrusion equipment is expensive. Extrusion cooking is one of several different processes to produce flours for infant foods. From a nutritional perspective, extrusion cooking allows inactivation of antinutritional factors, starch gelatinization, and protein denaturation thus increasing protein digestibility. The time and energy required to cook porridge from extruded starchy flours is considerably reduced as the flour is already cooked. The objective of the present study was to determine consumer acceptability of extruded light red kidney bean porridge.

MATERIALS AND METHODS

Whole dry light red kidney beans were purchased from Bayside Best Beans, LLC (Sebewiang, Mich., U.S.A.). Extrusion runs of raw ground red kidney beans were accomplished using a low-cost laboratory co-rotating twin-screw extruder model JS30A manufactured in China by Qitong Chemical Industry Equipment Co, Ltd. Sensory evaluation of extruded bean porridge was carried out at the National University of Rwanda hospital located in the southern of province of Rwanda. 75 panelists comprised mostly of adult low-income women evaluated bean porridge and compared it with a local porridge known as *sosoma*. Extruded bean porridge was prepared approximately in the ratio of 1:1:8(w/w) for extruded bean flour, sugar, and water respectively. Panelists were asked to rate their liking for color, texture, flavor and overall acceptability on a 1-9 hedonic scale (9=like extremely, 8=like very much, 7=like moderately, 6=dislike slightly, 5=neither like nor dislike, 4=dislike slightly, 3=dislike moderately, 2=dislike very much, 1=dislike extremely). A score of 5 or below was considered a limit of acceptability for all sensory attributes tested.

RESULTS

Panelists liked extruded bean porridge because all samples evaluated scored higher than 5 for the sensory attributes tested.



Sensory evaluation of sosoma and extruded bean porridges. Higher numerical values represent higher degree of acceptance.
Means with different letters are significantly different ($p \leq 0.05$)

CONCLUSIONS

Since this is completely a new product and 100 % beans, the sensory results from this study show that extruded bean porridge is a promising product.

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RHEOLOGICAL PROPERTIES OF CRANBERRY BEAN (*PHASEOLUS VULGARIS* L.) EXTRUDATE FLOUR

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INTRODUCTION

Legumes are the major source of proteins and fiber in the human diet particularly for vegetarians (Ramasamy Ravi 2007). Only they play an very important role in meeting the daily dietary requirement of the body (Sgarbieri 1989). The annual production of dry beans in the US is around 1.4 million metric tons of which Navy and Black Beans are the major contributors, while Cranberry, Kidney, Lima, Pink, Small Red, Blackeye and Great Northern beans are also contributed significantly. Nearly fifty per cent of the beans produced are being consumed domestically and another 50% are being exported. Cranberry beans, has a high protein and fibre content and also supplies important minerals such as Ca, Mg, Zn, K, Fe, P apart from vitamins like thiamine and niacin (Reyes-Moreno and Paredes-Lopez, 1993; Wu et al., 2004). Extrusion cooking is an advanced food processing technique to make variety of specialty food products. This technique modifies the functional properties or to inactivate anti-nutritional factors present in foods (Serna-Saldivar et al., 1988). Puffed extrudate products with unique porous and crunchy texture, can also be produced by using this high pressure extrusion cooking which is also shelf stable. Studying the rheological properties of the extrudate flours is important to make novel products like energy drinks, soups, porridge, concentrates etc., and also helps in designing equipments such as pumps involved in making liquid foods.

MATERIALS AND METHODS

Cranberry beans (*Phaseolus vulgaris* L.) was procured from the local market (Bayside Best Beans LLC , Sebewiang MI). The beans were soaked for 6 hours in water and dried overnight at 60 C.

Extrusion: Extrusion was done using a twin screw extruder (Model JS30A, Qitong Chemical Industry equipment Co., Ltd., China) with the screws are 30mm in dia and the barrel has a L and D ratio of 16. The extrudate were dried at 60°C overnight. The dried extrudate was ground by using a lab grinder (Nutrimill) to get the flour of 250 micron particle size.

Rheological measurement: The extrudate flour dispersion rheology was studied using a rheometer (Haake VT-550) with respect to shear rate (1-500 s-1), shear stress and app. viscosity. All measurements were done in duplicates and at 30°C. The power law model fitting was done using the shear rate – shear stress data using the MS-Excel spreadsheet.

RESULTS

Effect concentration: The dispersions of the extrudate flours, were studied at 10, 20 and 30% solids concentration (Fig.1). The power law constant, consistency index increased from 0.147 to 14.42 Pas, while the FWI decreased from 0.7379 to 0.4422 when the concentration increased from 10 to 30%, clearly indicating the shear thinning or pseudoplastic nature of the dispersions.

Effect temperature: The apparent viscosity decreased with increasing temperature as the extent of decrease was more with higher temperature (Fig. 2).The results of this study can be used to develop a novel foods based on extrudate flour dispersions.

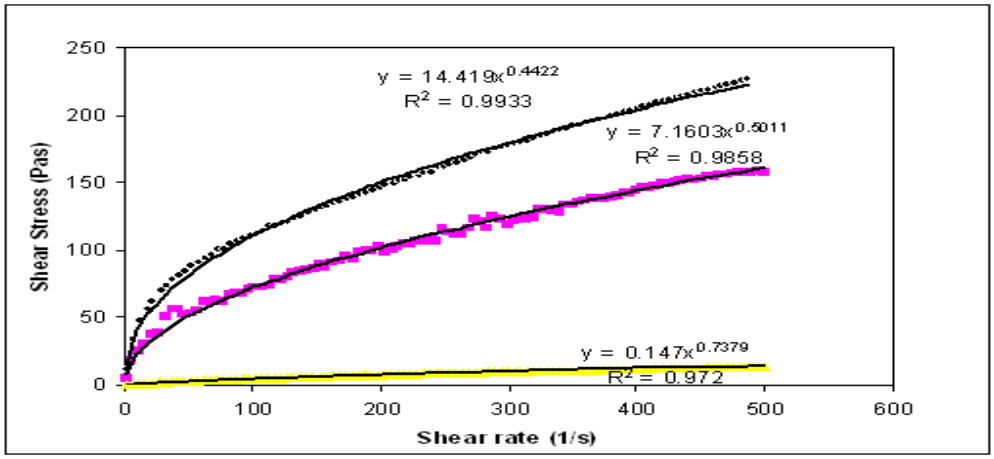

Fig. 1 Effect of concentration of on the shear stress and power law constants.

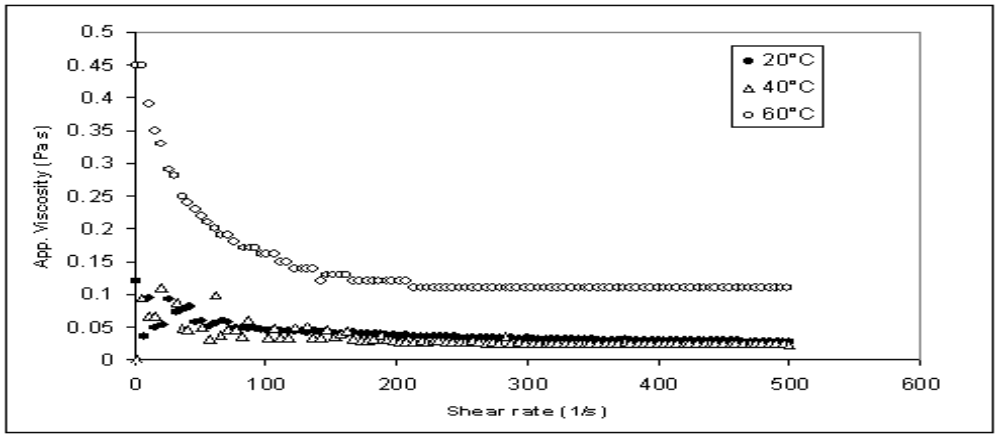

Fig. 2 Effect of temperature on the apparent viscosity of extrudate dispersion.

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PHYSICAL PROPERTIES OF CRANBERRY AND RED KIDNEY BEANS

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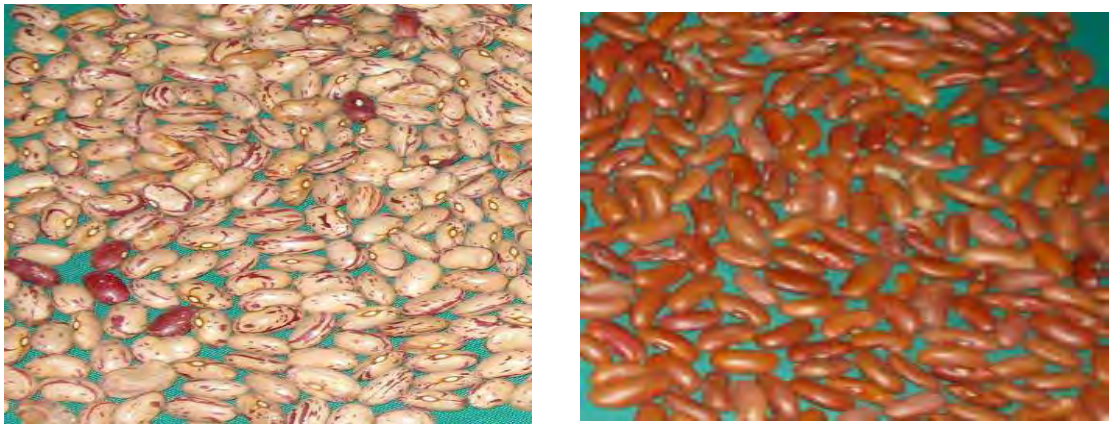
INTRODUCTION

On a global basis, around 80% of food energy and about 65% of food proteins are supplied by plant foods. Legumes are major group of plant foods that make a significant contribution to human and animal food supply (Sathe 2002). Michigan State is the largest cranberry bean producer in the US and nearly 9,000 mt of Cranberry beans are grown in the state alone. Cranberries are round in shape with red specks, which disappear on cooking. They have a creamy texture with a flavor similar to that of chestnuts. Red kidney beans were two types i.e light red and dark red and both are kidney shaped and pink in color, characterized with very firm texture and flavor. In the US, production of red kidney beans is around 100,000 mt harvest, of which 56 % Light Red and 44 % Dark Red kidney Beans. Generally beans are underutilized because of long soaking and cooking time needed to achieve required digestibility (Uebersax et al., 1991). Because of the high nutritional and health promoting properties, the development of value added bean based products for new market opportunities in the functional and nutraceutical industry is being promoted (Singh 1999) to a greater extent. Beans, as an ingredient for many novel food products featuring high dietary fiber and high antioxidant levels appear promising in particular for the ready to eat and snack food market (Anton et al 2007). The physical properties of beans are essential for the design of equipment, especially for handling, processing and storing beans. The purpose of this study was to examine various physical properties of cranberry beans and red kidney beans.

MATERIALS AND METHODS

Cranberry beans and light red kidney (*Phaseolus vulgaris* L.) beans (Fig.1) were procured from the local market (Bayside Beat Beans LLC , Sebewiang MI).



a) Cranberry beans b) Light Red kidney beans

Fig. 1 Color photograph of a) cranberry beans and b) Light kidney beans

Color measurement of beans: Color measurement of beans was done using Hunter colorimeter (Hunter associates Laboratories Inc., Reston, Virginia USA) with D25 L optical sensor. Hunter color parameters “L”, “a” and “b” were recorded and all measurements were done in triplicate.

Water absorption: Beans of 100 g from each variety, were soaked in water and water absorption were calculated at regular interval

RESULTS

As expected, the Hunter color parameters were distantly different for the cranberry and red kidney beans (Fig. 1) as the "L" values were higher for cranberry indicating lighter surface color compared to red kidney beans. The water absorption behaviors also were significantly different and red kidney beans were observed with high water absorption compared to cranberry beans (Fig. 2). Large surface area may be one of the reasons for the high water absorption of red kidney beans.

Table. 1. Physical characteristics of cranberry beans and red kidney beans.

	Cranberry beans	Red kindly beans
Individual seed weight, g	0.463±0.21	0.586±1.65
100 seeds weight, g	44.98±1.75	57.24±1.16
100 seeds volume, mL	41.66±2.88	50.66±1.15
True density, g/mL	1.07±0.16	1.13±0.15

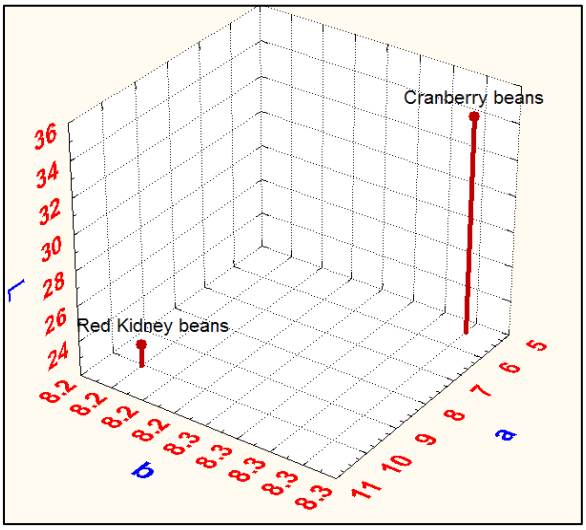


Fig. 1. Hunter color parameters

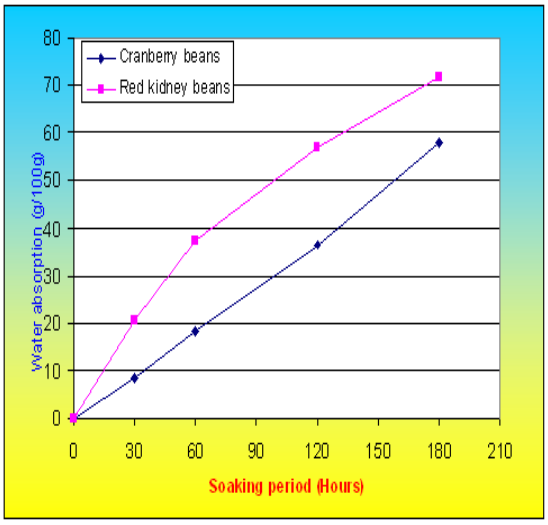


Fig.2. Hydration behavior of beans

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VALUE-ADDED PROCESSING OF FRUIT-BASED EXTRUDED PORRIDGE AND SNACKS

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INTRODUCTION

Michigan is one of the major dry beans and fruit producing states in the U.S. Beans and fruits, in addition to being low in fat and high in fiber, are also rich in many antioxidants. Because of their nutritional and health-promoting properties, the development of value-added bean-based products for new market opportunities is being promoted on an increased level (Singh, 1999). A review of approximately 200 studies on the relationship between fruit and vegetable intake and various cancers concluded, "major public health benefits could be achieved by substantially increasing consumption of these foods" (Block et. al., 1992). Diet is believed to play an important role in the four major health threats faced by our society—cardiovascular (heart and artery) diseases, cancer, hypertension, and obesity (Goldberg, 1999). The USDA food intake guidelines recommend 2-4 servings of fruits daily. To increase the current consumption level of fruits, there is a need to develop new, tasty, fruit-based products that are convenient to consume.

Our main objectives were: (1) to develop fruit-based products that are tasty, shelf-stable, nutrient-rich, virtually fat-free, and convenient to consume, and (2) evaluate these products different quality characteristics.

MATERIALS AND METHODS

Dry cranberry beans (*Phaseolus vulgaris* L.) were purchased from Bayside Best Beans, LLC (Sebewiang, MI) and ground in a hammer mill (WJ Fitzpatrick Company, Chicago, IL). Following fruits in diced/dried form were used for co-extrusion with bean flour: (i) Golden Delicious and (ii) Red Delicious apples, (iii) blueberries, (iv) cherries, (v) cranberries, and (vi) d'Anjou pears, using a low-cost twin-screw extruder (model JS30A, Qitong Chemical Industry Equipment Co, Ltd, China); shown in Fig. 1. Extruder screws are 30 mm in diameter and the barrel has a L/D (length/diameter) ratio of 16. Extrudates were dried overnight at 60 C and stored in sealed polyethylene bags until need for physico-chemical or sensory quality evaluation using standard lab procedures and equipment.

Figure 1. Twin-Screw Extruder

RESULTS

Sample fruit-based extrudates (snacks) made with a pilot-scale extruder are shown in Fig. 2. Addition of fruits, when compared to control, had minimal effect on extrudates density (data not shown). However, as shown in Table 1, hydration rate of the control extrudates was higher than those with added fruits except for one containing cranberries. Presence of pectin and sugars in the fruit tissue can affect the density of the extruded products thus resulting in lower hydration capacity. The hydration property of snacks plays a role in satiety by giving a feeling of 'fullness.'

Table 1. Hydration capacity of fruit-based extruded bean snacks

	Hydration Time (minutes)					
Bean Snacks with:	**15**	**30**	**45**	**60**	**75**	**90**
Control (no fruit)	33.3	39.3	41.8	45.1	47.3	48.9
Golden Delicious Apples	28.6	33.6	35.4	37	38.2	39.7
Red Delicious Apples	29.6	34.7	36.5	38.3	40.1	41.1
Blueberries	26.6	30.7	32	33.9	34.9	35.7
Cherries	34	40.1	42.6	44.5	46	47.5
Cranberries	30.9	35.7	37.5	38.3	39.8	40.8
D'Anjou Pears	30.6	36.1	38.2	39.7	41.1	42.6

As expected, the Hunter color "L", "a", and "b" values of fruit snacks differed significantly from the control (data not shown), as addition of pigment-rich fruits resulted in improved color of the final product. Data on fruit snack/strands texture (breaking force, Fig. 3); as compared to controls, except for the fruit snacks made with blueberries or cranberries, no significant differences were found among other fruit-added extrudates. Our results showed fruit-based bean snacks with acceptable quality can be prepared successfully using extrusion technology.

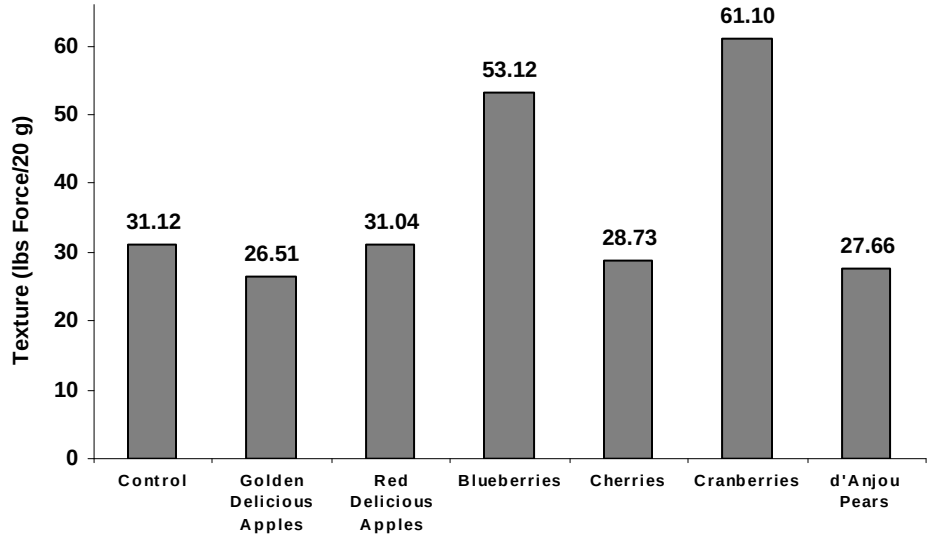

Figure 3. Instrumental texture values of fruit-based extruded bean snacks

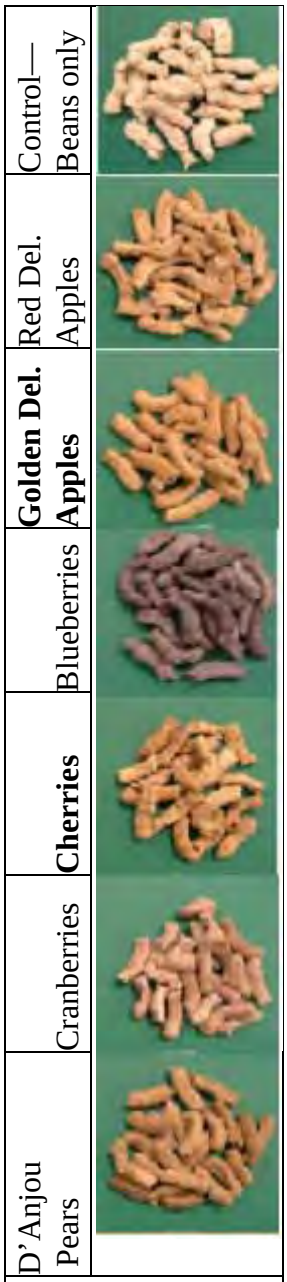

Figure 2. Fruit-based Bean Snacks.

ACKNOWLEDGEMENT

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DEVELOPMENT OF *CRAN-CRANBERRIES MINIS*© A CRANBERRY-BEAN (*PHASEOLUS VULGARIS* L.) SOFT MINI COOKIE

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INTRODUCTION

Cran-Cranberries Minis© are gluten-free soft mini cookies made with cranberry-bean (*Phaseolus vulgaris L.*), dried cranberries, and creamy white chocolate chunks. Through thoughtful ingredients selection and careful formulation, gluten-free cookies have been cleverly designed by combining nutrient-rich yet low-calorie, low-fat ingredients having equivalent flavor and texture properties of regular high-calorie, high-fat cookies. The growth in cookie market is predicted to be 1-3% per year to reach $6.1 billion in 2010 (not including crackers) led by demand for indulgent products and *healthier* versions of mass-market brands (AIB 2007; Heller 2006). These data lead to our target market of the population with celiac disease (gluten intolerant), which according to The National Institutes of Health is about 3 million people in total U.S. population (Bennett 2007). Further, the gluten-free category had an 86% increase in product launches and there is an increasing demand of gluten-free products in the market (Mintel 2006). *Cran-Cranberries Minis*© are poised to fill the market niche of being inexpensive yet nutritious and being a healthier snack compared to more expensive gluten-free products already in the market.

MATERIALS AND METHODS

Raw cranberry-beans containing approximately 13.5 % moisture were purchased from Bayside Best Beans, LLC (Sebewaing, Mich., U.S.A.). The beans were processed according to the method of Dolan et al. (2006). Beans were then soaked in a ratio of 1:5 beans:distilled water for eight hours. After soaking, beans were dried in the oven at 80^{o}C for 6 hours. Cranberry-beans were ground using a hammer mill to pass through 1-mm sieve. Extrusion of raw cranberry-bean flour was accomplished using a twin-screw extruder with 30-mm diameter screws and a 14:1 length-to-diameter ratio. Flour and water feed rates were manipulated such that the moisture content of the dough inside extruder barrel was 30 % wet basis. The extruder barrel was heated to 125 and 155^{o}C for feeding and mixing (closest to the die) zones, respectively. The extrudate exited the extruder through a 7-mm die and the extrudate was cut at 3-cm long. These extrudates were dried in a cabinet drier at 70^{o}C for 6 hours to obtain moisture content of 4-5 %. The dried extrudates were ground using a hammer mill to pass through 0.25-mm screen. This fine cooked cranberry-bean flour and other cookies ingredients were put together in a Hobart mixer to make cookie dough. The dough was chilled in the refrigerator before it was molded into 0.8-cm thick pieces. The cookies were baked at 325^{o}F for 25 minutes. After baking the cookies were allowed to cool on racks for before serving them in consumer acceptance sensory testing. The panelists (n = 48) were asked if they could detect the 'beany' flavor, purchase intention, and also to rate their likeness for appearance, aroma, texture, flavor, and overall quality on a 1-9 hedonic scale (1 = dislike extremely and 9 = like extremely). The panelists entered their responses on SIMS 2000, sensory computer software system. A score of 5 was considered the lower limit of acceptability for all sensory attributes tested.

RESULTS

Based on the responses obtained form the 48 panelists (general consumers), 90% said that they could not detect the bean flavor at all and they could not differentiate whether or not the cookies made out of bean flour. Ninety percent out of 48 panelists responded that they would purchase the cookies as healthier alternative to the regular cookies, and 10% said maybe purchase the cookie. The summary of consumer acceptance test is shown in Figure 1. A score of 5, that corresponded to "neither like nor dislike," or below was set as the limit of acceptability for sensory quality of the cranberry-bean cookies. The texture of the cookies, in this case, needed some improvement since it was the only attribute that scored the lowest (50% likeness).

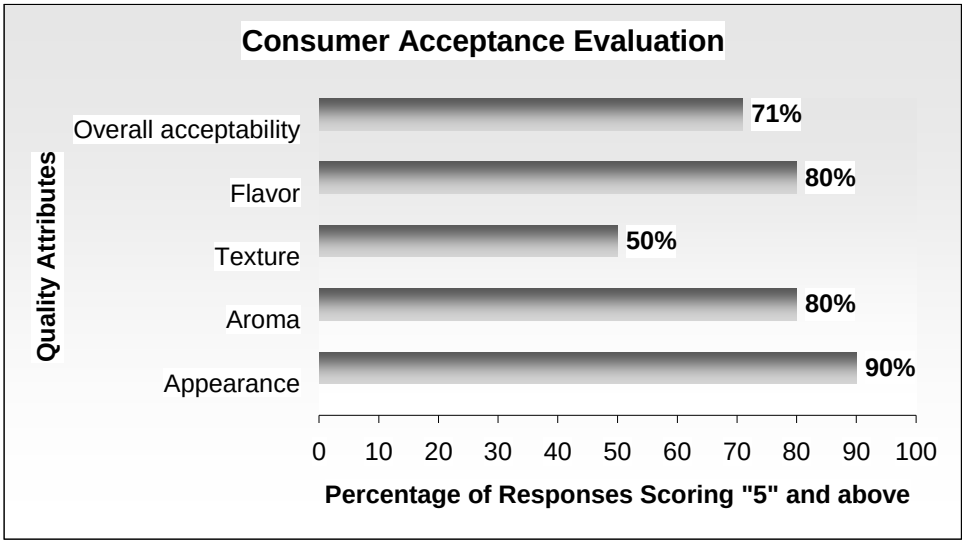

Figure 1. The consumer acceptance testing of *Cran-Cranberries Minis*©, cranberry-bean cookie (n = 48 panelists, hedonic scoring scale of 1-9: 1-dislike extremely, 9-like extremely)

CONCLUSIONS

Based on the consumer acceptance test results, it is concluded that *Cran-Cranberries Minis*© can find acceptability among consumers and offer potential for commercial production and marketing of such product.

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PILOY BEANS IN GUATEMALA

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Black beans (*Phaseolus vulgaris*) are the main bean market class consumed in Guatemala. Due to their important role in the Guatemalan diet, most dry bean research has focused on black beans. However, black bean prices tend to be lower than the prices of other less consumed bean types. As a result, farmers looking for higher profits are increasing their production of common beans of other market classes (e.g., small red, white) and non-*P. vulgaris* (known locally as "piloy") bean species (*P. coccineus, P. dumosus*) that command higher prices than black beans. "Piloys", which are primarily grown in the highlands, are sold in both highland markets and in open air markets in Guatemala City. Just as dry bean research has focused on *Phaseolous spp.*, market research and reports available from the Guatemalan Ministry of Agriculture are organized around providing timely price monitoring data on black, small red and white beans. Market information on "piloys" is virtually non-existent, despite their importance to farmers and consumers in Guatemala's western and central highlands. This study, conducted in summer 2006, collected and analyzed data on "piloys" sold by vendors located where they are mainly traded.

Key informants in the Agriculture Science and Technology Institute (ICTA) and bean market experts in the Inter-American Institute for Cooperation on Agriculture (IICA) were contacted to determine the general characteristics of the "piloy" subsector. First, consumption is highly linked to the indigenous population, which is mainly located in the 10 departments of Guatemala's western and central highlands. Second, "piloys" are climbing beans interplanted with maize mainly in May-June and harvested in December-January. Third, "piloys" are sold by common bean traders, but not all common bean traders sell "piloys", and vice versa.

Data Collection. Due to the non-availability of a "piloy" vendor lists to carry out random sampling, population data (a proxy for consumers' use of open air markets) provided by the National Association of Municipalities of Guatemala (ANAM) was used to identify 2-3 of the largest cities in the 10 target departments. In each market, the researcher entered the open-air market and interviewed all sellers of both common beans and "piloys". While 15 vendors per department was established as the target sample size, an average of 11 vendors per department (questionnaires n=119) provided completed data.

Key Findings. 1) Characteristics of Piloy Beans. While in most of the west/central highlands, non-*P. vulgaris* beans are known as "piloys". However, in a few markets in the central highlands they are refereed to as "furunas". Grain size varies from medium-to-large and color ranges from solid black or solid red to red with black speckles or black with white speckles. Vendors reported that consumers prepare "piloys" in dishes for special occasions, in combination with pork and other local spices (e.g. ground roasted pumpkin seed). Most consumers eat "piloys" regularly--once a week to once a month. Consumers value qualities such as freshness, absence of broken beans, and sorting by color. Vendors' displays emphasized these characteristics by displaying the reddest or the blackest "piloy" in the biggest sizes--although vendors indicated that during low-production months (June-November) all sizes are sold consistently.

2) Market Trends. The relative price of common beans vs. “piloys” varied among markets. In some regions, “piloys” were more expensive, while in other regions they were sold at the same or a higher price than common beans. The seasonal availability of “piloys” is determined by the rainy season--beans planted in May are harvested from the 1st week of December to the last week of January. About 89% of the vendors interviewed offer “piloys” 10 months a year. However, as supplies decline from June-November, prices double--from a mean low of Q2.84/lb to a mean high of Q5.14/lb (US$1=Q7.9). While prices were relatively uniform among venders in a single municipality market, they varied greatly among departments. While vendors could not provide detailed information regarding why this was the case, average prices were typically highest in markets far away from the major production areas, due to the presence of more intermediaries. For example, the highest mean price (Q7.0/lb.) was observed in Chimaltenango Department. Buyers in Chimaltenango bought from wholesalers, who gathered and sorted “piloys” from scattered growers in the Solola Department. In contrast, the mean price of “piloys” in Solola was only Q2.37/lb.

3) Vendors’ Supply Sources and Characteristics. Solola and Totonicapan Departments supply approximately 36% of the “piloys” traded in the highlands. Vendors’ purchases of common and “piloy” beans are cash transactions. Supply transactions for “piloys” are mostly between retailers and growers (71%), who bring their supplies to the market early on the market day. However, “piloys” are also traded by wholesalers located in the open markets. In contrast, vendors mainly obtain common beans from intermediaries. On average, vendors purchased (cash only) “piloys” once a month and tended to store them less than 2 months--despite venders assertion that “piloys” can be stored for 5 month before grain quality deteriorates (i.e., corrugated seed coat). Vendors reported making on average profit 7% on “piloy” sales—which suggests at least 70% of farmers supplying directly to wholesalers receive the benefits of the fluctuating market prices. Since off-season “piloys” could be an alternative for farmers with irrigation, this markup over purchase prices are key for further analysis.

4) Socio-Economic Characteristics of Vendors. While common beans and “piloys” are primarily sold by vendors with a permanent booth in the municipal open market, they are also sold by street venders. Most venders were women (64%), with an average age of 38 years, compared to 44 years for men. Typically, the vendors had limited education. Among the sample, all of the vendors sold both beans and several other products (e.g., spices, other grains).

Research Implications. This study suggests opportunities for increasing the incomes of “piloy” vendors, wholesalers, and producers. First, the large difference in prices across departments during the on and off seasons suggests a profitable opportunity for small farmers with access to irrigation to produce “piloys” during the off season. Second, the disparity of prices across departments during the on and off seasons suggests an opportunity for wholesalers in low-price areas to distribute “piloys” to areas with higher prices and where a more constant supply may encourage higher consumption of “piloys”. Finally, to date, no research has been conducted to identify and address farm level constraint to increasing the productivity of “piloys”. Given the strong consumer demand and high price that these species command, ICTA should initiate a farm-level survey designed to assess/prioritize production constraints and consider the feasibility of developing a research/breeding program to address these constraints.

ASSESSING THE IMPACT OF THE BEAN/COWPEA COLLABORATIVE RESEARCH SUPPORT PROGRAM'S (B/C CRSP) GRADUATE DEGREE TRAINING

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Since the early 1980s, with funding from USAID, the B/C CRSP has supported nearly 200 students for MS and PhD degrees at U.S. universities in the plant sciences, food sciences and the social sciences--fields critical to the development of bean/cowpea research capacity in host countries in Latin America and the Caribbean (LAC), Sub-Saharan Africa, and the U.S. The CRSP has invested more than US$69 million to support global bean/cowpea research, of which about US$7 million has been spent on training to enhance the capacity of agricultural research institutions in developing countries.

DATA COLLECTION.

A list of trainees and PIs was compiled by reviewing the Management Office's (MO) databases and searching the Internet. Survey questionnaires were developed and sent in 2006 to former trainees and PIs/scientists who supervised the trainees during their GDT (graduate degree training). An Internet search was conducted to collect additional evidence of impacts. A modified Kirkpatrick's Framework (impact of training on KSA; knowledge, skills, attitudes) was used to assess training impacts. The study results are based on analysis of surveys returned by 76 former trainees (41% of the target population) and responses from 25 former/current US-PIs. Case studies were also carried out in 2006 to highlight regional impact of CRSP investment in LAC (Escuela Agricola Panamericana - Zamorano (EPA)) and institutional impact in Tanzania (Sokoine University of Agriculture (SUA)). Face-to-face interviews were conducted with HC-PIs at each of these two universities.

KEY FINDINGS.

1) Survey of Former CRSP Trainees. CRSP Trainee Alumni reported that that their GDT was necessary for their professional development (100%), was highly relevant to their current work (92%); and that their CRSP research was necessary for their professional development (97%) and highly relevant to their current responsibilities (83%). Trainees considered the ability to "design/conduct/analyze scientific research" (87%) as the most important KSA acquired from their GDT. Most trainees shared their KSAs through publications (66%), seminar/conferences (70%), and the research supervision of students (66%). Most respondents with PhD degrees are now working at a university (52%) vs. 25% of MS graduates. The private sector employs 31% of MS graduates. Most respondents in the plant sciences are still active in bean/cowpea research (60%), vs. 41% for the social sciences, and 17% for the food sciences. The acquisition of a graduate degree greatly increased trainee salaries. Few U.S. trainees have had outside consultancies, while more than 55% of trainees from ESA and WA have been contracted by outside projects to augment income. Most the trainees (71%) reported changes in their personal lives, including improved financial status, greater self-confidence, an opportunity to learn a second language, and gaining new friends outside their home country. A high percentage of trainees (78%) attributed changes in their professional lives to improved KSAs. Most trainees (57%) reported the release of varieties, awards received for research, papers published, and positions held as important bean/cowpea-related achievements. The overwhelming majority of HC respondents (86%) returned home or to another developing country after receiving their highest degree. Furthermore, 79% of returnees

returned to the institution where they were employed prior to their GDT. A typical trainee profile included an earned PhD degree (86%), a specialization in the plant sciences, (69%), and employment in a government organization (36%) or a university (31%). Most returnees (72%) continue to work in a bean/cowpea related field, compared to 50% for non-returnees.

2) Survey of Former & Current US PIs. PIs (56%) praised the CRSP's commitment to long-term training, but cited (68%) the need for greater funding to support GDT, particularly at the PhD level. The main reasons PIs cited for providing full financial support to a trainee was because he/she was from a collaborating host country (31%) and that the trainee could not pursue GDT without full funding (27%). Alternatively, PIs indicated that trainees were partially supported if leveraged money was available from either a department (39%) or from an external source (25%). Problems PIs reported included delays in receiving funds (20%) from the CRSP--due to delayed funding from USAID--and the need for additional funds for GDT (16%). Most PIs (64%) reported significant jobs held by former trainees as an important bean/cowpea-related achievement. Several PIs cited trainees' contributions to their research area and noted publications/awards that resulted from their bean/cowpea-related research.

3) SUA Case Study. Ten of the 11 CRSP-supported trainees from Tanzania returned home after completing their GDT. Of these, a majority still work at SUA and are CRSP research collaborators. The Bean/Cowpea CRSP has played a major role in helping SUA develop its research and teaching programs, particularly in crop science. The CRSP's commitment to training has greatly enhanced SUA's capacity to train bean scientists for Eastern Africa plus contributed to making SUA a key institution in the national bean program. While SUA scientists have released four bean varieties, including two in 2007, farmer adoption has been greatly limited by constraints to seed production and multiplication. The institutional visit confirmed the hypothesis that former trainees are contributing to developmental impacts by teaching and supervising students who play strategic roles in NARS and successfully secure external funding for bean research, complementing that received for CRSP projects.

4) EAP (Zamorano) Case Study. The CRSP has not supported GDT for EAP staff being a small private agricultural university. However, the CRSP has had a significant impact on creating a strong regional bean research program, which has contributed to increased bean productivity in Central America. The CRSP has been a primary long-term funding source for the region's network of multi-locational varietal trials. In collaboration with national bean programs in Central America, EAP has developed many varieties that have been widely adopted throughout the region. The bean research program's excellent reputation has served to both recruit outstanding students from the region and encourage EAP students to major in plant science. With CRSP resources, EAP has provided students the opportunity to conduct research and develop skills in the use of research equipment purchased with CRSP funds. Upon returning home, these graduates have assumed key positions in national research programs and continue to collaborate with EAP on regional bean research. Key informants reported that the bean research program has greatly enhanced the reputation of EAP and thereby contributed to its success in obtaining external funding from other sources. The site visit affirmed that by enhancing EAP's capacity to train students and support varietal development, the CRSP has had a major impact on strengthening regional research capacity, increasing bean production, and increasing small farmers' incomes.

LESSONS LEARNED.

The B/C CRSP has played an important role in building capacity for teaching and conducting research on beans and cowpeas, thereby benefiting both U.S and host country agriculture. The CRSP should continue its commitment to GDT and give high priority to supporting HC trainees. USAID and other donors need to: 1) increase financial support for GDT, particularly for HC nationals; 2) recognize that almost all HC trainees return to their home countries after completing GDT; 3) recognize that the returned trainees play an important role in building capacity at HC institutions; and 4) recognize that the CRSP's GDT program has been highly successful in developing scientific capacity for conducting research in the US and host countries.

GENOTYPING OF COMMON BEAN CULTIVARS WITH MOLECULAR MARKERS LINKED TO DISEASE RESISTANCE GENES AS SUPPORT FOR GENE PYRAMIDING PROCESS

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One of the important factors hindering common bean productivity and performance is the occurrence of diseases. Angular leaf spot, caused by *Pseudocercospora griseola*, anthracnose, caused by *Colletotrichum lindemuthianum* and rust, caused by *Uromyces appendiculatus* are among the most important common bean diseases. In the integrated management of diseases, one of the strategies adopted is the use of resistant cultivars. They are usually developed by transferring resistance alleles from exotic sources to elite cultivars (ALZATE-MARIN et al., 2005). Cultivars with different resistance genes can be intercrossed to associate (pyramid) several resistance genes in the same genetic background. This is used to develop cultivars with durable and broad spectrum resistance. This strategy is extremely difficult to accomplish mainly due to limitations concerning the proper identification of resistant/susceptible plants after multiple inoculations with different pathogens. Molecular markers closely linked to resistant genes can be useful for the indirect selection of the resistance alleles, particularly in the initial and intermediate phases of the breeding process.

The objective of the present work was to genotype common bean cultivars used in bean breeding programs in Brazil with molecular markers linked to resistance genes to angular leaf spot, anthracnose and rust. This is a crucial step for the rational use of the gene pyramiding concept.

Leaf DNA samples were extracted (DOYLE & DOYLE, 1990) from 19 common bean genotypes and amplified with the following dominant SCAR markers $F10_{1050a}$, $BA08_{560a}$, $AA19_{651a}$, BA16, $N02_{950a}$, $H13_{520a}$, $Y20_{830a}$ and $AZ20_{845a}$ (Table 1).

The results shown in Table 1 demonstrate that different breeding strategies can be planned involving the genotypes tested. For instance, cultivar BRSMG-Talismã, which does not possess the alleles for angular leaf spot resistance (*Phg-ON* and *Phg-3*), presents the markers for these two allelles (BA16 and N02, respectively). These observations hinder the use of such markers for monitoring the introgression of these alleles into cv. BRSMG-Talismã. SOUZA et al. (2005) showed that cultivar BRSMG-Talismã is susceptible to several races of *P. griseola*, *C. lindemuthianum* and *U. appendiculatus*, indicating this cultivar does not possess many resistant alleles for these three pathogens. Other "carioca-type", as well as black and red seeded common bean cultivars tested also presented unexpected band patterns for some of the markers evaluated. This demonstrates the need for validation of each marker, case by case, aiming at different possibilities of intercrossing.

The results achieved in this work will give support for the design of future strategies of resistance gene pyramiding in breeding programs in Brazil.

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Table 1. DNA amplification of elite lines and cultivars of interest for strategies of gene pyramiding, using SCAR markers

Line/Cultivar	Group	Resistance Genes	Molecular marker and linked gene(s)							
			F10 *Ur-ON* and *Co-10*	BA08 *Ur-ON* and *Co-10*	AA19 *Phg-ON*	BA16 *Phg-ON*	N02 *Phg-3*	H13 *Phg-1*	Y20 *Co-4*	AZ20 *Co-6*
MAR-2		*Phg-4* and/or *Phg-5*^{2}	-	-	+	-	+	-	-	+
BAT 332		*Phg-6*^{2}	+	+	+	+	-	-	-	-
Mexico 54		*Phg-2* and/or *Phg-5* and/or *Phg-6*	-	-	+	-	+	-	-	+
Cornell 49-242		*Phg-3*	+	+	-	+	+	-	-	-
AND 277		*Phg-1, Phg-2*^{2}*, Phg-3*^{2} and *Phg-4*^{2}	-	-	+	+	+	+	-	-
Ouro Negro	Black	*Ur-ON, Co-10* and *Phg-ON*	+	+	+	+	-	-	-	+
Diamante Negro	Black	?	-	-	-	-	-	-	-	-
Valente	Black	?	+	+	-	-	-	-	-	+
BRSMG-Talismã	Carioca	?	-	-	-	+	+	-	-	-
Pérola	Carioca	?	-	-	-	-	+	-	-	-
Pérola “R”	Carioca	*Ur-ON, Co-4, Co-6, Co-10* and *Phg-1*	+	+	+	+	+	+	+	+
P-45-3-29-44	Carioca	*Ur-ON, Co-10* and *Phg-ON*	+	+	+	+	+	+	-	-
Rudá “R”	Carioca	*Ur-ON, Co-4, Co-6, Co-10* and *Phg-1*	+	+	+	+	+	+	+	+
MAR-138-1-11-4	Carioca	*Phg-4* and/or *Phg-5*^{2}	-	-	+	-	+	-	-	-
BAT-68-9-6	Carioca	*Phg-6*^{2}	-	-	+	+	-	-	-	-
MEX-37-3-6-3	Carioca	*Phg-2* and/or *Phg-5* and/or *Phg-6*	-	-	+	-	+	-	-	-
Rudá	Carioca	*?*	-	-	-	-	-	-	-	-
Ouro Vermelho	Red	?	-	-	-	-	-	-	-	-
Vermelhinho	Red	?	-	-	-	-	-	-	-	-

(+): presence of the band, (-): absence of the band; (?): genes not characterized or absent

MOLECULAR MARKER ASSISTED INTRODUCTION OF RESISTANCE GENES FOR RUST, ANTHRACNOSE AND ANGULAR LEAF SPOT INTO COMMON BEAN CULTIVAR BRSMG TALISMÃ

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The new common bean (*Phaseolus vulgaris* L.) cultivars released in Brazil must present a high disease resistance spectrum, as diseases are among the main causes for the low productivity of this crop in the country. BRSMG Talismã is a "carioca-type" cultivar which meets the market demands. The seeds are medium sized, beige with light brown stripes, with clear background, and colorless hylum). In addition, field evaluations demonstrate that BRSMG Talismã surpasses the most cultivated "carioca-type" varieties in Brazil by approximately 10%. However, this cultivar is highly susceptible to several races of *Uromyces appendiculatus*, *Colletotrichum lindemuthianum* and *Pseudocercospora griseola* (SOUZA et al., 2005). A "carioca-type" line Pérola "R" was developed by the Bean Breeding Program of BIOAGRO/UFV with the following resistance genes and respective linked molecular markers: *Ur-ON* ($SCF10_{1050a}$ and $SCBA08_{560a}$), *Co-4* ($SCY20_{830a}$), *Co-6* ($SCAZ20_{845a}$), *Co-10* ($SCF10_{1050a}$ and $SCBA08_{560a}$) and *Phg-1* ($SCH13_{520a}$). The objective of this work was to simultaneously transfer the resistance genes to rust (*Ur-ON*), anthracnose (*Co-4, Co-6* and *Co-10*) and angular leaf spot (*Phg-1*) present in Pérola "R" to the cultivar BRSMG Talismã with the aid of molecular markers.

In a previous work, DNA samples from BRSMG-Talismã and Pérola "R" amplified with those molecular markers showed they were polymorphic indicating that those markers could be used to assist the breeding process (Table 1). Crossings were made between the Pérola "R" and BRSMG Talismã in the greenhouse. The hybrid nature of the F_1 plants was confirmed with molecular marker $SCF10_{1050a}$. Molecular markers linked to the resistance genes were used in the F_2 and F_3 generations (Figure 1). For each generation analyzed, a leaf of each plant was collected and stored at -80°C for DNA extraction (DOYLE & DOYLE, 1990). From 17 F_1 plants, 476 F_2 seeds were produced. Out of those, 300 were sown, yielding 276 plants, which were inoculated with *P. griseola* race 63.23. Out of the 276 plants, 78 were susceptible to angular leaf spot, suggesting that they did not possess the resistance allele *Phg-1*. The 198 F_2 plants left were analyzed with the molecular markers mentioned in Table 1. These analyses showed that 18 plants harbored all the molecular markers for the three diseases. These plants were used to produce the F_3 generation. They were planted in a structure of families so progeny tests could be performed with the use of molecular markers. For the formation of each family, 15 seeds of each one of the 18 F_2 plants were sown. Two non-segregating families for all the molecular markers were identified.

The material to be reached at the end of the breeding process started in this work, after being tested for resistance and agronomic performance, may be released as a new cultivar and be used as a resistance gene source.

Table 1. Molecular markers linked to resistant genes to rust, anthracnose and angular leaf spot used in the transfer process

Marker	Distance[a] (cM)	Resistance gene	Resistance Sources	Reference
SCAR-Y20$_{830a}$	1.20	*Co-4*	TO	QUEIROZ et al. (2004b)
SCAR-AZ20$_{845a}$	7.10	*Co-6*	AB 136	QUEIROZ et al. (2004b)
SCAR-BA08$_{560a}$	2.20	*Co-10* and *Ur-ON*	Ouro Negro	CORRÊA et al. (2000)
SCAR-F10$_{1050a}$	6.50	*Co-10* and *Ur-ON*	Ouro Negro	CORRÊA et al. (2000)
OPX11$_{550a}$	5.80	*Co-10* and *Ur-ON*	Ouro Negro	FALEIRO et al. (2000)
SCAR-H13$_{520a}$	5.60	*Phg-1*	AND 277	QUEIROZ et al. (2004a)

[a]cM: genetic distance (centiMorgan) from the molecular marker to the resistance gene.

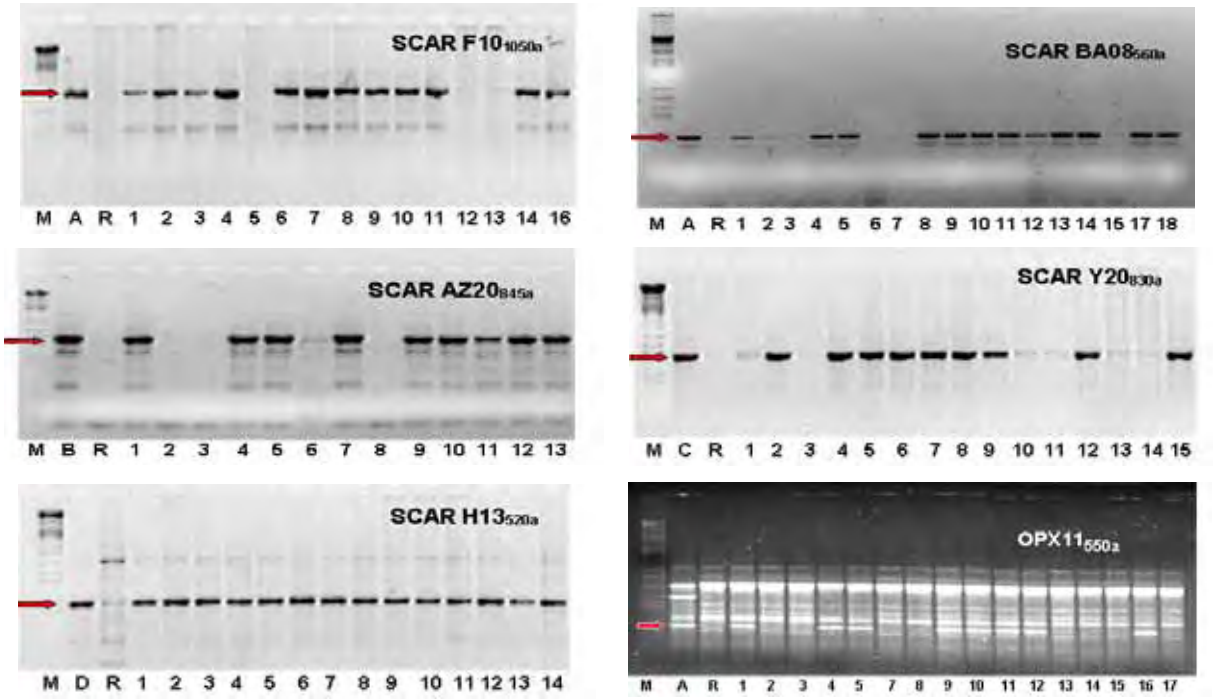

Figure 1. Amplification products obtained with markers SCARF10$_{1050a}$, SCBA08$_{560a}$, SCAZ20$_{845a}$, SCY20$_{830a}$, SCH13$_{520a}$ and OPX11$_{550a}$. Lane M: Lambda phage DNA digested with *Eco*RI, *Bam*HI, *Hin*dIII (size markers). Lanes R, A, B, C and D: Rudá, Ouro Negro, AB136, TO and AND 277, respectively, followed by part of the F_2 population (Pérola "R" x BRSMG Talismã). The arrows indicate the bands linked to the resistance loci.

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INTROGRESSION OF ANTHRACNOSE, ANGULAR LEAF SPOT AND RUST RESISTANCE GENES IN BEAN CULTIVARS VERMELHINHO AND OURO VERMELHO

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Brazil is the world largest consumer and the second largest producer of the common bean (*Phaseolus vulgaris*) (FAO, 2006). Although the "carioca-type" and black beans are the most commonly cultivated beans in the country, red beans are appreciated by consumers and farmers in the Zona da Mata Mineira (Minas Gerais state), where about 50% of the bean growing area is occupied by red grain beans.

'Vermelhinho' is a criollo cultivar widely planted in the Zona da Mata Mineira. The price of 'Vermelhinho' grains reachs up to twice the price "carioca-type" and black beans in this region of the country. 'Ouro Vermelho' is a high yielding red seeded cultivar recently developed by the Bean Breeding Program of the Universidade Federal de Viçosa. However, both cultivars are highly susceptible to anthracnose, rust, angular leaf spot, common bacterial blight and common mosaic virus, which impair productivity and grain quality.

The BIOAGRO/UFV Bean Breeding Program developed a bean line with "carioca-type" grains named Rudá "R", harboring resistance genes to anthracnose (*Co-6* and *Co-4*), rust (*Ur-ON*) and angular leaf spot (*Phg–1*) (RAGAGNIN et al., 2005). More recently, another line (Rudá "R1") was developed in which the allele *Co-4* was replaced by allele *Co-4*^{2} and the gene *Co-5* was added to the gene pyramid of Ruda "R". The main goal of this work was to transfer the resistance gene pyramid from Rudá "R" to cv. Vermelhinho and from Rudá "R1" to cv. Ouro Vermelho in a backcross process assisted by molecular markers.

For the cross Rudá ''R'' x 'Vermelhinho', F1 plants were selfed until F3 due to environmental constraints which prevented the beginning of the backcrossing process. In each generation DNA samples were extracted and amplified with the molecular markers $SCH13_{490a}$ (*Phg-1*), $SCAZ20_{940a}$ (*Co-6*), $SCF10_{1050a}$, $SCBA08_{560a}$ (*Ur-ON*), and $SCY20_{830a}$ (*Co-4*) (reviewed by MIKLAS, 2005). Out of 68 F3 plants obtained, eight presented at least four markers of interest. These plants were backcrossed to 'Vermelhinho', and 46 BC1F1 plants were obtained, out of which seven had at least four markers. Out of the 49 BC2F1 plants obtained, 20 presented at least three markers. These plants were selfed to obtain BC2F2 plants, three of which carried at least three markers. These plants were selfed to obtain enough BC2F3 seeds to be used in field trials. As the number of BC2F2 plants with at least three markers was too small, plants harboring one or two markers were also used to produce the next generation. All BC2F3 plants selected for the field trials presented bright red grains, similar to those of cv. Vermelhinho.

For the cross Rudá "R1" x 'Ouro Vermelho', F1 plants were used to begin the backcrossing process. For the marker assisted selection, molecular markers $SCH13_{490a}$ (*Phg-1*), $SCAZ20_{940a}$ (*Co-6*), $SCF10_{1050a}$, $SCBA08_{560a}$ (*Ur-ON*), $SCAS13_{950a}$ (*Co-4*^{2}), and $SCAB03_{400a}$ (*Co-5*) were used (reviewed by MIKLAS, 2005). Out of 49 BC1F1 plants, 16 had at least five markers. These plants were selfed and 20 BC1F2 plants were obtained that harbored at least four markers. The selected BC1F2 plants were backcrossed to 'Ouro Vermelho' producing 94 BC2F1 plants. Out of those, 28 had at least four markers and those with red grains were planted, generating 148 BC2F2 plants. Sixty-eight BC2F2 families with grain aspect similar to that of 'Ouro Vermelho' were selfed and multiplied in the field for future evaluations. The resulting BC2F3 plants are being used as donor parents to introduce the resistance genes of interest in other red bean cultivars of the UFV Bean Breeding Program.

ACKNOWLEDGEMENTS

The material developed in this work will be tested for productivity and inoculated with the pathogens of interest. Those with outstanding agronomic performance will be included in national field trials.

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MYSTERIOUS BEAN YELLOWING COMPLEX IN SRI LANKA

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INTRODUCTION

Bean (*Phaseolus vulgaris*) is one of the most important and popular vegetable in Sri Lanka. From recent past low yields from bean crops was reported due to a syndrome named as bean yellowing complex (BYC). Apparently, at the present, BYC has become the most serious threat for bean crop production in Sri Lanka.

BYC was first reported from farmer fields at Balangoda in 1999. Severity of the problem varied from location, field, age and season but lead even up to 100% loss of the crop. Studies so far have not given any clue of causal agents, causes or methodologies to control it. BYC was visible in plants even at 2 weeks after seeding to any stage of plants. Affected bean crops show higher rate of affected plants and the degree of its symptoms as the crop became older. Initial symptoms were chlorosis, light yellow or light green mosaic type patches in leaves. Subsequently all the developing leaves developed yellow or mosaic symptoms. Affected plants showed slower growth, smaller leaves, thin stems, stunting of plants and less flower and pod set. Symptoms seem to be spread only upward of the plant. These symptoms are similar with the symptoms of bean yellow mosaic virus disease (Hall, 1995). Two diseases by Mycroplasmalike organisms (MLO) in bean, Witche`s-Broom (Murayama, 1966) and Machismo (Granda, 1979) were identified but the symptoms were different to BYC. All the cultivated bean varieties in Sri Lanka and tested germplasm were susceptible to BYC.

The objectives of these experiments were to identify possible causal agent for this yellowing complex in order to find an appropriate control method.

MATERIALS AND METHODS

After preliminary observations, seed transmission, relationship with bean insects and the effect of sap inoculation studies were conducted in the field and plant houses at the institute (HORDI). Affected leaf tissue samples were sent to CABI, UK for ELISA and other tests for viruses. The number of plants showed the symptoms after 4 weeks and 8 weeks of plantings were recorded. Data were analyzed using Statistical Analysis System.

Seed transmission of BYC

Seeds from BYC affected and unaffected plants of three varieties were collected in the previous season. The three varieties included two pole bean type (“Keppetipola Nil” and “Lanka Butter”) and one bush type (“Top Crop”).

Three seeds of affected or unaffected were planted in 9 L plastic pots in a soil mixture. Half of the plants were kept inside the glass house protected with insect proof net. The other half of the plants were kept outside the glass house. The experiment was replicated three times. One experiment unit was consisted with four bean plants in two pots.

Sample testing for viruses

Infected leaf samples were sent twice (2005 and 2006) to Global Plant Clinic (GPC), CABI Bio science UK Center for virus testing. Six leaf samples from bean varieties PC 50 (bush dry bean), Lanka butter and KWG (pole bean) were collected in the morning and dried under the fan for four hours. Then 3 leaflets from each sample were wrapped with tissue papers. The samples packed in a

small regiform box were air freighted to CABI within 2-3 days. The second samples included varieties “Sanjaya” (bush Bean), “KWG” and “Lanka Butter” (pole bean). CABI carried out ELIZA for 11 viruses, tested with electron microscopy and sap inoculation to indicator plants.

RESULTS AND DISCUSSION

Seed transmission of BYC

Three factors were tested on BYC symptoms. All three varieties tested were infected with yellowing at the age 4 weeks after planting. No significant differences in number of infected plants were observed 8 weeks after planting. There was no significant difference between the number of plants affected with BYC symptoms obtained from infected and uninfected seeds. BYC is not transmitted through seeds as some viruses. Some viruses such as Bean Common Mosaic and Bean Yellow Mosaic are seed borne and transmitted through seeds (Hall, 1995). Third factor tested was the involvement of insect transferring the disease. Insect proof net in the glasshouse protected the movements of bean fly, plant hoppers, thrips, aphids and white flies. No symptoms were observed inside the glasshouse but in the open field showed insect relationship with the complex.

Test for viruses

Leaf mosaic yellowing symptoms are mostly associated with viruses. But the samples tested at GPC, CABI showed negative responses to Alfalfa Mosaic Virus (AMV), BLRV, BBWV-1, Broad Bean True Mosaic Virus (BBTMV), Tomato Ring Spot Virus (ToRSV), Tobacco Ring Spot Virus (TRSV), Tobacco Necrosis Virus (TNV), Tomato Spotted Wilt Virus (TSWV), INSV, Cucumber Mosaic Virus (CMV), and Bean Golden Mosaic Virus (Begomovirus), Virus particles could not be detected from any of the above samples with electron microscopy. Sap inoculations to indicator plants also proved the absence of related viruses in the tested samples. Relationship of bean insects (bean fly, whitefly, aphids) also tested and could not trace any link. Sap inoculation studies conducted at HORDI with infected bean leaf samples also showed that no virus transmissions involved and could not observed any symptoms on inoculated plants. Application of plant nutrients also showed no link with the problem.

CONCLUSION

BYC is becoming a devastating problem in bean growing areas. The yellowing mosaic symptoms are very similar to bean yellow mosaic virus diseases. Experiments conducted so far have not yielded any solution to the problem. However, it was revealed that the transmission of symptoms was not through the seeds but there must be insect involvement for the problem. The continuous application of chemicals for available insects on bean did not control the yellowing symptoms questioning the relationship between the yellowing and most common bean insects. The sample tested twice at CABI, UK clearly indicated there is no virus relationship with the yellowing with the extra tests of electron microscopy and sap inoculations. Though, the solution was not found, our findings will direct us on a proper path to find a lasting solution to this mysterious yellowing in beans.

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SNAP BEAN BREEDING LINE WITH RESISTANCE TO BEAN YELLOW MOSAIC VIRUS (BYMV) AND CLOVER YELLOW VEIN VIRUS (CYVV)

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Pioneering plant-breeding research conducted at Cornell University produced *Phaseolus vulgaris* lines with resistance to the potyviruses: bean yellow mosaic virus (BYMV) and clover yellow vein virus (CYVV) (Scully et al., 1995). These plant-breeding lines were used by our lab to develop indicator plants to diagnose if a bean plant was infected with BYMV, CYVV or both viruses (Shail et al., 2007). However, these lines were all dry bean types, while the focus of our project is on snap beans. The objective of this project was to assess snapbean varieties and breeding lines from the US seed industry for their resistance to both BYMV and CYVV. Lines with resistance to these potyviruses were further tested in the field in comparison with standard susceptible varieties.
Twenty-one snapbean varieties or breeding lines were provided by three seed companies and tested in the greenhouse. Inoculation methods were described in our earlier BIC paper (Shail et al., 2007). Disease ratings were made on each treatment.

Table 1. Responses of selected bean varieties to BYMV and CYVV inoculation

Source	Variety/Line	BYMV	CYVV
Harris Moran			
	Caprice	4	5
	Envy	3	4
	Hystyle	5	5
	HMX 5100	4	4
Seminis			
	Banga	5	5
	Cadillac	5	5
	Goldmine	4	5
	Spartacus	3	5
	Titan	4	5
	Zeus	3	5
	15340804	4	5
Syngenta			
	Hayden	5	5
	Herrera	4	5
	Masai	4	5
	Redon	2	5
	Summit	4	4
	SB 4285	4	5
	SB 4355	4	5
	SYN 36	3	5
	SYN 55	**1**	**1**
	SYN 75	3	5

BYMV disease severity index: 1 = no foliar symptoms, 2 = mild mosaic, no stunting, 3 = mosaic, stunting, 4 = severe mosaic, stunting and leaf roll, 5 = vein necrosis

CYVV disease severity index: 1 = no foliar or pod symptoms, 2 = mild mosaic, no stunting or apical necrosis, 3 = mosaic, stunting, 4 = severe mosaic, severe stunting, leaf roll or apical necrosis, 5 = vein and apical necrosis followed by death of the plant.

SYN 55 was the only variety or breeding line to receive a rating of "1" for both BYMV and CYVV, thus indicating resistance to both viruses. The surviving plants were subsequently inoculated with CMV, but were classified as susceptible to CMV infection.

A field test was conducted at the NYSAES, Geneva, NY on July 2, 2007. Hystyle, Spartacus and SYN 55 were sown in replicated plots and a portion of each plot was inoculated at the first trifoliate with CMV (legume strain), CYVV or BYMV. Plots were then covered with a row cover (Reemay) to exclude aphid transmission of viruses. In this manner, the impact of virus could be examined regardless of virus severity in the field. Yield, and grade were recorded. Gross profit per acre and percentage reduction in yield due to virus inoculation were calculated.

Table 2. The impact of CMV, BYMV and CYVV inoculation on yield.

		Tons per acre			Gross return	% Reduction
Variety	Virus	4's and less	5's and over	Total	$ per acre	in Yield
Hystyle	Nontreated	2.31	1.73	4.04	506	-
Hystyle	CMV	1.33	0.73	2.06	270	46
Hystyle	BYMV	1.27	0.73	2.00	264	48
Hystyle	CYVV	1.19	0.64	1.83	242	55
Spartacus	Nontreated	1.52	2.18	3.70	425	-
Spartacus	CMV	1.09	2.29	3.38	377	11
Spartacus	BYMV	0.89	1.67	2.66	302	28
Spartacus	CYVV	1.12	1.64	2.76	316	24
SYN 55	Nontreated	2.68	0.17	2.85	435	-
SYN 55	CMV	2.07	0.08	2.15	328	24
SYN 55	BYMV	2.56	0.11	2.67	408	4
SYN 55	CYVV	2.40	0.10	2.50	382	7

CMV, BYMV and CYVV virus inoculation had a severe impact on Hystyle resulting in approximately 50% reduction in yield. Spartacus was also negatively affected by viruses, but to a lesser degree than Hystyle. SYN 55 showed little reduction in yield due to BYMV or CYVV, with a 24% loss due to CMV only. Thus resistant breeding lines have the potential to reduce the deleterious impact of selected viruses on yield.

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RESPONSE OF BGMV AND BGYMV RESISTANT COMMON BEAN TO BEET CURLY TOP VIRUS

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INTRODUCTION

Beet curly top virus (BCTV, a leafhopper-vectored *Curtovirus*) and closely related species are endemic to the northwestern USA. Crop losses can be severe when susceptible large-seeded Andean dry and green bean cultivars are planted early in dry areas surrounded by abandoned or wastelands full of weeds or near semi-arid rangeland. Plant dwarfing, leaf puckering and chlorosis, and lack of pod production may result on plants infected at the seedling stage. Small and medium seeded Middle American cultivars, in general, are more tolerant.

There are five distinct *Curtovirus* species in the USA most of which can infect common bean. The ubiquitous presence of resistance in most great northern, pink, pinto, and red Mexican cultivars grown in the western USA may derive from the landraces California Pink and Common Red Mexican (see Miklas, 2000). 'Burtner' is also known to have contributed curly top resistance to green bean cultivars. A SCAR marker for the dominant gene, *Bct*, conferring resistance to BCTV, was generated and mapped to chromosome 4 (Larsen and Miklas, 2004). However, the marker is only useful for large-seeded Andean bean breeding.

Symptoms similar to BCTV may also be produced by *Bean dwarf mosaic virus* (BDMV, a whitefly transmitted geminivirus), *Bean golden mosaic virus* (BGMV, a whitefly transmitted geminivirus), and *Bean golden yellow mosaic virus* (BGYMV, a whitefly transmitted geminivirus). BDMV and BGMV occur in Argentina, Bolivia, and Brazil, whereas BGYMV is found in tropical and subtropical Central America, coastal Mexico, the Caribbean, and southeastern USA. Each of these viruses may also cause severe yield losses in susceptible cultivars in favorable conditions. Our objective was to determine the response to BCTV of selected common bean genotypes with resistance to BDMV, BGMV, and BGYMV.

MATERIALS AND METHODS

Sixty-five dry and green bean genotypes of diverse origin were planted in a farmer's field near Kimberly, Idaho in 2007. A randomized complete block design with two replicates was used. Each plot consisted of 1 m long spaced 0.56 m apart. An average of 20 seeds was planted in each plot. Viruliferous leafhoppers (*Circulifer tenellus* Baker) reared on susceptible sugar beet plants were released in the nursery approximately three weeks after emergence. Data on plot basis were recorded five weeks after infestation and verified at fully developed pod stage (R8). A 1 to 5 disease rating scale was used, where 1= healthy plants with no visible disease symptoms, 2=moderately resistant, 3=moderately susceptible, 4=susceptible, and 5=highly susceptible.

RESULTS AND DISCUSSION

Of 65 dry and green bean genotypes cranberry Capri and UI 51 were highly susceptible and G 5686 and Dragone were susceptible (Table 1). Beluga, Lassen, USWA 64, Morales (with *bgm-1*),

PR0247-49, Royal Red, Yakima, and Hooter were moderately susceptible. The moderately resistant group included A 195, Common Pinto, Common Red Mexican, GMR 2, G 122, Kimberly, PR 9771-3-2, Sawtooth, Tio Canela 75, UI 686, USWA 68, and Idelight. Dry bean genotypes A 429 (with *bgm-1*), DOR 390, DOR 500, and G 2402 (synonymous with Garrapato which is the original source of *bgm-1*) did not exhibit any symptoms of BCTV. In general, breeding lines and cultivars with known resistance to BGMV and BGYMV (except Morales) were either resistant or moderately resistant to BCTV. In 2008, our intension is to repeat the experiment to verify the results obtained thus far. Nonetheless, based on our past experience and published literature it may be concluded that the race Durango cultivars and landraces are sources of genes that impart resistance to most viruses attacking common bean including BCMV and BCMNV (e.g., UI 31, UI 34, UI 35, UI 59), BCTV (e.g., California Pink, Common Red Mexican), BYMV (e.g., UI 31, UI 59), and BDMV, BGMV, and BGYMV (e.g., Garrapato). Further molecular and genetics research would be required to understand the evolutionary origin of the virulence and resistance genes for these viruses.

Table 1. Reaction of some dry and green bean genotypes to sugar beet curly top virus (BCTV) evaluated using viruliferous leafhoppers at Kimberly, Idaho in 2007.

Genotype	BCTV score†	Genotype	BCTV score
A 195	2	Morales§	3
A 429‡	1	PR0247-49	3
Beluga	3	PR9771-3-2	2
Capri	5	Royal Red	3
Common Pinto‡	2	Sawtooth‡	2
Common Red Mexican‡	2	Tio Canela 75§	2
DOR 390§	1	UI 51	5
DOR 500§	1	UI 686	2
GMR 2	2	Yakima	3
G 122	2	Idelight	2
G 2402‡	1	G 5686	4
Kimberly‡	2	Hooter	3
Lassen	3	Dragone	4
USWA 64	3	USWA 68	2

†BCTV= *Beet curly top virus*. Scored on a 1 to 5 rating scale, where 1= healthy plants with no visible disease symptoms, 2=moderately resistant, 3=moderately susceptible, 4=susceptible, and 5=highly susceptible.
‡Race Durango landrace or cultivar.
§Race Mesoamerica cultivar.

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INHERITANCE STUDIES FOR ANTHRACNOSE RESISTANCE GENES OF COMMON BEAN CULTIVAR AND 277

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AND 277 [(Cargabello x (Pompadour Checa x Linea 17) x (Linea 17 x Red Cloud))], a common bean cultivar of Andean origin, is an important source for resistance to bean angular leaf spot in Brazil. This cultivar is used in the common bean breeding program assisted by molecular markers conducted at the Federal University of Viçosa, Viçosa, MG, Brazil (Niestche, 1997; Carvalho et al. 1998). AND 277 is resistant to twenty *C. lindemuthianum* pathotypes, including the pathotype 2047 (datum recently observed) and also possesses one gene allelic to *Co-1*, the *Co-1*^{4} (Alzate-Marin et al., 2003). However, fine studies are needed for complete anthracnose resistance characterization of this important source. The aim of this work was to determine the inheritance of anthracnose resistance gene(s) present in AND 277 by analyzing segregating populations derived from crosses with susceptible cultivar Rudá and Cornell 49-242.

MATERIAL AND METHODS

F_2 plants derived from crosses between resistant (AND 277) vs. susceptible (Rudá and Cornell 49-242) cutivars and 20 seeds of each parent were planted in a greenhouse. Seven days after sowing, the first leaf from each plant was inoculated with spores of *C. lindemuthianum* pathotypes 9, 73 and 453 according to Table 1. Spores (1.2 x 10^6 conidia/ml) were sprayed onto the plant leaves using a DeVilbiss apparatus. The plants were incubated and maintained in a mist chamber (20-22 ^oC, 95% relative humidity) for seven days. After this period, each plant was scored visually for disease symptoms using a 1 - 9 scale (Rava et al., 1993) in which 1 (one) is attributed to plants with no visible symptoms and 9 (nine) to severely diseased or dead plants. Resistant (R) phenotype was assigned to plants with no or limited symptoms (grades 1 to 3) whereas plants scored as 4 or higher were considered to be susceptible (S). To avoid cross-contamination, each experiment was conducted in a separate chamber. The observed values of resistant and susceptible plants were compared with the expected values, for each tested hypothesis, through the Chi-square test.

RESULTS AND DISCUSSION

Resistance of cultivar AND 277 to *C. lindemuthianum* pathotype 453 appears to be controlled by one single gene in the cross with Rudá (segregation ratio of 3:1 in the F_2 population, Table 1). For *C. lindemuthianum* pathotype 73, the resistance of AND 277 appears to be controlled by two independent genes in the crosses with Rudá (segregation ratio of 15:1 in the F_2 population, Table 1). However, F_2 populations derived from the crosses AND 277 x Cornell inoculated with *C. lindemuthianum* pathotypes 9 and 73 segregated according to the ratio 57:7 (Table 1). These results indicate that AND 277 carries two independent dominant genes for these pathotypes, and that one of

them can interact in a complementary way with a third gene depending on the cross and pathotype tested. Preliminary results of allelism tests of cultivar AND 277 with anthracnose differential cultivars and BAT 93 confirm that one of the genes present in AND 277 is located at the same locus occupied by previously characterized alleles *Co-1*, *Co-1*^{2} and *Co-1*^{3} (Alzate-Marin et al., 2003). The other gene of AND 277 is located at the same locus occupied by previously characterized allele *Co-9* (data not shown). Other new studies are currently being conducted aiming to characterize the gene of AND 277 that confers resistance to pathotype 2047.

Table 1. Inheritance study of the anthracnose resistance gene(s) present in cultivar AND 277, in F_2 populations of crosses with cultivars Rudá and Cornell 49-242.

Pathotype	Population F_2	Reaction	Expected ratio	Observed ratio (R:S)	χ^2	P %
73-497	Rudá x AND 277	S X R	15:1	108:11	1.8201	17.7293
453-457	Rudá x AND 277	S X R	3:1	79:24	0.1585	69.047
9-UFV1	AND 277 x Cornell 49-242	R x S	57:7	118:14	0.0148	90.2893
73-497	AND 277 x Cornell 49-242	R x S	57:7	149:25	2.1018	14.7119

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EVIDENCE THAT ANTHRACNOSE RESISTANCE IN COMMON BEAN CULTIVAR WIDUSA IS CONFERRED BY MORE THAN ONE GENE

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Widusa is one of the twelve international differential cultivars for common bean anthracnose, caused by *Colletotrichum lindemuthianum*. The genetic characterization of Widusa genes is controversial (Alzate-Marin et al., 2002; Gonçalves-Vidigal et al., 2006; Rodríguez-Suárez et al., 2008). Consequently, fine genetic studies are needed to elucidate this lack of agreement. In this sense, the present work aimed to determine the number of anthracnose resistance genes in Widusa using F_2 populations derived from crosses with susceptible cultivars Rudá, TO and Cornell 49-242.

MATERIAL AND METHODS

F_2 plants derived from crosses between resistant (Widusa) vs. susceptible (Rudá, TO and Cornell 49-242) cultivars and 20 seeds of each parent were planted in a greenhouse. These crosses are part of a common bean breeding program conducted at the Federal University of Viçosa, Viçosa, MG, Brazil. Seven days after sowing, the first leaf from each plant was inoculated with spores of *C. lindemuthianum* pathotypes 9, 73 and 453 according to Table 1. Spores (1.2×10^6 conidia/ml) were sprayed onto the plant leaves using a DeVilbiss apparatus. The plants were incubated and maintained in a mist chamber (20-22 ^oC, 95% relative humidity) for seven days. After this period, each plant was scored visually for disease symptoms using a 1 - 9 scale (Rava et al., 1993) in which 1 (one) is attributed to plants with no visible symptoms and 9 (nine) to severely diseased or dead plants. Resistant (R) phenotype was assigned to plants with no or limited symptoms (grades 1 to 3) whereas plants scored as 4 or higher were considered to be susceptible (S). To avoid cross-contamination, each experiment was conducted in a separate chamber. The observed values of resistant and susceptible plants were compared with the expected values, for each tested hypothesis, through the Chi-square test.

RESULTS AND DISCUSSION

Resistance of cultivar Widusa to *C. lindemuthianum* pathotype 73 appears to be controlled by one single gene in the cross with Rudá (segregation ratio of 3:1 in the F_2 population, Table 1). Alzate-Marin et al. (2002) observed that one gene confers resistance to *C. lindemuthiahum* pathotype 65 in crosses between Rudá x Widusa. Vidigal et al. (2006) also observed one gene conferring resistance in segregating populations derived from crosses between Widusa x BAT 93 and Widusa x Cornell 49-242 for pathotypes 65 and 73, respectively. However, F_2 populations derived from the crosses Rudá x Widusa, TO x Widusa and Widusa x Cornell 49-242 inoculated with *C. lindemuthianum* pathotypes 9 and 453 segregated according to 9:7 ratio (Table 1). These results indicate that Widusa carries two independent dominant genes for resistance to these pathotypes. The recessive allele of each

gene is epistatic in relation to the dominant allele of the other gene leading to susceptibility [9 R (AABB, AABb, AaBB, AaBb): 7 S (AAbb, Aabb, aaBB, aaBb, aabb)]. The results of the current work concerning resistance to race 453 in Widusa differ from those obtained by Vidigal et al (2006). In our work, we observed two genes conferring resistance to this pathothype in the same cross (TO x Widusa) (Table 1). Our data give support to the results obtained in previous inheritance studies that show that Widusa carries two dominant genes for resistance to *C. lindemuthianum* pathotype beta and gama (Bannerot, 1965), and two dominant genes, one conferring resistance to pathotypes 65, 73, 102 and 449 and the other to pathotype 38 (Rodriguez-Soarez et al., 2008).

Table 1. Inheritance study of the anthracnose resistance gene(s) present in cultivar Widusa, in F_2 populations of crosses with cultivars Rudá, TO and Cornell 49-242.

Pathotype	Population	Reaction	Expected ratio	Observed ratio (R:S)	χ^2	P %
9-UFV1	Rudá x Widusa	S x R	9:7	124:102	0.1755	67.5193
73-497	Rudá x Widusa	S x R	3:1	178:53	0.5209	47.0448
453-457	Rudá x Widusa	S x R	9:7	119:105	0.8888	34.5778
453-457	TO x Widusa	S x R	9:7	54:30	2.204	13.7645
9-UFV1	Widusa x Cornell	R x S	9:7	40:37	0.579	44.6682

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SQ4 SCAR MARKER LINKED TO THE CO-2 GENE ON B11 APPEARS TO BE LINKED TO THE UR-11 GENE

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INTRODUCTION

Anthracnose, caused by *Colletotrichum lindemuthianum*, is the most important widespread fungal disease affecting common bean (*Phaseolus vulgaris*) worldwide. Genetic resistance in the host is the most cost effective method to control the disease. Among the 11 resistance genes characterized (Kelly and Vallejo, 2004), the *Co-2* gene was one of the first resistance genes to be tagged and mapped to linkage group B11 (Adam-Blondon et al., 1994). Young and Kelly (1996) also identified two RAPD markers, $OQ4_{1440bp}$ and $B355_{1000bp}$ flanking the *Co-2* gene and these markers mapped at 2.0 to 5.4 cM in Andean and from 5.5 to 7.7 cM in Middle American backgrounds. Renewed interest in developing a SCAR marker for the *Co-2* gene is based on widespread deployment of these markers in bean breeding programs worldwide.

MATERIALS AND METHODS

The OQ4 marker was run in agarose gel excised the polymorphic band of 1440bp and converted to SCAR marker. The fragment was purified, cloned and sequenced as described by Melotto et al., (1996). SQ4 was developed and tested in several advanced lines and varieties known to carry the *Co-2* and *Ur-11* genes. The PCR protocol used to amplify the SQ4 marker consisted of 34 cycles of 10s at 94ºC; 40s at 59ºC, 2 min at 72ºC, followed by one cycle of 5 min at 72ºC. Sequence data of SQ4 was submitted to BLASTX search of *Phaseolus vulgaris* database.

RESULTS AND DISCUSSION

The SQ4 marker amplified a single polymorphic band of 1440bp in agarose gel. The marker was present in nine varieties possessing the *Co-2* gene (Figure 1A). Since the gene mapped to B11, where a major gene cluster for rust resistance (*Ur-11* and *Ur-3*) genes resides, another group of genotypes possessing the *Ur-11* gene were screened. These included the original PI181996, Belmineb, Belmidak and Beldakmi lines all known to carry the *Ur-11* gene but not the *Co-2* gene (Figure 1B). The marker was present in all genotypes with the Ur-*11* gene. The linkage between the SQ4 and *Ur-11* gene needs to be verified in segregating population(s).

The BLASTX search revealed several consensus sequences for nucleotide binding site-leucine rich repeat (NBS-LRR)-like proteins. Among these were cD7, cD8, cBA8, cJA78, cBA11 and cJA71 partial cDNAs (Creusot et al., 1999). The cD7 and cD8 showed the best match to SQ4 and they shared 42% amino acid identity with SQ4. These two candidates belong to the same NBS-LRR subfamily. The cD7 mapped 1 cM from OFR1 and co-segregated with *Co-2*, while cD8 mapped at 2 cM from *Co-2* (Creusot et al., 1999). The other NBS-LRR candidates cBA8, cBA11 from BAT93 and cJA71, cJA78 from JaloEEP558 showed less identity and mapped to B4 linkage group (Ferrier-Cana et al., 2003). The *Co-2* and *Ur-11* genes appear to be members of a resistance gene cluster on B11 that potentially share common LRRs. This is the first report in common bean of a marker linked

to a major gene conditioning resistance to one pathogen that shows linkage to another gene conditioning resistance to a different pathogen.

Table 1. Primer sequences and length for SCAR SQ4 marker linked to *Co-2* gene

Gene	SCAR	Primer sequence	Primer length
Co-2	SQ4 F	5'- CCTTAGGTATGGTGGGAAACGA-3'	22-mer
	SQ4 R	5'- TGAGGGCGAGGATTTCAGCAAGTT-3'	24-mer

Figure 1A. SQ4 marker present in bean genotypes carrying the *Co-2* gene.

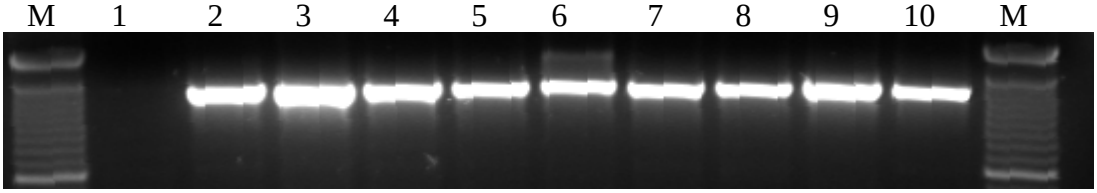

Figure 1A. Lines possessing the *Co-2* gene. M:100bp ladder; lane 1: Montcalm (*Co-1*); lane 2: Cornell (*Co-2*); lane 3: Chinook Select (*Co-2*); lane 4: Aresteuben (*Co-2*); lane 5: AC Centralia (*Co-2*); lane 6: Harofleet (*Co-2*); lane 7: Harokent (*Co-2*); lane 8: HR67 (*Co-2*); lane 9: AC Shetland (*Co-2*) lane 10: Blackhawk (*Co-2*); M:100bp

Figure 1B. SQ4 marker in bean genotpes with the *Ur-11* gene but lacking the *Co-2* gene.

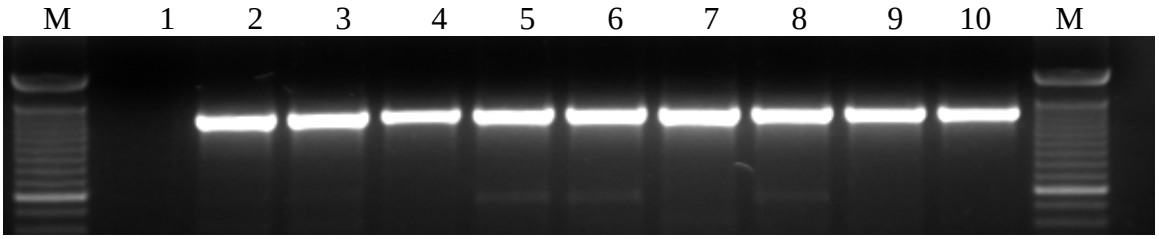

Figure 1B. Lines possessing the *Ur-11* gene. M: 100bp ladder; lane 1: Jenny (*ur-11*); lanes 2, and 3: PI 181996 (*Ur-11*); lane 4: PI 190078 (*Ur-11*); lane 5: BelMiNeb-RMR-8 (*Ur-11*); lane 6: BelDakMi –RMR-16 (*Ur-11*); lane 7: BelMiDak-RMR-10 (*Ur-11*); lane 8: BelMiNeb-RMR-13 (*Ur-11*); lane 9: BelDakMi-RMR-18 (*Ur-11*); lane 10: CNC (Ur CNC-1, 2?); M: 100bp ladder.

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VEGETATIVE COMPATIBILITY AND GENETIC ANALYSIS OF *COLLETOTRICHUM LINDEMUTHIANUM* ISOLATES FROM BRAZIL

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INTRODUCTION

The causal agent of common bean anthracnose, *Colletotrichum lindemuthianum*, presents a wide genetic and pathogenic variability that causes complications in the development of resistant cultivars. The aim of this study was to identify the variability within and between Brazilian pathotypes of *C. lindemuthianum* through the identification of vegetative compatibility groups (VGCs) and by randomly amplified polymorphic DNA (RAPD) analysis.

MATERIALS AND METHODS

Forty seven *C. lindemuthianum* isolates belonging to 13 different pathotypes were collected from naturally infected bean cultivars produced in various regions of Brazil during the period 2000 to 2006. The method described by Brooker et al. (1991) was applied to the development of *nit* mutants. Following identification, mutants were characterised phenotypically and classified as *nit1, nit2, nit3* and *nitM* according to their growth parameters (Correll et al. 1987). The vegetative self-compatibilities of different *nit* mutants of a single isolate and the cross-compatibilities between *nit* mutants of different isolates were tested by pairing mycelia plugs and the dishes were incubated in the dark at 22°C for at least 4 weeks. The growth of aerial mycelia in the contact zone between the two colonies and the formation of heterokaryons were monitored weekly. For the RAPD analysis, DNA was extracted from the isolates according to a modified version of the method of Raeder and Broda (1985). A dendrogram was produced from the similarity matrix thus generated using the unweighted pair-group method with arithmetic means (UPGMA) with the assistance of NTSYS-PC 2.1 software (Rohlf 2000).

RESULTS AND DISCUSSION

A total of 295 *nit* mutants (279 *nit3*, 15 *nit1* and one *nitM*) were obtained from 47 isolates. In complementation tests, six of the isolates were shown to be heterokaryon self-incompatible, whilst the cross-complementation observed among *nit1* and *nit3* mutants of the different isolates enabled 45 VGCs to be identified. The high frequency of formation of *nit3* mutants and the phenomenon of self-incompatibility observed in the present study has already been described in other studies (Brooker et al. 1991; Beynon et al. 1995). A correlation between the presence of L-asparagine or L-threonine in MM + chlorate and the recovery of *nitM* mutants has been established by some researchers (Leslie and Summerell 2006), but in the present study supplementation of the medium with these amino acids did not increase the frequency of *mitM* mutants. In the molecular analyses, 18 RAPD primers were employed and 111 polymorphic bands obtained. The estimates of genetic similarities, determined from the Sorence-Dice coefficient, ranged from 0.42 to 0.97, and the dendrogram obtained by cluster analysis revealed 18 separate groups of isolates (Figure1). The intra- and inter-genetic variability within the *C. lindemuthianum* population established by RAPD analysis is in agreement with previous findings (Mahuku and Riascos 2004; Talamini et al. 2006; Damasceno e

Silva et al. 2007). In the present study, groups within *C. lindemuthianum* have been identified through the generation of mutant strains and by RAPD analysis and the results confirm the immense diversity exhibited by this species. The findings underline the difficulties incurred in achieving long-term resistance against anthracnose in the common bean and emphasise the need to elucidate the mechanisms responsible for the large pathogenic variability shown by this phytopathogen.

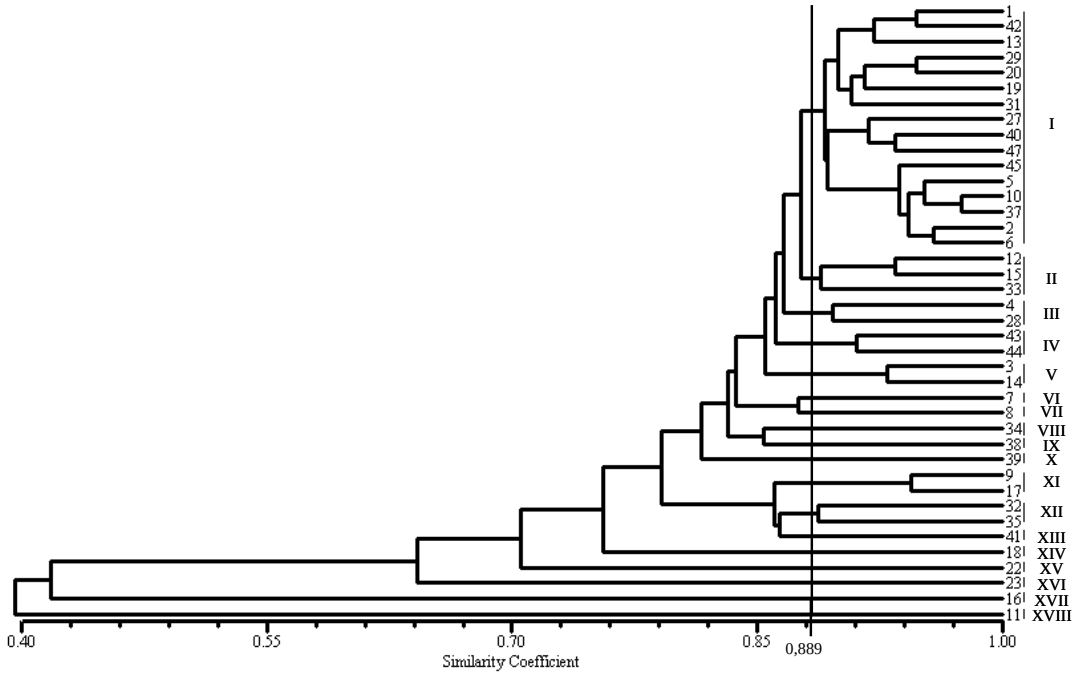

Fig. 1. Dendrogram showing the genetic similarity of isolates of *C. lindemuthianum* collected in different regions of Brazil.

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CHARACTERIZATION OF ACCESSIONS OF *PHASEOLUS VULGARIS* L. FOR REACTION TO THE FUNGUS *COLLETOTRICHUM LINDEMUTHIANUM*

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Anthracnose, incited by the fungus *Colletotrichum lindemuthianum*, is among the main diseases affecting the common bean crop. Losses caused by the disease may reach 100% when contaminated seeds are used in regions where appropriate conditions for the development of the pathogen prevail (PELOSO, 1992). Besides debilitating the plant or even causing its death, the disease leads to the occurrence of dark spots in the grains, which decrease their commercial value. The use of genetic resistance for controlling diseases like anthracnose is considered efficient and inexpensive, in addition it is ecologically sound. Therefore, studies leading to the identification of resistance sources of paramount importance. The objective of this work was to determine the reactions of 100 accesses of *P. vulgaris* of the Bean Germplasm Bank of the Universidade Federal de Viçosa, Minas Gerais, Brazil, to five *C. lindemuthianum* races.

C. lindemuthianum races 9, 64, 73, 89, and 453 characterized by RAVA et al. (1994) were used to inoculate the plants. Twelve seeds of each one of the 100 bean accesses and of cultivars Rudá (susceptible control) and Rudá "R" (resistant control) were used for each *C. lindemuthianum* race. The seeds were sown in the greenhouse in plastic trays (60 x 40 x 10 cm) containing a 4:1 mixture of soil and manure and 5 $kg.m^{-3}$ of NPK 4-14-8. The inoculum was prepared according to PIO-RIBEIRO & CHAVES (1975). Sporulation of the pathogen was achieved by transferring the mycelium from BDA medium to test tubes containing sterilized green beans pods partially immersed in agar-water medium. The test tubes were incubated for seven days at 23 °C. The final inoculum concentration used was 1.2×10^6 spores/mL. The inoculum was applied in both surfaces of the leaves with the aid of a DeVilbiss atomizer number 15 activated by an electric compressor. After inoculation and fast drying in the air, the plants were incubated for five days in mist chambers (20 ± 1^oC and >95% relative humidity), under a photoperiod of 12 hours. After this period, they were transferred back to the greenhouse, and after five days they were evaluated for the disease symptoms based on 1 to 9 scale (PASTOR-CORRALES, 1992). Accesses with average 3.6 or above were considered to be susceptible.

Out of 100 accesses evaluated, 33 were resistant to at least four of the five *C. lindemuthianum* races tested (Table 1). Out of these 33 accesses, 32 were simultaneous resistant to races 73 and 89, which are the most frequent races found in the state of Minas Gerais. It was possible to identify 12 accesses which were resistant to all five races tested (highlighted in Table 1). The 33 accesses mentioned (Table 1) can be potentially used as sources of resistance genes to anthracnose. Out of this group, Ouro Negro has already been widely used by bean improvement programs.

Table 1. Reactions of 33 common bean accessions to *Colletotrichum lindemuthianum* under artificial inoculation conditions

ACCESSION/CULTIVAR	RACE				
	9	64	73	89	453
RAÇA D	***1.00**	**1.00**	**1.57**	**1.00**	**1.14**
CNFC9444	1.66	8.00	2.28	1.00	1.40
CNFC8006	**1.00**	**1.50**	**2.21**	**1.53**	**1.06**
CNFC9466	1.20	3.83	1.00	1.00	1.00
CNFC9455	2.60	8.84	2.85	1.25	1.83
CNFC9454	1.13	3.77	2.83	1.06	2.63
CNFC9458	1.38	9.00	2.78	2.20	1.28
LM 95102682	1.00	1.00	1.65	1.53	8.78
MAJESTOSO	**2.41**	**1.00**	**1.66**	**1.20**	**1.07**
1862 SACAVEM 538	**2.00**	**1.00**	**1.00**	**1.00**	**3.46**
1864 SACAVEM 860	1.73	1.00	1.00	1.21	8.38
1868 SACAVEM 1061	**1.66**	**1.00**	**1.00**	**1.07**	**1.78**
3272	1.14	1.00	1.00	1.00	8.93
1843 55 G	1.00	1.00	1.26	1.00	6.35
1852 TAQUARI SARGES	1.73	1.00	1.00	9.00	1.14
1836S 464 VENEZUELA	**1.50**	**1.00**	**1.23**	**1.71**	**1.00**
P. 16 TRUJILLO 4	1.64	1.69	3.13	3.00	8.46
P 501(PUEBLA 199)	2.66	2.26	1.61	3.13	5.20
AN 911120	1.00	1.00	1.00	1.00	8.93
SC 9029935	**1.06**	**1.00**	**2.07**	**1.53**	**1.00**
OURO NEGRO	**1.14**	**1.00**	**2.53**	**1.00**	**1.08**
MEIA NOITE	1.57	1.00	1.07	1.00	8.53
VALENTE	1.00	1.00	1.40	1.00	5.53
LM 95103904	1.80	1.00	1.00	1.46	7.50
2970149	**1.40**	**1.00**	**3.06**	**1.00**	**1.00**
CNFJ10301	1.00	1.23	1.00	1.00	8.69
FEB-163	1.16	1.00	1.00	1.00	8.00
AN910522	3.41	1.00	3.36	2.26	8.42
1845 77 G	**1.00**	**1.25**	**1.00**	**1.00**	**1.00**
1849 FLORESTA 13041	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
1861 SACAVEM 486	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
OURO VERMELHO	1.00	1.00	1.00	1.00	5.16
VERMELHO 2157	1.57	1.00	1.00	1.00	7.61
[a]RUDÁ	9.00	4.25	9.00	9.00	8.92
[b]RUDÁ "R"	1.00	1.00	1.00	1.00	1.00

*Disease severity (average of 12 plants). Accessions with disease severity 3.6 or higher were considered susceptible; [a]susceptible control; [b]resistant control.

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VARIABILITY AMONG *COLLETOTRICHUM LINDEMUTHIANUM* ISOLATES BY RAPD MARKERS

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INTRODUCTION

Anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. & Magn.) Scribner is one of the most important diseases in common bean. Control strategies include, mainly, the development of resistant cultivars. However, the major limitation for developing durable resistance in common bean cultivars is the magnitude of variability in *C. lindemuthianum* which has been reported worldwide (Mahuku & Riascos, 2004; Talamini et al., 2004). Understanding the pathogenic variability is a fundamental point in breeding program. Combining virulence and molecular analysis will lead to a better understanding of the variability present in *C. lindemuthianum*. Therefore, the objective of this study was to analyze the pathogenic and genetic diversity of *C. lindemuthianum* isolates collected in Minas Gerais State, to generate data to be used in breeding programs for resistance to common bean anthracnose.

MATERIAL AND METHODS

48 isolates of *C. lindemuthianum* obtained from naturally-infected common bean cultivars were used in this study. Isolates were collected in four regions from Minas Gerais State, Brazil: Buriti, Coromandel, Monte Carmelo and Patos de Minas (Alto Paranaíba Region); Januária and Unaí (North of Minas Region); Lambari, Lavras and Luminárias (South of Minas Region); and Viçosa (Forest Zone Region), as shown in Table 1.

Table 1. *Colletotrichum lindemuthianum* isolates, pathotypes (P) and counties (C) of Minas Gerais State, Brazil.

Isolate	P	C[1/]	Isolate	P	C	Isolate	P	C	Isolate	P	C
1	65	BU	13	65	LM	25	81	VI	37	65	VI
2	65	BU	14	65	LM	26	81	VI	38	65	VI
3	69	BU	15	8	LV	27	81	VI	39	81	VI
4	81	BU	16	64	LV	28	81	VI	40	81	VI
5	65	CO	17	65	LV	29	81	VI	41	81	VI
6	65	MC	18	65	LV	30	89	VI	42	83	VI
7	65	MC	19	65	LV	31	89	VI	43	87	VI
8	81	PM	20	73	LV	32	65	VI	44	87	VI
9	81	PM	21	73	LV	33	65	VI	45	337	VI
10	87	PM	22[2/]	73	LV	34	65	VI	46	337	VI
11	65	JÁ	23[2/]	73	LV	35	65	VI	47	337	VI
12	81	UN	24[2/]	73	LV	36	65	VI	48	337	VI

[1/] County: BU: Buriti; CO: Coromandel; MC: Monte Carmelo; PM: Patos de Minas; JA: Januária; UN: Unaí; LM: Lambari; LV: Lavras; LU: Luminárias; VI: Viçosa.
[2/] Isolates of sexual stage (*Glomerella cingulata* f.sp. *phaseoli*)

C. lindemuthianum isolates were grown in liquid medium for 7 days (110 rpm at 20°C). The RAPD reactions were carried out with the *primers* OP A13, OP AQ11, OP AS19, OP BA03, OP BA06, OP BA08, OP BB01, OP BB03, OP BB05, OP BB08, OP BB12, OP BB13, OP BB15 and OP BB19 and

performed in a final volume of 14 µl containing 4 µl water, 35 ng of genomic DNA, 50 µM of each dNTP and 0.4 µM oligonucleotide *primer*, 50 mM Tris-HCl, pH 8.0, 2.0 mM $MgCl_2$, 20 mM KCl, and 0.6 units Taq DNA polymerase. Amplification was programmed for 1 initial desnaturation cycle (94°C for 2 minutes), followed by 38 cycles of 2 minutes at 94°C, 15 seconds at 37°C and 1 minute at 72°C and a final extension step of 2 minutes. Amplification products were separated by electrophoresis and visualized under UV light before to be photographed with the Kodak EDA – 290 photographic camera. The genetic similarities and clustering analysis were performed by using the Nei and Li coefficient and UPGMA, respectively. The analysis of molecular variance (AMOVA) was performed.

RESULTS AND DISCUSSION

A total of 64 polymorphic bands were used to analyze the 48 *C. lindemuthianum* isolates. An average of 4.57 bands was generated per *primer*. The genetic similarity among the isolates ranged from 0.65 to 0.99. The clustering showed the occurrence of five groups. For the AMOVA (Table 2), each region, except the North of Minas Region (only two isolates), was considered as a population. The AMOVA showed that the genetic differentiation among regions is significant (Φ_{ST} = 0.0394, p < 0.016), with 3.94% and 96.06% of the genetic variability being among regions and within regions (populations), respectively. The free exchange of seeds among regions may have contributed substantially to the increased variability within regions instead of among regions variance. Further clustering at pathotype level was performed, where each pathotype (except the 8, 64, 69, 83 and 89 pathotypes) was considered a population, and an analysis of molecular variance was carried out (Table 2). The greater part of the variability was detected within pathotypes (75.24%). It was also observed that the genetic variability among pathotypes was highly significant (Φ_{ST} = 0.248, p < 0,000).

Table 2. Summary of AMOVA of three regions (AP, SM and ZM), from Minas Gerais State and for seven pathotypes of fungus (*C. lindemuthianum*) evaluated by RAPD markers.

S.V.	DF	SS	Variance components	% Total	Φ_{ST}	P
Regions						
Among regions	2	23.592	0.3014	3.94	0.039	0.016
Within regions	43	315.625	7.3401	96.06		
Total	45	339.217	7.6415	100.00		
Pathotypes						
Among pathotypes	4	86.678	2.0727	24.76	0.248	0.000
Within pathotypes	37	232.989	6.2970	75.24		
Total	41	319.667	8.3696	100.00		

CONCLUSIONS

The existence of high variability, has been demonstrated which validated studies emphasizing the great potential of this fungus to generate variability, and the need to assess the mechanisms involved in obtaining this genetic variability.

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ANTHRACNOSE RESISTANT DRY BEAN CULTIVARS FROM THE MEXICAN HIGHLANDS, PATHOTYPE 1472

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Bean anthracnose is a widespread and highly variable disease in the Mexican highlands (Balardin *et al*., 1997; Gonzalez *et al*., 1998). Prior to 2006, 51 races were known in Mexico (Rodriguez *et al*., 2006) and seven new races have been recently identified in the southern state of Oaxaca (unpublished data). Race 1472 has been isolated in the states of Zacatecas, Hidalgo, Guanajuato and State of Mexico in sites around or above 2000 m in altitude, where it has been found only in rainy years. Race 1472 is a virulent race that defeats six resistance alleles carried by the differentials: Mexico 222 (*Co-3*), PI 207262 (*Co-4*^{3}, *Co-9*), To (*Co-4*) and AB 136 (*Co-6, co-8*). The main commercial bean classes in the Mexican highlands including shiny black and light colored seeded cultivars (pinto, cream, pink spotted, pink striped, etc.) and most landraces and bred cultivars grown in this wide region are susceptible to race 1472. Therefore, race 1472 was chosen to work with since it represents a high potential risk for most cultivars being grown in the highlands of Mexico and because any resistant genotype to this particular race, will be resistant to all the races that defeat all combinations of the alleles above mentioned, including the widespread race 448 (Rodriguez *et al*., 2006).

In Celaya, Guanajuato Mexico (20^{o}31'N, 100^{o}45' and 1765 masl) during 2007 more than one thousand lines (F_5 to F_7) and a set of 50 parental cultivars were grown under greenhouse conditions in 5 L pots filled with soil:peat moss (50:50v:v). Eight seeds were planted per pot and 15 d old plantlets inoculated with a spore suspension harboring 1.5 X 10^6 per ml of anthracnose race 1472 isolated in northern Guanajuato from infected tissue of a Flor de Mayo landrace. Inoculated plantlets were kept at 22±5°C and 90% relative humidity for 72 h and then grown under normal greenhouse conditions at the same temperature. Plantlets were scored 10 days after inoculation by using a scale from 0 to 4 as proposed by Garrido-Ramírez and Romero-Cova (1989).

Results showed a relatively low but important percentage of lines (10%) and even some commercial cultivars, i.e., Negro Otomi and Pinto Villa, which displayed a heterogeneous response to the inoculation. This response can be useful for: (1) saving resistant plants to purify the cultivar, a process we are conducting with the cultivars above, and (2) saving both, resistant and susceptible plants from individual lines to develop near isogenic lines. Around 35% of evaluated lines resulted resistant, in different proportions within each population derived from simple, three and four way cross; some of them including the parental genotypes shown in Table 1. Those lines were developed from individual plants selected at least during three generations under rainfed conditions from plants exposed to natural incidence of bean common blight, root-rots and rust.

Some important commercial cultivars that resulted susceptible to race 1472 include: Pinto Saltillo (Dgo. race), Flor de Mayo Anita (Jal.), Flor de Junio Marcela (Jal.), Flor de Durazno (NG) Bayomex (NG) Azufrado 26 (NG), ICA Palmar (NG), Negro 8025 (MA), and Negro Tacana (MA). Since some of these cultivars are in high demand by consumers we are incorporating resistance by conventional means (crossing and inoculating) by using as resistant donors local improved cultivars. In Table 1 we describe a set of parental genotypes that were resistant to race 1472. Two growth habits and diverse seed classes are represented among these resistant cultivars. The specific resistant

alleles in these cultivars need to be determined. Three cultivars are Andean in origin and many of other cultivars include Andean parents in their pedigree (Rosales-Serna *et al*., 2004).

Table 1. Dry bean cultivars from the Mexican highlands resistant to anthracnose pathotype 1472.

Cultivar	Growth Habit	Seed type	Race	Comments
Bayo Mecentral	III	Cream	Jalisco	Root-rot resistant
Flor de Mayo Noura	III	Pink spotted on cream	Jalisco	Rainfall adapted
Afn	III	Black stripped	-	Snap cultivar
Cacahuate 72[1]	I	Pink stripped on white	Nueva Granada	Early maturing
Canario 60	I	Cream	Nueva Granada	Early maturing
Canario 107	I	Cream	Nueva Granada	Early maturing
Bayo Zacatecas	III	Cream	Durango	Rainfall adapted
Bayo Victoria	III	Cream	Durango	Rainfall adapted
Flor de Junio Silvia	III	Pink stripped on cream	Jalisco	For irrigated conditions
FM 98116	III	Pink spotted on cream	Jalisco	Rainfall adapted
PT 98001	III	Brown spotted on cream	Durango	Rainfall adapted

[1] It was previously reported as heterogeneous in response to same anthracnose race (Rodriguez *et al*., 2006).

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PATHOGENIC VARIABILITY OF CAUSAL AGENT OF COMMON BEAN ANTHRACNOSE

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INTRODUCTION

The high variability of *Colletotrichum lindemuthianum* has resulted in continuous breakdown of resistance in commercial cultivars. Studies on the variability of *C. lindemuthianum* are needed to direct breeding efforts towards long-term resistance to anthracnose. The objective of this work was to investigate the pathogenic variability in isolates collected in different counties in Brazil in the last 4 years.

MATERIAL AND METHODS

Fifty three isolates collected in different counties in Brazil in the last four years (2004-2007) were inoculated on 12 differential cultivars proposed by CIAT (1990). Forty eight isolates were collected in the state of Minas Gerais (Lavras, Lambari, Nepomuceno, Ijaci, Madre de Deus, Patos de Minas, São Vicente de Minas, Cana Verde, Guarda Mor e Viçosa), three isolates in Paraná (Pinhão, Guarapuava e Turvo) and two isolates were from São Paulo (Campinas). Fungus was isolated from infected plant tissues. Monosporic cultures were grown in M3 medium. To obtain high sporulation, each one isolate was inoculated in pods culture medium and were incubated at 22± 2ºC for 10-15dias in darkness. Seedlings with fully expanded primary leaves were sprayed with the conidial suspension (1.2×10^6 conídios.mL^{-1}).

Inoculated plants were incubated in a humidity chamber (20 ± 2ºC) for 72 h with a 12 h photoperiod. After 7-10 days of inoculation, seedlings were evaluated for their disease reaction using a scale from 1 to 9 (Schoonhoven and Pastor-Corrales, 1987). Plants with disease reaction scores from 1-3 were considered resistant, whereas plants that were scored from 4-9 were considered susceptible.

RESULTS AND DISCUSSION

The 53 isolates were classified into 12 different pathotypes (Table 1). Pathotype 65 was the most frequent (43.4%) and widely distributed in Minas Gerais State. These results confirm the wide pathogenic variability in *C. lindemuthianum* in Brazil (Talamini et al., 2004; Damasceno e Silva et al., 2007). Talamini et al. (2004) used isolates collected between 2001-2002 and observed the pathotypes 65, 81, 337, 87, 73, 64, 0, 593, 83, 89 and 8. In the present study, we observed a higher frequency of pathotypes 65, 81, 73 and 64, but there was a reduction of pathotype 337. The pathotypes 83, 87, 89 and 593 were not observed. Different pathotypes that occurred in this state in the past (69, 83, 85, 87, 89 and 119) (Alzate-Marin & Sartorato, 2004) did not found in the present study. Similar results were obtained by Damasceno e Silva et al. (2007) that identified the pathotype 65, 81 and 73 were the most frequent of Minas Gerais. Isolates from Paraná were classified into pathotypes 321, 81 and 8. Alzate-Marin & Sartorato (2004) report pathotype 321 and 81 in this State. The two isolates from São Paulo belonging to pathotype 71 and 64. Knowledge of the variability of the fungus in each region is an important basis to establish a breeding program and to choose the main source of resistance.

Table 1. Pathotypes of *C. lindemuthianum* identified in the last four years (2004-2007) in Brazil.

	DIFFERENTIAL CULTIVARS[1]												
Pathotype	2^0	2^1	2^2	2^3	2^4	2^5	2^6	2^7	2^8	2^9	2^{10}	2^{11}	Number of isolates per Pathotype
0	-	-	-	-	-	-	-	-	-	-	-	-	1
8	-	-	-	+	-	-	-	-	-	-	-	-	1
9	+	-	-	+	-	-	-	-	-	-	-	-	1
64	-	-	-	-	-	-	+	-	-	-	-	-	7
65	+	-	-	-	-	-	+	-	-	-	-	-	23
71	+	+	+	-	-	-	+	-	-	-	-	-	1
72	-	-	-	+	-	-	+	-	-	-	-	-	2
73	+	-	-	+	-	-	+	-	-	-	-	-	5
81	+	-	-	-	+	-	+	-	-	-	-	-	8
321	+	-	-	-	-	-	+	-	+	-	-	-	1
329	+	-	-	+	-	-	+	-	+	-	-	-	1
337	+	-	-	+	-	-	+	-	+	-	-	-	2

[+] susceptible; [-] resistant; [1] Michelite (2^0), Michigan Dark Red Kidney (2^1), Perry Marrow (2^2), Cornell 49-242 (2^3), Widusa (2^4), Kaboon (2^5), México 222 (2^6), PI 207262 (2^7), TO (2^8), TU (2^9), AB 136 (2^{10}) and G 2333 (2^{11})

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GENETIC AND PATHOGENIC VARIABILITY WITHIN RACE 65 OF CAUSAL AGENT OF COMMON BEAN ANTHRACNOSE

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INTRODUCTION

The causal agent of common bean anthracnose, *Colletotrichum lindemuthianum*, presents a wide genetic and pathogenic variability that has complicated the development of resistant cultivars. In Brazil, more than 50 races have been identified, and the races 65, 73 and 81 are the most frequent in the last years (Silva et al. 2007). Some studies have underlined the wide dissemination and adaptation of race 65 in Brazil mainly in the State of Minas Gerais (Alzate-Marin & Sartorato 2004; Silva et al. 2007).

The objective of this study was to identify the variation within race 65, from isolates collected in State of Minas Gerais through RAPD markers and anastomosis groups and to evaluate the aggressivity in common bean cultivars in order to generate information for using in breeding programs for resistance to anthracnose disease.

MATERIAL AND METHODS

Thirteen isolates (LV 28, LV 29, LV 55, LV 57, LV 58, LV59, LV61, LV 73, LV 80, LV 89, LV 90, CL 837 and CL 844) from the race 65 collected in different counties of Minas Gerais State in Brazil, host cultivars and years, were used in RAPD and anastomosis analyses.

DNA manipulation and statistical analysis were made according to the methodology described by Silva et al. (2007). Anastomosis analysis was set up as described by Rodríguez-Guerra et al. (2003) with modifications and the genetic similarities among isolates were estimated by using the Russel & Rao coefficient (1940).

Six isolates (LV 29, LV 57, LV 58, LV 61, CL 837 and CL 844) were inoculated in seven commercial cultivars (Ouro Negro, OPNS-331, Pérola, Rosinha, Talismã, Valente and VC-3) in the 10^2, 10^3, 10^4, 10^5 and 10^6 spores.mL^{-1} concentrations like described by Silva et al. (2007).

RESULTS AND DISCUSSION

Twenty-four RAPD primers were used and amplified 83 polymorphic bands. The estimates of genetic similarities ranged from 0.54 to 0.82. The UPGMA cluster analysis allowed to identify 11 groups. Only two groups contained two isolates considered similar, group III (LV 57 and LV 58) and group V (LV 59 and LV 73), showing the high genetic variability within race. The anastomosis analysis were carried out considering as compatible when fusion of hyphae from the paired of isolates was observed. The proportion of compatible reactions for each isolate was estimated. Similarity estimates ranges from 0.28 to 0.85. Eleven groups of anastomosis were obtained and only the isolates LV 61, LV 73 e LV 58 were classified in the same anastomosis group, although these isolates were distinct by RAPD analysis. Both analyses showed the great variability within the race 65.

There is difference in the aggressivity of isolates from race 65, when inoculated in the commercial cultivars, being the CL 837 and CL 844 isolates, the most aggressive. Cultivars Pérola and Rosinha were the most susceptible at all concentrations and isolates. Cultivar Talismã, recommended for the State of Minas Gerais, was released as resistant to the races 65, 81 and 89 of *C. lindemuthianum*. In this study, it was possible to observe that the cultivar presented susceptibility reaction to the isolates Cl 844 and Cl 837 at 10^6 and LV 29 and Cl 844 at 10^5 spores.mL^{-1}. Cultivars OPNS-331 and VC-3 reacted similarly to race 65 isolates being susceptible to Cl 837 and Cl 844 isolates. Cultivar Valente is susceptible only to Cl 837 isolate. Among all commercial cultivars under study, Ouro Negro was the only one with resistance reaction to all race 65 isolates of *C. lindemuthianum*.

The isolates inoculated presented differences regarding pathogenicity in commercial cultivars, also showed to be genetically different by RAPD and anastomosis analyses. Thus it is obvious that the set of differential cultivars to identify the *C. lindemuthianum* races is inefficient to detect the difference within race 65, indicating that additional cultivars should be used.

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ARE COMMON BEAN *CO-3* AND *CO-7* RESISTANT ALLELES TO ANTHRACNOSE THE SAME?

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INTRODUCTION

Resistance is the best way of controlling the anthracnose caused by *Colletotrichum lindemuthianum* in common bean. Resistance major genes have been used in breeding programs and many of them are already known (Kelly and Vallejo 2004). Therefore, the resistance alleles need to be precisely identified for efficiently helping the breeder. Results from inoculation of parents and segregating populations with different pathotypes have pointed out *Co-3* and *Co-7* dominant alleles, considered different genes, as being the same (Pastor-Corrales et al. 1994, Young et al. 1998, Alzate-Marin et al. 2001, Pereira and Santos 2004). In this article the objective was to verify if *Co-3* and *Co-7* alleles are the same.

MATERIALS AND METHODS

Line H1 and the differential cultivar Mexico 222 (*Co-3*) were crossed, and the F_1, F_2 and F_3 generations obtained. Line H1 is derived from CI 140 (susceptible) x [ESAL 696 (*Co-5*) x G2333 (*Co-4*^{2}, *Co-5*, *Co-7*)] cross, it is susceptible to pathotypes 2047 and 73, and resistant to pathotype 1545, having therefore the *Co-7* allele (Pereira and Santos 2004).

Parents and the F_3 population were inoculated with pathotypes 8 and 65 of *C. lindemuthianum*. Each pathotype was previously characterized against the anthracnose differential cultivars to confirm its identity. Monosporic culture was the source of inoculum used for each pathotype, which grown on sterilized common bean pods and agar medium in glass tubes. Spores (1.2×10^6 conidia/ml) were sprayed on 10-day-old plants, and incubated in a mist chamber (20^0C $\pm 2^0$C, 95% - 100% relative humidity and 12/12 hours day/night) during 72 hours. Then the plants were moved to a greenhouse for seven days, with sprinkler irrigation every four hours. Disease symptoms were scored visually per plant using a 1-9 scale (Rava et al. 1993). Plants receiving 1-3 grade were considered resistant whereas plants graded 4 or greater were considered susceptible.

RESULTS AND DISCUSSION

The parents and 35 F_3 plants were 100% resistant to pathotype 8. Conversely, the parents and 80 F_3 plants were 100% susceptible to pathotype 65. Sixteen plants were inoculated per each parent and pathotype. These results suggest that both parents have identical genotypes for reaction to both pathotypes. One possibility for this identity is that the *Co-3* allele from Mexico 222 is the same allele present in line H1 derived from G2333, identified as *Co-7* so far.

The allele for resistance in the host and the allele for virulence in the pathogen can both be identified by the binary number (Robinson 1979, Kelly and Vallejo 2005). Hence Mexico 222 has the 64 resistance allele which is broken by the pathotypes 64, and 65 (64 + 1), although it is not broken by the pathotype 8. Then we can say that pathotype allele breaks resistant allele of the same number (Robinson 1979). The same reaction of line H1 (*Co-7*), derived from G2333 indicates that *Co-7* and

Co-3 are the same allele. Accordingly the segregation of 15:1 resistant/susceptible in F_2, from the SEL 1360 (*Co-5*) x G2333(*Co-4*^{2}, *Co-5*, *Co-7*) cross, after inoculating with pathotypes 521 (512 + 8 + 1) and 1545 (1024 + 512 + 8 + 1), indicates that the two resistant alleles are *Co-4*^{2} (2048) and *Co-7* (64) (Young et al. 1998). Identical segregation was observed by Alzate-Marin et al. (2001) in the F_2 from Rudá (susceptible) x G2333 cross, after inoculating with pathotypes 73 (64 + 8 + 1) and 89 (64 + 16 + 8 + 1), indicating in this case that *Co-4*^{2} and *Co-5* (512) are the resistant alleles. Based on these two results the *Co-7* (64) was not broken by the 1 or 8 virulence alleles, and was broken by the 64 allele confirming *Co-7* as the same *Co-3* allele.

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VEGETATIVE COMPATIBILITY AND DIPLOID FORMATION BETWEEN ISOLATES OF *COLLETOTRICHUM LINDEMUTHIANUM* RACE 65

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INTRODUCTION

Colletotrichum lindemuthianum (teleomorph: *Glomerella cingulata f. sp. phaseoli, G. lindemuthiana*) is the causal agent of anthracnose in common bean (*Phaseolus vulgaris* L.). This pathogen presents a high genetic variability that result in continuous overcoming resistance of commercial cultivars. Anthracnose control of common bean depends then, not only on development of resistant cultivars, but also on the comprehension of pathways that provides genetic variability in *C. lindemuthianum* pathogen. Hyphal anastomoses between isolates vegetatively compatible promote the exchange of genetic material in filamentous fungi, leading to the formation of heterokaryons vegetative, which characterize the first stage of the parasexual cycle (Saupe, 2000). Isolates which form stable heterokaryons between themselves comprise the same vegetative compatibility group (VCG). This study had as objective to examine the genetic variability of race 65 isolates of *C. lindemuthianum*, through the characterization of vegetative compatibility groups and the isolation of diploid strains throughout parasexual cycle.

MATERIAL AND METHODS

Seven isolates of race 65 of C. *lindemuthianum*, derived from different localities were utilized and maintained at a temperature of 5°C in BM (Castro-Prado et al., 2007). In order to obtain heterokaryons and diploids, mycelium plugs (5mm) of each *nit* mutant were paired equidistantly apart (approximately 1.0 cm) in petri dishes containing BM + $NaNO_3$ for vegetative complementation tests. Plates were incubated at 22°C for 12 to 21 days and then examined for prototrophic heterokaryotic growth. Prototrophic (*nit*+) and homogeneous fast-growing sectors, arising from heterokaryons grown in BM, were isolated and transferred to BM + benomyl (0.5 µg mL^{-1}) to detect the presence of unstable diploids. Mitotic segregants showing *nit* phenotype were recovered directly from diploid colonies.

RESULTS AND DISCUSSION

Stable *nit* mutants obtained in the presence of potassium chlorate were screened for their ability to utilize different nitrogen sources in order to determine their phenotypes and could be divided into three distinct phenotypic classes: *nit1* (nitrate non-utilizing), *Nit3* (nitrite and nitrate non-utilizing), and *NitM* (hypoxanthine and nitrate non-utilizing). There were obtained 459 nitrate non-utilizing mutants from the seven *C. lindemuthianum* isolates, in which 345 *nit1*, 79 *NitM* and 35 *Nit3*. After pairing of complementary *nit* mutants were identified: After pairing of complementary *nit* mutants were identified: VCG1 contained isolates 65-1, 65-7, 65-11, 65-12, 65-15 and 65-408, and VCG 2, that allocated the 65-8 isolate. Diploid colonies were recovered from heterokaryons growing in BM two or three weeks after pairing in BM. Diploids showed *nit*+ phenotype and growth rate similar to that of the original wild-type strains in BM (Figure 1A). When conidia or mycelium plugs from prototrophic sectors were transferred to BM or benomyl-supplemented BM, their mitotic instability could be identified by the production of auxotrophic segregants (or sectors), which showed parental

(*NitM* or *nit1*) phenotypes (Table 1, Figure 1B). The identification of different vegetative compatibility groups and the isolation of diploid strains formed with complementary *nit* mutants suggest that the parasexuality may be an important mechanism for generating genetic variability in *C. lindemuthianum.*

Table 1. Phenotypes of mitotic (parasexual) segregants derived from diploid colonies of *C. lindemuthianum*, race 65.

Diploid colonies	**Parasexual segregants**		
	Number of segregants	Phenotypes	
		nit1	*NitM*
NitM (65-15-14) // *nit1* (65-15-7)	28	22	06
NitM (65-15-14) // *nit1* (65-15-3)	156	51	105

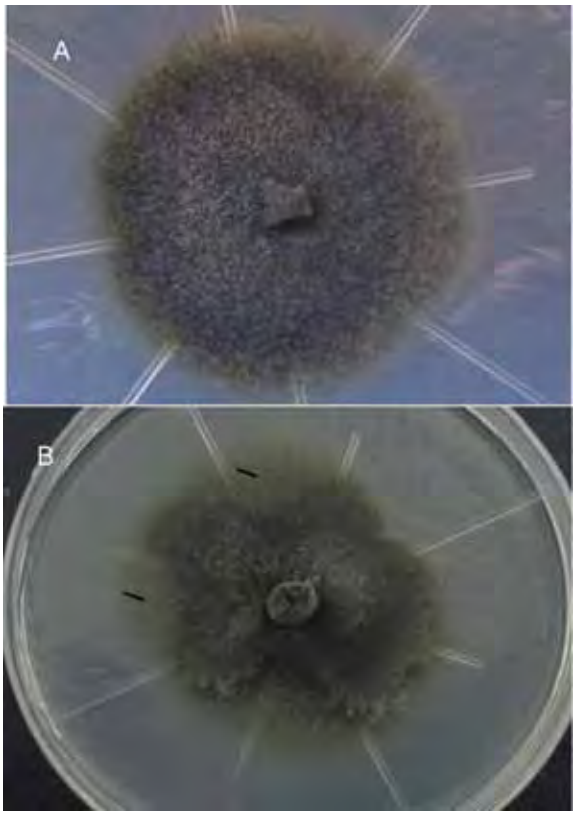

Figure 1. A. Prototrophic diploid strain 65-15//65-15 growing in Basal medium. B. Mitotic (parasexual segregants) originating from 65-16//65-16 diploid strain.

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NEW RACES OF *COLLETOTRICHUM LINDEMUTHIANUM* IN COMMON BEAN (*PHASEOLUS VULGARIS* L.) IN PARANÁ STATE, BRAZIL

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INTRODUCTION

Anthracnose is one of the most widespread and economically important worldwide common bean diseases (Balardin et al., 1997; Pastor-Corrales, 2005). This disease, which is caused by the fungus *Colletotrichum lindemuthianum* is particularly important and recurrent in sub-tropical and temperate bean production in the regions of the world. Bean anthracnose is exacerbated by the extensive virulence diversity of *C. lindemuthianum* and the seedborne nature of the pathogen (Menezes and Dianese, 1988; Pastor-Corrales and Tu, 1989; Thomazella et al., 2002; Damasceno e Silva et al., 2007). The occurrence monitoring of *C. lindemuthianum* races, present in regions of cultivation is necessary as a way to facilitate the using of pathogen genetic variability for effective control of this disease. The objective of this work was to characterize races of *C. lindemuthianum* current in crops of eight common bean producers from Paraná State.

MATERIAL AND METHODS

During the period of 2006 and 2007, a total of twenty samples of *C. lindemuthianum* were collected on pods from infected plants of commercial field-grown common bean in Paraná state, Brazil. Cultures from each sample were transferred to Petri dishes containing PDA (potato-dextrose agar) medium. The virulence phenotype of each monosporic isolate was confirmed by inoculating the standard set of 12 differential cultivars. Fourteen-days-old bean seedlings were sprayed with a concentration of 1.2×10^6 spores mL^{-1}. The plants were incubated and maintained in a mist chamber for seven days, at 22 ^oC and 90-100% relative humidity. Ten days after inoculation, the plants were scored as resistant (R) or susceptible (S) as described by (Pastor-Corrales, 1991), where 1 to 3 = resistant and 4 to 9 = susceptible.

RESULTS AND DISCUSSION

Twenty isolates of *Colletotrichum lindemuthianum* were analyzed based on virulence to 12 differential cultivars of *Phaseolus vulgaris* L. Fourteen distinct races were identified, six of which had not been reported previously in Paraná (Table 1). This is the first report of the occurrence of 10, 11, 17, 26, 27, 75 and 83 races of *C. lindemuthianum*. Races 73 and 89 were most common (15%). All isolates were incompatible with Kaboon, PI 207262, TO, TU, AB 136 and G 2333 cultivars. Some isolates infected not only differential cultivar of Mesoamerican origin, but also the ones of Andean origin.

Table 1. Reaction of differential cultivars to *C. lindemuthianum* isolates from Paraná state.

Isolate	**Differential Cultivars***												Race
	A	B	C	D	E	F	G	H	I	J	K	L	
1	S	R	R	S	R	R	S	R	R	R	R	R	73
2	R	R	R	R	R	R	S	R	R	R	R	R	64
3	S	R	R	S	R	R	S	R	R	R	R	R	73
4	S	S	R	S	S	R	R	R	R	R	R	R	27
5	S	S	R	S	R	R	S	R	R	R	R	R	11
6	S	S	R	R	S	R	S	R	R	R	R	R	83
7	S	R	R	R	S	R	S	R	R	R	R	R	81
8	S	R	R	S	R	R	S	R	R	R	R	R	73
9	S	S	S	R	S	R	S	R	R	R	R	R	87
10	S	R	R	S	S	R	S	R	R	R	R	R	89
11	S	R	R	S	S	R	S	R	R	R	R	R	89
12	R	S	R	S	S	R	R	R	R	R	R	R	26
13	S	R	R	S	S	R	S	R	R	R	R	R	89
14	R	R	R	S	R	R	S	R	R	R	R	R	72
15	S	S	R	S	R	R	S	R	R	R	R	R	75
16	R	R	R	R	R	R	R	R	R	R	R	R	0
17	R	S	R	S	R	R	R	R	R	R	R	R	10
18	S	S	R	S	R	R	S	R	R	R	R	R	75
19	S	R	R	R	S	R	R	R	R	R	R	R	17
20	S	R	R	R	S	R	S	R	R	R	R	R	81

*Common bean differential cultivars used to characterize races of *C. lindemuthianum* followed by there respective binary value (Pastor-Corrales, 1991): A- Michelite (1); B- Michigan Dark Red Kidney (2); C- Perry Marrow (4); D- Cornell 49-242 (8); E- Widusa (16); F- Kaboon (32); G- Mexico 222 (64); H- PI 207262 (128); I- TO (256); J- TU (512); K- AB 136 (1024); L- G 2333 (2048). S = Susceptible, R = Resistant.

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EPIDEMIOLOGY OF ANTHRACNOSE IN COMMON BEAN LINES "PER SE" AND IN MIXTURE

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INTRODUCTION

The various pathotypes of the fungus *Colletotrichum lindemuthianum* hamper improvement studies, since the useful lifetime of a newly developed cultivar is ephemeral. Alternatives are proposals to increase the durability of resistance. The most discussed is gene pyramiding (Alzate-Marin et al., 2001; Sartorato, 2002). This strategy is labor-intensive and cannot eliminate the possibility that the surge of a new pathotype would break the resistance again. Another option, used little in common bean, is the use of a multiline. In this case, lines with different resistance alleles are mixed. Theoretically, with the mixture, the selection pressure in the pathogen is lower and the durability of resistance tends to be greater (Mundt, 2002). To date, the technique of a multiline has not been exploited in common bean. This study had the objective to investigate the epidemiology of anthracnose in common bean lines "per se" and in mixture, aiming to verify whether a mixture of lines with different resistance alleles could provide more durable resistance.

MATERIAL AND METHODS

The experiment was conducted in the experimental area of the Departamento de Biologia of the Universidade Federal de Lavras (UFLA) (21º 14' S, 44º 59' W and mean altitude of 919 m asl), sown in February 2007. Six common bean lines of carioca grain were used, agronomically uniform with different resistance reactions to the *C. lindemuthianum* fungus. These were evaluated individually and in mixture of all in equal proportion, totaling eight treatments. The experimental design was of randomized blocks with six replications and the plots consisted of three rows of 3m , spaced 0.5m apart.

Twenty days after emergence, the seedlings were inoculated with a mixture of the races 65, 72, 81, 87, 89, and 337 of *C. lindemuthianum*, in order to ensure the presence of the pathogen in the experimental area. The first evaluation of disease symptoms was performed 10 days after inoculation using a 1 to 9 grade scale proposed by Rava et al. (1993), where 1 indicates absence of symptoms and 9, totally diseased plants. Seven such evaluations were performed every 10 days until the harvest. Equations of linear regression between the independent (date of evaluation) and the dependent variable (grade of anthracnose severity) were estimated for each one of the treatments. Grain yield data per plot were also obtained.

RESULTS AND DISCUSSION

Significant differences were observed between the mean performance of the treatments for the trait reaction to anthracnose in all evaluations, except in the first. The lowest mean grade, considering all

evaluations, was given to line MA-II-22, followed by the multiline, with a mean of 2.9. The highest mean of 6.9 indicated the susceptibility of line CI-107.

As expected, the means grades of anthracnose severity increased gradually throughout the evaluations in all lines, including in the multiline, as the positive estimates of the linear regression coefficients show (Table 1). Furthermore, a lower estimate of b_1 was observed for the multiline and for line MA-II-22, evidencing that the multiline was efficient in reducing the disease progress. The opposite was true for line CI-107 and cultivar Carioca, which is resistant to race 72 of *C. lindemuthianum* only. Cultivar MA-II-22 is resistant to race 65, which predominated in the experiment, i.e., in competition with the other races, it was the most adapted cultivar.

This reaction to the pathogen was reflected in the grain yield. The most resistant lines and the multiline performed best (Table 1). Yields were lowest in line CI-107 and Carioca, which had been expected, owing to the high pathogen incidence in these lines.

TABLE 1. Estimates of the coefficients of the linear regression equation between the independent variable (date of evaluation) and the dependent variable (anthracnose severity grade), and grain yield in g/plot, obtained in the lines and multiline evaluations.

Lines	b_0	b_1	Yield
Carioca	0.94	1.29(0.000)	306 b[1/]
Talismã	0.61	1.05(0.000)	723 b
RC-I-8	1.17	0.48(0.000)	1043 a
MA-II-8	1.23	0.44(0.000)	1030 a
MA-II-16	1.01	0.56(0.000)	1058 a
MA-II-22	1.36	0.28(0.000)	1108 a
CI-107	2.07	1.20(0.004)	302 b
Multiline	1.37	0.38(0.000)	907 a

[1/] Means followed by the same letter did not differ from each other in belong to the same group by the test of Scott & Knott (1974), at 5% probability.

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PHENOTYPICAL EVALUATION OF RESISTANCE SOURCES TO COMMON BEAN ANGULAR LEAF SPOT BY USING RACES OCURRING IN THE STATE OF MINAS GERAIS, BRAZL

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Angular leaf spot, incited by the fungus *Pseudocercospora griseola,* is one of the most important diseases affecting the common bean in Brazil. One of the major goals of the BIOAGRO/UFV Bean Breeding Program is to develop bean cultivars resistant to this pathogen. In previous works, five resistance sources were identified: AND 277, BAT 332, Cornell 49-242, MAR-2 and Mexico 54 (Table 1). The objective of this work was to determine the reactions of these five resistance sources to 17 isolates of the fungus collected in different bean growing regions of the state of Minas Gerais, Brazil.

Twelve seeds of each of the five resistance sources were sown in the greenhouse in 2.5 L pots (three plants per pot). Conidia suspensions were obtained by scrapping the agar surface in Petri dishes where the fungus was grown in a tomato juice medium. The suspensions were filtered through a gauze layer and the final conidia concentration was adjusted to 2.0×10^4 conidia/mL (PASTOR-CORRALES & JARA, 1995). The inoculations were performed in the abaxial and adaxial faces of the first trifoliolate leaves, using a De Vilbiss number 15 atomizer activated by an electric compressor. After the inoculations, the plants were transferred to a mist chamber (23 ºC and > 95% relative humidity), where they stayed for 48 hours, under a photoperiod of 12 hours. After this period, they were taken to the greenhouse, where they remained until symptom evaluation. The severity of the disease was visually evaluated 15, 18 and 21 days after the inoculations, using a nine-degree severity scale described by SCHOONHOVEN & PASTOR-CORRALES (1987). In this work, plants with grades 1 to 3 (absence of injuries with sporulations) were considered to be resistant, while plants with grades 4 to 9 (presence of injuries with sporulation) were considered to be susceptible.

Mexico 54, Cornell 49-242, BAT 332, AND 277 and MAR-2 were resistant to 11, 11, 8, 9 and 10 of the isolates, respectively (Table 2). SANGLARD et al. (2007) used four of these resistance sources to create a gene pyramid for resistance to angular leaf spot. Potentially the lines obtained by intercrossing these resistance sources will be resistant to most of the isolates tested in this work, except B_1 46 and B_7 50 which have been classified as race 63.63 (Table 2).

Pathogen characterization is an important step in the initial phases of the breeding process. It allows the identification of resistance sources and determination of the resistance genes inheritance modes. Our results clearly show that more common bean genotypes need to be characterized and new angular leaf spot resistance sources must be incorporated in the BIOAGRO/UFV Bean Breeding Program. None of the five sources used by the breeding program was resistant to all races collected in the state of Minas Gerais.

Table 1. Resistance sources to angular leaf spot with their respective genes

Resistance source	Genes (alleles)
Cornell 49-242	*Phg-3*
México 54	*Phg-2*, *Phg-5*, *Phg-6*
MAR-2	*Phg-4*, *Phg-5*[2]
BAT 332	*Phg-6*[2]
AND 277	*Phg-1*[a], *Phg-2*[2], *Phg-3*[2], *Phg-4*[2]

[a]Gene characterized by CARVALHO et al. (1998); the other genes were characterized by CAIXETA et al. (2002; 2005).

Table 2. Evaluation of resistance sources to angular leaf spot used by the BIOAGRO/UFV Bean Breeding Program

N°	Isolate	Race	Reaction of Resistance Sources				
			MAR-2	AND 277	México 54	BAT 332	Cornell 49-242
1	A_1 13	15.7	R	S	R	R	R
2	A_2 4	63.7	R	S	R	R	R
3	B_1 46	63.63	S	S	S	S	S
4	B_3 8	63.47	R	R	S	R	S
5	B_4 4	47.39	S	S	R	R	S
6	B_4 6	31.4	S	S	R	R	R
7	B_7 50	63.63	S	S	S	S	S
8	C_1 17	3.23	S	S	R	S	R
9	C_1 28	63.6	R	R	R	R	R
10	C_2 10	23.23	R	R	R	S	R
11	CM_1 2	63.63	S	R	S	S	S
12	CM_3 11	63.31	S	R	S	S	R
13	Coimbra 20	63.7	R	R	R	R	R
14	Coimbra 21	31.7	R	R	R	R	R
15	SM 32	63.23	R	R	R	S	R
16	Viçosa 3	63.23	R	R	R	S	R
17	Viçosa 7	63.63	R	S	S	S	S

R = Resistant, grades 1 to 3; S = Susceptible, grades 4 to 9.

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PATHOGENIC VARIABILITY AMONG ISOLATES OF *PSEUDOCERCOSPORA GRISEOLA* COLLECTED IN MINAS GERAIS STATE

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) can host several pathogens, including the *Pseudocercospora griseola* (Sacc.) Crous & U. Braun fungus, causal agent of angular leaf spot (ALS). This disease is responsible for significant crop damage in Brazil. Yield losses may reach up to 70%. The development of resistant cultivars is pivotal to any effective strategy used to control ALS. Constant evaluation of pathogenic variability is of crucial importance for the development of adequate pathogen resistant cultivars. The objective of this study was to evaluate the pathogenic variability of 48 isolates of *P. griseola*, collected in Minas Gerais State, Brazil.

MATERIAL AND METHODS

A collection of 48 isolates of *P. griseola* was obtained from naturally-infected bean leaves and pods collected in experimental fields in the counties of Lavras (27 isolates), Ijaci (19 isolates) and Alterosa (2 isolates), in the state of Minas Gerais, MG, Brazil. A set of 12 differential cultivars, plus the Rosinha G-2 cultivar (susceptible) and AND 277 cultivar (resistant), were used to classify *P. griseola* pathotypes. Seeds from differential cultivars were sowed in aluminum pots at a density of five seeds per pot containing 2.0 kg of soil. Spores for inoculation were obtained by culturing the fungus on bean leaf-dextrose-agar medium. Inoculum concentration was adjusted at the level of $2x10^4$ conidia.ml^{-1}. The first trifoliate leaf from each differential cultivars was inoculated (on both sides) at the V_3 development stage. The inoculated plants were incubated in a moist chamber, > 95% of RH, for 48 h (22±2ºC) with a 16-h photoperiod and then transferred to the greenhouse. Disease reactions were scored 14-18 days after inoculation according to the 1-9 descriptive scale developed at CIAT. Plants rated 1 to 3 presenting incompatible reaction, whereas plants with scores 4 or higher presenting compatible reaction.

RESULTS AND DISCUSSION

Isolates were classified into 10 pathotypes (Table 1). Pathotype 63-63 was the most widespread. Pathotypes 55-15 and 63-25 were identified just in the county of Lavras-MG. Pathotype 63-27 occurred only in the county of Alterosa-MG. Pathotypes 55-15, 63-15, 63-25 and 63-27 had not been previously reported in Minas Gerais State. Furthermore, this is the first report on the occurrence of pathotypes 55-15, 63-25 and 63-27 in Brazil. Pyramiding resistance alleles from both gene pools (Andean and Mesoamerican) can be an efficient control strategy, considering that ALS genetic resistance is monogenic (Mahuku et al. 2002; Sartorato, 2004). Taking into account that the ALS genetic resistance is inheritance more complex, the recurrent selection is a good alternative, since it provides an increasing number of favorable resistance alleles to the same lineage (Ramalho et al. 2001). The pathotypes (63-31 and 63-63) presented wide adaptation to different regions, generated by the free grain trade within the state. The high frequency of the pathotype 63-63 observed in this work poses a risk due to a wide pathogenicity spectrum, revealing the need for a continuous search

for new ALS resistance. The differential cultivars BAT 332 (*Phg-6*^{2}) and Cornell 49-242 (*Phg-3*) are important sources of resistance for a breeding program to control ALS.

CONCLUSIONS

A large variability among *P. griseola* isolates has been demonstrated, emphasizing the great potential of this fungus to generate variability. Information gained from this study has significant implications for regional ALS resistance breeding.

TABLE 1 Pathotype identification and reaction of differential cultivars to the isolates of *Pseudocercospora griseola* collected in Minas Gerais State.

Pathotype	Differential cultivars												Number of isolates
	Andean[a]						Mesoamerican[b]						
	2^0	2^1	2^2	2^3	2^4	2^5	2^0	2^1	2^2	2^3	2^4	2^5	
Lavras													**27**
55-15	+[c]	+	+	-[d]	+	+	+	+	+	+	-	-	1
63-15	+	+	+	+	+	+	+	+	+	+	-	-	1
63-25	+	+	+	+	+	+	+	-	-	+	+	-	1
63-31	+	+	+	+	+	+	+	+	+	+	+	-	6
63-47	+	+	+	+	+	+	+	+	+	+	-	+	1
63-63	+	+	+	+	+	+	+	+	+	+	+	+	17
Ijací													**19**
63-07	+	+	+	+	+	+	+	+	+	-	-	-	1
63-15	+	+	+	+	+	+	+	+	+	+	-	-	2
63-23	+	+	+	+	+	+	+	+	+	-	+	-	1
63-31	+	+	+	+	+	+	+	+	+	+	+	-	6
63-47	+	+	+	+	+	+	+	+	+	+	-	+	2
63-55	+	+	+	+	+	+	+	+	+	-	+	+	2
63-63	+	+	+	+	+	+	+	+	+	+	+	+	5
Alterosa													**2**
63-27	+	+	+	+	+	+	+	+	-	+	+	-	1
63-63	+	+	+	+	+	+	+	+	+	+	+	+	1
Total	48	48	48	47	48	48	48	47	46	44	40	28	48

[a] 2^0 - Don Timóteo; 2^1 – G11796; 2^2 – Bolón Bayo; 2^3 – Montcalm; 2^4 – Amendoin; 2^5 – G5686.
[b] 2^0 – Pan 72; 2^1 – G2858; 2^2 – Flor de Mayo; 2^3 – Mexico 54; 2^4 – BAT 332; 2^5 – Cornell 49-242.
[c] Compatible reaction (+).
[d] Incompatible reaction (-).

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EFFECT OF BEAN GENOTYPE ON THE OCCURRENCE OF BACTERIAL WILT SYMPTOMS CAUSED BY *CURTOBACTERIUM FLACCUMFACIENS* PV. *FLACCUMFACIENS*

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Leaf wilting, with or without interveinal necrotic lesions, leaf shriveling, stunted growth and plants death are the main symptoms of bacterial wilt on bean caused by the bacterium *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*. The pathogen is seed-borne and therefore the use of bacteria-free seeds and of resistant varieties is the only efficient means of disease control. A number of varieties with resistance reaction to bacterial wilt under field and greenhouse conditions have been identified (Coyne et al., 1963; Kiryakov and Genchev, 2000 and 2005; Maringoni, 2002; Hsieh et al., 2005). Different disease scales have been used for evaluation of the bacterial wilt resistance, most of them involving the degree of leaf wilting and plant stunting. Coyne et al. (1965) used a 5-degree scale to determine the resistance to bacterial wilt in the progenies of three crosses between resistant and susceptible genotypes. Plant stunting was a symptom of susceptibility – degrees 3 and 4. No correlation was observed between leaf wilting and growth stunting in some bean genotype (Kiryakov and Genchev, 2005). A data for the effect of the bean genotype on the degree of wilting and stunting symptoms expression are presented in this paper.

MATERIAL AND METHODS

A total of 285 common bean accessions from the core collection of DAI – General Toshevo were studied in a field experiment. The accessions were sown in 2 m rows, 0.5 m between the rows, in two replicates in randomized block design of the accessions. The plants were inoculated with bacterial suspension 10^8 cfu/ml from isolate CC 96212 (yellow strain) in the cotyledonary node, after removing the cotyledons at stage V_2 . Four plants per row were injected with sterile water as a check (Coyne et al., 1965). The bacterial wilt reaction of the plants was read 28 days after inoculation (R6). Two 9-degree disease scales were used: The first scale included interveinal necrotic lesions and wilting of trifoliates: 1 – no symptoms; 3 – single trifoliate leaves exhibit necrosis and wilting; 5 – several trifoliate leaves exhibit necrosis and wilting; 7 – more trifoliate leaves exhibit necrosis and wilting; 9 – plants are dead. The second scale included different degrees of plant stunt: 1 – vigorous growth; 3 – weak vigour; 5 – plant stunting about ¾ according to the check; 7 – plant stunting up to ½; 9 – plant stunting over ½. Plant wilt index (PWI) and plant stunting index (PSI) were calculated by the formula: PWI/PSI= $\Sigma(nw_{(s)})N$, where n = number of plants, $w_{(s)}$ = wilt/stunt rating (0-9), N = total number of plants.

RESULTS AND DISCUSSION

The 285 bean genotypes were grouped in 4 main classes on the basis of PWI and PSI values (Table 1). Class A included 21 accessions with high resistance to isolate CC96212 of *C.f.* pv. *flaccumfaciens*. Class B included 57 accessions with symptoms of wilting without plant stunting. In 6 of them PWI was over 5.0 – Ukraina 8 (PWI = 7.7), DG 96-1-2 (8.3), GN UI59 (6.0), etc. Class C included 19 accessions without symptoms of wilting and PSI within the range of 1.1 - 5.0 (Figure 1a). This group involved accessions MX 1834 (PSI = 4.5), Pukliv 1 (5.0), Tzaparevo 8 (4.3), Kavrikovo (5.0), Red Cloud (5.0), etc. Te rest of the accessions belonged to class D. This class included varieties and lines with different rating of disease symptoms expression. In 30 of the accessions PWI and PSI values were over 5.0 (Figure 1b).

Table 1. Clustering of 285 common bean accessions on the basis of their PWI and PSI values to *C.f.* pv. *flaccumfaciens* under field conditions.

Class	PWI	PSI	Number	%
A	1.0	1.0	21	7.37
B	up to 5.0	1.0	51	17.89
	over 5.0	1.0	6	2.11
C	1.0	up to 5.0	19	6.67
D	up to 5.0	up to 5.0	88	30.88
	over 5.0	up to 5.0	63	22.11
	up to 5.0	over 5.0	7	2.46
	over 5.0	over 5.0	30	10.53
Total			285	100

The resistance of common bean to bacterial wilt is controlled by two complementary recessive genes in crosses GN. Nebraska #1/PI 165078 и GN. Nebraska #1 sel. 27/PI 165078, and quantitatively in cross GN 1140/PI 165078 (Coyne et al., 1965). The results obtained allow the assumption that resistance to leaf wilting and growth stunting in the studied accessions are under different genes or QTL control.

A B

Figure 1. Bacterial wilt symptoms on common bean. A) Line MX 1834 with plant stunting symptoms (arrows). B) Variety Ludogorie with wilting and shriveling

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CHARACTERIZATION OF THE GENOMIC REGION CONTAINING A MAJOR QTL CONDITIONING COMMON BACTERIAL BLIGHT RESISTANCE IN COMMON BEAN

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ABSTRACT

A major QTL conditioning common bacterial blight (CBB) resistance was delimited to a 3.8 cM region on chromosome 6 of common bean (*Phaseolus vulgaris* L.) line HR45. Two contigs containing 18 BAC clones were assembled covering about 870 kb of the QTL region. *Hind* III digested DNA of four BAC clones from the tiling path was used as probes to screen cDNA library. Fifteen positive cDNA clones were identified. Northern analyses showed that a few cDNA clones were either up- or down-regulated at 12 hrs after infection. The cDNA clones were also used as probes to hybridize membranes with *Hind* III digested BAC DNA. Five promising cDNA clones ranging from 1.5 to 2.5 kb were completely sequenced and about 1.5 kb sequence of each of the rest was obtained. Through BLAST search, 11 cDNA have sequence similarity to various segments from 1.7 to 46.6 kb region of the BAC clone 71F18 of Mesoamerican wild line G02771, which is at the upstream of APA family. BAC-end and other cDNA clone sequences showed significant and high similarities at short segments (100-200 bp) with Zinc finger, gag-pol poly-protein, ATP synthesis. These results may indicate that the CBB resistance QTL genomic region is more complex and the CBB resistance mechanism may be different from those single dominant genes, such as *Co-2* and *I,* which confer anthracnose and bean common mosaic virus resistance and have similar TIR-NBS-LRR domains as other plant R genes.

INTRODUCTION

Common bacterial blight (CBB) is one of the major diseases causing yield and quality reduction in common bean. Planting resistant varieties is the economic and environmentally sound way to protect the common bean production. One major QTL providing resistance to CBB was transferred from *Phaseolus acutifolius* L. into *P. vulgaris* L. through interspecific crossing (Thomas and Waines 1984). Four PCR markers tightly linked to this QTL were developed (Yu *et al*. 2000, 2004; Liu *et al*. 2008). Partial physical map was constructed on this QTL. The objective of this research is to characterize the QTL genomic region using northern and southern analysis and to identify additional genes located at this region through cDNA library screening and sequencing.

MATERIALS AND METHODS

Construction of cDNA library: HR45 leaves were inoculated with CBB isolates at 10 days old then were harvested at a series of time from 4hrs to 5 days and pooled to extract total RNA and poly A^+ RNA was separated. Directional cDNA was synthesized using ZAP-cDNA synthesis kit from

Stratagene. The cDNA was cloned into E*coR*I and X*ho* I sites of Lamada Uni-ZAP XR vector. The primary library has over 97% recombinants and an average insert size of 2 kb.

cDNA library screening: Culture bacterial cells (XL1-blue) to plate cDNA library with a series of dilutions and calculate the titer of the library. cDNA clones were plated into 25,000 plaques per plate then transferred to nylon membrane. ^{32}P labeled HindIII digested target BAC DNA was hybridized onto the membranes. Second screening followed the same procedure to pick single positive cDNA clones.

Northern and southern analyses of target cDNA clones: target cDNA clone DNA was hybridized onto membranes of RNA extracted from leaf tissues growing at 6, 12, and 24 hrs after infection. These cDNA clones were also hybridized onto membranes with 18 different HindIII digested BAC DNA in the major QTL region.

Sequence analyses of target cDNA clones: target cDNA clones were sequenced and searched against public database to find DNA sequences or genes with high similarity.

RESULTS

Four BAC clones identified by at least one of the four markers that are tightly linked to the major QTL were digested with HindIII and to screen cDNA library. Fifteen positive cDNA clones were identified.

Northern analyses showed that some of the cDNA are either up- or down regulated at 12 hrs after inoculation, which is consistent with our findings using cDNA-AFLP analyses of CBB resistance.

Southern analyses confirmed that these cDNA clones are homologous to many other fragments of the18 BAC clones besides the four that were used to screen the cDNA library (Fig. 1)

Complete sequence was obtained from 5 target cDNA clones and about 1 kb sequence was confirmed for other target cDNA clones. Public database were searched using these cDNA clone sequence. Some cDNA clones are related to phosphate starved root EST or drought related mRNA while others are related to LHY protein which is confirmed by northern analyses. 11 cDNA have high similarity with various regions from 1.7 to 46.6 kb of a BAC clone from Mesoamerican wild line G02771, which is the upstream of APA gene family. This preliminary result showed that the CBB resistance QTL region in HR45 is fairly complex and the CBB resistance mechanism may be different from those single dominant R genes.

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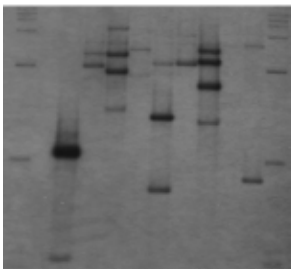

Fig.1 Southern blot analysis of 10 different BAC clones, digested with HindIII, using 12f cDNA as a Probe. Lanes 1 and 12 are standard. Lanes 2 to 11 are different BAC clones.

CHEMICAL CONTROL OF COMMON BEAN BLIGHT ON SUSCEPTIBLE PINTO CULTIVARS IN GUANAJUATO, MEXICO

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Common bean blight (CBB) (*Xanthomonas campestris* pv. *phaseoli*) is an important disease in the highlands of Mexico (Navarrete-Maya *et al*., 2006). The incidence and severity of CBB reduces seed yield and seed quality (Aggour *et al*., 1989), hampering the seed production of susceptible cultivars that are in demand due to other advantageous traits. For that reason, agrochemicals are considered as an option for the control of CBB despite the increase in cost of production. The aim of this work was to define the product, number of applications and efficiency of four agrochemicals in the control of CBB in two susceptible pinto bean cultivars.

The trial was conducted under natural disease occurrence at the Campo Experimental Bajío-INIFAP, Celaya, Guanajuato, Mexico during the rainy season of 2007. Ten rows 100 m length of each susceptible cultivar 'Pinto Durango' and 'Pinto Saltillo' were established under rainfed conditions. The crop was sown on August 3rd, 2007, and the recommended cultural practices for the crop in the area were applied. Experimental plots of 20 m were used per agrochemical, with 2 m plots marked and sampled for determinations. The commercial products: Folicur (fungicide), Cupravit mix, Bactrol and Agrimycin 500, were each applied one (single and double dosage) and three times per 20m single row, leaving untreated row between treatments. The applications started at the pre-flowering stage 43 days after sowing (das), and were done every seven days. Rates of individual applications corresponded to: Folicur 1.5 L ha^{-1}, Cupravix mix 3.0 L ha^{-1}, Bactrol 150 g ha^{-1} and Agrymicin 625 g ha^{-1}. Additionally, a foliar fertilizer treatment (triple 18-18-0, N-P-K plus minor elements, at a rate of two g per L of water) was applied twice to a single 20m row, coinciding with the last two applications made for the treatment with three applications. Determinations included scoring of all occurring diseases with emphasis on CBB using a 1 to 9 visual scale (Schoonhoven and Pastor-Corrales, 1987). Before the application of treatments, CBB was visually scored; a second reading was made at 64 das and a last one at physiological maturity (104 das) with the aim of scoring the incidence and severity of CBB on pods. Seed samples were taken to the laboratory in order to determine the transmission of CBB (Schaad and Roth, 1989).

Climatic conditions were conducive to the occurrence of several diseases including CBB. In the initial evaluation of CBB, the score across all plots on Pinto Durango was from 2.67 to 3.00 and from 1.33 to 1.67 in Pinto Saltillo, scores that increased to 5.75 and 4.0, respectively at the end of the trial. Pinto Durango showed a lower score with one application of Agrimycin 500 (3.25); the application of Cupravit and Agrimycin 500 were efficient to control rust (*Uromyces appendiculatus* var. *appendiculatus*) as well. Pinto Saltillo showed a lower score with one applicaton and the double dosis of Cupravit and Agrimycin 500, a similar result was observed with Folicur. The CBB score on all these treatments was half the score of untreated control plots. The application of foliar fertilizer showed an irregular response with some control of CBB. During the crop cycle other diseases were present, such as rust, root rots (*Fusarium* spp., *Rhizoctonia solani*), BCMV and BCMNV, halo blight (*Pseudomonas syringae* pv. *phaseolicola*), powdery mildew (*Erysiphe poligoni*) and the presence of Mexican bean beetle (*Epilachna varivestis*).

Regarding pod infection, Pinto Durango had a plant incidence from 66.5 to 100 % and the severity ranged from 2.8 to 4.3, whereas in Pinto Saltillo the incidence was from 52.5 to 98.7 % with a severity from 4.0 to 5.8. On pods, CBB was more aggressive on Pinto Saltillo. The products used controlled to certain extent CBB on foliar tissues, and did not display any effect on the pods. Perhaps the effect of the products had already being diluted by the time of pod formation, allowing for the bacteria to infect the pod walls. In pods, halo

blight (*Pseudomonas syringae* pv. *phaseolicola*) was also observed, with higher severity on cultivar Pinto Saltillo (data no shown).

Table 1. Number of colonies of *Xanthomonas campestris* pv *phaseoli* obtained from water used to rinse the seeds of each cultivar/treatment and plated on Nutritive Agar and percent of infected seeds plated on PDA.

Treatment/cultivar		Pinto Durango		Pinto Saltillo	
	Applications	Number of colonies[&]	% Infected seeds[1]	Number of colonies[&]	% Infected seeds[1]
Folicur	1	2.00	0.00	0.50	5.00
	2[2]	1.00	0.00	38.25	0.00
	3	27.00	0.00	0.00	0.00
Control		0.75	0.00	0.25	0.00
Cupravit mix	1	0.25	0.00	0.25	0.00
	2[2]	2.25	0.00	0.75	0.00
	3	0.00	0.00	0.75	0.00
Control		0.75	0.00	0.25	0.00
Bactrol	1	0.50	5.00	0.25	0.00
	2[2]	0.00	0.00	0.50	0.00
	3	1.00	5.00	0.25	0.00
Control		0.75	0.00	0.25	0.00
Agrimicyn 500	1	0.50	5.00	0.00	0.00
	2[2]	0.50	5.00	0.25	15.00
	3	1.50	5.00	0.00	0.00
Control		0.75	0.00	0.25	0.00
Foliar fertilizer		0.25	0.00	0.25	0.00
		27.50	0.00	0.25	5.00
		0.75	0.00	0.25	0.00
Control		0.75	0.00	0.25	0.00

[&] Seeds washed with sterile water and the water striated on Nutritive agar NA, [+]seeds disinfected with sodium hypochlorite 1% and incubated on Potato Dextrose Agar PDA, each datum is the average of four replicates. [2]2: sprayed once with double dosage.

In both cultivars all plants from the fertilized and control plots were surface contaminated with CBB, while the seed of Pinto Saltillo obtained from plants treated with three applications of Folicur and one and three of Agrymicin 500 resulted clean, as well as the seeds of Pinto Durango from plants treated with three applications of Bactrol (Table 1). The plants sprayed once with double dosage showed some toxicity symptoms few days after the product was applied (data not shown). On the seeds plated on PDA, Pinto Durango showed contaminated seeds under all treatments with Agrymicin 500 and two of bactrol, while for Pinto Saltillo the seeds from all treatments with Cupravit mix and Bactrol resulted clean. While true resistant cultivars are being developed, the use of some agrochemicals will allow for the production of clean seed of susceptible cultivars.

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DIFFERENTIAL INTERACTION OF *XANTHOMONAS AXONOPODIS* PV. *PHASEOLI* ISOLATES AND COMMON BEANS GENOTYPES

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Bacterial common blight caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*) affect common beans yields. Aiming to evaluate significant differential interaction 33 cultivars and lines of common bean were inoculated with six *Xap* isolates (*Xap* 1, *Xap* 2 *Xap* 3 *Xap* 4, *Xap* 5 e *Xap* 6) proceeding from Ponta Grossa-Paraná State. These inoculations were made under greenhouse conditions at Embrapa Rice & Beans, on a complete randomized block design, with three replicates. Inoculation was carried out by cutting the first trifoliate leaves using scissor previously plunged into a bacterial suspension (10^8 c.f.u. ml^{-1}) at 10 days after emergence. There were done two cuts facing each other at a distance of two cm on a perpendicular orientation in relation to the central vein. Evaluation of disease severity was done at eight and ten days after inoculation by using a severity notes scale varying from zero to six. Data were analyzed by variance analysis and it was used the mean of disease severity to perform a partial diallel analysis. The determination of the estimative of horizontal and vertical resistance of the genotypes and the virulence of *Xap* isolates was done according to the model proposed by Melo and Santos (1999). Races x genotypes interaction were significant being strongest for BRS Esplendor, BRS Executivo and BRS Pioneiro (Figure 1) when inoculated with six *Xap* isolates. Differences in virulence of the pathogen was observed, for example, the isolate *Xap* 2 was the most pathogenic for the cultivar BRS Esplendor and the isolate *Xap 4* showed the lowest virulence (Figure 1). The cultivars BRS Esplendor, BRS Pontal, Corrente, BRS Vereda, BRS Campeiro showed great horizontal resistance (Table 1). The pronounced interaction for races x cultivars concerning the association of virulence/vertical resistance in this pathosystem suggest the necessity of establishment of a differential series of common bean genotypes for the classification of *Xanthomonas axonopodis* pv. *phaseoli* races.

Table 1. Horizontal resistance and notes of the 10 common beans cultivars inoculated witch six Xap races.

Cultivar	Horizontal resistance	notes (mean)
BRS Esplendor	-0,90	2,40
BRS Pontal	-0,81	2,41
BRS Vereda	-0,63	2,68
Corrente	-0,57	2,67
BRS Campeiro	-0,38	2,78
BRS Marfim	-0,37	2,79
BRS Pitanga	-0,27	3,07
BRS Executivo	-0,22	3,07
Aporé	-0,21	3,02
Pérola	-0,18	3,03

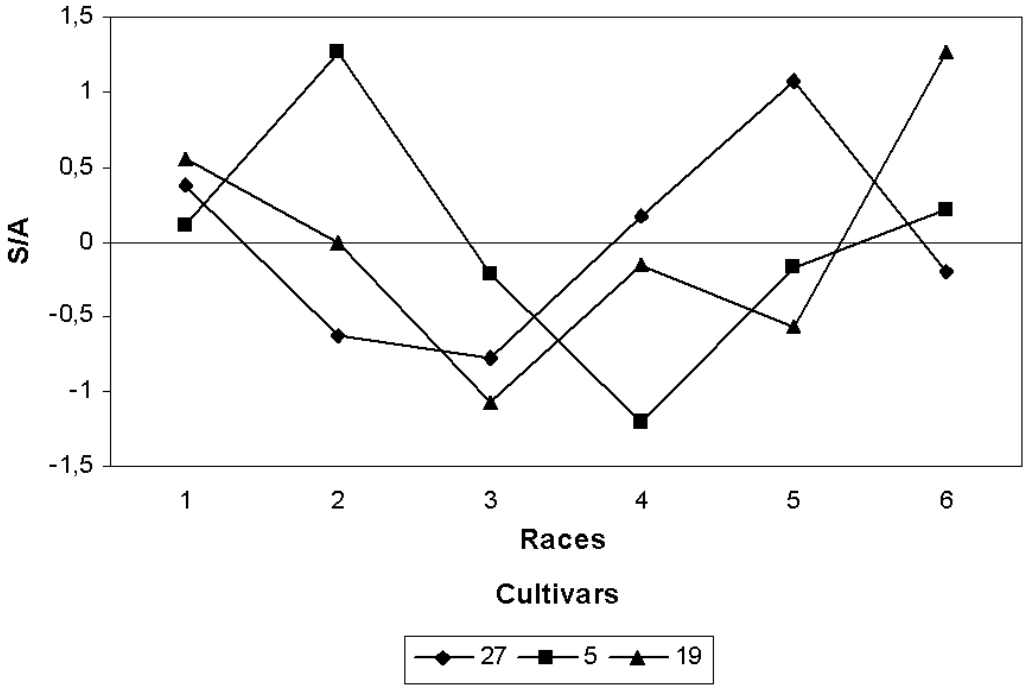

Figure 1. Differential interaction of the common bean cultivars (27- BRS Pioneiro, 5- BRS Esplendor e 19- BRS Executivo) inoculated with six *Xap* races.

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REACTION OF COMMON BEAN LINES TO PHAKOPSORA PACHYRHIZI IN BRAZIL

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INTRODUCTION

Soybean rust incited by the fungus *Phakopsora pachyrhizi* H. Sydow & P. Sydow has caused severe damages to soybean production in Brazil. The high severity and variability of the pathogen as well as its wide range of host species prevent the effective genetic control of the disease and identification of resistance sources. *P. pachyrhizi* also represents a potential threat to another economically important crop in Brazil, the common bean (*Phaseolus vulgaris*). Soybean rust has been reported in common bean varieties gown under field and controlled conditions (Preez *et al*., 2005; Pastor-Corrales *et al*., 2006; Miles *et al*., 2007). For this reason, many bean breeders are concerned because this pathogen may also become a serious problem to the common bean crop in endemic areas. The main goal of the present work was to identify soybean rust resistance sources among common bean lines maintained in the *P. vulgaris* germplasm bank of Bioagro/UFV.

MATERIAL AND METHODS

Ten plants from each one of 45 *P. vulgaris* lines and two susceptible soybean control cultivars (Table 1) used were inoculated with *P. pachyrhizi* spores. Among the *P. vulgaris* lines are resistance sources to common bean diseases, such as rust, anthracnose and angular leaf spot, Brazilian commercial cultivars with "carioca-type", "black-type" and "red-type" genetic backgrounds, and common bean elite lines developed by the Bioagro/UFV breeding program. The initial inoculum of *P. pachyrhizi* was obtained from soybean plants infected under field conditions in UFV experimental stations located in the cities of Viçosa, Coimbra and Tocantins, state of Minas Gerais, Brazil. The primary leaves and the first trifolium of all plants were inoculated about 15 days after sowing using a solution containing a pool of *P. pachyrhizi* spores (3.0×10^5 spores/mL). Disease intensity was evaluated at 15, 20 and 25 days after the inoculations based on a 1-to-5 scale modified from Stavely (1985), where 1 = no sporulation, 2 = sporulation present but less than 10% of fully sporulating lesions, 3 = sporulation present and 11 to 25% of fully sporulating lesions, 4 = sporulation present and 26 to 40% of fully sporulating lesions, and 5 = sporulation present and 65 to 100% of fully sporulating lesions.

RESULTS AND DISCUSSION

Out of the 45 common bean lines analyzed, 14 were considered resistant to *P. pachyrhizi* (mean of disease intensity ≤ 3.00) and the other 31 were considered to be susceptible (mean of disease intensity > 3.00) (Table 1). There were not immune lines to soybean rust among the 45 tested lines (Table 1, Figure 1). Three resistant bean lines, 'PI 181996', 'Pérola' and 'Redlands Pioneer', were considered to be promising sources for resistance to *P. pachyrhizi* (mean of disease intensity of 2.25 ± 0.26, 2.20 ± 0.41 and 2.20 ± 0.35, respectively) (Table 1). The resistance inheritance mode of these three lines is underway so they can be used as resistance sources in the Bioagro/UFV bean breeding program.

Table 1. Reaction of common bean lines and soybean cultivars to *P. pachyrhizi* expressed in mean of disease intensity (1-to-5 scores) with the respective standard errors (SE).

Common bean line	Reaction*		Common bean line	Reaction*	
	Mean	SE		Mean	SE
AB 136	3.75	0.35	P-49-8-2 (Bioagro/UFV)	3.65	0.24
AND 277	4.55	0.37	Pérola	2.20	0.41
Aurora	4.15	0.34	PI 181996	2.25	0.26
BAT 332	3.20	0.35	PI 260418	4.50	0.33
Brow Beauty	3.00	0.00	Pinto Olathe	4.70	0.42
CNC	3.75	0.26	R-127-4-13 (Bioagro/UFV)	2.85	0.24
Cornell 49-242	4.65	0.34	R-127-10-14 (Bioagro/UFV)	2.95	0.37
California Small White 643	4.65	0.34	R-97-13-5 (Bioagro/UFV)	3.80	0.26
Diamante Negro	4.55	0.37	R-97-13-6 (Bioagro/UFV)	2.95	0.16
Dorado	4.65	0.41	Redlands Pioneer	2.20	0.35
G 2333	4.15	0.24	Rudá	4.65	0.34
Golden Gate Wax	4.20	0.35	SEL 1308	4.60	0.32
IAPAR 14	2.95	0.44	Small White (UFLA)	4.60	0.39
IAPAR 16	2.95	0.16	Talismã	4.00	0.24
IAPAR 57	3.00	0.33	TO	3.80	0.26
Jalo EEP 558	2.75	0.42	TU	4.55	0.44
Mar 2	4.15	0.24	US Pinto 111	4.75	0.26
Mexico 235	4.15	0.34	Valente	2.75	0.35
Mexico 309	4.55	0.16	Vermelhinho	4.60	0.46
Mexico 54	4.65	0.41	Vermelho 2157	4.20	0.35
Montcalm	4.40	0.39	Vi-4899/Pioneiro (Bioagro/UFV)	3.00	0.33
Ouro Negro	3.35	0.24	**Soybean cultivar (susceptible control)**		
Ouro Vermelho	4.55	0.37	CAC-1	5.00	0.00
P-33-5-1 (Bioagro/UFV)	3.00	0.00	Cristalina	4.95	0.16

* Resistant: mean of disease intensity ≤ 3.00; susceptible: mean of disease intensity > 3.00.

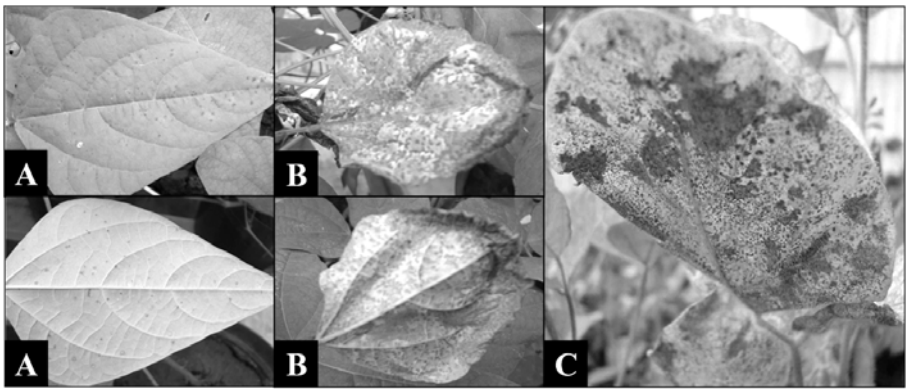

Figure 1. Soybean rust intensity on *P. vulgaris* and soybean plants. A: common bean resistant line PI 181996, mean score of 2.25 ± 0.26; B: common bean susceptible cultivar US Pinto 111, mean score of 4.75 ± 0.26; C: soybean susceptible cultivar CAC-1, mean score 5.00 ± 0.00.

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USE OF TRAP MARKERS TO MAP RESISTANCE TO A NEW RACE OF COMMON BEAN RUST IN MICHIGAN

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INTRODUCTION

Common bean rust, caused by the hypervariable fungal pathogen *Uromyces appendiculatus* (Pers.:Pers) Unger, severely limits common bean (*Phaseolus vulgaris* L.) production worldwide (Steadman et al., 2002). Many races of this pathogen have been characterized, but several resistance genes have also been identified and deployed. Due to co-evolution, both rust resistance genes and pathogen races can be grouped according to their gene pool of origin. Pyramiding multiple resistance genes into a single cultivar provides the most durable resistance to this pathogen, although few commercially available varieties possess such gene combinations (Stavely, 2000). Previously, the *Ur-3* gene has been widely used and provided adequate rust resistance to all rust races found in the state of Michigan. However, during 2007, rust was observed on the leaves and stems of several previously resistant varieties. Therefore the objectives of this study were to first evaluate an isolate of common bean rust collected in Michigan to confirm its ability to overcome *Ur-3*. Additionally, this isolate was used to map and tag a source of rust resistance segregating in a population of high yielding black beans.

MATERIALS AND METHODS

Samples of infected leaves from the varieties 'Jaguar', 'Merlot', and 'Vista' with sporulating pustules were collected by G.V. Varner in Tuscola county, MI in the fall of 2007. A spore suspension was prepared from these samples and used to inoculate these three varieties, along with the susceptible variety 'Othello' and the breeding line 115M in the MSU greenhouse. Spores were collected from these plants and increased further on 'Othello' to obtain additional inoculum to facilitate further evaluations. Preliminary characterization of this unknown race of common bean rust was accomplished by inoculating the differential series proposed by Steadman et al. (2002). A previously established population of 96 recombinant inbred lines (RILs) from the cross 'Jaguar' x 115M was also inoculated to enable mapping of the rust resistance segregating in this high yielding black bean population. A genetic map (unpublished) constructed with JoinMap 3.0 was available for this population and consisted of 119 SSR, SRAP, and TRAP markers. The targeted region amplified polymorphism (TRAP) marker F7R1, discussed below, was amplified according to the methods of Terpstra et al. (2006) from the following primer sequences: F7: CTT CAG CAG TGT CTC TCC R1: GCG AGG ATG CTA CTG GTT.

RESULTS AND DISCUSSION

This isolate of common bean rust induced a susceptible reaction in the varieties 'Jaguar', 'Merlot', and 'Vista'. These varieties carry the *Ur-3* resistance gene and have previously been resistant to all known races of rust in the state of Michigan. 'Aurora', the rust differential that possesses *Ur-3*, was also susceptible to this isolate. These data suggest varieties that rely on this single gene to condition rust resistance in Michigan could suffer significant yield reductions if environmental conditions favor development of the new rust race in future years. Breeders should be aware that integrating other rust resistance genes into their breeding programs will be necessary if this rust becomes established in bean producing regions of the state.

Based on prior knowledge of resistance to several races of rust (M.A. Pastor-Corrales, personal comm.), the breeding line 115M was included in the first inoculation. This rust isolate produced a small pustule resistant reaction on 115M. A previously established RIL population from the cross of 'Jaguar' and 115M, along with an accompanying genetic map provided an opportunity to map the resistance source present in 115M. Using the phenotypic data obtained from inoculating the population, the

resistance was mapped to the end of linkage group B4, approximately 3 cM from the TRAP marker F7R1 which produced a 150 bp fragment that co-segregated in coupling phase with this rust resistance. To our knowledge, this is the first report of a resistance gene tagged with a TRAP marker. This result supports the conclusion of Miklas et al. (2006a) who suggested the utility of TRAP markers to tag disease resistance genes and QTL of common bean. This marker also amplified the same fragment in Mex309 and B190, which carry the Ur-5 gene. Together, this information suggests that the rust resistance present in 115M co-locates with the resistance gene cluster on B4 containing *Ur-5*, *Ur-Dorado-108*, as well as several R genes for anthracnose (Miklas et al., 2006b). Further work is needed to determine the exact identity of the effective *Ur*-gene, although map location and pedigrees suggest the presence of *Ur-Dorado-108*. The complete results of the differential series (Table 1) showed that *Ur-3, 6,* and *13* genes produced susceptible reactions when challenged with this isolate. 'Montcalm', an important dark red kidney variety grown in Michigan, was also susceptible. Conversely, *Ur-11*, previously reported to confer resistance to all but one race in the USDA-ARS rust collection (Stavely, 2000) showed resistance to this isolate.

Table 1. Reaction of 12 common bean rust differentials inoculated with rust collected from Tuscola county, MI. Reactions scored from 1 to 6 according to Steadman et al. (2002).

Mesoamerican	R-Gene	Reaction	Andean	R-Gene	Reaction
GN1140	*Ur-7*	3,4	Early Gallatin	*Ur-4*	2,3
Aurora	*Ur-3*	6,5	Redlands Pioneer	*Ur-13*	4,3
Mexico 235	*Ur-3+*	3	Montcalm	unknown	4,5
Mexico 309	*Ur-5*	1	PC 50	*Ur-9, Ur-12*	2,3
CNC	*Ur-CNC*	2,3	Golden Gate Wax	*Ur-6*	5,6
PI181996	*Ur-11*	1	PI260418	unknown	2,3

CONCLUSIONS

Breakdown of previously effective resistance to any pathogen presents a challenge to breeders to identify alternative solutions. Although the *Ur-3* gene was overcome in Michigan bean fields during the 2007 growing season, the long term implications of this discovery remain to be determined. This knowledge should serve as a reminder that pathogen populations are continually evolving, and maintaining successful genetic resistance requires continual effort. Further work is needed to determine the precise identity of the Ur-gene conditioning resistance in 115M. Additionally, the TRAP marker F7R1, which produced a 150bp fragment shown to co-segregate with this resistance, should be considered as a candidate for conversion to a SCAR marker to facilitate marker assisted selection for rust resistance.

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INTROGRESSION OF QTL FOR WHITE MOLD RESISTANCE FROM COMMON AND SCARLET RUNNER BEAN

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White mold disease in common bean (*Phaseolus vulgaris* L.), caused by *Sclerotinia sclerotiorum* Lib de Bary, can significantly reduce seed yield (Schwartz et al., 1987). Currently, the primary means to control losses to white mold include disease avoidance by planting cultivars with upright architecture, fungicide use, and cultural practices that reduce disease pressure. While these methods are moderately effective, they are not completely effective and there is a need to develop cultivars with genetic resistance.

Common bean germplasm with partial physiological resistance to white mold has been widely reported (Dickson et al., 1982; Fuller et al., 1984; Schwartz et al., 1987; Kolkman and Kelly, 2003; Miklas et al. 2001, Ender and Kelly, 2005, Miklas, 2007). Most studies have reported resistance to be quantitatively inherited and have low to moderate heritability. Park et al. (2001) reported nine candidate quantitative trait loci (QTL), Miklas et al. (2001) reported a major QTL for resistance in the Andean line G122, and we reported five QTL that conferred partial resistance (Maxwell et. al., 2007). The highest levels of resistance occur in scarlet runner bean (*P. coccineus* L). Abawi et al. (1978) and Schwartz et al. (2006) reported single dominant gene resistance to white mold in *P. vulgaris* x *P. coccineus* interspecific crosses. Gilmore (2007) reported six QTL for reaction to white mold in *P. coccineus*. Our objectives were to combine resistance genes from common and scarlet runner beans to achieve higher levels of physiological resistance to white mold than what is currently available.

We developed an interspecific inbred backcross line (IBL) population to combine resistance derived from common and scarlet runner bean. The interspecific inbred backcross line (IBL) population was made by crossing WM67, a moderately resistant common bean line that possessed resistant QTL from G122, with scarlet runner bean PI255956 that has high levels of resistance to white mold reported by Gilmore (2007). Six QTL reported by Maxwell et al. (2007) possessed by WM67 and one QTL possessed by PI 255956 were tested for their effect in the IBL population. One molecular markers previously reported by Gilmore (2007) contributed by scarlet runner bean parent PI 255956 accounted for 7.0 ($P<0.05$) of the phenotypic variation in resistance, and two markers previously reported by Maxwell et al. (2007) contributed by common bean parent WM67 accounted for 10.8 ($P<0.01$) and 12.8% ($P<0.01$) of the phenotypic variation. The remaining markers previously reported in the parents could not be evaluated for their associations with WM resistance because they were not polymorphic in the IBL population. However, four additional genomic regions based on markers from PI225956 were found to be associated with ($P<0.05$) resistance in the IBL.

Only 16 of the 65 IBL were polymorphic for markers from the two parents. The lack of polymorphisms was likely due to the low level of recombination between common and scarlet runner bean chromosomes, during the development of IBL. We observed severe segregation distortion for every molecular markers ($P \leq 0.001$), and only 16 of the 65 IBL possessed any markers from the scarlet runner parent. Introgression of scarlet runner alleles was limited because most early generation IBL were highly self sterile and many had crippled phenotype. To determine if any crossover events occurred between chromosomes from common and scarlet runner bean, we

evaluated for the presence of recombination at loci mapped to the same linkage group based on the core common bean map. Only two recombination events were detected. One recombination occurred on linkage group B2 between SSR markers BM152 and BM160 (1.5%), and a second on linkage group B7 between SSR marker BM160 and the *Phs* SCAR marker (15.4%). The results from this study suggest that QTL associated with white mold resistance from common and scarlet runner bean can be combined in an IBL population, however recombination between common and scarlet runner bean chromosomes is limited. Our results suggest that MAS to improve resistance to white mold in common bean by introgressing QTL from scarlet runner bean may be a viable method, however to enhance recombination, intermating among selected IBL or further backcrossing will be needed.

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USE OF MULTI-SITE SCREENING TO IDENTIFY PARITAL RESISTANCE TO WHITE MOLD IN COMMON BEAN IN 2007

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(University of Nebraska-Lincoln)

Data also from S. Singh (ID), P. Miklas (WA), J. Kelly (MI), J. Myers (OR),
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There is no complete resistance to *Sclerotinia sclerotiorum*, cause of white mold, in common bean. The development of bean cultivars with partial resistance and/or avoidance to white mold (WM) would reduce disease losses at no cost to producers. The objective of the study was to identify bean germplasm with broad partial resistance to white mold. To accomplish this, putative sources of resistance developed by bean breeders were evaluated by greenhouse and field screening methods at multiple sites.

Field tests consisted of two rows of each entry and a common susceptible genotype, resulting in a three-row plot 4.6 m (15 ft.) long replicated three times in a randomized complete block design. There were 13 screening tests at 9 locations, 6 field and 7 greenhouse (straw test). Many field screening sites were eliminated because of flooding in 2007. The greenhouse screen tested 17 bean lines this year, and the field screen tested 12 bean lines. The straw tests were analyzed by using the mean rating of each entry from each location determined by the modified Petzoldt and Dickson scale (Teran et al, 2006) (Table 1). The field tests were evaluated by calculating the percent white mold infection of the field screen bean lines (Table 2). Spearman and Pearson correlations were used to compare entry WM ratings in the greenhouse test, and entry WM percentages in the field tests.

All of the results of the field screening locations (excluding Nebraska and North Dakota due to low white mold infection) were positively correlated, ranging from r=0.65, p=0.021; Oregon and Idaho to r=0.92, p=<.0001; Oregon and Washington. In the greenhouse tests, Idaho and Nebraska screening results were positively correlated with results from all other greenhouse screening locations. The Washington, New York, and Wisconsin results were correlated with all sites except Colorado and Oregon.

Bean lines A195 (bayo), VA 19 (LRK), Cornell 606 (BLK), Cornell 605 (LRK), and PS02-029C-40 (GN) were identified as more resistant than the other bean lines in the greenhouse screening tests. G122 (CRAN), Cornell 606 (BLK), Cornell 604 (BLK), B05055 (BLK), VA 19 (LRK), Cornell 605 (LRK), and PS02-037-7-B2 (GN) were identified as having partial white mold resistance in the field. The multi-site field tests combined with greenhouse results helped to identify disease escape or avoidance in B05055 that is similar to that previously identified in Bunsi. B05055 ranked among the susceptible lines in the greenhouse but was more resistant in the field.

In the 2007 screening season all ranking of greenhouse and field data was eliminated; thus the evaluation for greenhouse sites (the straw test) and field screening sites was consistent. Due to differences found in isolates of *S. sclerotiorum* used to screen for white mold resistance (unpublished), a common isolate(s) should be selected to use in greenhouse screening tests across locations in 2008. Changes have been made annually to improve multi-site white mold resistance screening to provide bean breeders and pathologists with the most consistent and informative results.

Table 1. Mean straw test rating* with t grouping**used to measure white mold resistance in bean lines at 7 greenhouse screening locations.

Entry	CO	NY	NE	ID	WI	WA	OR	Mean	t Grouping
A195	2.9	4.3	4.6	3.8	3.3	3.2	7.8	4.3	A
VA 19	2.8	5.2	4.8	4.7	4.3	3.5	5.8	4.4	A B
Cornell 606	2.5	4.4	5.0	4.3	4.9	4.7	5.8	4.5	A C B
Cornell 605	3.0	5.6	4.7	5.9	3.3	3.9	5.7	4.6	A C B
PS02-029C-40	2.2	6.7	5.0	5.1	3.3	4.5	5.7	4.7	A C B
G122	3.9	7.7	4.4	4.9	4.4	4.8	4.7	5.0	A C B
PS02-011A-39	5.5	6.0	5.3	6.5	3.3	3.5	6.0	5.2	A C B
PS02-029C-26	4.0	7.5	5.8	5.9	3.8	3.5	6.4	5.3	A C B
Cornell 604	4.7	6.7	5.8	5.2	3.1	4.3	7.9	5.4	D C B
WM 67	7.9	5.9	5.6	6.3	3.0	4.0	6.0	5.5	D C E
PS02-037-7-B2	4.4	6.8	7.5	7.8	4.9	5.2	8.2	6.4	D F E
PS02-0361-10-B3	3.1	8.1	7.6	7.4	6.4	5.8	6.4	6.4	D F E
Bunsi	4.7	8.6	7.9	6.6	5.0	5.0	7.8	6.5	F E
PS02-006D-15	4.3	9.0	7.7	8.1	5.8	6.3	6.9	6.9	F
B05055	4.8	8.9	6.6	6.3	8.1	6.1	8.1	7.0	F
B04316	3.9	8.3	6.8	7.4	8.1	7.1	8.7	7.2	F
Beryl	7.9	6.8	8.9	8.8	5.5	6.2	7.3	7.3	F

*ST Rating (1-3=resistant, 4-6=intermediate, 7-9=susceptible); **Alpha=0.05, LSD=1.08

Table 2. The mean percent infection and t grouping** in field screening plots from 6 white mold resistance screening locations.

Entry	ID	WA	MI	OR	NE*	ND*	Mean	t Grouping
G122	21	11	19	22	2	5	18.3	A
Cornell 606	33	12	33	24	0	3	25.5	A
Cornell 604	32	30	11	33	0	3	26.5	A
B05055	45	30	14	31	1	2	30.0	A B
VA 19	29	23	30	39	2	7	30.3	A B
Cornell 605	42	26	26	34	1	10	32.0	A B
PS02-037-7-B2	65	17	26	23	1	0	32.8	A B
PS02-036A-10-B3	53	41	52	31	1	3	44.3	B C
B04316	40	78	44	55	2	5	54.3	D C
Bunsi	92	50	56	47	1	4	61.3	D C
WM 67	77	58	70	42	3	0	61.8	D
Beryl	100	92	93	87	16	4	93.0	E

*Nebraska and North Dakota had low WM infection in the field, and were not figured into the grand mean;
**Alpha=0.05, LSD=17.02

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REACTION OF TWENTY-TWO COMMON BEAN ACCESSIONS AGAINST THREE LOCAL ISOLATES OF WHITE MOLD

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INTRODUCTION

White mold, caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, is a frequent and destructive disease in common bean, being a serious problem in mild and wet areas like northern Spain. The resistance against this pathogen has quantitative nature and is very complex, implicating both avoidance and physiological mechanisms. At least 10 independent quantitative trait loci (QTL) conditioning resistance have recently been described (Miklas, 2007). So far, the virulence of local isolates and the presence of pathogenic variants in this fungus have not been investigated. Our objectives were i) to investigate the differences among three local isolates of the pathogen, and ii) to identify potential resistance sources in a set of well-know common bean cultivars or lines.

MATERIALS AND METHODS

Isolations were made from tissues of naturally infected bean plants with sclerotia symptoms. One sclerotia was germinated on potato dextrose agar medium in Petri plates for 3 o 4 days at 23ºC in order to produce inoculum. Resistance tests were developed using the straw method (Petzoldt and Dickson, 1996). Plants were evaluated 8-10 days after inoculation considering the invasion in the main stem and the disease reactions were assessed using a 1 - 9 severity scale, where 1= no symptoms and 9 =total plant collapse (Miklas, 2007). The accessions were classified in three main types according to the average in the disease reaction obtained from three independent evaluations: resistant accessions (disease reaction < 4), susceptible accessions (disease reaction > 6), and accessions showing an intermediate reaction (disease reaction between 4 and 6).
A total of 22 accessions maintained in the SERIDA collection were evaluated: the anthracnose differential cultivars 'Widusa', 'Cornell 49242', 'Michelite', 'TU', 'MDRK', 'AB136', 'Perry Marroy', and 'Kaboon'; resistance sources against potyvirus or anthracnose, BRB57, BRB130 and IVT7214, 'Sanilac', 'Catrachita', SEL1308, SEL1360, A55, A321, A252 and A493. The cultivar 'Andecha' (market class fabada), the local accession V203 (market class canellini), and the cultivar 'Xana' (market class fabada, derived from the cross Andecha x V203) were also included in this evaluation.

RESULTS AND DISCUSSION

Three isolates were obtained from three different local cultivars, in three different localities in Asturias (Spain): Isolate 1 obtained from the cultivar 'Xana' at Villaviciosa, Isolate 2 obtained from the cultivar 'Andecha' at Navia, and Isolate 3 obtained from a landraces (named verdina) at San Tirso de Abres. After inoculating the plants with these three isolates, the typical white mold symptoms were developed: a white and cottony mycelium and black sclerotia on the infected tissue.
The reaction of twenty two materials against the three local isolates of white mold (average of three independent evaluations) is shown in Table 1. Two types of reactions were identified in the evaluated materials: intermediate and susceptible reaction. Significant differences for the reaction against the there isolates were found in four materials (IVT7214, SEL1308, Widusa; and AB136)

suggesting the possibility of a pathogenic variability. However, these differences were not high (< 3 units of the severity scale) and they did not implicate a clear differentiation between resistant and susceptible reaction. Considering the average obtained from the reactions against the three isolates, a total of eight materials showed an intermediate reaction, V203; BRB57 and BRB130 having the highest resistance levels. 'Xana', derived from the cross 'Andecha' x V203, showed resistance levels intermediate between those of their parents. The results reveal the possibility of increase the resistance levels in the market class fabada.

Table 1. Disease reactions of twenty–two common bean against three local isolates of white mold using straw test. Significant differences were revealed by means of a single-factor analysis of variance (ANOVA). I = Intermediate reaction, S = susceptible reaction.

Line or	Isolate 1			Isolate 2			Isolate 3				Total	
Cultivar	Nº	Mean	SE	Nº	Mean	SE	Nº	Mean	SE	ANOVA*	mean	Reaction
Sanilac	19	**6,2** ±	0,3	14	**6,0** ±	0,2	24	**6,6** ±	0,2	**ns**	6,3	**S**
A321	28	**6,6** ±	0,2	27	**6,7** ±	0,1	25	**7,1** ±	0,2	**ns**	6,8	**S**
BRB57	11	**4,9** ±	0,4	15	**4,7** ±	0,2	14	**4,1** ±	0,2	**ns**	4,6	**I**
BRB130	17	**4,8** ±	0,3	19	**5,1** ±	0,2	18	**4,8** ±	0,3	**ns**	4,9	**I**
IVT7214	20	**6,9** ±	0,2	22	**5,5** ±	0,2	27	**6,6** ±	0,2	**s**	6,3	**S**
Catrachita	26	**5,5** ±	0,3	21	**4,7** ±	0,3	20	**5,7** ±	0,5	**ns**	5,3	**I**
SEL1308	21	**6,1** ±	0,1	26	**5,3** ±	0,2	19	**6,1** ±	0,3	**s**	5,9	**I**
Kaboon	19	**4,9** ±	0,3	20	**5,1** ±	0,2	19	**5,7** ±	0,3	**ns**	5,2	**I**
Widusa	30	**6,4** ±	0,2	18	**5,8** ±	0,2	26	**7,0** ±	0,2	**s**	6,4	**S**
A55	27	**8,0** ±	0,3	17	**7,9** ±	0,4	23	**8,0** ±	0,2	**ns**	8,0	**S**
Perry Marrow	18	**6,4** ±	0,5	22	**6,0** ±	0,4	24	**6,1** ±	0,4	**ns**	6,2	**S**
SEL1360	20	**8,3** ±	0,2	25	**7,6** ±	0,3	21	**7,6** ±	0,3	**ns**	7,8	**S**
A493	27	**7,3** ±	0,3	25	**7,4** ±	0,2	20	**7,0** ±	0,5	**ns**	7,2	**S**
A252	33	**5,9** ±	0,2	19	**6,6** ±	0,3	20	**6,3** ±	0,2	**ns**	6,3	**S**
Cornell 49242	34	**8,8** ±	0,1	34	**8,5** ±	0,1	31	**8,7** ±	0,1	**ns**	8,6	**S**
AB136	19	**4,4** ±	0,3	14	**4,9** ±	0,3	14	**5,7** ±	0,3	**s**	5,0	**I**
MDRK	12	**6,3** ±	0,5	18	**6,1** ±	0,2	15	**5,9** ±	0,4	**ns**	6,1	**S**
Tu	16	**7,9** ±	0,3	18	**7,9** ±	0,2	19	**8,5** ±	0,2	**ns**	8,1	**S**
Michelite	6	**7,2** ±	0,2	16	**6,3** ±	0,2	17	**6,7** ±	0,2	**ns**	6,7	**S**
Andecha	17	**6,7** ±	0,6	9	**6,1** ±	0,3	10	**5,8** ±	0,6	**ns**	6,2	**S**
V203	21	**4,4** ±	0,2	22	**4,1** ±	0,2	18	**4,9** ±	0,3	**ns**	4,5	**I**
Xana	32	**5,0** ±	0,3	27	**4,7** ±	0,3	21	**5,8** ±	0,4	**ns**	5,2	**I**
Mean	473	6,3 ±	0,2	448	6,0 ±	0,2	445	6,4 ±	0,2	ns		

***s= significant diferences (*p* <0.05) ns=not significant diferences (*p* >0,05)**
****SE=standard error**

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RESPONSE OF DRY BEAN GENOTYPES WITH DIFFERENT LEVELS OF RESISTANCE TO *SCLEROTINIA SCLEROTIORUM* TO THREE INOCULATION METHODS

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INTRODUCTION

Several methods of inoculation with *Sclerotinia sclerotiorum* (Lib.) de Bary (the cause of white mold) have been used for germplasm screening, genetics, and breeding in the common bean and other crops over the past 35 to 40 years. Singh and Terán (2008 this issue) briefly discussed different indirect and direct screening methods and modifications in the straw-test or cut-stem method since reported by Petzoldt and Dickson in 1996. The reaction of common bean genotypes may vary depending upon the inoculation methods and the field or greenhouse environments used. Our objective was to compare the effectiveness of the three inoculation methods, namely (1) infected oat seed on the basal internode, (2) infected flowers put in the angel of main stem and branch, and (3) cut-stem or the straw test.

MATERIALS AND METHODS

Four common bean genotypes, namely A 195, 'ICA Bunsi' (synonymous with ExRico 23), VCW 54, and 'Othello' were evaluated in the greenhouse at the University of Idaho, Kimberly Research and Extension Center, Kimberly, Idaho in 2008. Breeding line A 195 has large seeds, determinate growth habit Type I, and a high level of resistance to white mold (Singh et al., 2007a). ICA bunsi is among the first group of common bean cultivars identified with moderate levels of resistance to white mold in the field. The interspecific breeding line VCW 54 was derived from a common and scarlet runner bean cross (Singh et al., 2007b). Breeding line VCW 54 possesses a high level of resistance to white mold. Pinto Othello is highly susceptible to white mold. The four genotypes were planted in a randomized complete block design with three replicates. Each replicate had three 6-inch pots each with two plants. Plants in each plot were inoculated two times, namely on January 12 and 19, 2008 using the three inoculation methods, namely infected oat seed on the basal internode, infected flowers put in the angel of main stem and branch, and (3) cut-stem or the straw test. Data on single-plant basis were recorded at 16, 23, and 33 days post inoculations. All data were analyzed using the SAS statistical package and the mean and LSD (P=0.05) values were determined for appropriate comparisons.

RESULTS AND DISCUSSION

The mean squares due to replicates, replicates x genotypes, and inoculation methods x genotypes interactions were non-significant (P>0.05). In contrast, there were large significant (P<0.05) differences among genotypes and inoculation methods. Interspecific breeding line VCW 54 followed by A 195 had the lowest white mold scores across the three inoculation methods at all three dates of evaluation (Table 1). Othello had the highest disease scores irrespective of the inoculation method and evaluation date. The disease score tended to increase with delayed evaluations. Among the inoculation methods, the infected oat seed inoculation was the least effective and it was not possible

to differentiate among the resistant, susceptible, and intermediate genotypes. In contrast, the cut-stem method produced most severe disease. Nonetheless, differences between the cut-stem and infected flower inoculation methods were non-significant for highly resistant genotypes such as A 195 and VC 54 or highly susceptible Othello. For the moderately resistant ICA Bunsi, the infected flower inoculation method had consistently significantly lower disease score than the cut-stem method.

Table 1. Mean score for white mold reaction of four common bean genotypes and three inoculation methods evaluated in the greenhouse at UI-Kimberly, Idaho in 2008.

Genotype	Method	WM16DAI	WM23DAI	WM33DAI
A 195	Cut-stem	6.3	6.7	6.8
	Infected flowers	5.6	5.8	5.9
	Infected oat seed	1.7	1.8	1.8
	Mean	4.5	4.8	4.9
ICA Bunsi	Cut-stem	9.0	9.0	9.0
	Infected flowers	6.0	7.1	7.1
	Infected oat seed	1.0	1.0	1.0
	Mean	5.3	5.7	5.7
VCW 54	Cut-stem	5.2	5.5	5.7
	Infected flowers	5.4	5.9	6.0
	Infected oat seed	1.4	1.8	1.8
	Mean	4.0	4.4	4.4
Othello	Cut-stem	9.0	9.0	9.0
	Infected flowers	8.0	8.2	8.5
	Infected oat seed	3.6	3.7	3.8
	Mean	6.9	7.0	7.2
Overall Mean		5.2	5.4	5.5
LSD (0.05)[1]		0.8	1.1	1.0
LSD (0.05)[2]		1.5	1.6	1.3

[1]To compare among the mean of genotypes over the three inoculation methods.
[2]To compare genotypes across inoculation methods.

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QTL ANALYSIS OF WHITE MOLD RESISTANCE IN AN INBRED BACKCROSS MAPPING POPULATION DERIVED FROM A WILD MEXICAN BEAN

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INTRODUCTION

White mold, caused by *Sclerotinia sclerotiorum*, is a serious disease of common bean (*Phaseolus vulgaris*) that results in substantial yield loss and reduced seed quality. Resistance to white mold in bean is a complexly inherited, quantitative trait that is highly influenced by environment, which makes selection for resistance difficult. Field resistance to white mold is partial and involves physiological resistance and morphological avoidance mechanisms. QTL associated with white mold resistance have been reported in diverse bean genotypes (Ender and Kelly, 2005; Kolkman and Kelly, 2003; Maxwell et al., 2007; Miklas et al., 2001; Park et al., 2001). The present work was undertaken to identify novel QTL from undomesticated wild bean germplasm.

MATERIALS AND METHODS

This study was conducted in a population of 89 BC2F3:4 inbred backcross lines (IBL) derived from the cross between the Mexican black bean 'Tacana,' the recurrent parent, and the wild Mexican accession PI 318695. Tacana is recognized as having white mold avoidance and PI 318695 has shown resistance to white mold in greenhouse straw tests (Miklas et al., 1999). Phenotypic data was also collected on a subset of 30 lines in 2004-2006 in naturally infected field plots rated when the plants reached physiological maturity on a scale of 1 to 9 as described by Miklas et al. (2001). Four replicated straw tests were also conducted on the population and rated on a scale of 1-9 as described by Petzoldt and Dickson (1996).

The population was genotyped using both SSR, SRAP and TRAP markers (Blair et al., 2003; Gaitan-Solis et al., 2002; Li and Quiros, 2001; Yu et al., 2000). 106 SSR, SRAP and TRAP markers that were polymorphic between the parents were genotyped on the entire population. The markers were mapped using Joinmap and single marker analysis and composite interval mapping was conducted using QTL Cartographer.

RESULTS AND DISCUSSION

72 SSR, SRAP, and TRAP markers were mapped to ten anchored and seven unanchored linkage groups of common bean. One QTL for resistance to white mold in the straw test was identified on B9 near the SSR marker BM148 and SRAP marker F6R8.600. This QTL was significant in three of the four straw tests with SMA and twice with CMA (R^2=11.1 and 18.1%). The QTL on B9 was not detected in one run of the straw test. This is not surprising, as this run was inoculated when the greenhouse where the inoculations took place was unseasonably cold.

A QTL for resistance was identified in all three years of field screenings on B3 near SSR marker AG1 (R^2=20.2, 33.1, and 17.7%). This region of B3 was also significantly associated with agronomic traits, including days to flowering, days to maturity, lodging, and plant height, indicating that the QTL on B3 is associated with avoidance because of the colocalization with QTL for architectural traits previously associated with avoidance to white mold (Kolkman and Kelly, 2003). Park et al. (2001) identified a minor QTL for white mold resistance on the same region of B3 from PC 50. This QTL on B3 is also located near the defense related gene *P. vulgaris* pathogenesis-related gene, PvPR-1. PvPR-1 is one of two small acidic pathogenesis related proteins in bean that are induced by fungal elicitors (Walter et al., 1990). The other, PvPR-2 is located on B2 and has been associated with 'Bunsi' derived QTL for white mold resistance (Kolkman and Kelly, 2003; Ender and Kelly, 2005). This region of B3 was previously identified as

containing a QTL for resistance to oxalate (R^2 = 11.4%) in the 'Bunsi' x 'Newport' population, but was not associated with field resistance to white mold (Kolkman and Kelly, 2000).

Another QTL for white mold resistance was identified on a separate region of B3 in two of the three field environments. In the IBL population this QTL colocalized with QTL for days to flowering, days to maturity, plant height, and desirability as well as yield and seed size across all years, indicating that it is also associated with architectural avoidance. A QTL in this region was also identified in the 'Bunsi' derived ND88-106-04 navy breeding line (Miklas et al., 2007). A QTL for disease incidence on B9 was detected in 2005 with CIM near SRAP marker F1R8.300 and SSR marker BMd-46. The region of B9 associated with disease incidence was also significantly associated with several agronomic traits across all years, and the QTL for disease incidence is likely associated with architectural avoidance. Maxwell et al. (2007) identified a QTL in the same region of B9 for field disease severity in a G122 x CO72548 mapping population. This QTL for disease incidence maps to a different region of B9 compared to the straw test QTL detected on B9 near marker F6R8.600 and the straw test QTL was not significantly associated with white mold resistance in any field environment. This may be due to the small subset of individuals from the IBL population that was included in the field analysis. Alternatively, the architectural traits associated with avoidance that segregate in the population may play a more significant role in overall white mold resistance in a field setting and effectively mask any effects of the physiological resistance that may otherwise be observed in the straw test.

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AFLP MARKERS ASSOCIATED WITH MACROPHOMINA PHASEOLINA RESISTANCE IN BEAN

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Expedite development of common bean (*Phaseolus vulgaris* L.) cultivars with enhanced resistance to *Macrophomina phaseolina* (Tassi) Goid.] (Mp) has been delayed due to reduced knowledge about resistance heritability (11). Resistance to Mp in *Phaseolus* has been found in genotypes from Mesoamerica race (8, 9). Resistance to Mp in BAT 477 is governed by two dominant genes with complementary epistatic effects (7, 11). In addition, other minor genes appear to influence its resistance as well (7, 10). Marker-assisted selection increases efficiency for incorporating Mp resistance genes into widely-cultivated and susceptible common bean germplasm such as pinto beans ('Pinto Americano', 'Pinto Villa', 'Pinto Saltillo'). The aim of this work was to identify AFLP molecular markers associated with Mp resistance in common bean BAT 477 bred line.

One hundred F_2 plants were obtained by self-pollination of F_1 plants derived from cross between BAT 477 (resistant to Mp) x Pinto UI-114 (susceptible) were obtained. F_2 plants plus parents were grown in greenhouse and cotyledonal leaves were obtained to 15 days after planting. Detached leaves were inoculated with a highly virulent Mp isolate (6). Mp severity ratings were registered 5 days after inoculation. Disease severity scores in leaf tissue were used to classify plants as resistant (1 - 30% of infected tissue) or susceptible (>30%). Resistant:susceptible ratios were subjected to chi-squared goodness-of-fit tests. Total genomic DNA from F_2 plants and parents was isolated (2) and subjected to AFLP analysis (12). Products were separated by electrophoresis in acrylamide gels 6% and revealed by silver staining (Promega©, Madison, WI). AFLP loci were mapped using MapMaker/Exp version 3.0 (5) based on the mapping function of Kosambi (1944) (4). Linkage map was drawn using MMDrawer version 0.2.0.0 (2003. Emboss Co. Auckland, New Zealand). QTL mapping by intervals was done using R/QTL version 1.00. LOD critical values were calculated by permutation tests. QTL analysis based on single markers included was performed by regression analysis as well as permutation tests (1, 3) with Mathematica (13).

Data confirmed that genetic resistance to Mp found in BAT 477 is governed by two dominant genes with double-recessive epistasis (Table 1) (7, 11). Under field conditions, quantitative resistance to Mp has been also reported (7, 10). Importance of 'detached leaf' method for measuring plant reaction to Mp in common beans was corroborated. This method conserves valuable germplasm which could be used in later genetic resistance studies and common bean breeding. Twenty most polymorphic AFLP primer combinations produced 294 bands, from which 50% showed distortion. The 130 less distorted loci were used for mapping and eight LGs including 38 loci were obtained (Fig. 1). QTL mapping indicated that none LOD was higher than critical value for genome-wide level using 1000 permutations (7.23). The highest LOD (5.112) was found in LG1. Chromosome-wide permutation tests for LG1 produced LOD=5.5. Although statistical analysis did not assume the presence of QTLs, LOD critical value suggests the possible presence of one QTL. QTL analysis

based on single markers produced a critical F=14.36 using 1000 permutations. Locus ATA/AGT-19 showed the highest F (9.329, P=0.0029). Significant P value was found using conventional regression analysis but not significance was obtained in permutation test. Therefore, the association between the locus and genetic resistance will need a higher simple size to be statistically reliable and then can be used in marker-assisted selection strategies for breeding of Mexican common bean germplasm.

Table 1. Chi-squared test for observed ratios of *M. phaseolina* reactions in common bean plants *in vitro*.

Classes	Observed	Expected	Deviations	$(O-E)^2$	$(O-E)^2$-0.5/E
Resistant	59	56.25 (9/16)	2.75	7.56	0.13
Susceptible	41	43.75 (7/16)	-2.75	7.56	0.16
	100	100	0		0.29NS

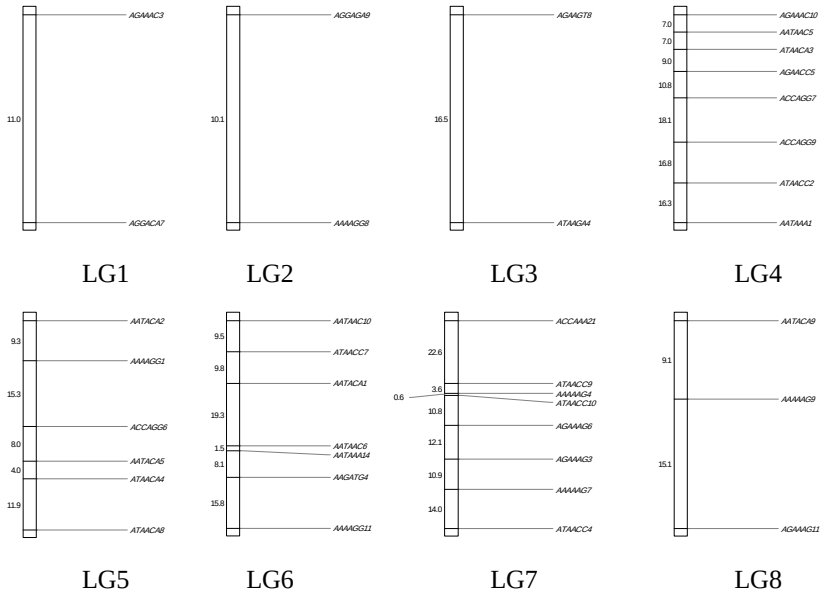

Fig. 1. Linkage groups (LG) formed by AFLP analysis from BAT 477 x Pinto UI-114.

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ROOT ROT FUNGI ASSOCIATED TO COMMON BEANS IN DURANGO, MEXICO

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Root rots caused by fungi (*Fusarium* sp., *Pythium* sp., and *Rhizoctonia solani* among others) reduce common bean (*Phaseolus vulgaris* L.) production and grain quality at northern Mexico (1, 3, 4). Identification, epidemiological and predominance studies of root rot fungi are important activities in order to establish management strategies to reduce disease problems (2, 5). Fungi genetic and pathogenic diversity analyses are also important for disease control in wide producing areas of Mexico. The objective was to recover and identify fungi species associated to root rots in common beans grown in Durango, Mexico in order to implement control methods.

Twenty root samples were taken during 2007 in 15 common bean commercial fields distributed along regions 'Los Llanos', and valleys of 'Poanas', 'Guadiana' and 'Canatlán' in the state of Durango, México. Samples were surface disinfested with NaOCl 2% during 2 min, rinsed with sterile distilled water and dried at room temperature. Root sections were placed in potato-dextrose-agar acidified with lactic acid. Four replications (one petri dish with a root piece) per location were cultured. Petri dishes were incubated in the dark for 24 h at 27 ± 1 °C. Fungi isolates were identified using macroscopic and microscopic morphological traits (2, 5, 6).

Seventy five fungi strains were isolated from which 54% were identified as *Fusarium* sp., 10% *Macrophomina* and 2% *Pythium* (Table 1). *Fusarium* was the most widely distributed and was found in all the sampled locations. Three species were identified: *F. oxysporum*, *F. solani* and *F. graminearum* (data not shown). Predominance of *Fusarium* was found in 93% of sampled locations and 75% was found as single genus. *Macrophomina* was detected in four samples and predominated over *Fusarium* in one sampling site and close associated to cv. Pinto Saltillo. *Phytium* was found in one site located on the slope of a rocky hill where landrace of 'Flor de Junio' commercial class was grown. Results corroborated previous findings which demonstrated close relationship between *Fusarium* genus and common bean root rots in the state of Durango (7). *Fusarium* genus affected common bean cultivars despite of seed commercial class while *Macrophomina* infected pinto beans mainly. Fungi phenotypic characterization will be ratified by further molecular analysis. Pathogenic and genetic diversity studies need to be done in order to implement an efficient management method. Use of *Trichoderma* will be also evaluated as management strategy of *Fusarium* species found in Durango in wide-common bean producing areas.

Table 1. Sampling sites and fungi genera isolated from root samples obtained during 2007 in Durango, Mexico.

Location	Altitude (masl)	Genus (%)
Campo Experimental Valle del Guadiana	1880	*Fusarium* sp. (100)
Olegario Méndez-Pánuco de Coronado	2051	*Fusarium* sp. (100)
Campo Auxiliar Francisco I. Madero	1966	*Fusarium* sp. (80) - *Macrophomina* (20)
Guadalupe Victoria	1994	*Fusarium* sp. (100)
Alfredo Cabello Mesta-Cuauhtémoc	2194	*Fusarium* sp. (100)
Cuauhtémoc-Antonio Amaro	2186	*Fusarium* sp. (100)
Antonio Amaro-Poanas	2111	*Fusarium* sp. (75)- *Macrophomina* (25)
Antonio Amaro-Poanas	2122	*Fusarium* sp. (66) - *Phytium* sp. (33)
Cieneguilla, Poanas	1948	*Fusarium* sp. (100)
Pino Suárez-Mezquital	1909	*Macrophomina* (66) - *Fusarium* sp. (33)
Nombre de Dios-Vicente Guerrero	1924	*Fusarium* sp. (100)
Nombre de Dios-Vicente Guerrero	1887	*Fusarium* sp. (100)
Venustiano Carranza-Flores Magón	1979	*Fusarium* sp. (100)
Flores Magón	2026	*Fusarium* sp. (100)
J. Guadalupe Aguilera-V. Carranza	1953	*Fusarium* sp. (60) - *Macrophomina* (40)

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POPULATIONAL DYNAMICS OF *FUSARIUM SOLANI* F. SP. *PHASEOLI* IN SOIL AND DISEASE SEVERITY ON LEGUMES AND CEREALS

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INTRODUCTION

Fusarium solani f. sp. *phaseoli* (FSP) is among the most important soilborne pathogens in Brazil. Symptoms of Fusarium root rot are frequently observed on production areas of common bean in the State of Minas Gerais, Brazil. This fungus produces structures of resistance, named clamidospores, which are able to maintain the fungus in soil even in host absence. The objective of this study was to evaluate the populational dynamics of FSP in soil cultivated with legumes and cereals species, aiming to know their effects on fungus survival.

MATERIALS AND METHODS

From December 2006 to November 2007, two field trials were carried out in an area in Oratórios, State of Minas Gerais, previously cultivated with common bean, where plants with severe symptoms of Fusarium root rot had been observed. The following treatments were tested: 1. hand-weeding plot; 2. common bean (*Phaseolus vulgaris* cv. Pérola); 3. *Cajanus cajan*; 4. *Crotalaria juncea*; 5. *Canavalia ensiformis*; 6. *Zea mays*; 7. *Brachiaria decumbens*; and 8. *Pennisetum glaucum*. A randomized complete-block design with six replicates was used. Each plot had four 2 m-long rows, 0.5 m apart. Once a month, the populational dynamics of FSP was monitored in soil based in colonies forming units (CFU) grown in PCNB-peptone-agar (PPA) selective medium. Moreover, hypocotyls and roots of some legumes and cereals species commonly used as green manures and/or in no-tillage systems was also studied to determine the disease severity according to a 1 to 9 scale (Abawi and Pastor-Corrales, 1990). With these data, the index of McKinney (IM) was calculated according this equation:

$$IM\,(\%) = \frac{\sum (score \times number\ of\ plants\ with\ this\ score)}{(total\ number\ of\ plants \times greater\ score)} \times 100$$

RESULTS AND DISCUSSION

Owing to similar results of the trials, just results of one trial are showed. Successive cultivation of common bean in areas with FSP increased the fungus population in soil (Figure 1), because it was the most susceptible species to the fungus (Figure 2). Fusarium root rot was less severe on *C. cajan*, *C. juncea* and *C. ensiformis* than on common bean. Thus, CFU countings for green manure legumes were always lower during the trial. Keeping the soil weeding or cultivated with cereals (species not susceptible to the fungus) reduced the fungus population to almost zero. Our results also indicated that higher populational densities of FSP in soil and higher severity of disease coincided with periods of elevated temperatures from December to April (Figure 2).

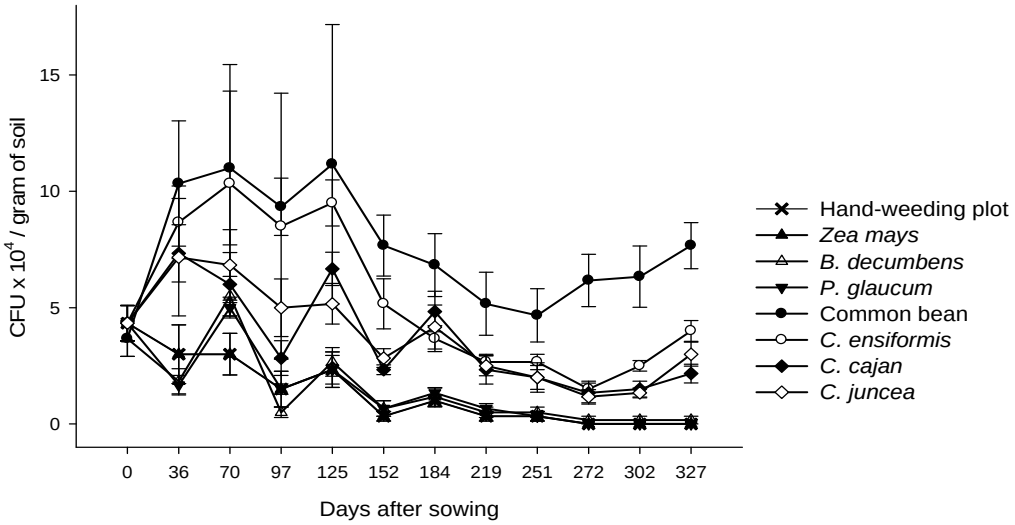

Figure 1. Populational dynamics of *Fusarium solani* f. sp. *phaseoli* (± SE) in soil cultivated with legumes and cereals species. Data expressed in CFU (colonies forming units).

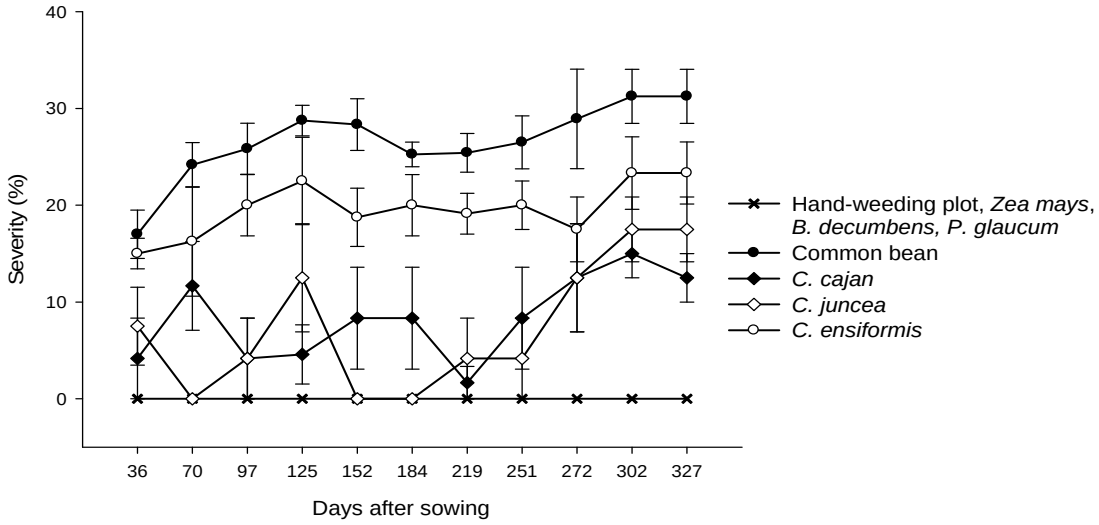

Figure 2. Evaluation of Fusarium root rot severity (± SE) based in a 1 to 9 scale in different legumes and cereals species.

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SEED YIELD UNDER RAINFED CONDITIONS AND CANOPY TEMPERATURE DEPRESSION IN COMMON BEAN

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INTRODUCTION

Drought and high temperature stress occur often during the growing season in rain-fed environments hampering crop yield. An approach that may help improve seed yield under heat and water stress is the integration of physiological traits into the selection criteria. Drought susceptibility index (DSI) has been successfully used to select wheat lines under water stress i.e., genotypes with low values for the susceptibility index are considered drought resistant (Fischer and Maurer, 1978). Canopy temperature depression (CTD) i.e. the difference between air temperature and crop canopy temperature, is an estimate of stomatal conductance and photosynthesis rate at a canopy level; for that reason, this physiological trait has been used to select wheat lines in heat stressed environments (Reynolds *et al*., 2001). The objective of this research was to determine whether variation in the DSI and CTD, might be useful to identify bean genotypes with higher seed yield and drought resistance under drought conditions.

MATERIALS AND METHODS

Two field experiments were carried out, one under irrigated and the other under rain-fed conditions. Both were established at Montecillo, State of Mexico (19º21'N and 98º55'W, 2250 m above sea level) in June 21th, 2004. Seven commercial bean (*Phaseolus vulgaris* L.) cultivars: six from the Flor de Mayo seeded type and one Flor de Junio seeded type, plus the landrace Michoacan 128 were included. A complete random block design with four replicates was used. Plots were two 4-m rows separated at 0.8 m. Thirty days after sowing (das), 40 kg ha^{-1} of nitrogen and 40 kg ha^{-1} of phosphorous fertilizers were applied. A second application of 40 kg of nitrogen was carried out at 68 das. The irrigated experiment was watered every two weeks from sowing to physiological maturity and the rain-fed experiment received 402 mm of rainfall during the growing season. Herbicides were applied to control weeds at 60 das. Insecticides were applied to control whitefly at 40 and 68 das. Collected data were analyzed using the statistical analysis system (SAS, 2000). Least Significant Difference of Tukey (LSD, $p \leq 0.05$) was calculated for comparison of means.

RESULTS AND DISCUSSION

Seed yield and its components - Significant differences were observed among cultivars in seed yield and its components (expressed in m^{-2}, except 100 seeds weight) were significant in both experiments. Cultivars Flor de Mayo Bajío, Flor de Mayo M38 and Flor de Junio Marcela showed the highest seed yield (SY) in both experimental conditions. Flor de Mayo Bajío had a high final above-ground biomass (BM), normal pods (NP), normal seeds (NS) and 100 seed weight (100SW). Flor de Mayo M38 had high NP, NS and 100SW, and Flor de Junio Marcela had high BM and 100SW under rain-fed conditions (Table 1). Results indicated that BM, NP, NS and 100SW were important traits contributing towards a high SY under rain-fed conditions and that these traits might be useful in a selection program to increase seed yield under intermittent water stress.

Drought susceptibility index - There were significant differences in the DSI (calculated with data from irrigated and rain-fed yields) among cultivars; Flor de Mayo Corregidora showed low DSI for SY, BM and NP; Flor de Mayo Sol for SY and NP and Flor de Mayo Anita for SY and NP (Table 2). These responses to rain-fed conditions showed that SY was an important trait followed by its components BM, NP and NS. This may indicate that selection criteria in rain-fed environments should include low DSI values for SY and its components BM, NP and NS in order to increase yield under those conditions.

Table 1. Cultivars with highest seed yield and their components (seed yield m^{-2} (SY, m^{-2}), final above-ground biomass m^{-2} (BM, m^{-2}), normal pods m^{-2} (NP m^{-2}), normal seeds m^{-2} (NS m^{-2}) and 100 seed weight (100SW)) in full irrigation (I) and rain-fed (R) environments.

	Irrigated (I) and Rain-fed (R)				
Cultivar	SY (g m^{-2})	BM (g m^{-2})	NP m^{-2}	NS m^{-2}	100SW (g)
Flor de Mayo Bajío	IR	IR	IR	IR	-
Flor de Mayo M38	IR	I	IR	R	IR
Flor de Junio Marcela	IR	IR	I	-	IR

Canopy temperature depression - Variation in CTD among cultivars was significant; Flor de Mayo Bajío, Flor de Mayo M38, Flor de Junio Marcela, Flor de Mayo RMC, Flor de Mayo Sol, Flor de Mayo Anita and Flor de Mayo Corregidora had higher CTD than Michoacan 128 in the irrigated environment, whereas cultivars Flor de Mayo Bajío, Flor de Mayo M38, Flor de Junio Marcela and Flor de Mayo Sol had higher CTD than Flor de Mayo RMC, Flor de Mayo Anita, Flor de Mayo Corregidora and Michoacan128 in the rain-fed environment (Table 2). The high CTD of Flor de Mayo Bajío, Flor de Mayo M38, Flor de Junio Marcela and Flor de Mayo Sol might indicate that these genotypes maintained high stomata conductance and photosynthetic rates in both drought and irrigation environments. This suggests that Flor de Mayo Bajío, Flor de Mayo M38, Flor de Junio Marcela and Flor de Mayo Sol may have deeper and branched root systems that will help maintain high transpiration and photosynthetic rates in water stress conditions.

Table 2. Drought susceptibility index for seed yield m^{-2} (DSI_{SY}), final above-ground biomass m^{-2} (DSI_{BM}), normal pods m^{-2} (DSI_{NP}), and normal seeds m^{-2} (DSI_{NS}), and canopy temperature depression of eight cultivars under irrigation and rain-fed environments.

	Drought susceptibility index				**Canopy temperature depression (ºC)**	
Cultivar	**DSI_{SY}**	**DSI_{BM}**	**DSI_{NP}**	**DSI_{NS}**	**Irrigation**	**Rain-fed**
Flor de Mayo Bajío	0.98	1.00	0.95	1.00	2.5	-0.3
Flor de Mayo M38	1.09	0.97	1.09	1.08	2.7	-1.4
Flor de Junio Marcela	0.98	1.06	1.06	1.02	2.7	-1.5
Flor de Mayo RMC	1.21	1.06	1.17	1.10	2.1	-2.4
Flor de Mayo Sol	0.90	0.98	0.93	0.99	2.4	-1.7
Flor de Mayo Anita	0.86	1.00	0.94	0.86	2.1	-2.9
Flor de Mayo Corregidora	0.96	0.89	0.93	0.97	1.7	-2.8
Michoacan 128	1.01	0.97	0.99	0.98	1.6	-2.6
LSD ($p \leq 0.05$)	0.24	0.11	0.23	0.20	1.0	0.9
General average	**1.00**	**0.99**	**1.01**	**1.00**	**2.3**	**-1.9**

CONCLUSIONS

Using the DSI and CTD we identified bean cultivars with high seed yield, and low canopy temperature depression under rain-fed conditions. This also allowed for identifying the yield components that most contributed to high seed yield in drought stress conditions.

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EFFECT OF SOWING DATE ON SEED YIELD OF EARLY AND LATE DRY BEAN CULTIVARS AT THE HIGHLANDS OF MEXICO

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INTRODUCTION

Dry beans are an important source of protein in the diet of most people in Mexico. Currently, the average consumption is about 12 kg per person per year. Thus, common bean is the second most important crop in our country. However, almost 65% of the total area planted with dry beans is cultivated under rainfed conditions, where precipitation is limited during crop cycle. Therefore, crop is generally subjected to drought stress, which in turn affect grain yield. On the other hand, the area cultivated with dry beans under irrigated conditions has declined during the last 10 years because scarcity of irrigation water. In the states included in the Highlands of Mexico, about 80 thousand hectares were sowed with dry beans in the summer of 2000, as compared to only 58 thousand sowed in 2007 (1). For this reason, it is important to evaluate different agronomic practices to increase water use efficiency, as well as using new genotypes to maintain dry bean productivity. The objective of the present study was to evaluate the effect of different sowing dates on the seed yield of early and late dry bean cultivars under irrigated conditions.

MATERIALS AND METHODS

The study was conducted at the Experimental Station of Pabellón (22° 09´ N; 102° 17´ W and 1912 masl) located in Aguascalientes state, during the summer of 2003 and 2004. Sowing dates evaluated were: May 12th and June 5th in 2003 and April 29th and June 22^{ed} in 2004. The dry bean cultivars included in the study were: Flor de Mayo Sol "FMSol" and Negro Vizcaya "NGViz", the former is considered as early cultivar with about 90 to 95 days to maturity and neutral response to photoperiod, while the second is a late cultivar with 110 to 115 days to maturity and it is sensible to photoperiod. Both cultivars were obtained at the dry bean genetic improvement program of INIFAP having a Type III habit and are suitable to be used at the semiarid highlands (2). The experimental unit consisted of ten rows of 30.0 m long and 0.76 m apart. Meteorological data (rainfall and temperature) were registered at daily bases from a near climatological station in each growing season. Plant traits recorded in each plot were days to flowering "DF" and maturity "DM", total aerial biomass (leaves excluded), seed yield and harvest index (HI=grain yield/total aerial biomass). Leaf area index (LAI) was also measured during the growth cycle using a lineal ceptometer (DECAGON DEVICES INC. ACCUPAR Ver. 4.1) which provides the LAI values directly.

RESULTS AND DISCUSSION

Considering the four environments, average days to flowering and maturity were 45.7 and 91.7 in FMSol, while in NGViz were of 53.3 and 109.5, respectively. It was observed a reduction of the growth cycle in the second sowing date in both cultivars. NGViz showed the most drastic reduction of the growth cycle in 2004, having 112 days to maturity in the first sowing date and only 100 days to maturity in the second sowing date. Maximum values of LAI were observed in NGViz, with and average of 5.6, as compared to 4.0 in FMSol. Several authors reported previously (3) that critical

values of LAI to reach 95% of light interception are between 3.0 and 4.0. This suggests that the dry bean cultivars did not have restriction on this physiological component. Nevertheless, regression coefficients were not significant for the relationship between LAI and seed yield (data no shown). Total biomass and seed yield were higher in NGViz than in FMSol, however HI showed greater values in the second cultivar (Table 1).

Table 1. Seed yield and seed yield components of two dry bean cultivars evaluated under different sowing dates at the Experimental Station of Pabellón in Aguascalientes, Mexico.

Genotype	Year	Sowing date	Total biomass kg ha^{-1}	Seed yield kg ha^{-1}	HI %	W100 seeds g	DF	DM	Maximum LAI
FMSol	2003	May 12	5770	3503	60.4	28.0	44	95	4.85
		June 05	6877	4018	58.4	25.3	42	90	3.29
	2004	April 29	4379	2827	64.7	23.5	52	95	3.97
		June 22	5803	3389	58.3	25.7	45	87	--
	Mean		**5707**	**3432**	**60.5**	**25.6**	**46**	**92**	**4.0**
NGViz	2003	May 12	11087	5349	48.2	30.8	52	116	5.96
		June 05	9324	4534	48.5	27.6	50	110	4.22
	2004	April 29	7604	4066	53.5	24.1	61	112	6.63
		June 22	6204	3229	51.8	29.2	50	100	--
	Mean		**8554**	**4294**	**50.5**	**28.0**	**53**	**109**	**5.6**

HI= Harvest Index; W100 seeds= Weight of 100 seeds; DF= Days to Flowering; DM=Days to Maturity

It was observed a differential effect of the sowing dates on seed yield of both dry bean cultivars. Flor de Mayo Sol showed an increase on the seed yield at the second sowing date in both years, whereas NGViz, had an opposite response showing higher seed yield in the first sowing date (Table 1). These results suggest that late cultivars such as NGViz, could have greater seed yield when sowed during first half of May. In contrast, an early cultivar such as FMSol seems to have better response when sowed at the begging of June. These findings are important because the establishment of the rainy season at the region of study is usually at the end of June. Then, irrigation water can be saved by avoiding one or two irrigations, without decreasing seed yield. On the other hand, early sowing dates besides not to increase seed yield, crops need more water. Thus water use efficiency may be increased by managing sowing date along with the use of improved dry bean cultivars best adapted to that environment.

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KINETICS OF WATER UPTAKE IN ITALIAN COMMON BEAN ECOTYPES

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In the last decades, the development of cultivars with superior agronomic traits, high production and resistance towards pest and diseases has been the main objective of improvement in common bean. Unfortunately, these goals have take in poor consideration the technological traits of the developing cultivars. Fast water uptake, short cooking time and seed appearance after cooking are very important traits being strictly related to the cultivar acceptance by consumers and food industry. On the other hand, the technological traits have to be taking in high consideration also when an ecotype would be re-proposal as niche product. It is known that the grain water uptake depends on physical characteristics of seed coat such as the degree of coat adherence to the cotyledons (Powell 1998) as well as the presence on the coat of small cracks detectable with scanning electron microscopy (Ma et al 2004). Some studies related slow water uptake to high sowing quality of seeds. Of course, this contrasts with the fast water uptake required by consumers and food industry. Still today, genotypic variation in the rate of water uptake and cooking time within the common bean market classes (white seed, white bicoloured seed, cream seed, etc.) has been little investigated. Taking these aspects in consideration, a portion of common bean ecotypes cultivated in Italy were analysed to acquire information about differences in the water absorption rate within this germplasm.

The study was conducted on 98 populations belonging to 62 Italian common bean ecotypes (Table 1). They were classified into different market classes according to Santalla et al (2002). About 150 g of dry seeds for each population were acquired for two years consecutively by farmers who traditionally grow it. Eleven commercial cultivars were included as references. The kinetic of water uptake was monitored for 8 h consecutively by measuring the seed weight increase of 30 seeds randomly selected. The hydration index, defined as the ratio of the seed weight increase at time t and the initial weight, was calculated and plotted against the time of soaking for each tested sample. The percentage of hard grains (seeds without the capacity of absorbing water) was quantified after 24 h of soaking. The coat percentage was determined as previously described (Piergiovanni et al 2000).

The comparison of the hydration index plots relative to the 98 tested samples evidenced the existence of three distinguishable groups. The first one (Fig.1, lowest trace) correspond to very slow water uptake being the hydration index after 5h of soaking inferior to 30 %. The second group (Fig. 1, intermediate trace) was characterised by intermediate water uptake (hydration index at 5h ranging from 30 to 50%), while the third one comprised the samples with very fast water uptake being the hydration index at 5h more than 50% (Fig. 1, upper trace). Polynomial equations of different degree fitted the curves with a good correlation ($R^2 > 0.996$). Equations of second, third and fourth order needs to be used to fit slow, intermediate and fast water uptake, respectively. As expected, different populations belonging to the same ecotype showed different hydration index values during the test but this did not affect the attribution of the ecotype to one of the three groups. Similarly, the trend of water uptake (slow, intermediate or fast) did not changed by the year-to-year. Among the tested ecotypes only 8 (Table 1) were characterised by significant year-to year variation so that their assignation to one group remained uncertain between to groups (slow-intermediate or intermediate-fast water uptake). No significant correlations were detected between the lying in one of the three groups and the coat percentage or the seed density. Finally, the presence of hard grains was more frequently observed for samples with coloured coat.

Generally, ecotypes belonging to the same market class were assigned to different water uptake groups (Table 1). At the opposite, any variation was observed among the cultivars belonging

to the same market class. This lack of variation is probably a consequence of the breeding process from which the cultivars were obtained.

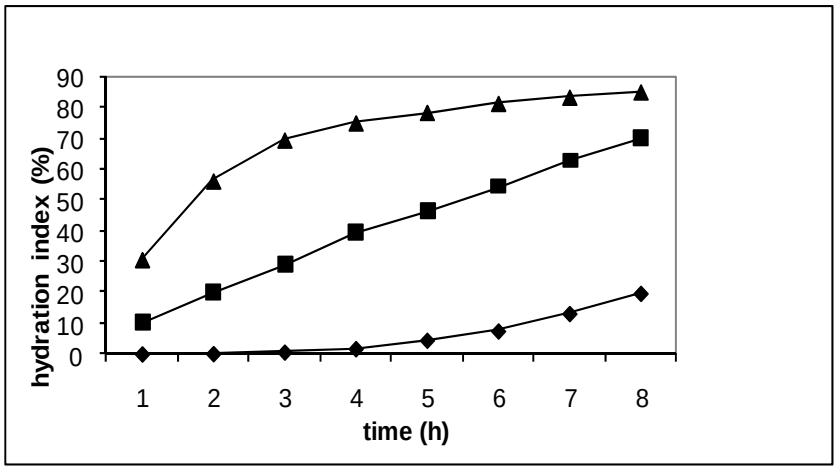

Figure 1. Hydration index plots relative to the three identify groups. From the top to the bottom: fast; intermediate and slow water uptake.

Table 1. Distribution of tested common bean ecotypes and cultivars among the three water uptake groups identify. The number of cultivars belonging to each group is reported in brackets.

Market class	Slow	Intermediate	Fast	Unclassified	Total
White seed	2	4	7 (3)	4	17 (3)
White with pattern around hilum	---	---	3	---	3
White bicoloured seed	6	4	1	---	11
Borlotto type	9 (8)	---	7	3	19 (8)
Cream seed	2	2	1	---	5
Yellow seed	---	---	1	---	1
Brown seed	---	---	2	---	2
Green seed	1	---	1	---	2
Violet seed	---	---	1	1	2
Total	*20 (8)*	*10*	*24 (3)*	*8*	*62 (11)*

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CARBOHYDRATE DEPLETION IN ROOT, STEM AND LEAVES OF SALT-STRESSED *PHASEOLUS* SPECIES

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Carbohydrates protect plants from stress through different ways, including contribution to cellular osmotic adjustment, detoxification of reactive oxygen species, protection of membrane integrity, and stabilization of enzymes/proteins (Bohner and Jensen 1996). Carbohydrate changes are of particular importance because of their relationship with physiological processes such as photosynthesis, translocation, respiration, and growth (Kerepesi and Galiba 2000). Surprisingly, in cultivated *Phaseolus vulgaris*, which can be included among the most sensitive crops to salinity and in other *Phaseolus* species, which have a wide range of salt tolerance (Bayuelo-Jiménez et al. 2002), little information is available on carbohydrate changes during salt stress (Bahena-Betancourt et al. 2006). The study reported here represents a contribution to this approach.

MATERIALS AND METHODS - Two wild and two cultivated species of *Phaseolus* differing in salt tolerance were used in this study: *P. vulgaris* PI325687, a wild salt-tolerant type (WT); *P. acutifolius* G40169, a wild salt-sensitive type (WS); *P. vulgaris* G04017, a cultivated salt-sensitive type (CS); and *P. acutifolius* G40142, a cultivated salt-tolerant type (CT). Plants were grown in nutrient solution under greenhouse conditions at Universidad Michoacana de San Nicolás de Hidalgo, Mexico between April and July 2006. Seedlings were allowed to grow with no salinity stress until the emergence of the first trifoliate leaf, when several NaCl treatments were added to the solutions (0, 60 and 90 mM). A randomized complete block design with a split-plot arrangement of salt treatments and four replications was used. Root, stem and leaves samples were freeze-dried, frozen and then ground, before storage at -20^{0}C. An enzymatic assay method for non-structural carbohydrate was used (Gomez et al. 2007). Soluble sugars were extracted from 15 mg of fine ground plant powder, in the presence of 4 mL methanol: water, followed by 100 µL chloroform. Two liquid phases were separated from the plant powder after centrifugation (IEC Model GP8R., Needham, MA). Right after evaporation under vacuum (CentriVap Labcondo Model 75100, Kansa, MO, USA), the dried pellet was returned to its soluble form by agitation in water at 4^{0}C. The aqueous extract was then combined with 15 mg polyvinyl pyrrolidone (PVP) to eliminate any residual phenols. After repeated shaking, the supernatant was analyzed using the MP plate (The Multtiskan Ascent MP Systems, Labsystems Thermo Fisher Scientific, Finland). Glucose, fructose and sucrose concentrations were successively quantified, by measuring the production of NADH (Gomez et al. 2007). Data were analyzed using GLM procedure (SAS, 2002).

RESULTS AND DISCUSSION - Plants treated for 20 days with 90 mM NaCl showed 46 to 66 % reduction in hexose (glucose plus fructose) concentration compared to controls (Table 1). In both wild and cultivated *P. acutifolius*, the increase in hexose content was linear over salt treatment, attaining values about twice as high for those in control plants. Sucrose content was also affected by salinity where total sucrose on *P. vulgaris* species decreased linearly in salt treatments. In *P. acutifolius*, however, no significant differences were detected under both salinity levels. Saline-induced changes in soluble carbohydrate content were also highly dependent upon the species and plant organ. Hexose accumulation in leaves and stems occurred with salinization in all species. However, these accumulations decreased in *P. vulgaris* PI325687 and salt-sensitive G04017. In both *P. acutifolius* G40169 and G40142 species, similar hexose contents were found in leaves and stems in both salt treatments. Hexose content in roots also increased with salinity in all species except for *P. vulgaris* G04017. Sucrose content sharply increased in relation to stress intensity in the leaves and stems in both *P. acutifolius* G40169 and G40142 species and remaining constant over the whole period. On *P. vulgaris* species, however, sucrose accumulation decreased with salinity stress. Our results demonstrated that salt-tolerant *Phaseolus* species accumulate more carbohydrates (hexose and sucrose) than the salt-sensitive, but that carbon is accumulated in different ways. Salt-tolerant G40142 accumulates more carbohydrate on leaves and stems and less on roots than the salt-sensitive ones, thus showing a different behavior concerning the shoot/root carbon partitioning under saline conditions.

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Table 1. Total concentration of sucrose, fructose and glucose of *Phaseolus* species following a 20-day salinization period.

	(mmol Kg^{-1} DW)		
Species/NaCl	**0 mM**	**60 mM**	**90 mM**
***P. vulgaris* PI325687 (WT)**			
Root Glucose	4.9 c	9.4 b	13.5 a
Leaf Glucose	4.6 b	8.6 a	0.9 c
Stem Glucose	10.4 a	9.7 a	2.8 b
Root Fructose	9.6 b	24.8 a	28.0 a
Leaf Fructose	5.6 b	12.1 a	1.0 c
Stem Fructose	14.9 b	19.3 a	2.8 c
Root Sucrose	36.1 a	38.9 a	37.1 a
Leaf Sucrose	46.4 a	37.9 b	4.2 c
Stem Sucrose	64.1 a	51.5 a	5.5 b
***P. vulgaris* G04017 (CS)**			
Root Glucose	4.9 b	14.7 a	1.6 b
Leaf Glucose	6.1 b	16.6 a	1.8 c
Stem Glucose	20.7 b	36.0 a	9.6 c
Root Fructose	7.9 b	34.2 a	3.9 b
Leaf Fructose	6.5 b	22.4 a	2.1 b
Stem Fructose	25.2 ab	43.9 a	14.6 b
Root Sucrose	49.5 a	27.8 b	3.7 c
Leaf Sucrose	42.0 a	32.5 b	3.2 c
Stem Sucrose	49.9 a	39.2 b	6.1 c
***P. acutifolius* G40169 (WT)**			
Root Glucose	4.3 c	10.1 b	14.1 a
Leaf Glucose	11.5 b	21.7 a	24.6 a
Stem Glucose	12.8 b	13.9 b	21.7 a
Root Fructose	11.7 c	33.3 b	44.9 a
Leaf Fructose	11.9 b	26.0 a	29.2 a
Stem Fructose	13.1 b	21.7 ab	29.3 a
Root Sucrose	47.8 a	33.6 b	40.0 ab
Leaf Sucrose	40.5 a	32.9 b	37.6 a
Stem Sucrose	47.3 a	49.6 a	44.3 a
***P. acutifolius* G40142 (CS)**			
Root Glucose	10.0 b	8.4 b	15.4 a
Leaf Glucose	12.2 b	24.3 a	23.9 c
Stem Glucose	21.9 a	20.8 a	20.5 a
Root Fructose	14.6 c	32.8 b	48.2 a
Leaf Fructose	15.1 b	29.4 a	28.2 a
Stem Fructose	24.4 a	25.1 a	25.6 a
Root Sucrose	50.8 a	29.6 b	38.1 b
Leaf Sucrose	33.7 a	32.6 a	31.5 a
Stem Sucrose	47.2 a	40.0 b	41.8 b

Values in each column noted with different letters differ significantly a $P \leq 0.05$.

OSMOTIC ADJUSTMENT OF *PHASEOLUS* SPECIES UNDER SALT STRESS: CONTRIBUTION OF INORGANIC IONS AND SOLUBLE CARBOHYDRATES

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Salt excess in soil solution affects almost all plant functions and growth, either through osmotic inhibition of root water uptake or by specific ion effects (Munns 2002). Maintaining osmotic homeostasis requires an increase of osmotica in cells either by soil solutes uptake or by the synthesis of metabolically compatible compounds (Kerepesi and Galiba 2000). The role of carbohydrates and ionorganic ions as active osmolytes has been studied in many plant species with contrasting results depending on genotype, severity and duration of the stress, or tissue studied (Munns, 2002). However, there is little information about carbohydrate status as determinant of the level of osmolyte accumulation and the active role in plant stress responses in *Phaseolus* species (Bahena-Betancourt et al. 2006). The study reported here represents a contribution to this approach.

MATERIALS AND METHODS

Two wild and two cultivated species of *Phaseolus* differing in salt tolerance were used in this study: *P. vulgaris* PI325687, a wild salt-tolerant type (WT); *P. acutifolius* G40169, a wild salt-sensitive type (WS); *P. vulgaris* G04017, a cultivated salt-sensitive type (CS); and *P. acutifolius* G40142, a cultivated salt-tolerant type (CT). Plants were grown in nutrient solution under greenhouse conditions between April and July 2006. Seedlings were allowed to grow with no salinity stress until the emergence of the first trifoliate leaf, when several NaCl treatments were added to the solutions (0, 60 and 90 mM). A randomized complete block design with a split-plot arrangement of salt treatments and four replications was used. Root, stem and leaves samples were freeze-dried, frozen and then ground, before storage at -20^0C. An enzymatic assay method for non-structural carbohydrate was used (Gomez et al. 2007). Tissue was ashed at 500^0C for 8 h, followed by dissolution in 1 mM hydrochloric acid (Basta and Tabatai 1985). Sodium and potassium concentrations were determined by flame emission using an Atomic Absorption Spectrometer (Varian SpectrAA-220FS). Free chloride was extracted from 3 mg of ground material with 50 ml of deionized water (Beke and Selles 1993). Chloride concentration was determined calorimetrically using an UV/BIS Spectrometer (Lamda 40 Perkin Elmer). The osmotic potential of each solute (Ψ_π) was estimated by van´t Hoff equation, and summed up to obtain the calculated osmotic potential (Ψ_π), or expressed as percentage of the total measured Ψ_π. The measured and calculated Ψ_π were corrected for maximum turgor. Data were analyzed using GLM procedure (SAS, 2002).

RESULTS AND DISCUSSION

The contribution of different organic and inorganic solutes to osmotic potential differed among species (Table 1). Total amount of N^+, Cl^- and K^+ accumulated in the plant treated with 60 and 90 mM NaCl contributed in 54 and 48 % and 62 and 55 % of the measured osmotic potential in the salt-tolerant and salt-sensitive *Phaseolus* species, respectively. Among the soluble carbohydrates, hexose had the highest contribution to leaf osmotic potential. However, this contribution decreased with increased

salt stress, except for the salt-tolerant G40142. At 60 mM of NaCl the total hexose contribution accumulated was 15 and 33 % of the measured osmotic potential in both salt-tolerant and salt-sensitive *Phaseolus* species, respectively. There is substantial evidence that glycophytes adjust to high salt concentrations by lowering tissue osmotic potentials with an increase of inorganic ions and/or compatible solutes (Munns 2002). This generalization appears to hold for *Phaseolus* species because of accumulation of high levels of inorganic ions in their leaves. In these leaves, osmotic potential decreased as the concentration of total inorganic rose, and therefore contributing for a significant proportion (51 and 47 %) of the measured decrease in leaf osmotic potential (-1.13 and -1.24 MPa) at 60 and 90 mM NaCl. The salt-induced hexose accumulation had a key contribution to leaf osmotic potential of all *Phaseolus* species, particularly at 60 mM NaCl. The net increase in hexose contributed with an important proportion (24 %) of the measured decrease in leaf osmotic potential. Moreover, in salt-tolerant G40142, total hexose concentration may still contribute, in part, to the decrease of leaf osmotic potential at 90 mM NaCl (28 %). This would also help to maintain root water absorption and its flux to the shoot under salt stress conditions.

Table 1. Contribution of soluble carbohydrate and inorganic ions to osmotic potential ($\Psi_{\Pi,}$ in MPa and %) of control and salt-stressed *Phaseolus* species. $\Psi_{\Pi\ control,}$ osmotic potential of the control plants; $\Psi_{\Pi\ stress}$ osmotic potential of the stressed plants.

Species/Genotype	$\Psi_{\Pi\ control}$ (MPa)	$\Psi_{\Pi\ stress}$ (MPa)		Contribution to the osmotic potential (%)	
NaCl (mM)	0	60	90	60	90
***P. vulgaris* PI325687 (WT)**					
Sucrose	-0.49	0.01	0.05	-1.2	-4.3
Hexose	0.12	-0.14	-0.05	12.3	4.4
Total inorganic ions	-0.94	-0.73	-0.87	65.8	71.9
***P. vulgaris* G04017 (CS)**					
Sucrose	-0.09	0.23	0	-19.9	-0.4
Hexose	0.02	-0.37	-0.17	32.7	13.9
Total inorganic ions	-0.99	-0.60	-0.76	53.1	62.1
***P. acutifolius* G40169 (WS)**					
Sucrose	-0.09	0.12	0	-32.2	-0.4
Hexose	-0.02	-0.39	-0.06	33.5	4.4
Total inorganic ions	-1.04	-0.49	-0.67	41.9	48.7
***P. acutifolius* G40142 (CT)**					
Sucrose	-0.07	0.30	0.29	-27.4	-24.9
Hexose	-0.05	-0.19	-0.33	17.8	28.2
Total inorganic ions	-0.90	-0.44	-0.61	41.9	51.9

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PHENOLOGY, GROWTH ANALYSIS AND YIELD OF BEANS IN ALKALINE SOILS

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INTRODUCTION

The most prominent nutritional disorder of crop plants grown in alkaline soils (pH>7) is iron deficiency and is often enhanced by poor soil aeration caused by compaction or high water content and low soil temperatures which keep the soil wet for longer . The crop species or cultivars within species which grow in alkaline soils without developing symptoms of chlorosis are called iron efficient. Those that became chlorotic are called iron inefficient (Brown and Jones,1976). The differences in iron efficiency are basically related to differences in acquisition of iron by the root. The responsible mechanisms have been reviewed elsewhere (Romheld and Marschner, 1986). The differences obtained in resistance to chlorosis between soybeans and peanut cultivars when growth on calcareous soils provide a classical example of genetically controlled mineral nutrition in general and iron nutrition in particular (Froenlich and Fehr,1981). The knowledge of growth analysis and their relation with the yield of the crops in alkaline soils is the importance for generate strategies for get highest yield. This was the aim of the present study.

MATERIALS AND METHOD

The study was conducted during rainy season in Montecillo Mex. (19º N,98º W and 2250 m of altitude) of semiarid climate. Three cultivars of bush bean *Phaseolus vulgaris* L. Bayomex, Criollo Tequexquinahuac of indeterminate type, and Canario 107 of determinate type and one cultivar of *P.coccineus* L. "Ayocote" of indeterminate type were sown at population density of 6.25 (80*25 cm) plants m^{-2} on June 19, 2000 in a dry clay soil with pH 8. The experimental design was a randomized block with 4 replications. All experiments were fertilized with 100-100-00 NPK. The phenology was registered and harvest of two plants by treatment-replication were realized to 24, 53 and 83 days after sowing (das) for calculated the mean of: leaf area (LA,dm^2 $plant^{-1}$); biomass (B,g $plant^{-1}$); specific leaf area (SLA,dm^{-2} g^{-1}); leaf area ratio (LAR,dm^2 g^{-1});leaf weight ratio (LWR, g g^{-1}) according with Escalante and Kohashi (1993) .The absolute growth rate (AGR, g day^{-1}) and net assimilation net rate (NAR, g dm^{-2} day^{-1}) were calculate with the regression analysis between biomass and das; biomass and leaf area, respectively. The final harvest the biomass (BIO; g $plant^{-1}$), seed yield (SY, g $plant^{-1}$), biomass per day (BD) and seed yield day^{-1}(SYD) were evaluated.

RESULTS AND DISCUSSION

Genotypes emerged 10 days after sowing (das). Flowering occurred at 42 das for Ayocote and Canario, and 51 and 73 days for Bayomex and Criollo. Physiological maturity was reached 90 and 118 das by Canario and Bayomex, and 132 das by Ayocote and Criollo. In the table 1 that presents the indexes of analysis of growth, the biomass and yield, significant differences are observed among cultivars. The biomass and higher yield was in Ayocote with 36.4 g $plant^{-1}$ and 16.3 g $plant^{-1}$, respectively and Bayomex with 32.6 g $plant^{-1}$ and 18.9 g $plant^{-1}$, respectively. Also, Ayocote showed the LA, SLA, LAR, AGR, NAR higher followed by Bayomex and Criollo. The biomass, yield, LA, SLA, LAR and AGR lower corresponded Canary that showed a chlorosis (iron inefficient,

Brown and Jones, 1976). In contrast, the NAR was also higher in this cultivar. However, it was not reflected in a high production of biomass due yield to leaf area lower and shorter cycle of growth (90 days). On the other hand, highest LWR belonged to Criollo followed by Bayomex, Canario and Ayocote. The regression analysis presented in the table 2, indicates that the biomass and seed yield showed a high (R^2=0 .96 * *) relationship than suggests that to achieve a high yield in bean we should look for plants with more biomass and in turn with a higher (R^2=0 .98 * *) AGR, LA (R^2=0 .85 *) and SLA (R^2=0 .96 * *). These results indicate that to achieve a high yield in alkaline soils, the bean cultivars should be looked for with more biomass, seed yield per day, absolute growth rate, leaf area and specific leaf area.

Table 1. Biomass, seed yield and growth analysis indexes of cultivars of *P. vulgaris* L. and *P. coccineus* L. growth in alkaline soil. Montecillo, Méx.

GAI	AYOCOTE	BAYOMEX	CRIOLLO	CANARIO	F PROB.	TUKEY 0.05
LA (dm^2)	12.9 a	10.6 b	1.4 c	1.7 c	***	2.0
SLA (dm^2 g^{-1})	2.4 a	1.9 b	1.9 b	1.7 b	***	0.3
LAR (dm^2 g^{-1})	0.9 ab	0.9 ab	1.0 a	0.8 b	**	0.1
LWR (g g^{-1})	0.39 c	0.48 b	0.54 a	0.43 b	***	0.05
AGR (g day^{-1})	0.7 a	0.59 b	0.47 c	0.34 d	***	0.1
ANR (g dm^{-2} day^{-1})	1.99 a	1.85 b	1.18 c	1.99 a	**	0.13
BD (g day^{-1})	0.303 a	0.326 a	0.192 b	0.137 b	*	0.058
SYD (g day^{-1})	0.136 a	0.189 a	0.078 b	0.065 b	***	0.053
BIO (g $plant^{-1}$)	36.4 a	32.6 a	23.1 b	11.0 c	***	11.0
SY (g $plant^{-1}$)	16.3 a	18.9 a	9.4 b	5.2 b	***	5.5

,* P>0.01, 0.001, respectively. GAI=Growth analysis indexes.

Table 2. Coefficient of determination (R^2) and F probability between biomass, seed yield and growth analysis indexes in beans. Montecillo Méx.

VARIABLES	R^2	F PROBABILITY
Seed yield vs. Biomass	0.96	**
Seed yield vs. Biomass day-1	0.98	***
Seed yield vs. Seed yield day-1	0.92	**
Biomass vs. AGR	0.96	**
AGR vs. LA	0.85	*
AGR vs. SLA	0.96	**
Biomass vs. Biomass day-1	0.90	**

*, **,*** F probability 10,5, 1 %, respectively.

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YIELD ADJUSTMENT FOR STAND VARIATION IN COMMON BEAN GENETIC BREEDING EXPERIMENTS

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INTRODUCTION

Cultivar yield evaluation requires precision and validity in order that phenotypic observations truly reflect the joint effects of genotype and environment. Plant loss is one of the most common factors to affect the quality of field experiments. To compensate for stand disuniformity, seed yield adjustment for differences in final stand has been a common practice (Vencovsky and Cruz, 1991). The objective of this work was to evaluate of yield adjustment for stand in common bean yield trials and suggest an adjustment procedure based on covariance analysis.

MATERIAL AND METHODS

Data from 35 Rio Grande do Sul Common Bean State Trial, from 33 environments, comprising 16 location and eight cropping years (1987/88 to 1994/95) were analyzed. Genotypes varied according to years. For each environment the covariance analysis of seed yield and final stand was performed to verify the existence of linear-quadratic polynomial relationship, to choose the adequate model (linear or linear-quadratic). The analysis of variance of final stand was also carried out in order to test for significance of genotype. Based on the results of these analyses, four adjustment models have been proposed:

Model 1: Absence of yield adjustment for stand, according to the equation:

$y_{ij} = \mu + b_j + g_i + e_{ij}$, i=1,2,...,g; j=1,2,…,b, where y_{ij}: genotype i observed yield in the j rep; μ: expected general mean; b_j: random effect of rep j; g_i: fixed effect of genotype i; e_{ij}: deviation between the observed and the expected yield at the plot ij. This model is adequate when linear or quadratic effects of stand on yield are not significant.

Model 2: Yield adjustment for stand general mean, following the equation:

L: $y_{ij} = \mu + b_j + g_i + \beta(x_{ij} - \bar{x}) + e'_{ij}$ or LQ: $y_{ij} = \mu + b_j + g_i + \beta_1(x_{ij} - \bar{x}) + \beta_2(x_{ij} - \bar{x})^2 + e'_{ij}$, i=1,2,...,g; j=1,2,…,b, where β, β_1 e β_2: regression coefficients; x_{ij}: observed stand for genotype i in rep j: $\bar{x}$: stand general mean; e'_{ij} deviation between the observed and the expected yield at the plot ij. This model is adequate when the final stand variation is due solely to the experimental error, as, for example, to initial stand differences. However, this model is not adequate to situations where the final stand is affected by genotype effects.

Model 3: Yield adjustment for genotype stand mean, following the equation:

L: $y_{ij} = \mu + b_j + g_i + \beta(x_{ij} - \bar{x}_i) + e''_{ij}$ or LQ: $y_{ij} = \mu + b_j + g_i + \beta_1(x_{ij} - \bar{x}_i) + \beta_2(x_{ij} - \bar{x}_i)^2 + e''_{ij}$, i=1,2,...,g; j=1,2,…,b, where $\bar{x}_i$: genotype i stand mean; e''_{ij}: deviation between the observed and the expected yield at the plot ij. This model is adequate when stand variation among genotypes occurs solely from genotype effects.

Model 4: Yield adjustment for genotype group stand mean, following the equation:

L: $y_{ij} = \mu + b_j + g_i + \beta(x_{ij} - \bar{x}_k) + e'''_{ij}$ or LQ: $y_{ij} = \mu + b_j + g_i + \beta_1(x_{ij} - \bar{x}_k) + \beta_2(x_{ij} - \bar{x}_k)^2 + e'''_{ij}$, i=1,2,...,g; j=1,2,…,b, where $\bar{x}_k$: stand mean for genotype group k; e'''_{ij} : deviation between the observed and the expected yield at the plot ij. This model takes into account genotype differences due to solely experimental error. It is adequate when the stand variation among genotypes is due exclusively to genotype group effects. Grouping of genotypes according to their effects on stand can be performed with any appropriate clustering method, such as the test of Scott and Knott.

The choice of the appropriate model should be based on the knowledge about the origin of the genotype stand variation, which can be due to genotype effects or to characteristics linked to genotypes. In the present study, available information on environments did not permit the identification of this variation.

RESULTS AND DISCUSSION

In 70% of the environments tested, the effects of stand upon yield where linear or quadratic. In 85%, it was detected genotype effects on stand. Stand compensation was observed at different intensities at different environments. Results suggest that yield adjustment can contribute to the improvement of common bean genotype evaluation. However, the non-significant effects of stand on yield, for some of the environments, also suggest the existence of a compensatory effect when the stand reduction is not too drastic (Adams, 1967; Shimada et al., 2000; Ribeiro et al., 2004). In such cases adjustments are not necessary. It can be concluded that seed yield can be affected by final stand variation, and that final stand reflects genotype variation. Thus, yield adjustment for stand variation, under the consideration of genotype effect on stand, is important in breeding experiments. The record of information that would allow discerning the origin of stand variation is very important.

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GRAIN YIELD OF FOUR NEW BEAN CULTIVARS BASED ON PLANT DENSITY

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INTRODUCTION

New types of beans were a good way to add value to the end production, only with the new choice of cultivar for sowing. This demand took some bean programs to dedicate also to the other commercial types, beyond the "carioca", that already are available. However, to make possible the effective utilization of these new cultivars by farmers, has necessity to test them in different soil and climate conditions, mainly with relation to the plant densities. Aiming to study the agronomic behavior of four news beans cultivars in different plant densities in the north region of the Minas Gerais State, Brazil, were conducted two field experiments on Mocambinho and Jaíba localities.

MATERIAL AND METHODS

The experimental design was randomized blocks with three replications and a 4x5 factorial scheme involving four cultivars and five plant densities (100, 200, 300, 400 and 500 thousand plants.ha^{-1}). The cultivars were BRS-Radiante (determinate growth, habit type I and large mottled grain), Ouro Vermelho (indeterminate, type II and small red grain), Bolinha (indeterminate, type II and small yellow grain) and Novo Jalo (indeterminate, type II and large yellow grain) (Ramalho & Abreu, 2006). Each plot had four rows with 5.0 m length and spacing of 0.5 m between rows. The manual sowing was carried on July and the harvest, on October/2007. At sowing, all the plots had received identical fertilization, determined by the soil analysis interpretation. The N fertilization at covering was carried at to the 21 days after emergency, using 30 kg.ha^{-1} of N, urea source. The experiments were lead under irrigation, using the conventional aspersion at Mocambinho and microaspersion at Jaíba. In the harvest, samples of 10 plants for determination of the pod number per plant, grain number per pod and one hundred grains weight were collected. The grain yield was determined by the total grain weight of the each plot.

RESULTS AND DISCUSSION

The bean cultivars differed in relation to all the evaluated characteristics and this effect varied with the localities. The grain yield of the four cultivars varied from 1962 kg ha^{-1} at 3410 kg.ha^{-1}. The bean cultivar BRS Radiante showed the bigger grain yield on the two localities, but on Mocambinho it did not differ from the cultivar Bolinha (Table 1). The increment of the plant density in the interval of 100 the 500 thousand plants.ha^{-1} reduced the grain number per pod (Figure 1) and the pod number per plant, this last differentially on the two localities (Figure 2) and on each cultivar (Figure 3). The grain yield, however, was not influenced by the plant densities.

TABLE 1 – Averages values of the pod number per plant, grain number per pod, one hundred grains weight (g) and grain yield (kg ha^{-1}) of four bean cultivars grown at two locations Jaiba and Mocambinho.

Cultivars	Pods.plant^{-1}	Grains.pod^{-1}	100 grain weight	Grain yield
Jaiba	16	4,0	33,4	2566
BRS Radiante	17 **b**	3,4 **c**	38,6 **a**	3410 **a**
Novo Jalo	12 **d**	3,9 **b**	38,7 **a**	2492 **b**
Bolinha	14 **c**	4,0 **b**	35,1 **b**	2399 **b**
Ouro Vermelho	20**a**	5,0 **a**	21,3 **c**	1962 **b**
Mocambinho	11	4,0	31,8	2452
BRS Radiante	36,6 **b**	13**a**	3,3 **b**	2739**a**
Novo Jalo	40,0**a**	7 **c**	3,6 **b**	2311 **b**
Bolinha	31,5 **c**	10 **b**	3,9 **b**	2530**a**
Ouro Vermelho	19,1 **d**	14**a**	5,3**a**	2227 **b**

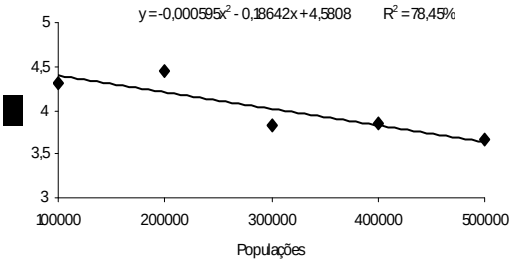

FIGURE 1 – Grain number per pod of the bean crop in relation to the plant densities (averages from four cultivars and two localities). Winter season 2007.

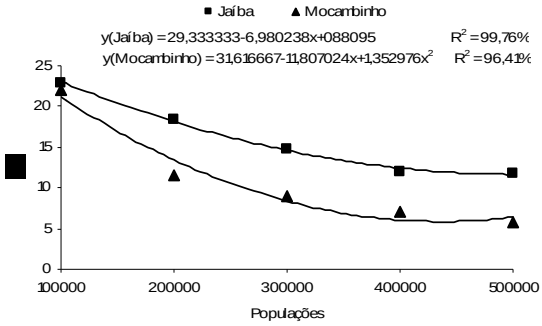

FIGURE 2 – Pod number per plant of the bean crop in relation to the plant densities at two localities (averages from four cultivars). Winter season 2007.

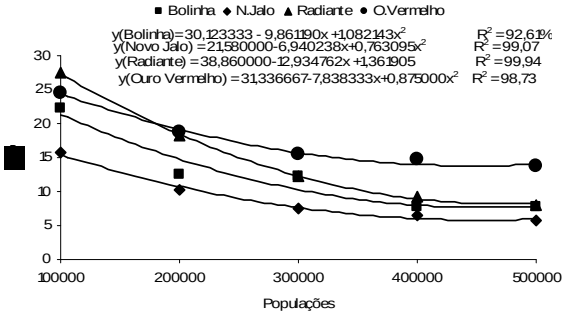

FIGURE 3 – Pod number per plant of the bean crop in relation to the plant densities by four cultivars (averages from two localities). Winter season 2007.

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CROP GROWTH OF FOUR NEW BEAN CULTIVARES BASED ON PLANT DENSITY UNDER CONVENTIONAL AND NO TILLING SYSTEMS

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INTRODUCTION

Amongst other factors, the ideal plant population for beans production is function of the cultivar and, more specifically, of the plant architecture. Populations advanced with the too much production factors are a practical technology, of easy acceptance, without additional cost and that they can promote economic profits. Aim to determine the main effects of the plant density on the plant height and plant dry weight of four news beans cultivars of erect architecture, two field experiment were carried out on a conventional (CO) and no-tillage crop system under *Brachiaria* sp. straw (NT). The sowing was accomplished at 2006/2007 spring-summer, in a typical dark red latossol at experimental area of the Departamento de Agricultura, Universidade Federal de Lavras, Minas Gerais State, Brazil.

MATERIAL AND METHODS

The experimental design was randomized blocks with three replications and a 4x5 factorial scheme involving four cultivars and five theoretical plant densities (100, 200, 300, 400 and 500 thousand plants.ha^{-1}). The cultivars were BRS-Radiante (determinate growth, habit type I and large mottled grain), Ouro Vermelho (indeterminate, type II and small red grain), Bolinha (indeterminate, type II and small yellow grain) and Novo Jalo (indeterminate, type II and large yellow grain) (Ramalho & Abreu, 2006). Each plot had four rows with 5.0 m length and spacing of 0.5 m between rows. The manual sowing was carried on November/2006 and the harvest, on February/2007. At sowing, all the plots had received identical fertilization, determined by the soil analysis interpretation. The N fertilization at covering was carried at to the 21 (conventional system) or 30 (no-till system) days after emergency (DAE), using 30 kg.ha^{-1} of N, urea source. The experiments had not been irrigated. Every 10 days, samples of 10 plants for determination of the plant height and dry weight were collected. The plant height was measured from the soil-level at the last leaf insertion on principal shoot. The collected material was dry under air circulation to 65-70^{o}C, even constant weight.

RESULTS AND DISCUSSION

The final populations had been inferior to the waited ones. In both the sowing systems, CO and NT, the cultivars differed in relation to the plant height, presenting the following decreasing sequence: Ouro Vermelho > Novo Jalo > Bolinha > BRS Radiante (Figures 1 and 3). All the cultivars showed increase of height with the increment of the plant population, except the New Jalo cultivar in the CO system, that reduced the plant height with the increase of the plant density (Figures 2 and 4). The dry matter accumulation grew linearly until end of the cycle (Figures 5 and 7). In general way, the cultivars showed increase of the dry matter accumulation with the increase of the plant density, except the BRS Radiante cultivar in NT system (Figures 6 and 8).

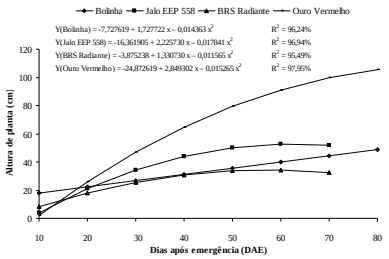

FIGURE 1 – Plant height (cm) of four bean cultivars in relation to the days after emergency, on conventional system. Lavras, spring-summer, 2006/2007.

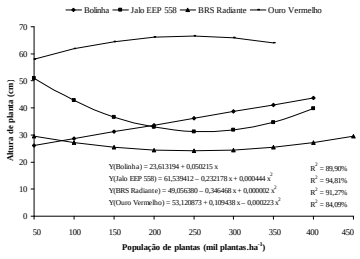

FIGURE 2 – Plant height (cm) of four bean cultivars in relation to the plant densities, on conventional system. Lavras, spring-summer, 2006/2007.

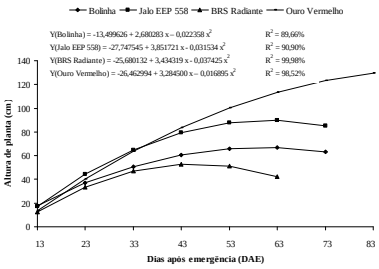

FIGURE 3 – – Plant height (cm) of four bean cultivars in relation to the days after emergency, on no till system. Lavras, spring-summer, 2006/2007.

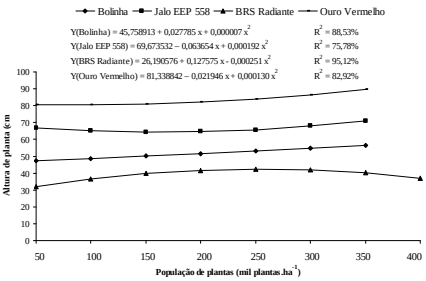

FIGURE 4 – Plant height (cm) of four bean cultivars in relation to the plant densities, on no till system. Lavras, spring-summer, 2006/2007.

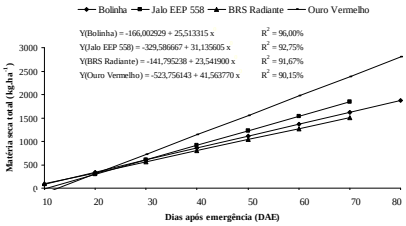

FIGURE 5 – Dry weight (kg.ha^{-1}) of four bean cultivars in relation to the days after emergency, on conventional system. Lavras, spring-summer, 2006/2007.

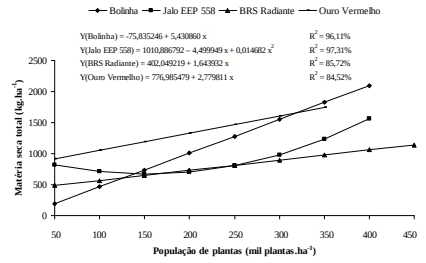

FIGURE 6 – Dry weight (kg.ha^{-1}) of four bean cultivars in relation to the plant densities, on conventional system. Lavras, spring-summer, 2006/2007.

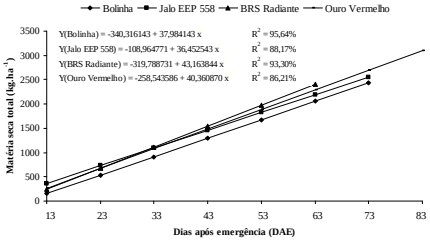

FIGURE 7 – Dry weight (kg.ha^{-1}) of four bean cultivars in relation to the days after emergency, on no till system. Lavras, spring-summer, 2006/2007.

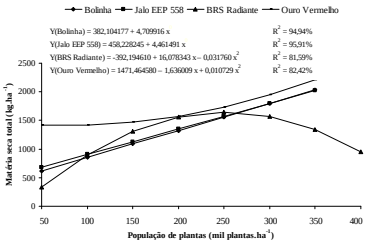

FIGURE 8 – Dry weight (kg.ha^{-1}) of four bean cultivars in relation to the plant densities, on no till system. Lavras, spring-summer, 2006/2007.

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BRAZILIAN LAND RACE GERMPLASM YIELD POTENTIAL

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INTRODUCTION

In order to accomplish with urban population demand for common bean (*Phaseolus vulgaris* L.), a staple food in Brazil, the Embrapa Temperate Climate Research Center – CPACT - common bean breeding program has selected as main goals the improvement of both agronomic performance and nutritional profile. Such accomplishment would suffice not only producer needs, but also consumer ones. Among the available germplasm for breeding purposes, land races represent a very important source. This importance is due to the high genetic variability present as result of the different environments in which common bean is grown, that exert distinct evolutionary forces, understanding that the plant population simultaneously exert environmental changes, ending up in a co-evolution of plants and environments. In order to find answers on the potential of land race germplasm as source of genes for yield improvement as well as new cultivars for direct use, and as such summing up elements to adjust improvement for agronomic performance, a comparison on yield of land races and CPACT-derived breeding lines, was conducted.

MATERIAL AND METHODS

Common bean land races obtained both through donation by farmers and field collections, and CPACT–derived breeding lines, were compared. Land races have their origin from all production regions of Rio Grande do Sul State, in Southern Brazil, and some of them have been cultivated for more than 50 years. Mostly of the breeding lines were at F_5 generation and resulted from selection performed at the common bean breeding program of CPACT. The land races were sown in individual test rows 4m long, 0.5m apart, with a seed density of 12 seeds m^{-1}. At each ten line-group, an individual line of both cultivars BRS Expedito and BRS Campeiro were sown and used as a parameter for performance comparison. BRS Expedito is a black seeded cultivar released by the CPACT common bean breeding program that presents a high yielding potential, a good field resistance to anthracnose, and a high protein content, being suitable to direct harvest. BRS Campeiro, is also a black seeded cultivar, released by Embrapa Rice and Common Bean Research Center, with broad adaptation and high yielding potential. The trials were carried out in 2005/06 and 2006/07 at Embrapa Temperate Climate, located in Pelotas, Rio Grande do Sul State, Brazil. Fifty land races and 56 CPACT-derived breeding lines were tested in 2005/06 cropping year, whereas 57 land races and 60 CPACT breeding lines were tested in 2006/07. Sowing dates were, October 27 and November 13, in 2005 and 2006, respectively. The comparison of line behavior was based on the yield mean of the check cultivars for each group of ten lines.

RESULTS AND DISCUSSION

Results have shown that in 2005/06, 28,0% of the land races tested outyielded the mean of the check cultivars as compared to 69.0% for the breeding lines. The check cultivars mean was 1,940.1 kg ha^{-1}. The same way, 28% of the land races presented yields above check cultivars yield mean, in 2006/07, compared to 31% of the breeding lines. The check cultivars mean in 2006/07 was 1,421.2 kg ha^{-1}. Another important observation refers to the fact that in the 2005/06 cropping year, 14% of the land races have shown a yield performance superior to the general mean for the best check cultivar for each ten-lines group. For the same comparison, 30.3% of the breeding lines have shown a better performance in relationship to the best check cultivar mean yield. In 2006/07, the values for the comparison with the best check cultivar mean, were 12.3 % for the land races and 29.0% for the breeding lines. It was also observed that the mean yield of the land races, decreased 41.9% from 2005/06 to 2006/07, as compared to the breeding lines, which did not suffer any decrease in mean yield. This result suggest that the breeding lines, that were selected at the site where the trials were carried out, have a greater yield stability for this site, while land races, in opposition to the breeding lines, are originated from diverse environments possessing specific adaptation to these sites. It is recognized that the land race germplasm under study show a very promising picture as new cultivars as well as new sources of genes for the common bean yield improvement program.

BULGARIAN LANDRACES AND LINES OF COMMON BEAN (*PHASEOLUS VULGARIS* L.) WITH RESISTANCE TO BACTERIAL WILT

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Curtobacterium flaccumfaciens pv. *flaccumfaciens* (Hedges) Collins & Jones is a bacterial pathogen on common bean (*Phaseolus vulgaris* L.) that is found in all continents of the world except Antarctica (CABI/EPPO, 1999). In the field, diseased bean plants exhibit wilting, with or without interveinal, necrotic lesions surrounded by bright yellow borders, or stunted growth (Kiryakov et al., 2008). Breeding for resistance is the most efficient means for control of bacterial wilt. In the past few years researches are being carried out in DAI – General Toshevo, aimed at breeding of bean cultivars resistant to *Curtobacterium* pv. *flaccumfaciensi*, by using land races and selected lines as resistance donors (Genchev et al., 1998; Kiryakov and Genchev, 2000; Hsien et al., 2005).

MATERIAL AND METHODS

285 accessions from Dobroudja Agricultural Institute – General Toshevo core collection were tested for physiological resistance to bacterial wilt. The plants were sown in two rows, each 2 m long, in two replications, in randomized design of the accessions in the trial field of DAI – General Toshevo. The plants were inoculated with bacterial suspension 10^8 cfu/ml from isolate CC 96212 (yellow strain) in the cotyledonary node, after removing the cotyledons at stage V_2. The last four plants in the rows were injected with sterile water and used as checks. The bacterial wilt reaction of the accessions was rated at stage R_6, 28 days after inoculation, according to the following two scales. The first scale included interveinal, necrotic lesions and wilting of trifoliates: 1 – no symptoms; 3 – single trifoliate leaves exhibit necrosis and wilting; 5 – several trifoliate leaves exhibit necrosis and wilting; 7 – more trifoliate leaves exhibit necrosis and wilting; 9 – plants are killed. The second scale included different degrees of plant stunt: 1 – vigorous growth; 3 – weak vigour; 5 – plant stunting about ¾ according to the check; 7 – plant stunting up to ½; 9 – plant stunting over ½.

RESULTS AND DISCUSSION

Table 1 shows eight landraces Trakia, Trudovets, Zornitsa, Grozevo 1, Grozevo 2, Pirina, Kresna 23 and Bello Polle 1) and two selection lines (Elixir and DG 91-10-lan) with immune reaction (score 1) under field conditions. According to Kiryakov et al. (2008), the symptoms of wilting and stunted growth in common bean (*Phaseolus vulgaris* L.) are controlled by different genes. The above accessions possessed resistance both to the reaction of wilting and stunted growth. Seven donors of resistance had habit type Ia, and habit types IIa, IIIb and IV had each one accession. With the exception of Pirina, the rest of the accessions were characterized with short vegetation (80-86 days). All accessions had medium to large seeds (36 – 67 g/100 seeds).

The combining of high resistance to both wilting and stunted growth with I and II habit type, early maturation and large seeds makes the accessions from Table 1 very suitable for breeding of II type varieties, early maturing, with large seeds, erect habit and suitable for direct harvesting.

Future work should investigate the performance of common bean land races and breeding lines with regard to the other breeding traits. These resistant parents should be useful for future genetic improvement of multiple disease resistance and productivity of common bean.

Table 1. *Bean accessions with immune reaction (score 1) to C. f.* pv. *flaccumfaciens under field conditions.*

Accessions			[z]Leaf necrosis and wilting	[y]Stunted growth	[x]Growth habit	Vegetation period, days	100 seeds weigth,g
Elixir			1.0	1.0	IIIb	82	41
DG 91-10-lan			1.0	1.0	IIa	90	36
Trakia			1.0	1.0	Ia	85	51
Trudovets			1.0	1.0	Ia	85	49
Zornitsa			1.0	1.0	Ia	84	44
Grozdevo 1			1.0	1.0	Ia	84	48
Grozdevo 2			1.0	1.0	Ia	83	46
Pirina			1.0	1.0	IVa	95	67
Kresna 23			1.0	1.0	Ia	86	37
Bello polle 1			1.0	1.0	Ia	85	43

[z]Resistant reaction, scores by first scale : *1 – immune reaction and 9- very susceptible reaction.*
[y]resistant reaction, scores by second scale: *1 – immune reaction and 9- very susceptible reaction.*
[x]Growth habit**:** *I – Determinate growth habit; II – Indeterminate growth habit, vegetative terminal bud on main stem and branches, both main stem and branches strong and upright; III – Indeterminate growth habit, branches relatively weak and open, semi-prostrate or twining.*

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COMMON BEAN (*PHASEOLUS VULGARIS L.*) DISEASES IN BELARUS

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The production of common bean (both for dry seeds and green pods) is stably increasing in Belarus. The reasons for such high interest to this crop are the realization of the state programs on general increasing of vegetables production and economical benefits of common bean cropping. Cool rainy climate conditions at summer and during seed harvesting, and plant diseases are the major reasons which reduce yield. Therefore the objective of our research was to identify the most spread common bean diseases in Belarus and identify the sources of resistance to include them as parental components into breeding programs.

The general inspection of the common bean fields both in smallholdings and large-scale agricultural farms have shown the wide spread occurrence of the bacterial diseases. Halo blight (HB, caused by *Pseudomonas syringae* pv. *phaseolicola*) is a predominant type of such diseases. Common bacterial blight (CBB, caused by *Xanthomonas campestris* pv. *phaseoli)* and brown spot (BS, caused by *Pseudomonas syringae* pv. *syringae*) were also found. But harm caused by these 2 last diseases is not too serious because, first, there spread is limited and, secondly, CBB begins to develop at the end of summer and beginning of autumn when the forming of the seeds of the improved and local varieties is finishing. Fungal diseases like anthracnose, white mold, and fusarium wilt, and viral infection which sometimes can be detected in germplasm are scarce and have no serious economical effect at the present time. The investigation of the level of the plants infection by Halo blight has shown that sometimes 100 % of susceptible plants can be infected. In general, we observe the ordinary symptoms and development of Halo blight on common bean plants in Belarus.

Our further goal was to isolate the pathogens and to identify races of the *P. syringae pv. phaseolicola* by using of classical methods of Mohan and Schaad, (1987); Taylor et al., (1996). As a result we have identified 2 races of Halo blight (1 and 2) from the samples of local varieties collected from different parts of Belarus. We also have detected all the known races except 3 and 6 on the plants of germplasm samples.

Genetic resistance is the most effective control method of bacterial blights. Our next study examines the potential of local samples in susceptibility to Halo blight. As we re-sowing all the germplasm samples annually thus we have the results of plants tests in the natural infectious conditions. And as a result we have selected a number of the collection and local samples which are never demonstrated the symptoms of Halo blight both on leaves and pods. But the infection of the plants in this case was casual. Therefore we have selected 30 high productive landraces to determine their resistance to Halo blight using artificial infection of the plants by the our own isolates of races 1 and 2 in the field conditions on the stage of beginning of flowering (leaves reaction) and after beginning of the pods formation (green pods reaction) in 2005 and 2006. Selected population of landraces has shown the high diversity in reaction of the leaves and pods. And the samples LV-10, LV-35, LV-61, LV-65, LV-98 were most resistant to both races. All these bush type lines originated from the northern regions of Belarus, has the brown, black and buff colored seeds and very short period of vegetation (90-95 days from sowing to seeds harvesting). All these samples were included into our breeding programs.

But breeding of the new varieties needs a long period of time. Thus we looked at ways of chemical control of Halo blight. And the preparation on the base of poly-hexamethyl-guanidin-hydrochloryde (named ISAR (10 % water soluble concentrate), registered in Belarus) in concentration 0.2 % was the most effective as for seeds treatment (30 ml/kg of seeds), as a spray of the plants after appearance of the first trifoliate leaf for prophylaxis. We have not yet investigated the effect of this preparation to the next inoculation of the roots by Rhizobium, but seeds treatment by ISAR is a good way to exclude the seeds infection for recovery of some very susceptible to Halo blight samples.

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UTILIZATION OF GERMPLASM OF TWO BEAN SPECIES (*PHASEOLUS VULGARIS L., PHASEOLUS COCCINEUS L.*) FOR BREEDING IN BELARUS

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The accurate characterization of germplasm accessions of a crop species under consideration of all available morphological, phenological, and molecular information is the basis of every sensible breeding program. We have started the collection and evaluation of *Phaseolus vulgaris* and *coccineus* samples in 1996. Germplasm collection of the common bean consist of 1183 accessions now and more than half of them are originated from the collection of Cambridge University and transferred to Belarus by Adrian J. Shirlin in 1996. Most part (~ 80 %) of *Phaseolus coccineus* collection (total 92 accessions) are the local samples, other accessions were selected as matured in Belarusian conditions from received from different European genebanks. Including of new samples into collections is carried out after 1-year field tests. We test and add the new samples of the common and runner beans into our germplasm annually.

The botanical and agronomical evaluation of the collection samples is carried out under "Handbook on evaluation of Phaseolus germplasm" by C. De La Cuadra, A M. De Ron and R. Schachl with some modification for seed colour. We have not a possibility for the long term saving of the seeds, therefore we re-sowing the collection samples annually with full estimation of the traits. Thus, these our activities during 12 years have permitted us to collect the present germplasms with high variability of the investigated traits.

Common bean cropping in Belarus, especially in the south regions, have shown socially and economically importance both in small farmers (mainly, dry bean) and farm industry (both dry and French bean). Small farmers usually use the wide range of the landraces. There is only 1 cultivar of the dry bean registered in Belarus in 1972. There are few varieties of the French bean registered in Belarus and all of them are originated from Europe (mainly, from Poland and Russia). Thus, genetic breeding research is largely responsible for the development of this crop in Belarus, because there is no really good adopted to Belarusian condition varieties with stable productivity now. Thus, we have started the selection of the parental pairs and hybridization in 1997 and in 1999 we have carried out the hybridization of 8 varieties of Navy and Small white types (Snowbounting, Fleetwood, Upland, Edmund, Harofleet, Mela, Adrian, Albion - most perspective commercial types of common bean in Belarus) by diallel scheme to estimate the combine abilities of the parental samples and adaptive abilities of the hybrid population to determine the best parent components for the future breeding programs. As a result we have now some perspective high productive lines of Navy type very adaptive to Belarusian conditions.

We have received a number of hybrid populations of common bean and some new lines from Cambridge (UK) together with germplasm samples. As a result of the next breeding work we have selected some very productive and adaptive lines and one from them (named Riche, Navy type) now is tested in State Commission for varieties testing. Thus, such international cooperation permitted us

to produce new varieties with high seeds productivity (up to 3,4 t/ha in Riche (+20 % to standard variety after 2-years state trials).

There is not any variety of *P. coccineus* registered in Belarus, but interest for this crop is very high because the seeds are very large. The most part of the samples from European genebanks is too late matured and has very long pods. Some from the collected white-seeds climbing local samples have short pods and seeds are located very close. Also the most part of the local samples are fully matured before end of September (the closing date to harvest bean seeds in Belarus). Another challenge problem for the breeding of new varieties of *P. coccineus* for Belarus is the absence of samples with bush determinant type of the plants which combine with short vegetation period in the collected germplasm. Using of some varieties with determinant bush type (but with long period of vegetation) by way of the source of such habitat and some white-seeds early matured local samples we have created a hybrid populations with wide range of the traits. Some lines from these populations can be perspective to be registered as new varieties.

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EVALUATION OF THE CARIOCA AND BLACK BEANS GROUP LINES INOCULATED WITH *RHIZOBIUM TROPICI* STRAINS

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INTRODUCTION

Studies with bean plant in soils with low N availability indicate the need to obtain segregating families seeking to get efficient cultivars to the biological nitrogen fixation (BNF). Due to the great genetic variability among beans cultivars, the identification of more efficient genotypes to BNF will make possible the selection of more efficient symbiotic systems, reducing or eliminating the Nitrogen fertilization (Hardarson et al, 1996; Bliss, 1993). The objective of the work was to evaluate the crop production of advanced lines of Carioca and Black bean groups, inoculated with two *Rhizobium tropici*.

MATERIAL AND METHODS

Two trials were carried out in Ponta Porã district, MS, Brazil (22º32` S e 55º42` W), in a low fertility clay oxisoil area. The experimental design was a randomized block with three replications. The tested treatments resulted of a 14x2 factorial for the Carioca bean group and a 12x2 factorial for the Black bean group, being 14 and 12 the beans genotypes and 2 the levels of nitrogen covering fertilization (0 and 60 kg ha^{-1} of N-urea), applied 30 days after the sowing. In the treatment without N fertilization the seeds were inoculated with a *Rhizobium tropici* bacteria mixture (SEMIA 4077 (CIAT 899) and SEMIA 4080 (PRF 81)) with density of 10^9 cells g^{-1} of turf in the proportion of 1000 g of inoculate for 50 kg of seeds. Before sowing the experimental area was fertilized with 320 $kgha^{-1}$ of the formulated 00-20-20 ($N\text{-}P_2O_5\text{-}K_2O$). The obtained datas were submitted to variance analyses and the averages compared by Tukey test at 5%.

RESULTS

Significant differences ($p<0.05$) of crop production were observed among the lines tested for the two bean groups, indicating significant genetic variability among the genotypes. This is a satisfactory result, because indicates the possibility of selection of more productive lines for the Carioca and Black bean group. However, significant effect for N fertilization on crop production was observed only for lines of Black bean group. In this case the observed effect occurred due to covering N fertilization. When the seeds inoculation with *Rhizobium tropici* bacteria strains was used, seeking the nitrogen biological fixation, significant effects were not observed among the lines, independently of the bean group considered (Table 1). In agreement with GRAHAN (1981) factors as carbohydrates supply for the nodules and N absorption should been contributed to the genotypic variability of the lines that presented rates of BNF that supplied the N plant demand and guaranteed similar crop production with those obtained in the treatments with N covering fertilization. In the black bean group stood out the lines CNFP-10104, CNFP-10109 and BRS-Grafite, that presented medium grains production of 1732, 1754 and 1773Kg ha^{-1}, respectively. Among the lines of Carioca group the prominence were to the lines CNFC-10438 and BRS-Pontal, that presented grains production of 1550 and 1741 kg ha^{-1}, respectively. These values for grains production are higher

than national average that is of 800 kgha^{-1} and they indicate high capacity of BNF and great productive potential of these lines.

Table 1. Medium crop production values of Carioca and Black bean groups in function of the inoculation and N fertilization.

Bean Carioca Group*		Bean Black Group	
Inoculated	N fertilization	Inoculated	N fertilization
Crop production (kg ha^{-1})			
1255 a	1.360 a	1545 b	1658 a

* Averages followed by the same letter for each Bean group, do not differ by Tukey test at 5%.

CONCLUSIONS

- The lines of Carioca bean group presented high capacity of biological nitrogen fixation and high grains production but did not differ from those obtained in the treatments with N fertilization. Outstanding lines CNFC-10438 and BRS-Pontal, generated grain production of 1550 and 1741 kgha^{-1}, respectively.

- The highest grains crop productions, among bean Black group lines, were obtained when the N fertilization was used. However the CNFP-10104, CNFP-10109 and BRS-Grafite lines when only inoculated presented medium grains production of 1732, 1754 and 1773 kgha^{-1}, respectively.

- The high crop productions obtained by some inoculated lines of Carioca and Black bean groups, indicate the possibility of selection of the high performance cultivate and independent of the N fertilization.

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ANSWER OF TWO CARIOCA BEANS CULTIVAR TO THE CHEMICAL AND ORGANIC FERTILIZATION

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INTRODUCTION

The bean plant (*Phaseolus vulgaris* L.) is cultivated in different regions of the Brazil. It is an important protein source, especially for smaller budge populations. The brazilian dry bean production in 2006 was about 3.5 million tons and Paraná state participated with 23.7% of this production (IBGE, 2007). In Brazil the utilization of urban residues *in natura* or composed in soil organic fertilization is a frequent agricultural practice. This work was carried out with the objective to evaluate the effect of the *in natura* urban residues (the grinding of tree branches) and chemical fertilization, on the components of production and the grain yield of two carioca beans cultivar.

MATERIAL AND METHODS

The experiment was carried out at the experimental farm of the State University of Londrina, Londrina, Paraná, Brazil. The experimental design was a randomized block with treatments distributed in a 5x2 factorial arrangement, which the factors were five doses (0, 15, 30, 45 and 60 Mg ha^{-1}) of urban organic residues (the grinding of tree branches) and two chemical fertilization levels (without and with inorganic fertilization (80, 50 and 30 kg ha^{-1} of N, P_2O_5 and K_2O, respectively)), with three replications. Two bean cultivar of the Carioca (Colibri and Eldorado) were tested. The evaluated variables were: pods number/plant (**PNP**), grain mass/plant (**GMP**), the 1000 grains mass (**M1000**) and grain yield. The obtained data was submitted to variance analyses and the averages were compared by Tukey test at 5% or adjusted to regression equations.

RESULTS AND DISCUSSION

The **PNP** and **GMP** medium values of the two carioca beans cultivars in the chemical fertilization treatments were significantly higher than those observed in the treatments without fertilization. Only for Eldorado cultivar, the **M1000** was influenced by chemical fertilization (Table 1). However, the observed values (230.0 g) were lower than regional average (IAPAR, 2007).

Table 1. Pods number/plant (**PNP**), grain mass/plant (**GMP**) and 1000 grain mass (**M1000**) for Colibri and Eldorado bean cultivars in response to chemical fertilization.

Variables	Colibri		Eldorado	
	With fert.	Without fert.	With fert.	Without fert.
PNP	**9.8 a**	**6.5 b**	**9.3 a**	**5.5 b**
GMP (g)	**9.6 a**	**6.1 b**	**6.6 a**	**3.4 b**
M1000 (g)	**n.s**	**n.s**	**186.0 a**	**167.0 b**

Averages followed by the same letter in the lines for each cultivar, do not differ by Tukey test at 5%.

The grain yield of the Eldorado cultivar was significantly influenced by chemical and organic residue fertilizations. In the absence of chemical fertilization, the grain yield reduced with the

increase of the residues doses ($y = 2686.5 - 86.621x + 1{,}129\ x^2$, $r^2 = 0{,}88$ and minimum grain yield in 38,5 Mg ha^{-1}).

In the treatments with chemical fertilization, the significant effects ($p < 0{,}05$) were observed only for residue doses which was above than 15 Mg ha^{-1}, however for all the treatments the grain yield were below of the regional average of 2948 kg ha^{-1} (IAPAR, 2007).

Except for grain yield of Eldorado cultivar, the organic residues did not affect the values of the other studied variables. The highest values were observed to treatments with chemical fertilization. These results are in agreement with those obtained by Andrade et al. (2005).
Only for the Colibri cultivar, were obtained significant and positive correlation among the variables PNP and GMP and the grain yield ($r=0{,}75$ and $r=0{,}79$, respectively).

CONCLUSIONS

The fertilization with organic residues reduced the grain yield of Eldorado bean cultivar, but did not have a significant effect on the Colibri bean cultivar

The highest values for grain yield, pods number per plant and grain mass per plant were obtained for the Colibri bean cultivar when inorganic fertilizer was used.

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NITROGEN FERTILIZATION AND GROWTH OF TWO DRY BEAN CULTIVARS IN NO-TILLAGE SYSTEM

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INTRODUCTION

The appropriate management of nitrogen fertilization is fundamental to increase dry bean yield. On no-tillage system the N dynamics is modified by organic material decomposition, which is concentrated on soil surface, providing a temporary immobilization of the N available to plants from fertilization by part of microorganisms. Thus, it requires the using of high N doses in occasion of crops seeding (Sá, 1998). This work was carried out with the objective to evaluate the nitrogen fertilization effect, applied on seeding and fractioning, upon growth and development of two dry bean cultivars on no-tillage system.

MATERIAL AND METHODS

Field experiments were carried out in Apucarana and Maringá counties (summer crop season 2005/2006), Paraná State, Brazil, in a red nitosoil. Two dry bean cultivars IPR Juriti (Carioca type) and IPR Uirapuru (Black bean) were submitted to different nitrogen (N) levels. The used experimental design was a randomized block, with nine treatments and five replications. Treatments were: T_1 (0+0); T_2 (50+0), T_3 (25+25), T_4 (100+0), T_5 (50+50), T_6 (150+0), T_7 (75+75), T_8 (200+0) and T_9 (100+100) kg N per hectare at seeding and topdressing, respectively. The leaf area index, leaves dry matter, canopy dry matter, plant height, root collar diameter and plants final stand were evaluated.

RESULTS AND DISCUSSION

Data submitted to statistical analysis showed that the growth of two dry bean cultivars were dependent on N fertilization level and also on the environment conditions. Both dry bean cultivars showed a superior plant growth in Maringá (Table 1, 2 and 3).

Table 1 – Mean values of treatment, local, interaction treatment versus local for the leaf area index (LAI), leaves dry matter (LDM), canopy dry matter (CDM) and root collar diameter (RCD) of IPR Juriti common bean cultivar under to nitrogen fertilization. Apucarana and Maringá, PR, BR. 2005/2006.

	LAI			LDM (g pl^{-1})			LDM (g pl^{-1})			RCD (mm)		
Treatments	Treat.	Apuc.	Mgá.	Treat.	Apuc.	Mgá.	Treat.	Apuc.	Mgá.	Treat.	Apuc.	Mgá.
0+0	2.00b	1.48	2.51b	2.98b	2.55b	3.42b	5.28b	4.74b	5.83b	5.06b	4.57	5.54b
50+0	2.52a	1.87	3.17a	3.96a	3.53a	4.38a	7.56a	6.94a	8.17a	5.59a	5.04	6.13a
25+25	2.62a	2.14	3.09a	3.81a	3.42a	4.21a	7.07a	6.16a	7.98a	5.58a	5.17	5.98a
100+0	2.65a	2.56	2.75b	4.00a	4.26a	3.74b	7.34a	7.96a	6.72b	5.40a	5.12	5.67b
50+50	2.68a	2.08	3.27a	3.91a	3.46a	4.35a	7.51a	6.82a	8.20a	5.57a	5.07	6.07a
150+0	2.61a	2.01	3.21a	3.91a	3.42a	4.39a	7.56a	6.55a	8.56a	5.66a	5.02	6.30a
75+75	2.83a	2.21	3.45a	4.19a	3.83a	4.55a	7.94a	7.41a	8.48a	5.62a	5.26	5.97a
200+0	2.52a	2.09	2.96a	3.72a	3.52a	3.92b	7.19a	6.92a	7.47a	5.60a	5.05	6.14a
100+100	2.82a	2.41	3.24a	4.21a	4.03a	4.38a	8.08a	7.79a	8.38a	5.77a	5.23	6.31a
Mean	2.58	2.09B	3.07A	3.86	3.56B	4.15A	7.28	6.81B	7.75A	5.54	5.06B	6.01A
C.V.(%)	15.98			15.75			17.00			6.28		

The equal small letters on the column and the equal capital letters on the line indicate that the mean are not different among them by Scott – Knott Test (P>0.05).

Table 2 – Mean values of treatment, local, interaction treatment versus local for the leaf area index (LAI), leaves dry matter (LAI), canopy dry mater (CDM) and root collar diameter (RCD) of IPR Uirapuru common bean cultivar under nitrogen fertilization. Apucarana and Maringá, PR, BR. 2005/2006.

Treatments	LAI			LDM (g pl^{-1})			CDM (g pl^{-1})			RCD (mm)		
	Treat.	Apuc.	Mgá.	Treat.	Apuc.	Mgá.	Treat.	Apuc.	Mgá.	Treat.	Apuc.	Mgá.
0+0	1.41b	0.86	1.97c	1.76b	1.39b	2.13b	3.26b	2.63b	3.89b	4.39c	3.92b	4.85c
50+0	2.24a	1.48	3.00b	3.00a	2.57a	3.43a	5.87a	4.97a	6.78a	5.37a	4.65a	6.09a
25+25	2.18a	1.21	3.14b	2.65a	2.00b	3.30a	4.97a	3.77b	6.18a	4.95b	4.27b	5.63b
100+0	2.26a	1.41	3.12b	3.09a	2.46a	3.73a	5.95a	4.63a	7.23a	5.36a	4.68a	6.04a
50+50	2.46a	1.25	3.67a	3.06a	2.17a	3.96a	5.91a	4.22a	7.61a	5.57a	4.58a	6.55a
150+0	2.42a	1.47	3.36b	3.15a	2.49a	3.81a	6.05a	4.60a	7.51a	5.38a	4.73a	6.03a
75+75	2.57a	1.35	3.79a	3.26a	2.33a	4.19a	6.24a	4.48a	8.02a	5.47a	4.63a	6.31a
200+0	2.52a	1.56	3.47a	3.37a	2.66a	4.09a	6.30a	4.94a	7.67a	5.60a	4.79a	6.40a
100+100	2.57a	1.50	3.63a	3.39a	2.57a	4.21a	6.57a	4.99a	8.14a	5.65a	4.81a	6.49a
Mean	2.29	1.34B	3.23A	2.97	2.29B	3.65A	5.68	4.36B	7.00A	5.30	4.56B	6.04A
C.V.(%)	19.46			19.06			19.23			8.14		

The equal small letters on the column and the equal capital letters on the line indicate that the mean are not different among them by Scott – Knott Test (P>0.05).

Table 3 – Mean values of treatment, local, interaction treatment versus local for the plant height (PH) and plants final stand (FS) of IPR Juriti e IPR Uirapuru dry bean cultivars under nitrogen fertilization. Apucarana and Maringá, PR, BR. 2005/2006.

	IPR JURITI						**IPR UIRAPURU**					
	Characteristics											
	PH (cm)			FS (1000 pl.)			PH (cm)			FS (1000 pl.)		
Treatments	Treat.	Apuc.	Mgá.	Treat.	Apuc.	Mgá.	Treat.	Apuc.	Mgá.	Treat.	Apuc.	Mgá.
0+0	23.39b	20.15b	26.63b	172,7b	219,6a	125,8b	22.65b	19.83	25.48b	187,3	223,1	151,6
50+0	27.88a	24.98a	30.82a	193,8a	217,8a	169,8a	27.84a	23.90	31.77a	194,7	216,4	172,2
25+25	26.35a	23.55a	29.15a	189,3a	221,3a	157,3a	27.76a	23.38	32.15a	198,2	228,6	168,0
100+0	26.01a	22.70a	29.32a	168,9b	194,2b	143,6b	27.83a	23.60	32.05a	190,9	218,2	163,6
50+50	27.46a	24.20a	30.72a	186,9a	217,8a	156,6a	28.30a	23.98	32.62a	199,6	220,4	178,6
150+0	26.55a	22.80a	30.30a	178,9b	197,3b	160,4a	27.18a	22.50	31.87a	181,8	214,2	149,3
75+75	27.56a	24.58a	30.58a	174,9b	198,7b	151,1a	28.06a	22.67	33.45a	190,2	221,8	158,7
200+0	26.29a	22.50a	30.08a	164,9b	193,8b	136,0b	26.20a	21.27	31.23a	183,3	203,6	163,1
100+100	26.94a	23.35a	30.52a	181,1a	203,1b	159,1a	28.23a	22.63	33.83a	198,4	224,9	172,0
Mean	26.49	2319B	29.80 A	179,0	207 A	151,0 B	27.12	22.6B	31.60 A	191,6	219A	164,1B
C.V.(%)	6.98			9.68			8.48			9.0		

The equal small letters on the column and the equal capital letters on the line indicate that the mean are not different among them by Scott – Knott Test (P>0.05).

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NITROGEN FERTILIZATION AND PRODUCTIVITY OF TWO DRY BEAN CULTIVARS IN NO-TILLAGE SYSTEM

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INTRODUCTION

Common bean is a crop that responds to nitrogen fertilization, and the quantity of N to be used depends on the type of existing vegetal residues on soil surface. In no-tillage system it has been observed N deficiency in common bean crop which makes necessary the anticipation of fertilization and the use of higher quantities of N, due to insufficient availability of N for plants. This occurs mainly when crop is carried out after grass planting or irrigated environment (Soratto et al., 2001; Soratto et al., 2004). Therefore, the objective of this work was to evaluate the N application on seeding and fractioning, upon yield components as well as yield of two common bean cultivars on no-tillage system.

MATERIAL AND METHODS

Field experiments were carried out in Apucarana and Maringá counties (summer crop season 2005/2006), Paraná State, Brazil, in a red nitosoil. Two dry bean cultivars IPR Juriti (Carioca type) and IPR Uirapuru (Black bean) were submitted to different nitrogen (N) levels. The used experimental design was a randomized block with nine treatments and five replications. Treatments were as follows: T_1 (0+0); T_2 (50+0), T_3 (25+25), T_4 (100+0), T_5 (50+50), T_6 (150+0), T_7 (75+75), T_8 (200+0) and T_9 (100+100) kg ha^{-1} of N applied at seeding and topdressing, respectively. The evaluation involved number of pods per plant, number of grains per plant, 100 seeds mass and grains yield.

RESULTS AND DISCUSSION

Data submitted to statistical analysis showed that the behavior of two dry bean cultivars in relation to the N fertilization were dependent on N fertilization level and on the environment conditions. IPR Juriti cultivar had a higher grain yield in Apucarana at a level from 100 to 200 N kg ha^{-1} applied totally during seeding period. On the other hand, in Maringá the highest grain yield was obtained at the level of 50 kg N ha^{-1} applied in the seeding period (Table 1). IPR Uirapuru also had the higher grain yield in Apucarana with 150 kg N ha^{-1} or 200 kg N ha^{-1} that was all applied during seeding (Table 2). The average of grains per pod, 100 grain mass and grain yield were higher in Apucarana for both cultivars.

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Table 1 – Mean values of treatment, local, interaction treatment versus local related to a number of pods per plant (NPP), number of grains per plant (NGPP), 100 seeds mass and grain yield of IPR Juriti common bean cultivar under nitrogen fertilization. Apucarana and Maringá, PR, BR. 2005/2006.

	NPP			NGPP			100M			YIELD		
Treatments	Treat.	Apuc.	Mgá.	Treat.	Apuc.	Mgá.	Treat.	Apuc.	Mgá.	Treat.	Apuc.	Mgá.
0+0	8.14d	7.78d	8.50c	3.31b	3.54b	3.08b	21.90b	24.20	19.60b	924b	1369b	480b
50+0	10.92c	11.00c	10.84b	3.64a	3.57b	3.71a	21.90b	24.20	19.60b	1090b	1499b	680a
25+25	12.61b	12.16b	13.06a	3.57a	3.58b	3.56a	22.20b	24.30	20.10b	1284a	1668b	899a
100+0	12.52b	13.38b	11.66b	3.81a	3.89a	3.72a	22.10b	24.30	19.90b	1391a	1924a	859a
50+50	11.89b	11.48c	12.30a	3.61a	4.05a	3.16b	22.05b	24.40	19.80b	1380a	1834a	925a
150+0	14.50a	15.46a	13.54a	3.81a	3.76b	3.87a	22.65a	24.40	20.90a	1483a	2010a	956a
75+75	13.72a	13.32b	14.12a	3.71a	4.08a	3.34b	22.30a	24.30	20.30a	1517a	1968a	1066a
200+0	13.04b	14.70a	11.38b	3.68a	3.90a	3.47a	22.55a	24.40	20.70a	1409a	1986a	832a
100+100	13.41a	13.50b	13.32a	3.84a	3.92a	3.75a	22.40a	24.40	20.40a	1471a	1841a	1100
Mean	12.31	12.08A	12.53A	3.67	3.81A	3.52B	22.23	24.31A	20.14B	1327	1789A	866B
C.V.(%)	13.61			8.46			2.55			14.27		

Table 2 – Mean values of treatment, local, interaction treatment versus local related to number of pods per plant (NPP), number of grains per plant (NGPP), mass of 100 seeds and grain yield of IPR Uirapuru common bean cultivar under nitrogen fertilization. Apucarana and Maringá, PR, BR. 2005/2006.

	NPP			NGPP			100M			YIELD		
Treatments	Treat.	Apuc.	Mgá.	Treat.	Apuc.	Mgá.	Treat.	Apuc.	Mgá.	Treat.	Apuc.	Mgá.
0+0	5.43b	7.78	3.08b	3.46b	3.74b	3.18	18.55b	20.00	17.10b	503c	696c	309b
50+0	6.67b	9.36	3.98b	3.56b	4.21a	2.91	18.95b	21.20	16.70b	808b	1259b	357b
25+25	7.79a	9.10	6.48a	3.69b	4.36a	3.02	19.45b	21.00	17.90b	888b	1264b	512b
100+0	7.19a	9.86	4.52b	3.54b	4.05b	3.03	19.60b	21.90	17.30b	823b	1266b	381b
50+50	7.14a	8.46	5.82a	3.82a	4.34a	3.30	19.85a	21.50	18.20b	946b	1256b	636a
150+0	7.53a	8.98	6.08a	3.86a	4.32a	3.39	20.10a	22.00	18.20b	867a	1244b	489b
75+75	8.50a	10.30	6.70a	3.67b	4.33a	3.01	20.20a	21.80	18.60b	1204a	1712a	696a
200+0	8.37a	10.92	5.82a	3.98a	4.81a	3.15	20.00a	22.30	17.70b	1117a	1609a	625a
100+100	7.92a	9.58	6.26a	3.79a	4.38a	3.21	21.45a	22.20	20.70a	1123a	1497a	749a
Mean	7.39	9.37A	5.42B	3.71	4.28A	3.13B	19.79	21.54A	18.04B	920	1311A	528B
C.V.(%)	22.09			9.71			5.79			14.77		

The equal small letters on the column and the equal capital letters on the line indicate that the mean are not different among them by Scott – Knott Test (P>0.05).

Table 3 – Mean values of treatment, local, interaction treatment versus local related to N leaf content location of IPR Juriti and IPR Uirapuru common bean cultivar under nitrogen fertilization. Apucarana and Maringá, PR, BR. 2005/2006.

	N leaf content (g kg^{-1})					
	IPR JURITI			IPR UIRAPURU		
Treatments	Treat.	Apuc.	Mgá..	Treat.	Apuc.	Mgá.
0+0	49.83b	38.00b	61.67	48.50d	44.00b	53.00c
50+0	56.00a	55.00a	57.00	60.33b	62.33a	58.33b
25+25	49.67b	39.00b	60.33	66.17a	65.67a	66.67a
100+0	56.33a	54.33a	58.33	55.67c	45.00b	66.33a
50+50	58.33a	53.33a	63.33	53.83c	63.00a	44.67d
150+0	49.00b	40.00b	58.00	56.00c	46.33b	65.67a
75+75	51.17b	39.00b	63.33	55.00c	64.00a	46.00d
200+0	50.17b	40.67b	59.67	67.50a	66.33a	68.67a
100+100	49.67b	39.33b	60.00	56.67c	66.00a	47.33d
Mean	52.24	44.30B	60.19A	57.74	58.07A	57.41A
C.V.(%)	5.03			4.34		

The equal small letters on the column and the equal capital letters on the line indicate that the mean are not different among them by Scott – Knott Test (P>0.05).

INDIRECT SELECTION FOR COMMON BEAN LINES TOLERANT TO LOW NITROGEN AVAILABILITY

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INTRODUCTION

Although common bean is a leguminous crop, the plant nutrient demand is not sufficiently met by symbiotic nitrogen (N) fixation (Alves, 2002). Nitrogen application is therefore in most cases indispensable. In Brazil common bean is grown by farmers who use no nitrogen fertilizers as well as by rural entrepreneurs who apply high quantities. It is important to identify lines tolerant to low N availability and/or that are responsive to the applied nutrient. It is desirable to verify whether selection under high N can also identify lines tolerant to low nitrogen availability.

MATERIAL AND METHODS

The experiments were conducted at two sites in the southern state of Minas Gerais: Lavras (21º 14' S, 44º 59' W and mean altitude of 919 m asl) and Ijaci (21º 10' S, 44º 55' W and mean altitude of 805 m asl), sown in February 2007.

One hundred lines of common bean of the Universidade Federal de Lavras (UFLA) were evaluated in two different but contiguous experiments, with the same cultural treatments, differing only in nitrogen fertilization. In the first, 80 kg ha^{-1} of P_2O_5, 80 kg ha^{-1} of K_2O and no N was applied. In the second, the same quantity of phosphorus and potassium, plus 100 kg ha^{-1} of N was split-applied: 1/3 at sowing, 1/3 on the 20^{th} day after sowing (DAS), and the last 1/3 on the 30^{th} DAS. The experimental design was a 10 x 10 triple lattice and each plot consisted of two rows of two meters.

The grain yield data were processed by analysis of variance per N level at each location, and by joint variance analysis per location. The genetic and phenotypic parameters were estimated based on the yield data. With the data means, the nitrogen response index (α_i) was estimated by the expression proposed by Thung (1990), that is: $\alpha_i = (N_{1i} - N_{2i})/Q$, where: N_{1i} and N_{2i}: mean yield of line i in the presence and absence of nitrogen , respectively. Q: quantity of applied N (Q = 100 kg ha^{-1}).

RESULTS AND DISCUSSION

Significant differences were detected between the lines ($P \leq 0.10$) in all experiments. The estimates of genetic variance (σ^2_G) reinforce the existence of variation between the same (Table 1). The F test of the source of variation levels was also significant ($P \leq 0.01$). In the mean, the response of the lines was 7.3 kg grain per kg of applied N. This estimate varied from -5.5 - 16 kg grain per kg of applied N.

The interaction N levels-by- lines was also significant ($P \leq 0.01$), indicating that the performance of the lines was not coincident in presence and absence of the nutrient. This interaction can also be

visualized by means of estimates of the genetic correlation (r_{Gxy}). Especially in Ijaci, these estimates were of small magnitude (Table 2).

The existence of variability in the lines and the h^2 estimates is an indicator of the success with selection. The selection gain estimates with N (SG_x) as well as without N (SG_y) were higher than 5%. The estimate of correlated response ($RC_{y/x}$) in the environment without N by the selection performed in the environment with N was also significant, but lower than the estimate of direct selection (Table 2). The reason was that the estimates of the genetic correlation with and without N were of small magnitude (Falconer & Mackay, 1996). These results are similar to those of Banziger et al. (1997), indicating that the gain would be greater if selection for N lack- tolerant lines was performed in low N- environments.

Table 1. Estimates of the genetic variance (σ^2_G) in the lines and heritability (h^2) in the environments with (h^2_x) and without (h^2_y) nitrogen, at the two evaluation sites.

Environments	σ^2_G	LL[1/]	UL[1/]	h^2 (%)	LL	UL
Lavras with N	1109.132	855.025	1496.764	20	-15	43
Lavras without N	5348.251	4122.945	7217.416	57	38	69
Ijaci with N	4987.790	3845.067	6730.977	45	21	61
Ijaci without N	1022.109	787.939	1379.327	33	4	53

[1/]LL and UL - lower and upper limits of the of confidence intervals, at 5% probability.

Table 2. Estimates of genetic correlation (r_{Gxy}) between the performance of lines in the environment with and without N, expected selection gains in the presence (SG_x) and absence (SG_y) of nitrogen and correlated response by indirect selection ($RC_{y/x}$), at the two evaluation sites.

Locations	r_{Gxy}	SG_x (%)	SG_y (%)	$RC_{y/x}$ (%)
Lavras	0.57	5.26	24.96	8.49
Ijaci	0.44	13.43	7.48	3.83

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RHIZOBIUM SELECTION FROM MATO GROSSO DO SUL SOILS FOR DRY BEAN INOCULATION

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Over the last few years, there has been a decrease in the production of the dry bean crop in Brazil, which has occured mainly due to the low technological level employed. (Merchant et al., 1999; Straliotto et al., 2002). Accordingly, nitrogen has been a major limiting factor to the productivity in Brazil. The dry bean plants associated with rhizobia through the symbiotic process can take advantage of this supply of nitrogen, without the cost burden of culture production, establishing an environmentally sustainable process. Although the strains of rhizobium currently recommended for inoculation of bean plants can produce significant increases in productivity, its potential of biological nitrogen fixation (BNF) still is limited to certain environmental conditions and cultivars of bean. This study aimed to evaluate the nodulation and efficiency of symbiotic bean plants inoculated with different isolates of rhizobium soil obtained from various regions of Mato Grosso do Sul, Brazil.

MATERIAL AND METHODS

The evaluation was performed in 46 isolates of rhizobia, using as a comparison, two treatments corresponding to the inoculation with the strains CIAT 899 (= 4077 SEMIA BR = 322) and PRF 81 (= SEMIA 4080), *Rhizobium tropici*, which are amongst those commercially recommended for the production of inoculum trade in Brazil, in addition to two treatments used as control without inoculation: fertilization with N-urea (NH4NO3) and the other without nitrogen fertilization (control). The experimental design was in randomized blocks, with three replications. The nodulation and the symbiotic rhizobia efficiency of the bean plants inoculated, cv. Carioca, were evaluated, using pots of sterilized "Leonard", containing a mixture of sand and vermiculite (1:1, v: v). The growth of rhizobium isolates were also evaluated in the folowing conditions: in Luria-Bertani medium LB, at 28ºC, and in TY medium (tripton-of yeast extract), at 40 ° C.

RESULTS AND DISCUSSION

The results showed a correlation between the growth of the rhizobium isolates in LB medium and its tolerance to temperature of 40°C (Fig. 1). Concerning the number of nodules, about 52% of the isolates under evaluation were superior to with the inoculation treatment of strain CIAT 899 and 30% showed superiority to inoculation with the strain PRF 81. As for dry nodules weight, 58% of the isolates of rhizobium from the soils of Mato Grosso do Sul, proved to be superior to the strain CIAT 899 and 48% were superior to the inoculation with the strain PRF 81, as shown in Fig 2. The production of shoot dry matter of the shoot bean plants inoculated with the isolated rhizobia were superior in 50 and 56% when compared with the strains CIAT 899 PRF and 81, respectively.

CONCLUSION

Insulated from rhizobia, as CPAO 60.2F3, CPAO 84.2F, CPAO 33.3F3, CPAO 32.3F3, CPAO 41.12F3, CPAO 7.5F, CPAO 13.3F3 and CPAO 21.2F, showed a great symbiotic potential for the inoculation in bean plants, opening prospects to obtain more effective inoculants for this culture.

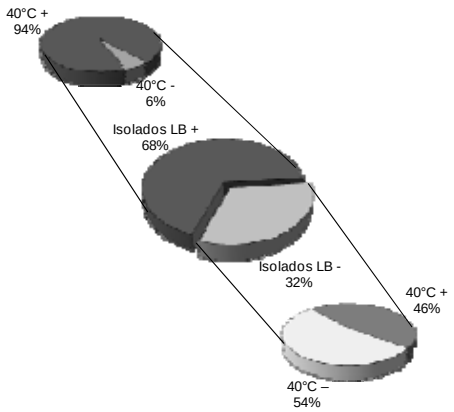

Fig 1. Assessment of growth of 50 isolates of Rhizobium sp. In LB medium ("Luria-Broth") at 40°C and in TY medium (tripton-of yeast extract).

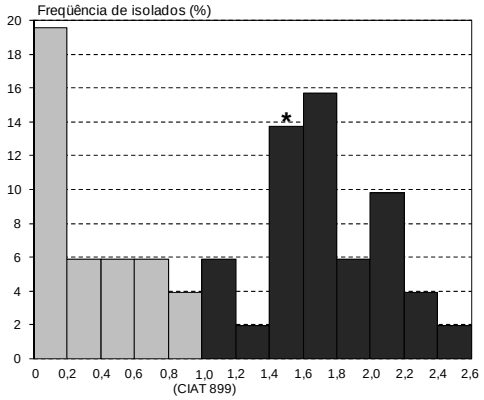

Fig 2. Dry matter of bean plant nodules, cv. Carioca, inoculated with 50 isolates of rhizobium. Values regarding the strain CIAT 899. Averages of three replications.
*Includes the strain PRF 81.

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RED BEAN BREEDING AT THE UNIVERSIDADE FEDERAL DE VIÇOSA (UFV)

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The common bean is a Brazilian staple food cultivated nationwide. The "carioca" and black bean types are the most important and have attracted more attention of breeding programs. However, other bean types such as “Jalo" (yellow, large-sized seeds), purple, cranberry and red are regionally important. Bean culture in Brazil has been undergoing great technological advancements, especially after the introduction of the irrigated bean, though it is still mainly grown by small farmers with low technology level (Borém & Carneiro, 2006). This scenario can be easily observed at the "Zona da Mata" region, state of Minas Gerais, where red bean predominates for the higher prices obtained, compared to that of other bean types, like "carioca" and black. This region is characterized by family farmers that cultivate bean in small-scale. The bean cultivars available do not meet the family farmers’ needs due to their susceptibility to major diseases or to unacceptable commercial quality of grains.

The Bean Breeding Program at the Universidade Federal de Viçosa (UFV) has been developing research on this specific type of bean. The objective of this work was to evaluate the inbred lines derived from the first cycle of recurrent selection (C_0), being developed at the UFV. These lines were obtained from the selection of 154 plants among the best families $F_{3:7}$ derived from different zero cycle populations (C_0). The evaluations were carried out during the 2006 and 2007 “drought" season (Mar. - Jun.) at the Experimental Station of Coimbra, located at 690 m altitude, 20°45’ S latitude and 42°51’ W longitude. A triple lattice 13x13 was used. Each plot had two 2m-long rows, 0.5m apart. Grain yield (kg/ha), grain aspect and plant architecture were evaluated. There was a significant difference among inbred lines ($P \leq 0.01$) for all the variables in the individual analyses. However, in general, the inbred lines of a same population did not differ in grain yield and plant architecture. On the other hand, for source of variation among populations (groups of inbred lines), there was a significant effect ($P \leq 0.01$) for all variables, and some populations had a better performance. Considering the combined analyses, the inbred lines x years interaction was significant ($P \leq 0.01$), indicating that the inbred lines did not present a consistent performance over different years. This fact shows the importance of evaluations being made in different environments. The interaction between population’s lines x years was not significant in most cases but the interaction between source of variation among populations x years was significant ($P \leq 0.01$). The results obtained were as expected, since an intensive selection was conducted during generation advancement or family evaluation. A smaller difference was observed among inbred lines within populations than among lines from different populations. The most outstanding populations were 11 (Vermelhinho/Vermelhinho/IAPAR81) and 15 (Vermelhinho/LR720982/Vermelhinho/AB136) (Table 1). These populations also presented the highest number of inbred lines among the most productive, especially population 15, which presented 4 inbred lines among the most productive out of the 10 lines evaluated. Thus, these populations inbred lines are candidates for future Cultivation and Use Value trials, aiming at recommendation of new bean cultivars for the state of Minas Gerais.

Table 1 - Mean, upper limit (UL), lower limit (LL) and variation range (R) of grain yield (kg/ha), number of lines (NL) per population among the fifty (50+), twenty (20+) and ten (10+) most productive ones, obtained in the 2006 and 2007 evaluation in Coimbra, MG.

	Grain Yield (kg/ha)				Number of Lines (NL)			
Population	Mean	UL	LL	R	Evaluated	NL (50+)	NL (20+)	NL (10+)
Population 15	3103	3344	2866	479	10	7	4	2
Population 11	3029	3250	2500	750	8	5	3	2
Population 8	3006	3229	2723	506	4	3	1	0
Population 14	2983	3428	2522	906	9	5	2	1
Population 17	2973	3253	2560	693	9	3	1	1
Population 12	2963	3213	2669	545	9	4	1	0
Population 7	2949	3190	2680	510	8	3	1	0
Population 4	2944	3282	2581	701	10	4	2	2
Population 6	2918	3229	2551	678	10	3	1	0
Population 2	2884	3243	2586	657	20	7	2	1
Population 9	2843	3226	2606	621	9	2	1	0
Population 10	2834	3346	2607	739	9	1	1	1
Population 3	2811	3076	2427	650	8	2	0	0
Population 13	2791	3072	2611	461	7	1	0	0
Population 1	2791	2960	2663	297	7	0	0	0
Population 16	2708	2890	2446	443	8	0	0	0
Population 5	2341	2671	1917	754	9	0	0	0
Lines	2876							
Controls	2555							
Ouro Vermelho	2778							
Vermelho 2157	3105							
Ouro Negro	2780							

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POTENTIAL OF SEGREGATING POPULATIONS OF "CARIOCA" TYPE BEANS

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Brazil stands out as the largest producer and consumer of common beans (*Phaseolus vulgaris*) followed by India and China (FAO, 2007). However, it still presents one of the lowest world yield averages, around 800 $kg.ha^{-1}$ (CONAB, 2007). One of the factors responsible for this low yield is the occurrence of several diseases that affect the culture. Anthracnose (*Colletotrichum lindemuthianum*), rust (*Uromyces appendiculatus*) and angular leaf spot (*Pseudocercospora griseola*) are very important due to the damage that they cause and also due to the high variability of the pathogens (Paula Júnior and Zambolim, 2006). Among several strategies used to control these diseases, the use of resistant cultivars is one of the most efficient. In Brazil, however, it has been difficult to obtain "carioca" type cultivars with ample resistance to such pathogens.

An effective tool for plant resistance is the use of assisted selection by molecular markers associated to resistance genes. Thus, studies conducted at BIOAGRO/UFV (Alzate Marin et al., 2005) led to identification of several resistance gene markers and the introgression of these genes in the Ruda cultivar. The inbred line obtained with a resistance gene pyramid was denominated Rudá-R. The utilization of this inbred line as a source of resistance genes for crossings with other commercial cultivars could be an interesting strategy. Thus, this study aimed to transfer resistance genes to *C. lindemuthianum*, *U. appendiculatus*, and *P. griseola* of Rudá-R inbred line into elite inbred lines of "carioca" type beans. Rudá-R inbred line is originated from the BIOAGRO/UFV program and contains a pyramid with genes resistant to anthracnose (*Co-4*, *Co-6* and *Co-10*), angular-spot (*Phg-1*) and rust (*Ur-ON*).

Five segregating populations were selected starting with the crossings of elite inbred lines of "carioca" type beans with "Rudá-R": UTF-0013 x Rudá-R (FP1), CNFC 9437 x Rudá-R (FP2), OPS-82 x Rudá-R (FP3), Carioca 1070 x Rudá-R (FP4) and GEN 12-2 x Rudá-R (FP5), whose parents showed polymorphism with Rudá-R for some SCAR and RAPD molecular markers, previously identified in studies developed at BIOAGRO. Around 150 F_4 plants of each of these populations were genotyped and identified with those containing the molecular markers corresponding to the resistance genes of interest. Ninety families originated from the selected plants and ten controls were evaluated in the Coimbra Experimental Station-UFV during the wet season/2006, dry season/2007 and wet season/2007, using triple 10x10 lattice designs. Yield, plant architecture (scores from 1 to 5) and grain aspect (scores from 1 to 5) were evaluated. The data were submitted to individual and joint variance analysis involving the three seasons.

A significant effect on populations and families within populations was observed, indicating a wide variability for the characteristics evaluated, not only between but also within populations. Overall, season/year interactions were significant, highlighting the importance of evaluations in different environments. The FP1 and FP2 populations stood out from the others in yield potential and plant architecture and showed a higher number of families among the 30, 20 and 10 superior ones (Table

1). However, all populations presented high-potential families with yield levels higher than that of the controls, which produced 3088 kg.ha^{-1} (Table 2). Regarding grain aspect, the FP5 population stood out from the others, with 16, 13 and 8 families among the 30, 20 and 10 superior ones, respectively. However, all populations showed families with good grain aspect and superior to the controls, which presented an average score of 2.5. These results show the potential of these populations to obtain inbred lines of "carioca" type beans and high yield.

Table 1. Number of families among the 30, 20, and 10 superior ones per population for grain yield, plant architecture and grain aspect.

Families[1]	Yield (kg.ha^{-1})			Plant architecture			Grain aspect		
	30 +	20 +	10 +	30 +	20 +	10 +	30 +	20 +	10 +
FP1[2]	13	10	7	11	9	5	5	1	0
FP2	10	6	2	10	7	5	9	6	2
FP3	4	3	1	1	0	0	0	0	0
FP4	0	0	0	8	4	0	0	0	0
FP5	3	1	0	0	0	0	16	13	8

[1] eighteen families per population were evaluated.
[2] FP1 = UTF-0013 x Rudá-R; FP2 = CNFC 9437 x Rudá-R; FP3 = OPS-82 x Rudá-R; FP4 = Carioca 1070 x Rudá R and FP5 = GEN 12-2 x Rudá-R.

Table 2. Means of grain yield, (kg.ha^{-1}), plant architecture and grain aspects of ninety segregating families of "carioca" type beans and controls. Coimbra - MG.

Families and controls	Yield (kg.ha^{-1})	Plant architecture	Grain aspect
FP1	3507 (3098 - 3775)[1]	3.1 (2.2 - 3.7)	2.4 (2.1- 2.7)
FP2	3337 (2493 - 3701)	3.2 (2.1 - 4.1)	2.2 (1.8 - 2.8)
FP3	3200 (2803 - 3620)	3.7 (3.1 - 4.3)	2.7 (2.4 - 3.1)
FP4	2948 (2680 - 3401)	3.3 (2.9 - 3.9)	2.7 (2.3 - 2.9)
FP5	3180 (2534 - 3483)	3.8 (3.4 - 4.2)	1.9 (1.7 - 2.5)
Controls	3088 (2330 - 3564)	3.3 (2.1 - 4.2)	2.5 (1.4 - 3.5)

[1]Values within parenthesis indicate the inferior and superior limits of each population to the correspondent characteristic.

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RUNNER BEAN (*PHASEOLUS COCCINEUS* L) PRODUCTION IN CHILE

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Common bean (*Phaseolus vulgaris* L.) is the only one bean planted in Chile, however, there are small home-made vegetable gardens with runner bean (*Phaseolus coccineus*), called locally "poroto pallar", in the central-southern area as a climbing bean that grow on poles. An interesting point is that related with genetic diversity with large size seed, especially those of white colour because of its excellent cooking quality and high demand after they are tasted. For this reason, the Grain Legume Breeding Program of Agricultural Research Institute of Chile (INIA, tested a system of runner bean production after selection of a landrace called Quilapallar, with a white and large seed size. This system recommends a production method without support, similar to common bean. Despite that runner bean has indeterminate growth habit with high foliar production and respond to use of support, it is less expensive to crop without support, especially in large scale.

As the runner bean is unable to reach total maturity in the central-southern area of Chile, and they have to be harvested at the end of summer, middle of march, no matter if there are plants with pods in different development stages, flowers, and new leaves in that moment. At the harvest time, each plant has 6 to 8 mature pods, and they are cut and harvested about 7 days later. Farmer production with this system can yield about 1200 to 2000 kg ha^{1}.

The most expensive production factor in this system was cost of seeds because of its large size, about 184 g per 100 seeds. Experiments with different seed rates have been done in order to increase profit margin. Table 1 shows the main results of this experiment, and they indicates that higher seed rates had greater yield; however, the best profit was with 153 kg/ha as seed rate, with 20 cm intra row and 60 cm inter row.

Table 1. Distance intra rows, seed rate, plant density, pod per plant, seeds per pod, weight of 100 seeds, and yield of runner bean landrace "Quilapallar". Chillán, Chile 2006.

Distance intra row (cm)	Seed rates (kg ha^{1})	Plant density (plants m^{2})	Pods per plant	Seeds per pod	Weight of 100 seeds (g)	Yield (kg ha^{1})
5 cm	613	29,17	3,17	2,16	168	3891
10 cm	307	17,50	5,57	2,23	174	3344
15 cm	204	11,67	7,64	2,29	179	3286
20 cm	153	8,33	10,42	2,49	167	3248
25 cm	123	7,50	7,11	2,58	171	2951
CV, (%)		21,59	9,88	8,76	15,27	5,2
LSD (.05)		3,55	4,15	ns	ns	250

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RELEASE OF 'CROISSANT' PINTO BEAN

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The Colorado Agricultural Experiment Station announces the release of 'Croissant', pinto bean (*Phaseolus vulgaris* L.) variety. Croissant was developed at Colorado State University and tested in the Western Regional Bean Trials, Midwest Regional Performance Nursery, and Colorado State University Dry Bean Variety Testing Program as CO23704.

Croissant was derived from a single F_5 plant selection in 2001 from the pedigree BelDakMi-RR-3/CO07010-2//WM2-93-5. BelDakMiRR-3 is a pinto line released by the USDA-ARS for resistance to rust; CO07010-2, is a pinto line from the Colorado State University Breeding Project that has semi-upright architecture, resistance to rust caused by *Uromyces appendiculatus*, and excellent pinto seed quality; and WM2-93-5 is an experimental pinto line from Dr. Dermot Coyne, University of Nebraska Dry Bean Breeding Project. WM2-93-5 possesses resistance to rust, field tolerance to common bacterial blight caused by *Xanthomonas campestris* pv. *phaseoli* and has semi-upright architecture. Hybridization of parental lines was made at the Colorado State University greenhouse, Fort Collins, CO. The line was selected at the Agricultural Research, Demonstration, and Education Center, Fort Collins, and pure seed was increased at the Western Colorado Research Center, Fruita, Colorado.

Croissant has semi-upright architecture (IIb) in most environments however, in high yield environments it expresses semi-vine architecture (IIIa). It possesses resistance to the prevalent races of rust in the High Plains and BCMV caused by *Bean common mosaic virus* (a potyvirus), and medium harvest maturity (92 to 95 d). The specific rust resistant gene(s) has not been characterized but appears to be conditioned by either the Ur-3 allele from WM2-93-5 or UR-11 from BelDakMi-RR3. Resistance to BCMV appears to be conditioned by the recessive allele $bc2^2$. Mean seed yield was 2900 and 2915 kg ha^{-1} over four locations in the Midwest Regional Performance Nursery and Western Regional Bean Trials, and mean seed weights were 37.1 and 34.8 g 100 $seed^{-1}$, respectively. In Colorado, mean seed yield was 2841 kg ha^{-1} averaged over two locations in 2007.

Foundation seed of Croissant will be released to seed producers in May 2008. Application for Foundation Seed can be made to Mr. Fred Judson, Western Colorado Research Center, 3168 B 1/2 Road, Grand Junction, Colorado 81503-9621. Plant Variety Protection under Title V will be sought. A "Technology Fee" paid to Colorado State University, collected by the Certification agency in the state of production will be assessed on all Registered and Certified seed produced. Seed for testing is available from Mark Brick, Department of Soil and Crop Sciences, Colorado State University, Fort Collins, CO 80523, 970-491-6551 or Mark.Brick@Colostate.edu.

BRS AGRESTE - NEW BEIGE SEEDED COMMON BEAN CULTIVAR WITH ERECT PLANT TYPE AND HIGH YIELD POTENTIAL

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INTRODUCTION

The Brazilian bean consumer is demanding for bean culinary quality and grain type. Besides, in the Northeast region, there is a demand for the beige bean seed coat color. In this area bean takes over an expressive socioeconomic importance due to its widespread cultivated area and for offering, to the low income population, a low cost vegetable protein. To attend this demand, Embrapa Rice and Bean is releasing the BRS Agreste bean cultivar for the States of Sergipe, Alagoas, Bahia, Goias and Federal District, enabling the farmers to offer a better quality product to the final consumer and to obtain better revenue with this crop.

Origin and cultivar development

BRS Agreste was obtained in the bean breeding program of Embrapa Rice and Bean, in 1993, from the single cross between CB 912052 and AN 9022180. From F2 to F5 generations plants were inoculated in the field with pathotypes 55, 89, 95, 453 and 585 of *Colletotrichum lindemuthianum* and selected by the modified mass selection method when the susceptible plants were eliminated. In F5 the remaining resistant plants were harvested individually giving origin to families in the F6 generation which were then inoculated under artificial conditions with the same five races of the pathogen. The resistant lines were evaluated in the field, for architecture, lodging, yield and post-harvest grain type. Among those lines LM 96200224 was selected for preliminary evaluation trials (EPL). In the year of 1999, this line was evaluated in the Preliminary beige seeded trials and in 2001 in the Intermediary beige seeded trials. This line was then tested in the Regional trials (VCU) in 48 different environments together with 10 other lines and three controls, in a randomized complete block design with three replications (each plot consisted of four rows of 4 m). All recommended technologies used in the State of Goias, Sergipe, Alagoas, Bahia and Federal District were used. The joint analysis of grain yield data and other agronomic characteristics provided the elements to promote the line to be selected with the pre-commercial denomination of CNFM 7958.

RESULTS

Yield

In 48 Regional trials (VCU), from 2003 to 2007, conducted during the rainy and dry (under irrigation) sowing seasons in the State of Goias and in the Federal District and in the rainy sowing season in the States of Sergipe, Alagoas and Bahia, the line CNFM 7958 showed to be 5.2% superior in an average yield when compared to the average yields of the controls BRS Marfim, Corrente and IPA 6 (Table 1).

Table 1. Yield of BRS Agreste cultivar in the States of Goias (GO), Sergipe (SE), Alagoas (AL), Bahia (BA) and Federal District (DF) compared with control averages in the Regional trials (VCU) from 2003 to 2007.

State	Sowing season	BRS Agreste average yield (kg/ha)	Control average yield (kg/ha)	Relative yield (%)	Number of tested environment
GO/DF	wet	2.585	2.589	99.8	8
GO/DF	winter	2.366	2.706	87.4	13
SE/AL/BA	wet	2.259	2.091	108.0	27
Total average yield		2.342	2.227	105.2	48

Morphophysiologic, technologic and industrial characteristics

This cultivar presents an erect growth habit, a growing cycle from sowing to maturity of 75 to 85 days, 41 days to flowering, white flower, yellow to light red pods at maturity, beige grain color with no brightness and resistance to lodging. Besides BRS Agreste have a very uniform grain color and size, 100 grain weight of 25 g and cooking time of 32 minutes (Table 2).

Table 2. Technological and industrial grain qualities of BRS Agreste cultivar compared to the controls BRS Marfim and IPA 6.

Cultivar	Cooking time (min.)	Protein (%)	100 grain weight (g)
BRS Agreste	32	21	25
BRS Marfim	28	17	27
IPA 6	27	19	24

Disease reaction

Under artificial inoculation BRS Agreste was resistant to Bean common mosaic virus and to the pathotypes 23, 55, 71, 89, 89-AS, 95, l27, and 453 of *Colletotrichum lindemuthianum*, the causal agent of anthracnose. In the field trials it showed a intermediary reaction to angular leaf spot and was susceptible to Bean golden mosaic virus.

CONCLUSION

Due to its erect plant type, high yield potential and resistance to anthracnose, the common bean cultivar BRS Agreste with beige grain color is recommended to be cultivated during rainy and dry (under irrigation) seasons in the State of Goias and Federal District and in the rainy season in the States of Sergipe, Alagoas and Bahia.

Institutions involved in the cultivar evaluation:

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2. Universidade Luterana do Brasil (ULBRA) - Itumbiara/GO;
3. Universidade Estadual de Goiás (UEG) - Ipameri/GO;
4. Centro Federal de Educação Tecnológica (CEFET) - Urutaí/GO;
5. Centro Federal de Educação Tecnológica (CEFET) - Morrinhos/GO;
6. Embrapa Arroz e Feijão - Santo Antônio de Goiás/GO;
7. Embrapa Cerrados - Planaltina/DF;
8. Embrapa Tabuleiros Costeiros - Aracaju/SE;
9. Fundação de Ensino Superior de Rio Verde (FESURV/ESUCARV) - Rio Verde/GO; and
10. Empresa Baiana de Desenvolvimento Agrícola (EBDA) - Salvador/BA.

BRS EMBAIXADOR - DARK RED KIDNEY COMMON BEAN FOR INTERNATIONAL MARKET

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INTRODUCTION

Nowadays, the demand for beans with accepted grain type has been growing in the international market. In this way, the program of bean genetic improvement at Embrapa Rice & Beans is focused on the development of new cultivars to attend this new market request, enabling farmers to offer a better product value and to obtain better revenue with the crop. As a result of this effort, the Dark Red Kidney bean cultivar BRS Embaixador has been released for the dry season, under irrigation, in the State of Goias.

Origin and cultivar development

BRS Embaixador was obtained from the single cross between XAN 42 and G 13922 accomplished at International Center for Tropical Agriculture (CIAT), in 1983. The selected line DRK 18 was evaluated at Embrapa National Rice & Beans, under field conditions for plant type, lodging, yield and pos-harvest grain type. In 2000 this line was evaluated together with 22 other lines plus three controls and in the year of 2001 it was evaluated with three other lines and two controls in a completely randomized block design with four replications (each plot consisted of four rows of 4 m) using all cropping practices recommended for the different bean planting systems. Evaluations were realized at Santo Antonio de Goias, Santa Helena de Goias and Anapolis in the years 2000 and 2001 during the dry season, under irrigation, performing a total of 14 experiments in the State of Goias.

RESULTS

Yield

In 14 Regional trials (VCU) conducted in the years of 2000 and 2001 during the dry season in the State of Goias, the cultivar BRS Embaixador showed to be 19.1% superior in an average yield when compared to the average yields of the controls Irai and Jalo Precoce (Table 1). Although the controls present a different grain type but, a similar 100 grain weight, they were used because there are no beans such as the BRS Embaixador grain type registered in Brazil.

Table 1. Yield of BRS Embaixador in the State of Goias compared to the average controls in the Regional trial (VCU) during 2000 and 2001.

State	Sowing season	BRS Embaixador yield (kg/ha)	Control average yield (kg/ha)	Relative yield (%)	Number of environments tested
GO	dry	2,214	1,859	119.1	14

Morphophysiologic, technologic and industrial characteristics

This cultivar belongs to the Dark Red Kidney bean group and presents a growing cycle from sowing to maturity of 75 to 85 days, 33 days to flowering, rose and violet colored flower, green-yellow pods at maturity, dark red grain color with intermediary brightness, erect plant type and is resistant to lodging. Besides BRS Embaixador have a very uniform grain color and size, 100 grain weight of 63g and cooking time of 28 minutes (Table 2).

Table 2. Technological and industrial grain qualities of BRS Embaixador bean cultivar.

Cultivar	Cooking time (min.)	Protein (%)	100 grain weight (g)
BRS Embaixador	28	19	63
Irai	37	22	44
Jalo Precoce	25	24	36

Disease reaction

BRS Embaixador presented intermediary reaction to anthracnose after inoculation under artificial inoculation and susceptibility to angular leaf spot, rust, common bacterial blight, bean common mosaic virus, mildew and bean golden mosaic virus under field condition.

CONCLUSION

The bean cultivar BRS Embaixador due to its Dark Red Kidney grain type, erect plant type and the agronomic characteristics is an option to farmers interested in producing beans for the international market during the dry season, under irrigation, in the State of Goias.

BRS EXECUTIVO - CRANBERRY COMMON BEAN FOR INTERNATIONAL MARKET

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INTRODUCTION

Nowadays, the demand for beans with accepted grain type has been growing in the international market. In this way, the program of bean genetic improvement at Embrapa Rice & Beans is focused on the development of new cultivars to attend this new market request, enabling farmers to offer a better product value and to obtain better revenue with the crop. As a result of this effort, the Cranberry bean cultivar BRS Executivo has been released for the dry season, under irrigation, in the State of Goias.

Origin and cultivar development

BRS Executivo was obtained from the single cross between A 192 and BAT 1274 accomplished at International Center for Tropical Agriculture (CIAT), in 1983. The selected line SUG 33 was evaluated at Embrapa National Rice & Beans, under field conditions for plant type, lodging, yield and pos-harvest grain type. In 2000 this line was evaluated together with 11 other lines plus three controls and in the year of 2001 it was evaluated with three other lines and two controls in a completely randomized block design with four replications (each plot consisted of four rows of 4 m) using all cropping practices recommended for the different bean planting systems. Evaluations were realized at Santo Antonio de Goias, Santa Helena de Goias and Anapolis in the years 2000 and 2001 during the dry season, under irrigation, performing a total of 14 experiments in the State of Goias.

RESULTS

Yield

In 15 Regional trials (VCU) conducted in the years of 2000 and 2001 during the dry season in the State of Goias, the cultivar BRS Executivo showed to be 5.2% superior in an average yield when compared to the average yields of the controls Irai and Jalo Precoce (Table 1). Although the controls present a different grain type but, a similar 100 grain weight, they were used because there are no beans such as the BRS Executivo grain type registered in Brazil.

Table 1. Yield of BRS Executivo in the State of Goias compared to the average controls in the Regional trial (VCU) during 2000 and 2001.

State	Sowing season	BRS Executivo yield (kg/ha)	Control average yield (kg/ha)	Relative yield (%)	Number of environments tested
GO	dry	1,644	1,563	105.2	15

Morphophysiologic, technologic and industrial characteristics

This cultivar belongs to the Cranberry bean group and presents a growing cycle from sowing to maturity of 85 to 95 days, 39 days to flowering, rose and violet colored flower, yellow pods with red stripes at maturity, beige grain color with dark red points and stripes, semi-erect plant type. Besides BRS Executivo have a very uniform grain color and size, 100 grain weight of 76g and cooking time of 26 minutes (Table 2).

Table 2. Technological and industrial grain qualities of BRS Executivo bean cultivar.

Cultivar	Cooking time (min.)	Protein (%)	100 grain weight (g)
BRS Executivo	26	25	76
Irai	37	22	44
Jalo Precoce	25	24	36

Disease reaction

BRS Executivo presented intermediary reaction to anthracnose after inoculation under artificial inoculation and susceptibility to angular leaf spot, rust, common bacterial blight, bean common mosaic virus, mildew and bean golden mosaic virus under field condition.

CONCLUSION

The bean cultivar BRS Executivo due to its cranberry grain type, semi-erect plant type and the agronomic characteristics is an option to farmers interested in producing beans for the international market during the dry season, under irrigation, in the State of Goias.

'MSHINDI' KABLANKETI DRY BEAN FOR EAST AFRICA

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Dry beans provide a significant source of protein and calories to the majority of Tanzanians. Yields are often below 500 kg ha^{-1} whereas yield potential with agricultural inputs can be up to 2,500 kg ha^{-1}. Yields remain low in part because of biotic and abiotic stresses (Wortmann et al., 1998). The Bean/Cowpea CRSP supported varietal development at Sokoine University of Agriculture (SUA) in Tanzania from 1981 to 2007. The program focused on yield stability, adaptation to low to mid altitude (<1000 masl) environments and disease resistance.

Kablanketi is an important bean market class in southern and central Tanzania. The original 'Kablanketi' landrace had seed with fine purple flecking superimposed over a cream background, and a type III indeterminate growth habit. Kablanketi was probably first introduced from Zambia into the southern Tanzania highlands about three decades ago (C. Madata, Uyole Research Station, personal communication). Its diffusion throughout Tanzania was very rapid compared to formal bean variety introductions because of its highly desired consumer qualities.

Origin: Mshindi was derived from the cross 'Rojo' x Kablanketi made in 1992-93. Rojo is a large red-seeded variety released by SUA in 1997 with *I bc-1*[2] BCMV/BCMNV resistance, race specific angular leafspot resistance, and moderate common bacterial blight and halo blight resistance. Rojo was derived from 'SUA 90' x 86EP5034-B, the latter of which has the pedigree [(Blue Mountain x NY76) x (Cornell49-242 x Montcalm)]. SUA 90 was developed at CIAT (accession number G5476; Hillocks et al., 2006) and distributed in Africa with the designation TMO 216. Blue Mountain is a snap bean released in 1983 (Silbernagel and Drake, 1983). 'Montcalm' is a dark red kidney bean released in 1974 by Michigan State University. Cornell 49-242 is a small black from Venezuela, and the origin of NY 76 is unknown.

The Rojo x Kablanketi cross was advanced through the F_1 and F_2 generations in 1993. In 1994, farmers evaluated seed and plant characters of about 1,000 F_3 progeny rows at the SUA Mifiga research farm (Michael Butler et al., 1995). The F_4 generation was advanced without farmer participation. In the F_5, 60 single plant derived progeny rows were again evaluated by farmers. Selected F_6, lines were placed in preliminary trials and 16 F_7 lines were evaluated in advanced trials. Eight F_8 lines were evaluated, and on-farm trials were initiated in 2001 and 2002. Advanced and on farm trials were repeated in 2005. Mshindi was also tested in 2005 by the National Bean Program at the Selian Research Center in Arusha. On-farm trials were conducted in Msongozi and Maharaka Village in Morogoro rural district in 2001, 2002, 2005, and Dihinda Village in 2002, 2004, 2005 in Mvomero rural district.

Description: Mshindi, previously tested as EG 21, means "Winner" in Swahili. Mshindi out-yielded the local check ('Kenya') in 10 of 12 trials and had an overall yield advantage of 117%. Similarly, Mshindi out-yielded SUA 90 by 107% with higher yields in 67% of the trials. When compared to Rojo, Mshindi yielded 98% of the check and had higher yields in 53% of trials. Yields for Mshindi ranged from 975 – 1783 kg ha^{-1} at the Mafiga and Selian sites and from 363 – 1665 kg ha^{-1} in farmer's fields at Msongozi, Maharaka and Dihinda. Kablanketi was not included in the bush trials,

but in climbing trials conducted at SUA in 1999-2000, 2005-2006, yields ranged from 376 - 1495 kg ha^{-1}.

Mshindi averages 33 d to 50% flower, and 77 d to 85% buckskin pods. It was similar to 'Kenya', 'SUA 90' and 'Rojo' with days to 50% flower of 34, 33, and 32, and days to maturity of 77, 78, and 77 days, respectively. 'Kablanketi' matured in 70 d in SUA trials.

Mshindi has erect determinate bush (Type I) growth habit averaging 49 cm in height. Flowers are pink but other plant parts lack anthocyanin.

Seed of Mshindi was 28.9 g·100 $seeds^{-1}$ averaged over 12 trials. Kablanketi, SUA 90, Rojo, and Kenya averaged 27.5, 26.9, 38.9, and 36.4 g·100 $seeds^{-1}$, respectively. Mshindi seed is dull with a pink background overlain by a fine purple flecking. Mshindi seed differs from Kablanketi in its pink rather than cream background. This novel color combination was identified by farmers as being potentially desirable because it combined the colors of two preferred market classes (Michael Butler et al., 1995).

BCMV/BCMNV symptoms were never observed on Mshindi in field trials. Mshindi was evaluated in 15 trials for reaction to ALS, 12 trials to CBB, and three trials to bean rust. Based on trials with moderate disease pressure, we classify Mshindi as moderately susceptible to ALS, and resistant – moderately susceptible to CBB. The three check varieties were moderately susceptible to CBB and ALS. Rust infection was too light to determine resistance reaction.

Mshindi cooks in 29 min as determined by Mattson cooker (Mattson, 1946) over gas and 112 min over charcoal (aver. two villages). SUA 90, Rojo and Kablanketi had similar cooking times while Kenya cooked in 23 min. Kenya had a shorter cooking time (98 min) over charcoal whereas Rojo and SUA 90 were longer (130 and 120 min, respectively).

Seventy-two farmers from Maharaka and Msongozi villages evaluated Mshindi and checks for dry seed color, cooking time, broth and soup suitability, and taste. Fifty-two farmers also evaluated marketability. More than half found the color to be suitable or highly suitable, similar to SUA 90 and lower than Rojo and Kenya. Two-thirds thought that Mshindi was highly suitable or suitable for cooking time, taste and marketability. Sixty-one percent of farmers found the taste to be suitable or highly suitable.

Mshindi was approved for release by the Tanzania National Variety Release Committee with no restrictions on propagation and use. Small quantities of seed for research purposes are available from the bean breeding program at SUA.

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'PESA' LARGE RED DRY BEAN

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In much of sub-Saharan Africa, dry beans (*Phaseolus vulgaris* L.) provide a staple source of protein, carbohydrates, and some minerals and vitamins. In Tanzania, dry bean is an important subsistence and cash crop. Official yield figures for Tanzania are typically below 500 kg ha^{-1} whereas yield potential with agricultural inputs is up to 2,500 kg ha^{-1}. Yields remain low in part because of biotic and abiotic stresses (Wortmann et al., 1998). The Bean/Cowpea CRSP supported varietal development at Sokoine University of Agriculture (SUA) in Tanzania from 1981 to 2007. The program focused on yield stability, adaptation to low to mid altitude (<1000 masl) environments and disease resistance.

In eastern and southern Africa, large seeded types from the Andean center of domestication are preferred. Many seed colors are used, but among the most wide-spread are the reds as typified by landrace varieties such as 'Canadian Wonder', 'Bwana Shamba', and 'Kenya'. This type has a preferred broth color and is considered to be a highly marketable cash crop.

'Pesa' large red seeded dry bean is being released as a high yielding variety with a preferred seed color type for market and good cooking quality. Reflecting its cash crop status, the name Pesa means "money" in Swahili.

Origin: Released in 2006, Pesa was derived from 'Rojo' x 'Kablanketi' made in 1992-93. Rojo is a large red-seeded variety released by SUA in 1997. Rojo was derived from 'SUA 90' x 86EP5034-B, the latter of which has the pedigree [(Blue Mountain x NY76) x (Cornell49-242 x Montcalm)]. SUA 90 was developed at CIAT (accession number G5476; Hillocks et al., 2006) and distributed in Africa with the designation TMO 216. Blue Mountain is a snap bean released in 1983 (Silbernagel and Drake, 1983). 'Montcalm' is a dark red kidney bean released in 1974 by Michigan State University. Cornell 49-242 is a small black from Venezuela, and the origin of NY 76 is unknown. Kablanketi is a popular local landrace (see release notice for 'Mshindi', this issue, for more information).

The Rojo x Kablanketi was advanced through the F_1 and F_2 generations in 1993. In 1994, farmers evaluated seed and plant characters of about 1,000 F_3 progeny rows at the SUA Mifiga research farm (Michael Butler et al., 1995). The F_4 generation was advanced without farmer participation. In the F_5, 60 single plant derived progeny rows were again evaluated by farmers. Selected F_6, lines were placed in preliminary trials and 16 F_7 lines were evaluated in advanced trials. Eight F_8 lines were evaluated, and on-farm trials were initiated in 2001 and 2002. Advanced and on farm trials were repeated in 2005. Pesa was also tested in 2005 by the National Bean Program at the Selian Research Center in Arusha. On-farm trials were conducted in Msongozi and Maharaka Village in Morogoro rural district in 2001, 2002, 2005, and Dihinda Village in 2002, 2004, 2005 in Mvomero rural district.

Description: Pesa was previously tested under the experimental number EG 44. Pesa out-yielded 'Kenya' in nine of 12 trials and had an overall yield advantage of 109%. Yields were similar to SUA 90 with Pesa at 99% of 'SUA 90' and having higher yields in six of 15 trials. When compared to Rojo, Pesa yielded 91% of the check and had higher yields in 40% of trials. Overall yields were

similar in researcher's plots and on-farm trials. Yield over 15 environments averaged 978 kg ha^{-1} at the Mafiga and Selian sites and 424- 1934 kg ha^{-1} in farmers' fields at Msongozi, Maharaka and Dihinda.

Pesa averages 33 d to 50% flower and 78 d to 85% buckskin pods; similar to Kenya, SUA 90 and Rojo with 34, 32 and 33 d to 50% flower and 78, 78 and 77 d to maturity. Pesa has determinate bush (Type I) growth habit 44 cm in height. Flowers are pink but other plant parts lack anthocyanin. Pods average 13 cm long, 1 cm wide and contain four ovules on average.

Pesa seeds averaged 36.8 g·100 $seeds^{-1}$ compared to 36.4, 26.9 and 38.9 g·100 $seeds^{-1}$ for Kenya, Rojo, and SUA 90. Seed of Pesa are similar to Rojo, but shaped more spherically.

BCMV and BCMNV symptoms have never been observed on Pesa in field trials. Pesa was evaluated in 15 trials for reaction to ALS, 12 trials to CBB, and three trials to bean rust. Based on trials with moderate levels of disease, Pesa was moderately susceptible to ALS, and CBB. Rust infection was too light to determine resistance reaction.

Pesa cooks in 42 min as determined by Mattson cooker (Mattson, 1946) over gas and 108 min when cooked over charcoal (average of two villages). Kenya, SUA 90 and Rojo, had shorter times of 23, 29, and 28 min, respectively. Kenya had a shorter cooking time (98 min) over charcoal whereas Rojo and SUA 90 were longer (130 and 120 min, respectively).

Seventy-two farmers from Maharaka and Msongozi villages evaluated Pesa and check varieties at harvest for color of the dry seed, cooking time, broth and soup suitability, and taste. Fifty-two farmers evaluated marketability. Seventy-five percent found the color to be suitable or highly suitable. Cooking time was perceived as suitable or highly suitable by 81% of farmers. Broth suitability and taste were rated as suitable or better by 74 and 64% of farmers, respectively. Eighty-five percent of farmers thought that Pesa was suitable to highly suitable for market. Overall, farmers found Pesa to meet their criteria for a palatable variety with high potential marketability.

Availability: Pesa was approved for release by the Tanzania National Variety Release Committee with no restrictions on propagation and use. Small quantities of seed for research purposes are available from the bean breeding program at Sokoine University of Agriculture, Morogoro, Tanzania.

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AVALANCHE, A NEW NAVY BEAN FOR THE NORTHERN PLAINS

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ABSTRACT

Avalanche is a medium maturing, high yielding navy bean, with very good seed size, shape, and appearance. Avalanche has white flowers, dark green leaf color, is erect (Type II, short vine), with good lodging resistance. Avalanche exhibits good synchronous plant dry-down prior to harvest (both plant and pods mature concurrently). Maturity is earlier than Vista and Mayflower. The upright plant structure, combined with its synchronous dry-down, suggests that this line may be suitable for direct combining, given appropriate equipment and operator care.

PEDIGREE AND BREEDING HISTORY

Avalanche (previously coded as ND012103) is a navy bean line selected from the cross 96-177-01-01//Voyager/Black Knight, which was made in 1999. It was the final step of a hybridization series that involved crosses made back in 1985. This cross was an attempt to combine several traits such as good yield, earliness, erect architecture, desirable seed characteristics, and multiple disease resistance. 96-177-01-01 is a line from the NDSU dry bean breeding program involving several crosses with lines from Michigan State University (N90618, N90616, and N85007), ICA Bunsi, Northland, Norstar, and some other experimental lines from the NDSU breeding program. Ica Bunsi, N91618 and N90616 have some degree of resistance to white mold (Tu and Beversdorf, 1982; Kelly, personal comm.). Additionally, several of the lines involved in this cross have upright architecture. Voyager is a navy bean released by Rogers® in 1995. Black Knight is a black bean jointly released by the Florida, Idaho, and Cornell Agricultural Experimental Stations in 1998 (Halseth et al., 1998).

In the summer of 1999, the F_1 seed from this cross was grown in the field in Fargo, ND. Obtained F_2 seeds were then planted in spaced-plant rows at Hatton, ND in 2000 and individual plants were selected and harvested. Seed from each plant was grown in individual rows at the Puerto Rico winter nursery in 2001. The line coded as ND012103 was selected and F_4 seed was bulk harvested and sent back to ND. Given its excellent visual appearance for architecture, yield potential (pod load), maturity, plant growth habit, and lack of disease symptoms, the line was directly included in the navy preliminary yield trials (NPYT) at two locations in ND (Erie and Hatton) in the summer of 2001. Avalanche was one of the best lines in these trials and therefore it was moved up to the navy advanced yield trials (NAYT) for additional testing across years and locations. From 2002 to 2007, Avalanche has been tested at more than 24 environments across ND as well as other states. Avalanche has shown excellent performance across most of the environments tested, with yields superior to other navy commercial varieties (Table 1). Avalanche posses an upright structure with a type II growth habit (Kelly, 2000).

Avalanche is resistant to bean common mosaic virus (BCMV), but it is susceptible to the necrotic strain (BCMNV). Avalanche is moderately resistant to rust and it is being tested for anthracnose resistance. Canning tests made at Michigan State University showed that Avalanche was close to Vista in terms of visual appearance and quality. Limited amounts of seed can be obtained from the corresponding author.

ACKNOWLEDGEMENTS

The NDSU dry bean breeding program is able to release this navy line thanks to the support and effort of several people and institutions, including, the program staff, and other bean breeding programs such as Michigan State University for allowing germplasm exchange and field and disease testing. A special acknowledgement for all the long term support given by Northarvest Bean Growers Association, and the North Dakota Dry Edible Bean Seed Growers Association (NDDEBSGA). We also thank Drs. Phil Miklas and Marcial (Talo) Pastor-Corrales for their help with disease testing.

Table 1. Comparison of Avalanche with commercial check cultivars for agronomic and disease reactions summarized from several tests in ND.

Trait	Avalanche	Vista	Mayflower
Yield (kg ha^{-1})[1]	2402	2211	2166
Maturity (d)	102	103	107
100-weight seed (g)	18.4	18.2	18.7
Growth Habit[2]	II	II	II
Pl. Height (cm)	53	55	58
Lodging (0-9)[3]	2	1	1
Rust[4]	MR	R	R
BCMV[4]	R	R	R

[1] Average seed yield across 21 environments.
[2] Growth Habit = CIAT scale where I = determinate bush; II = upright, short vine (IIb tendency toward floppiness); III = prostrate vine (IIIa will be erect in certain environmental conditions); IV = indeterminate climber.
[3] Lodging scores 0 = 100% erect, 9 = no erect plants
[4] Rust and BCMV: R= Resistant, MR= Moderately Resistant, S=Susceptible

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RELEASE OF 'LARIAT' AND 'STAMPEDE' PINTO BEANS

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ABSTRACT

Lariat and Stampede are medium-early maturing, high yielding pinto bean, with very good seed size, shape, and appearance. Both cultivars have white flowers and seed with a very light background, light brown mottle and a yellow hilar ring. Lariat and Stampede growth habit is Type II upright, short vine, with very good lodging resistance. However, Lariat is usually taller and narrower than Stampede. Both lines exhibit very good synchronous plant drydown prior to harvest (both plant and pods mature concurrently). The improved plant structure, combined with its synchronous drydown, suggests that these lines may be suitable for direct combining, given appropriate equipment and operator care. Both lines are resistant to BCMV, rust, and anthracnose (Race 7).

PEDIGREE AND BREEDING HISTORY

Lariat pinto bean (previously coded as ND020069) is a selection from a hybridization series that which began in 1996: T1255/'Aztec'//'Winchester'/3/P96753/4/'Maverick'. It was an attempt to combine earliness with erect architecture, desirable seed characteristics, and disease resistance. T1255 and P96753 were pinto bean breeding lines obtained from Dr. James D. Kelly, Michigan State University. Line T1255 possess extremely erect architecture, but has late maturing. P96753 was both early and erect, but seed characteristics were not that of a typical pinto bean. Aztec, released by The Michigan Agricultural Experiment Station is a short, erect, early bean with very good seed quality, but low yield potential and susceptible to both BCMV and rust (Kelly et al., 1992). Winchester pinto bean (Rogers®) combines erect architecture with disease resistance (*I*-gene for BCMV, rust resistance, and some degree of white mold resistance), but possessed the 'green stem' trait (poor drydown) and has poor seed quality. Maverick pinto bean has excellent seed quality, is early with good drydown, and has shown considerable yield stability in North Dakota. Maverick is resistant to rust, but is susceptible to BCMV (Grafton et al., 1997). The final cross, designated 99-031 was made in the fall 1998 greenhouse season.

Stampede pinto bean (previously coded as ND020351) is a selection from the cross: 94-029-01-01/BDM-RMR-14.Line 94-029-01-01 is an NDSU breeding line selected from the cross: 88-075-08-01C//4-91-1/92BG-141. Line 88-075-01C is derived from the cross: 87-070-01/87-049-01, two breeding lines in the NDSU breeding program. These lines were developed from the following crosses: 87-070-01 = T295//T667/5-375; and 87-049-01 = CO81-12034/T295. Lines 4-91-1 and 5-375 were breeding lines from the USDA-ARS program at Beltsville, MD, to develop broad spectrum resistance to bean rust in pinto bean market class. Line 92BG-141 was a line from the USDA-ARS genetics program at Tropical Agricultural Research Service, Mayaguez, Puerto Rico. This line was derived from a population improvement program involving interspecific hybridization, including *Phaseolus coccineus*, which is a source of white mold resistance. Lines T295 (pinto) and T667 (navy) were breeding lines obtained from Dr. James D. Kelly, Michigan State University. Pinto bean breeding line CO81-12034 was obtained from Dr. Mark A. Brick, Colorado State University. BDM-RMR-14 is a germplasm release from USDA-ARS, Beltsville, MD, North Dakota State University, and Michigan state University, combining broad spectrum rust resistance and bean

common mosaic virus resistance (BCMV) into the pinto bean market class. This hybridization series, which began in 1996, was an attempt to combine erect architecture of navy bean and unique disease resistance genes with desirable seed characteristics in pinto bean. The final cross, designated 99-182, was made in the fall 1998 greenhouse. Stampede is homozygous for the dominant *I* allele, which confers resistance to bean common mosaic virus. In greenhouse rust tests using a collection of rust made locally, Stampede expressed a necrotic reaction typical of the reaction conferred by the dominant resistance alleles *Ur-3* and *Ur-11*. After many selections in several trials in and out of North Dakota, both Lariat and Stampede were entered into Preliminary Yield Tests in ND in 2002. From 2002 to 2007, both lines were tested at more than 35 environments across ND as well as other states. They showed excellent performance across most of the environments, with yields superior to other pinto commercial varieties (Table 1). Limited amounts of seed of both lines can be obtained from the corresponding author. We thank Northarvest Bean Growers Association and North Dakota Dry Edible Bean Seed Growers Association for their financial support. We also thank Drs. Phil Miklas and Marcial (Talo) Pastor-Corrales for their help with disease testing.

Table 1. Comparison of Lariat and Stampede with commercial check cultivars for agronomic and disease reactions summarized from several locations in North Dakota.

Trait	Lariat	Stampede	Buster	GTS-900	Maverick
Yield (kg ha^{-1})[1]	2800	2780	2716	2605	2460
Maturity (d)	100	96	94	99	95
100-weight seed (g)	38.6	37.9	36.5	35.2	34.3
Growth Habit[2]	II	II	IIIa	IIb	IIIa
Plant Height(cm)	66	62	51	57	57
Lodging (0-9)[3]	1	2	7	4	8
Rust	R	R	R	R	R
BCMV[4]	R	R	R	R	S
Anthracnose (7)[4]	R	R	R	R	R
(73)	S	S	S	S	S

[1] Average seed yield across 17 environments.
[2]Growth Habit = CIAT scale where I = determinate bush; II = upright, short vine (IIb tendency toward floppiness); III = prostrate vine (IIIa will be erect in certain environmental conditions); IV = indeterminate climber.
[3] Lodging scores 0 = 100% erect, 9 = no erect plants
[4] Rust and BCMV: R= Resistant, MR= Moderately Resistant, S=Susceptible

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RELEASE OF 'ZORRO' BLACK BEAN

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The Michigan Agricultural Experiment Station announces the release of 'Zorro', black bean (*Phaseolus vulgaris* L.) variety. Zorro was developed at Michigan State University and tested in Production Research Advisory Board Statewide Trials and Michigan State University Dry Bean Variety Testing Program as B04554. http://www.css.msu.edu/VarietyTrials/DryBean_HomePage.html

B04554 black bean breeding line was developed from the cross: B00103*/X00822 with one backcross to B00103. B00103 is a F4-generation sib of the black bean cultivar Condor and possesses many of same traits as Condor for yield, disease resistance and canning quality. The MSU black breeding line X00822 (B98311/VAX5) was selected to possess the drought tolerance of B98311 and the common bacterial blight resistance of VAX 5. The purpose of the cross was to introduce common bacterial blight resistance into high-yielding erect black beans and combine the resistance with anthracnose, virus, and white mold resistance and retain the good canning quality of future erect black bean varieties. B04554 was developed through pedigree selection combined with two seasons of mass selection in winter nursery trials and was entered into yield trials as $F_{4:6}$ breeding line in 2004.

Yield Performance:

B04554 has been tested for four years (2004-07) over 20 locations by MSU in cooperation with colleagues in Michigan and Washington. Over 20 locations, B04554 yielded 25.5 cwt/acre and significantly exceeded the yield of all entries except 115M at the locations tested. Yield ranged from a high of over 41 cwt/acre in Saginaw to a low of 14 cwt/acre in Presque County in 2006. Over the locations tested B04554 significantly outyielded all the commercial check varieties: Jaguar (13%), Condor (15%), T-39 (18%), Eclipse (15%), and Domino (18%). No significant yield difference was observed with breeding line 115M (Black Rhino) over 16 locations. Under the narrow row (20") width testing combined with direct harvest used at the Bean & Beet Farm in Saginaw, B04554 has yielded from 39-41 cwt/acre in 2005 and 2006 and appears well suited to this increasingly popular management system. B04554 topped yield tests in Saginaw in 2005 and 2006 and continued to show the same potential in 2007. It significantly outyielded a total of 643 black bean breeding lines trialed in the same four years of testing by11%.

Agronomic Features:

B04554 exhibits the type-II upright short vine (indeterminate) growth habit combined with resistance to lodging (<2). Plants average 51 cm in height, are more upright than Condor, and exhibit an overall upright appearance similar to Jaguar. B04554 is a mid-full season bean maturing 94 days after planting. The range in maturity is from 88-99 days depending on season and location. It matures with T-39, and Jaguar, is one day earlier than Condor and three days earlier than Domino. B04554 has demonstrated the same uniform maturity and dry-down as Jaguar. B04554 has a high agronomic acceptance rating due to its upright habit, resistance to lodging and excellent pod load and favorable high pod placement in the plant canopy.

Disease Resistance:

B04554 possesses the single dominant hypersensitive *I* gene which conditions resistance to seed-borne Bean Common Mosaic Virus (BCMV) but is sensitive to the temperature-insensitive-necrosis-inducing strains of BCMNV like NL 3 and NL 8. B04554 exhibits improved levels of tolerance to white mold [caused by *Sclerotinia sclerotiorum* (Lib.) de Bary] compared to Condor and has expressed levels of avoidance similar to Jaguar. B04554 is partially resistant to common bacterial blight [CBB caused by *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Dye]. Resistance was inherited from the X00822 parent that possessed resistance from VAX 5. Resistance was confirmed by colleagues at the Tropical Agriculture Research Station –TARS in Puerto Rico using the cut-leaf assay with two strains of CBB (strain #3353, 484A); [Mean CBB score =4.4; VAX 6 (R) =1.5; Morales (S) = 7.3]. At North Platte, NE B04554 was inoculated with two CBB isolates LB72 and SC4A; [Mean CBB score =5.5; XAN159 (R) =4.0; Othello (S) = 8.5]. In trials in Washington in 2007, B04554 exhibited resistance to root rot pathogens (likely *Pythium* and *Rhizoctonia*) present at Othello where plant stands were near perfect compared to other genotypes being tested. B04554 is resistant to race 7 but is susceptible to race 73 anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. et Magnus) Lams.-Scrib. B04554 is susceptible to rust [incited by *Uromyces appendiculatus* (Pers.:Pers.) Unger] race 53, similar to reaction of Jaguar and Condor.

Quality Characteristics:

B04554 has a typical small opaque black bean seed averaging 21 g/100 seeds and size ranges from 19-24 g/100 seeds. The seed is equivalent to T-39 in size, shape and color. In canning trials, B04554 has been subjectively rated by a team of panelists as being above average in cooking quality. B04554 rated 3.8 on a scale of 1 to 7 where 7 is best and 4 is mid scale (neither acceptable nor unacceptable). This evaluation is based upon whole bean integrity (no splitting or clumping); uniformity of size (uniform water uptake); black color (limited leaching); clear brine (no starch extrusion into canning liquid). Data on cooked color exhibited no differences between B04554 and other commercial black bean varieties. No data were collected on hydration and drained weight ratios as the canning procedure was modified to reduce color leaching and the blanch process was altered. The texture of 55 kg/100g was equivalent to other commercial black bean varieties except Domino that had a firmer texture (69 kg). Values are within the acceptable range of 45 to 75 kg/100 g for processed black beans. B04554 exhibited acceptable color retention compared to most commercial black bean varieties. Within the commercial black bean class, B04554 was equivalent to Jaguar in visual appearance, whereas Condor demonstrated the best overall canning quality.

Naming and Release Procedure:

Black bean breeding line B04554 was released as the variety **Zorro**. The recommendation is that Zorro will be available under license from Michigan State University, with the option that the variety be sold for seed by **name only** under the Foundation and Certified Seed classes. The variety will only be sold commercially as a class of Certified Seed under the three-class system used in Michigan. A royalty will be assessed on each hundredweight unit of Foundation Seed sold. Breeder Seed will be maintained by the Michigan Agricultural Experiment Station under license with the Michigan Crop Improvement Association. Plant Variety Protection (PVP) is anticipated, and parties interested in licensing Zorro may contact MSU Technologies.

RELEASE OF 'SANTA FE' PINTO BEAN

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The Michigan Agricultural Experiment Station announces the release of 'Santa Fe' pinto bean (*Phaseolus vulgaris* L.) variety. Santa Fe was developed at Michigan State University and tested in Production Research Advisory Board Statewide Trials and Michigan State University Dry Bean Variety Testing Program as P04205 http://www.css.msu.edu/VarietyTrials/DryBean_HomePage.html.

P04205 pinto bean breeding line was developed from the cross: P99119/G99750. MSU breeding line P99119 is an upright pinto with avoidance to white mold. It was derived from cross of P94211 and Matterhorn great northern. MSU breeding line G99750 (BDM-RMR-11/Matterhorn) is an upright GN line with avoidance to white mold. The purpose of the cross was to combine sources of avoidance to white mold and incorporate yield potential and desirable agronomic characteristics of Matterhorn GN into the pinto seed type. P04205 was developed using pedigree selection and one generation of mass selection in winter nursery trial and was entered into yield trials as a $F_{4:6}$ breeding line in 2004.

Yield Performance:

P04205 has been tested for four years (2004-07) over 15 locations by MSU in cooperation with colleagues in Michigan, North Dakota, Colorado, Nebraska (MRPN), Idaho and Washington. Over all 15 locations, P04205 yielded 22.6 cwt/acre. Yield ranged from a high of over 34 cwt/acre in Montcalm County to a low of 14 cwt/acre under stress in Presque Isle County in 2006. Under the narrow row (20") width testing combined with direct harvest used at the B&B Farm, P04205 yielded 23 cwt/acre in 2007 and appears well suited to this increasingly popular management system. No significant differences in yield were observed between P04205 and other pinto cultivars. Buster and Othello were slightly lower yielding but this may be a reflection of 2007 data as plots were direct harvested. The new La Paz pinto was higher yielding but was only compared at two locations in 2007. No significant differences were detected in yield in comparisons with Matterhorn.

Agronomic Features:

P04205 exhibits the type-II upright short vine growth habit combined with resistance to lodging. P04205 exhibits an overall upright appearance similar to Matterhorn and is more erect than either Buster or Othello pintos. Othello have a type III prostrate vine habit and Buster has an intermediate type IIb plant structure. The differences in erectness are reflected in the higher lodging scores for both Buster (3.1) and Othello (3.3) when compared to P04205, Matterhorn and La Paz (2.0). Plants of P04205 average 49 cm in height and are taller than Buster and Othello but slightly shorter than La Paz. P04205 is a mid-season bean maturing 90 days after planting, 2d later than Buster, 5 d later than Othello and 6 d earlier than La Paz. The range in maturity is from 88-92 days depending on season and location. P04205 has demonstrated the same uniform maturity and dry-down as Matterhorn but has a higher agronomic acceptance rating due to its upright habit, resistance to lodging and excellent pod load and favorable high pod placement in the plant canopy. The differences in plant structure and erectness between P04205 and other pintos are reflected in the higher visual scores for P04205.

The new La Paz variety exhibits many of the same desirable architectural traits but it is significantly later in maturity.

Disease Resistance:

P04205 possesses the single dominant hypersensitive *I* gene which conditions resistance to seed-borne Bean Common Mosaic Virus (BCMV) but is sensitive to the temperature-insensitive-necrosis-inducing strains of BCMNV like NL 3 and NL 8. P04205 is highly resistant to rust conditioned by the *Ur-3* gene and is essentially immune to the indigenous rust [incited by *Uromyces appendiculatus* (Pers.:Pers.) Unger] races prevalent in Michigan. P04205 exhibits avoidance to white mold [caused by *Sclerotinia sclerotiorum* (Lib.) de Bary] when compared to traditional prostrate pinto varieties. Over three years of testing under white mold pressure, P04205 yielded 28.8 cwt/a compared with yield of 14.3 cwt/a for the susceptible variety Beryl. In fact the highest yields for P04205 have been recorded under white mold pressure at the Montcalm test site. P04205 is susceptible to race 73 anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. et Magnus) Lams.-Scrib. P04205 has a similar level of susceptibility to common bacterial blight [CBB caused by *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Dye] as other commercial pinto bean varieties.

Quality Characteristics:

P04205 has a large pinto bean seed averaging 42 g/100 seeds and size ranges from 38-48 g/100 seeds. The seed is larger than other commercial pinto varieties: Buster (40g), Othello (37g), and La Paz (35g). In canning trials, P04205 has been subjectively rated by a team of panelists as being above average in cooking quality. P04205 rated 3.0 on a scale of 1 to 7 where 7 is best and 4 is mid scale (neither acceptable nor unacceptable). This evaluation is based upon whole bean integrity (no splitting or clumping); uniformity of size (uniform water uptake); color (no after darkening); clear brine (no starch extrusion into canning liquid). Data on cooked color, suggest that seed is slightly darker than Buster or Othello. The hydration and drained weight ratios exhibited no differences between P04205 and other commercial pinto bean varieties. Hydration ratios (2.0) were high while drained weight ratios (1.2) were low as the beans are blanched overnight to remove the color pattern. P04205 is similar to Othello in texture, whereas Buster is firmer in texture. Texture of 73 kg/100g is well within the acceptable range of 50 to 80 kg/100 g for processed pinto beans. P04205 has acceptable visual score compared to commercial pintos. Within the commercial pinto bean class, Othello demonstrated the best overall canning quality whereas Buster consistently exhibits inferior canning quality.

Naming and Release Procedure:

Pinto bean breeding line P04205 was released as the variety **Santa Fe**. The recommendation is that Santa Fe will be available under license from Michigan State University, with the option that Santa Fe be sold for seed by **name only** under the Foundation and Certified Seed classes. The variety will only be sold commercially as a class of Certified Seed under the three-class system used in Michigan. A royalty will be assessed on each hundredweight unit of Foundation Seed sold. Breeder Seed will be maintained by the Michigan Agricultural Experiment Station under license with the Michigan Crop Improvement Association. Plant Variety Protection (PVP) is anticipated and parties interested in licensing Santa Fe may contact MSU Technologies.

RELEASE OF 'FUJI' OTEBO BEAN

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The Michigan Agricultural Experiment Station announces the release of 'Fuji', Otebo bean (*Phaseolus vulgaris* L.) variety. Fuji was developed at Michigan State University and tested in Production Research Advisory Board Statewide Trials and Michigan State University Dry Bean Variety Testing Program as G05922 http://www.css.msu.edu/VarietyTrials/DryBean_HomePage.html.

OTebo ("Tebo") is a specialty bean class from Japan that is used to produce the confectionary known as 'An' bean paste. Tebo has a medium ovoid/round white seed (25-30 g/100seed) and a determinate (Type I) growth habit, but is highly susceptible to bean common mosaic virus (BCMV) and to certain races of anthracnose (caused by *Colletotrichum lindemuthianum*). In the year 2001 all production fields of Tebo in Michigan were infected with BCMV. The virus observed in Tebo seed produced in the state of Washington appears to be a new NL-4 type strain of BCMV. A crossing program was initiated to introduce BCMV resistance into the Tebo bean class by selecting for the *I* resistance gene from Matterhorn. Matterhorn GN was chosen as the donor parent since it was a medium sized white bean not dissimilar to the Tebo class in seed size and color.

The original cross between the commercial Hime Tebo variety and Matterhorn great northern was made in the greenhouse in E. Lansing, MI in 2001. The selection strategy of marker-assisted backcrossing (MABC) was used to screen the segregating populations assumed to carry the *I* gene as the SW-13 marker is tightly linked to the *I* gene. A major limitation to direct inoculation and selection for the *I* gene using the NL 3 strain of BCMNV is the top necrosis reaction and death of those plants carrying the *I* gene. Early generation crossing and selection was restricted to the greenhouse during the fall and spring seasons. Four backcross generations to Hime Tebo were conducted using MABC and following the last backcross the BC_4F_2 lines were screened for the SW-13 marker and selfed two generations in the greenhouse to produce seed for field evaluation. G05922 entered yield trials in 2005 and was trialed for three seasons in comparison with other BC lines and the commercial Hime Tebo. The highest-yielding lines were advanced based on similarity to Hime Tebo. Resistant to BCMNV, strain NL 3 was confirmed by inoculation in the greenhouse in E. Lansing using remnant seed of the $BC_4F_{4:6}$ lines. In addition resistance to race 73 of anthracnose was confirmed in all advanced lines including G05922. Based on BCMV resistance, consistent yield performance, and similarity to Hime Tebo in maturity, growth habit and appearance, G05922 was released as the variety **'Fuji'**. A seed sample was sent to Japan for evaluation and suitability in the 'An' paste product.

COMBINED DATA OVER 3-YEARS TEBO BEAN TRIALS

ENTRY	YIELD Cwt/acre	100 SEED WT. G	DAYS TO FLOWER	DAYS TO MATURITY	LODGING 1-5	HEIGHT cm	DES. SCORE	ANTRAC Race 73	BCMV NL 3
G05922	23.8	23.5	41.0	81.5	3.0	45.9	4.5	R	R
HIME TEBO	23.3	23.9	41.6	81.5	3.0	45.4	4.5	R	S
MEAN (30)	22.2	23.2	40.4	82.1	3.0	46.5	4.6		
LSD 0.05		1.1	0.7	0.9	0.1	1.0	0.6		
CV (%)		3.3	1.2	0.8	2.5	1.6	9.2		

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BEAN IMPROVEMENT COOPERATIVE
2007 FINANCIAL STATEMENT

BALANCE ON HAND January 1, 2007		$12,820.17
INCOME		
2007 Dues	3,773.00	
2007 Dues CD	853.00	
Back Issues	80.00	
Bic Meeting	0	
Bank Interest	284.86	
TOTAL INCOME	4,960.86	
EXPENSE		
Postage, Copy Charges and Office Supplies	1,753.45	
Printing Volume 50	2,895.00	
2007 BIC Annual Meeting – Loan	1,393.22	
Bank Charges	17.00	
TOTAL EXPENSE	6,058.67	
BALANCE ON HAND December 31, 2007		11,722.36